S.K. Gupta *Editor*

Technological Innovations in Major World Oil Crops, Volume 1

Breeding

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Editor
S.K. Gupta, Ph.D. (P.A.U.) P.D.F. (Cal)
Division of Plant Breeding and Genetics
Sher-e-Kashmir University of Agricultural Sciences
and Technology of Jammu, Chatha
Jammu and Kashmir, India
guptaskpbg@rediffmail.com

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Preface

Over the past decades, the production and the trade of the major world oil crops has increased tremendously in response to growing world population and rising living standard. Technological innovations in breeding major oil crops have led to higher yield and nutritionally superior edible oil. Despite of the fact that recent technological advances made in all the major oil crops, the need and opportunities to increase the production and oil yield are as great today as they have ever been. Realizing the importance of these crops in India, Canada, China, USA, Germany, Polland, Spain, Sweden, France, Australia and rest of the countries of the world, there is urgent need to upto date the knowledge of the recent technologies developed so far in enhancing the production at global level. The objective of editing this volume is to provide the latest references for those interested or involved in the genetic manipulation of these crops. This volume covers 13 chapters which have been well prepared by the leading scientists of the world with long experience and intensive knowledge of the subjects. It also contains the technological innovations not only related to breeding but also to nutritionists, biotechnologists and industrialists as well.

S.K. Gupta
Jammu and Kashmir, India

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Contributors

W.N. Ajambang Specialised Centre for Oil Palm Research of La Dibamba, Douala, Cameroon

V. Arondel Laboratoire de Biogenèse Membranaire, CNRS Université Bordeaux, Bordeaux Cedex, France

Aurora Díaz Instituto de Biología Molecular y Celular de Plantas-CSIC/ Universidad Politécnica de Valencia, Laboratory 0.08 Ciudad Politécnica de la Innovación, Ingeniero Fausto Elio, s/n-Escalera 8G, Valencia, Spain

José M. Fernández-Martínez Institute for Sustainable Agriculture (CSIC), Córdoba, Spain

B. Lalu Firman PT Astra Agro Lestari TBK, Jakarta, Indonesia

S.K. Gupta Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu and Kashmir, India

Lori Hinze USDA-ARS, Southern Plains Agricultural Research Center, TX, USA

G. Jilani Department of Agronomy, PMAS Arid Agriculture University, Rawalpindi, Pakistan

Sinisa Jocic Institute of Field and Vegetable Crops, Novi Sad, Serbia

Richard C. Johnson USDA-ARS, Western Regional Plant Introduction Station, Washington State University, Pullman, Washington, DC, USA

Yalcin Kaya Trakya Agricultural Research Institute, Edirne, Turkey

Theodore J. Kisha USDA-ARS, Western Regional Plant Introduction Station, Washington State University, Pullman, Washington, DC, USA

Russell Kohel USDA-ARS, Southern Plains Agricultural Research Center, TX, USA

P. Koona Specialised Centre for Oil Palm Research of La Dibamba, Douala, Cameroon

Jitendra Kumar Crop Improvement Division, Indian Institute of Pulses Research, Kanpur, India

Dragana Miladinovic Institute of Field and Vegetable Crops, Novi Sad, Serbia

M.Y. Mirza Crop Sciences Institute, National Agriculture Research Centre, Islamabad, Pakistan

T. Mohapatra National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, India

A.K. Mondal Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India

A. Mothilal Regional Research Station, Tamil Nadu Agricultural University, Vridhachalam, Tamil Nadu, India

A.K. Mubashir Crop Sciences Institute, National Agriculture Research Centre, Islamabad, Pakistan

U. Najeeb Crop Sciences Institute, National Agriculture Research Centre, Islamabad, Pakistan

Institute of Crop Science, Zhejiang University, Hangzhou, China

G.F. Ngando-Ebongue Specialised Centre for Oil Palm Research of La Dibamba, Douala, Cameroon

S.A.C.N. Perera Coconut Research Institute, Lunuwila, Sri Lanka

K.V. Prabhu Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Aditya Pratap Crop Improvement Division, Indian Institute of Pulses Research, Kanpur, India

Manmohan Sharma Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India

Naveen Singh Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

R.K. Solanki Crop Improvement Division, Indian Institute of Pulses Research, Kanpur, India

Sujata Vasudev Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Leonardo Velasco Institute for Sustainable Agriculture (CSIC), Córdoba, Spain

D.K. Yadava Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

W.J. Zhou Institute of Crop Science, Zhejiang University, Hangzhou, China

Chapter 1
Production and Trade of Major World Oil Crops

Manmohan Sharma, S.K. Gupta, and A.K. Mondal

Abstract Oilseeds are an important group of crop plants whose oil can be used for human consumption. There are about 40 different oil seeds whose oil can be consumed but only a few are significant in the total world trade. Oil crops are grown world over under varied agroclimatic situations and are vital commodities in the trade and commerce of many economies. The increase in production has occurred mainly due to rising demand for oilseed products and it has been possible mainly due to increase in area under the crop, as well as due to breeding of high yielding varieties. This has been supplemented with the advanced scientific production technologies which have resulted in high levels of per unit productivity, particularly in countries with high standards of agricultural production. Among the oilseed crops, soybean is the major contributor in world oilseed economy followed by rapeseed mustard, cotton, peanut and sunflower. The most important tropical oilseeds are the coconut, palm kernels and groundnut. The major oilseed producing areas are in the temperate zones. America and Europe together account for more than 60% of the world production of oil seeds whereas substantially small production (<5%) comes from tropical areas such as Africa, Malaysia and Indonesia. Both oilseed and oil production have consistently increased over the years to meet the ever increasing demand of vegetable oils. Among the oil seeds, soybean is the chief oil seed crop. *Brassica* species are the second largest oilseed crop after soybean (*Glycine max* (L.) Merr.) in the world oilseed production, surpassing peanut (*Arachis hypogaea* L.), sunflower (*Helianthus annuus* L.) and cottonseed (*Gossypium hirsutum* L.) during the last two decades (FAO (2010) Agricultural Outlook 2010–19). Palms are grown predominantly in the tropical areas of the world as perennial trees and are an important source of vegetable oil. About two-thirds of the total fat oil production is supplied by oilseeds, with palm oil having maximum share of 33%. Copra, cotton, palm, peanut, rapeseed, soybean and sunflower are the oilseed

S.K. Gupta (✉)
Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus, Chatha, Jammu and Kashmir 180 009, India
e-mail: guptaskpbg@rediffmail.com

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crops which dominate the international markets for trade purpose. Both imports and exports of oilseeds and their oils have shown a rising trend due to increased demand and supply of these commodities. China is a dominant importer for oilseeds as well as vegetable oils while the USA is a major exporter for oilseeds, and Indonesia and Malaysia for vegetable oils.

Keywords Oil crops • Agricultural production • World production • Vegetable oils • International markets • Imports and exports

1 Introduction

Plants are useful to human beings in a variety of ways. The crop plants are particularly useful for human consumption. These include cereals, pulses, oilseeds, vegetables, condiments, spices, medicinal and aromatic plants, etc. They are important because they are vital for sustaining human life, besides a variety of animals, birds, insects and many other living creatures. Oilseeds are an important group of crop plants whose oil can be used for human consumption. There are about 40 different oil seeds whose oil can be consumed (Lennerts 1983), but only a few are significant in the total world trade and supply of oilseeds. Oil crops are grown world over under varied agroclimatic situations and are vital commodities in the trade and commerce of many economies. Oil seed crops are of three distinct categories: the annual or biennial group which includes soybeans, sunflower, groundnuts and rapeseed; the perennial tree crops include coconuts and oil palms. Cotton and corn germ belong to third group in which embryo is a by-product. Other oilseeds are of minor importance in the world trade but they do play a significant role in local markets or serve as raw materials for special products. These include castor, safflower, linseed, tung nut, etc. Besides consumption in the form of oils as ingredients of human food, many of the oilseeds are used unprocessed in animal feed as well as in processed form as cakes or meals which are an important source of dietary proteins for animals.

2 Production Trends

There has been an increasing trend in average annual production of oilseed over years from 244.35 million metric ton (mmt) in 1997 to 395.13 mmt in 2008–2009 registering about 38% increase during this period. The increase in production has occurred mainly due to the rising demand for oilseed products and it has been possible mainly due to the increase in area under the crop, as well as due to the breeding of high yielding varieties. This has been supplemented with the advanced scientific production technologies which have resulted in high levels of per unit productivity, particularly in countries with high standards of agricultural production. Among the oilseed crops, soybean is the major contributor in world oilseed economy with production of 210.86 mmt in 2008–2009 accounting for 55–60% of the total oilseed

Table 1.1 Average annual production of major world oilseeds

	Year wise production (mmt)			
Oilseed crop	2005–2006	2006–2007	2007–2008	2008–2009
Copra	5.60	5.27	5.72	5.88
Cotton	43.47	46.00	45.91	41.26
Palm kernel	9.97	10.18	11.10	11.74
Peanut	33.22	30.72	32.29	34.15
Rapeseed	48.50	45.09	48.52	58.21
Soybean	220.67	237.12	221.14	210.86
Sunflower	30.04	29.84	27.02	33.03
Total	391.45	404.18	391.79	395.13

Source: FAO (2010)

Table 1.2 Average annual production of major world vegetable oils

	Year wise production (mmt)			
Oilseed crop	2005–2006	2006–2007	2007–2008	2008–2009
Coconut	3.46	3.22	3.53	3.63
Cotton	4.90	5.13	5.22	4.84
Olive	2.66	2.91	2.84	2.97
Palm	35.83	37.23	40.94	42.41
Peanut	4.97	4.51	4.90	4.97
Rapeseed	17.30	17.01	18.33	20.37
Soybean	34.62	36.36	37.54	36.09
Sunflower	10.60	10.61	9.85	11.73
Total	118.72	121.45	128.05	132.14

Source: FAO (2010)

production (Table 1.1). It is followed by rapeseed mustard (14.73%), cotton (10.44%), peanut (8.64%) and sunflower (8.34%). The most important tropical oilseeds are the coconut, palm kernels and groundnut.

However, with respect to oil production, palm oil dominates the world vegetable oil economy with production touching 42.41 mmt during 2008–2009 (Table 1.2). It contributes about 36% of the total world oil production. It is followed by soybeans and rapeseed which have a share about 29 and 15%, respectively, to the total vegetable oil production.

3 Supply and Distribution

The major oilseed producing areas are in the temperate zones. America and Europe together account for more than 60% of the world production of oil seeds whereas substantially small production (<5%) comes from tropical acres such as Africa, Malaysia and Indonesia. Table 1.3 indicates that the USA is a major contributing country in world oil seed production which contributes 20–25% of share to the total oil seed production. Brazil, Argentina, China and India are the other major contributors. These five countries produce 70% of the total oilseed production.

Table 1.3 Major oilseeds: world production and distribution (FAO 2010)

	Year wise production (mmt)			
Country	2005–2006	2006–2007	2007–2008	2008–2009
USA	95.97	96.84	82.45	89.20
Brazil	59.13	62.02	64.18	59.47
Argentina	45.03	53.16	51.71	35.69
China	56.80	55.23	53.35	57.79
India	30.70	29.92	33.95	33.70
Others	104.12	107.01	106.15	119.28
Total	391.45	404.18	391.79	395.13

Table 1.4 Major vegetable oils: world production and distribution

	Production trends (mmt)			
Country	2005–2006	2006–2007	2007–2008	2008–2009
Indonesia	18.25	19.37	20.98	22.73
Malaysia	17.50	17.20	19.73	19.41
China	14.76	14.27	14.69	16.02
EU-27	12.80	13.66	14.28	15.42
USA	10.38	10.41	10.53	9.65
Argentina	7.63	7.71	8.48	7.65
India	6.85	6.43	7.01	6.80
Others	30.55	32.42	32.36	34.48
Total	118.72	121.45	128.14	132.14

Source: FAO (2010)

Both oilseed and oil production have consistently increased over the years to meet the ever increasing demand of vegetable oils. With respect to oil production, Indonesia ranks first with production of around 22.73 mmt during 2008–2009. It is followed by Malaysia, China, EU, USA, Argentina and India. These countries together account for 70–75% of the total vegetable oil production (Table 1.4).

4 Oilseed Crops

4.1 Soybean

Among the oil seeds, soybean (*Glycine max* (L.) Merr.) is the chief oil seed crop. Increased demand for soybean has occurred due to the rising consumption of soya oil, and rapid growth in meal demand. Worldwide it was grown over an area of 96.66 mha with production of 210.86 mmt during 2008–2009 (Table 1.5). The USA is a major contributor (38%) of soybeans in world oilseed economy. It is followed by Brazil, Argentina, China, India, Paraguay and Canada. These countries together contributed about 96% to the total soybean production during 2008–2009. Turkey (35.71 q/ha), Italy (34.45 q/ha) and Egypt (30.26 q/ha) too contribute

Table 1.5 Soybean seed: production and distribution

	2006–2007		2007–2008		2008–2009	
Country	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)
USA	30.19	87.00	25.96	72.86	30.21	80.75
Brazil	22.05	59.00	20.57	61.00	21.27	57.00
Argentina	15.13	48.80	15.98	46.20	16.38	32.00
China	9.30	15.97	8.75	14.00	9.13	15.50
India	8.33	7.69	8.88	9.47	9.60	9.10
Paraguay	2.20	5.85	2.40	6.90	2.65	3.90
Canada	1.20	3.47	1.17	2.70	1.20	3.33
Others	6.62	9.34	6.18	8.01	6.22	9.28
Total	95.02	237.12	89.89	221.14	96.66	210.86

Table 1.6 Soybean oil: production and distribution

	Year wise production (mmt)			
Country	2005–2006	2006–2007	2007–2008	2008–2009
USA	9.25	9.29	9.33	8.50
China	6.15	6.41	7.04	7.31
Argentina	5.99	6.42	6.63	6.12
Brazil	5.43	5.97	6.16	6.24
EU-27	2.46	2.64	2.67	2.31
India	1.07	1.18	1.46	1.34
Mexico	0.67	0.68	0.64	0.61
Others	3.59	3.76	3.61	3.64
Total	34.61	36.35	37.54	36.07

substantially because of higher levels of productivity. Table 1.6 indicates that of total world soybean oil production, which amounted to 36.08 mmt, 30.48 mmt (84.50%) was contributed by USA (23.56%), China (20.27%), Argentina (16.96%), Brazil (17.30%) and EU (6.40%).

4.2 Rapeseed Mustard

Brassica species are the second largest oilseed crop after soybean (*G. max* (L.) Merr.) in the world oilseed production, surpassing peanut (*Arachis hypogaea* L.), sunflower (*Helianthus annuus* L.) and cottonseed (*Gossypium hirsutum* L.) during the last two decades (FAO 2010; Raymer 2002). Of the 37 species in the *Brassica* genus, the four most widely cultivated species for oilseed and vegetable production are *Brassica rapa* L., *Brassica juncea* (L.) Czernj and Cosson, *Brassica napus* L. and *Brassica carinata* A. Braun (Raymer 2002; Rakow 2004; Sovero 1993). The world's *Brassica* commerce consists mainly of seed produced from the two species *B. napus* and *B. rapa* in Canada and Australia (Rakow 2004; Raymer 2002). Rapeseed is the most favoured vegetable oil, in Europe for the manufacture of biodiesel and is

Table 1.7 Rapeseed mustard area and production in world

	2006		2007		2008	
Country	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)
Canada	5.24	9.00	6.33	9.60	6.49	12.64
China	5.98	10.97	5.64	10.57	6.59	12.10
India	7.28	8.13	6.79	7.44	5.75	5.83
Germany	1.43	5.34	1.55	5.32	1.37	5.15
Ukraine	0.39	0.61	0.80	1.05	1.38	2.87
Poland	0.62	1.65	0.80	2.13	0.77	2.11
UK	0.58	1.89	0.68	2.11	0.60	1.97
Australia	1.05	0.57	1.06	1.07	1.17	1.62
Others	2.97	6.93	3.78	9.23	4.11	13.92
World total	25.54	45.09	27.43	48.52	28.23	58.21

Source: FAO (2010)

in great demand there. It is the third leading source of vegetable oil in the world after soy and palm and is also the world's second leading source of protein meal. In Europe, rapeseed is primarily cultivated for animal feed due to its very high lipidic and medium proteinic content, and for the production of vegetable oil for biodiesel. Canola is a specific variety of rapeseed bred to have a low erucic acid content. Processing of rape seed for oil production provides a rapeseed animal meal as a by-product. The by-product is a high-protein animal feed.

Rapeseed was cultivated over an area of 28.23 mha with production of about 58.21 mmt making it the third most important oil plant in the world after palm oil and soybean. The leading producers in 2008 were Canada, China, India, Germany and Ukraine having production of 12.64, 12.10, 5.83, 5.15, 2.87 mmt and estimated areas of 6.49, 6.59, 5.75, 1.37 and 1.38 ma, respectively (FAO 2010). These countries along with Poland, UK and Australia contributed about 77% of the total rapeseed mustard production of the world during 2008 with Canada as the largest producer contributing 22% (Table 1.7). Germany has the highest productivity of rapeseed (37.60 q/ha) followed by United Kingdom (32.98 q/ha) and Czech Republic (29.38). Because of its high yields, European Union was the leading producer of rapeseed oil in 2008. Rapeseed mustard seems to be the fastest growing world source of edible oilseeds and is one of the few species with potential to meet the growing edible oil needs of many countries in Asia, Africa and America.

Winter type *B. napus* is the main rapeseed crop in most of Europe, in parts of China and also in the eastern United States. Spring type *B. napus* is produced in Canada, Northern Europe and China. Where winters are mild enough (e.g., southeastern United States) spring type *B. napus* can be grown. Spring type *B. rapa* occupies approximately 50% of the Canadian rapeseed area and is also grown in Northern Europe, China and India. Winter type *B. rapa* has largely been replaced by a more productive winter type *B. napus* and spring crops in its traditional production areas and has no significant impact on the world's rapeseed production at the present.

Only spring types exist in *B. juncea*. It is the leading *Brassica* oilseed in India and also produced in Canada and Europe but only for condiment use. Recently, low erucic acid, low glucosinolate types of *B. juncea* have been developed and it is

possible that in the future it will be an important oilseed crop for the more arid areas of Canada and the northern United States. The transition from high erucic to low erucic rapeseed, and the simultaneous rapid growth in the global rapeseed production began in Canada in 1968, with commercial release of single low cultivar "Oro" followed by several other single low cultivars and the first canola Cultivar "Tower" in 1974. In Europe, the transition started later with the release of the first single low cultivars in 1974. Almost all rapeseed produced in Canada and Europe is canola. The introduction of low erucic rapeseed is now underway in China and India. This change in crop quality has created a need for specialized production of industrial rapeseed. The oil cake is a better feed for cattle and poultry due to less quantity of glucosinolates (<30 μmoles/g oil free meal). It has been found to be at par with soybean meal with good potential of developing high value protein food and feed.

4.3 Cotton

Cotton (*Gossypium* spp.) is a major fibre crop of global importance and has high commercial value. Four out of 50 recognized cotton species in the world are cultivated. Two of them (*Gossypium arboreum* and *Gossypium herbaceum*) are diploid and the remaining two (*G. hirsutum* and *Gossypium barbadense*) are tetraploids. More than 80% of the world cotton area is covered by *G. hirsutum* and *G. barbadense*. However, diploid cottons are also in cultivation in Asia and Middle East. In India, all cultivated species and some of their hybrid combinations are commercially grown.

Cotton is cultivated in 70 countries worldwide with total coverage of about 34 mha and production of around 41.26 mmt of cotton seed and 4.72 mmt of cotton seed oil in 2008–2009 (FAO 2010). Over a quarter of the world cotton area is in India, followed by USA (16%), China (14%) and Pakistan (8%). The remaining production comes from Turkey, Australia, Greece, Brazil and Egypt.

The cotton is a dual purpose crop, producing both seed and fibre as valuable primary agriculture products. In the process of ginning the cotton boll, the fibre is separated from the seed and used in textile industry. The separated cotton seed is fuzzy at this stage and can be directly used as cattle feed or processed to obtain the cotton seed oil.

4.4 Palms

Palms are grown predominantly in the tropical areas of the world as perennial trees and are an important source of vegetable oil having utility as cooking oil besides a variety of uses in food and allied industries. Production of palm kernel was around 11.74 mmt; however, with respect to oil production, palm oil dominates the world vegetable oil economy with production touching 42.41 mmt during 2008–2009. It contributes about 36% of the total world oil production. The major palm oil producing countries are Indonesia, Malaysia, Thailand, Nigeria and Colombia which together contributed about 93% of total world palm oil production in 2008–2009 (Table 1.8).

Table 1.8 Palm oil: production and distribution

	Production (mmt)			
Country	2005–2006	2006–2007	2007–2008	2008–2009
Indonesia	15.56	16.60	18.00	19.50
Malaysia	15.49	15.29	17.57	17.26
Thailand	0.78	1.17	1.05	1.20
Nigeria	0.80	0.81	0.82	0.82
Colombia	0.69	0.77	0.83	0.76
Others	2.51	2.59	2.67	2.87
Total	35.83	37.23	40.94	42.41

Table 1.9 Groundnut: production and distribution

	2006		2007		2008	
Country	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)
China	3.98	12.81	3.97	13.08	4.62	14.34
India	5.62	4.86	6.29	9.18	6.85	7.34
Nigeria	2.22	3.83	2.23	3.84	2.30	3.90
USA	0.49	1.58	0.48	1.70	0.61	2.34
Myanmar	0.73	1.02	0.65	1.00	0.65	1.00
Others	8.43	6.62	7.68	3.59	9.49	5.23
World	21.45	30.72	22.30	32.39	24.52	34.15
Average world productivity		14.32		14.52		13.92

4.5 Groundnut

Groundnut (*A. hypogaea* L.) is the most important oilseed and a highly explored agricultural commodity. Groundnut is grown in about 84 counties on an area of 24.52 mha with the production of 34.15 mmt and average productivity of 13.92 q/ha. Among the groundnut producing nations, India sows the highest area (around 28% of total world area) under this crop and is the second largest producer, next only to China. In India, groundnut occupies about 6.85 mha area with a production of about 7.34 mmt (Table 1.9). China, India, Nigeria, USA and Myanmar contributed about 85% of the total world production of groundnut in 2008 (Table 1.8). China (31.02 q/ha), USA (38.29 q/ha), Nicaragua (36.10 q/ha), Turkey (34.33 q/ha), Egypt (33.99 q/ha) and Syrian Arab Republic (31.87 q/ha) are the countries having very high average yields and thus contribute significantly to the world pool of groundnut.

4.6 Sunflower

Among the oilseed crops, sunflower (*H. annus* L.) occupies fourth position in area and production in the world after soybean, rape seed mustard and groundnut. World harvest of sunflower was 33.03 mmt from an area of 18.98 mha during 2008 (Table 1.10).

Table 1.10 Sunflower: production and distribution

Country	2006		2007		2008	
	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)
Russian Federation	5.94	6.74	5.00	5.67	5.98	7.35
Ukraine	3.91	5.32	3.41	4.17	4.28	6.53
Argentina	2.17	3.76	2.35	3.50	2.58	4.65
India	2.12	1.23	1.88	1.46	2.05	1.11
China	0.99	1.80	0.72	1.19	1.04	1.85
Others	2.98	10.95	2.67	11.03	3.05	11.54
World	18.11	29.8	16.03	27.02	18.98	33.03

Table 1.11 Olive: production and distribution

Country	2006		2007		2008	
	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)	Area (mha)	Production (mmt)
Spain	2.48	5.68	2.60	6.22	2.60	6.22
Italy	1.17	3.42	1.16	3.43	1.21	3.51
Greece	0.80	2.43	0.80	2.44	0.80	2.44
Turkey	0.65	1.77	0.62	1.08	0.77	1.46
Tunisia	3.00	1.22	2.50	1.00	3.00	1.18
Others	2.37	4.21	2.42	3.37	2.45	3.51
World	10.47	18.73	10.10	17.54	10.83	18.32
Average world productivity (q/ha)		22.36		23.35		22.32

World sunflower production has remained almost stable over the last decade. Russian Federation, Ukraine, Argentina, India and China are the top five countries in the world with respect to production (21.49 mmt) and area under crop (15.93 mha). Europe alone accounts for about 50% of world area and total production. Asia and Africa share about 21 and 6% of the total sunflower area in the world, respectively. Average yield of the crop is significantly very high in countries like Crotia (31.03 q/ha), Austria (29.73 q/ha) and Switzerland (29.00 q/ha). USA, Romania, Bulgaria and Hungary are the other countries which have high potential for this crop.

4.7 Olive

The olive tree is the sixth most important oil crop in the world. World olive oil production is around 2.9 mmt, the Mediterranean countries being the major contributors (International Olive Council 2009, http://www.internationaloliveoil.org/downloads/production1_ang.PDF). Spain has largest area (2.60 mha) and highest production (6.22 mmt) followed in production by Italy, Greece, Turkey, Tunisia and Greece. These five countries accounted for 80.84% of the total world production (18.32 mmt) in 2008 (Table 1.11). Egypt has the highest productivity (63.60 q/ha) followed by

Peru (55.46 q/ha) and Australia (54.40 q/ha). USA, Chile, Mexico, Argentina and Slovenia are some of the other countries having higher productivity levels.

5 Trade and Supplies

The two important products obtained from oilseeds are oils/fats and meal/cake. Both the products are of great commercial value and hence the oilseeds are the commodities of great economic value. The proceeds from the sale of oil and cake cover the price of seed, which the miller has to pay, plus the processing costs. The profits obtained depend to a larger extent on the crushing capacities in relation to seed supply and product demand. Thus, oil and oilseed industry depends on commerce and business for three different types of markets, i.e. oil seed market, oil/fat markets and oilcake/meal markets. The government policies and laws, particularly with respect to import and export, are of great importance in day-to-day trading activities. The production of oilseeds is given in Table 1.1, but not all seeds are processed to obtain fat/oil. Part of oilseeds produced is used for sowing seed, fed unprocessed to animals or used directly for human consumption. Losses occur during storage and handling also. As a result 15–20% of seed produced remains unprocessed. About two-thirds of the total fat oil production is supplied by oilseeds, with palm oil having maximum share of 33%. In the production of vegetable oils, three annual crops, soybean, sunflower and rape and one tree crop, palm, predominate, accounting for 84% of all vegetable oils produced.

A review of Table 1.12 indicates that copra, cotton, palm, peanut, rapeseed, soybean and sunflower are the oilseed crops which dominate the international markets for trade purpose. Eighty to eighty-five percent of imports and exports are comprised of soybean trade alone. Both imports and exports of oilseeds have shown rising trends due to increased demand and supply of these commodities. The net import of oilseeds was 93.87 mmt during 2008 which was almost equivalent to the net export of 94.04 mmt during the same year. Oilseeds products such as protein cakes/meals for animals and specific by-products such as fatty acids increase the commercial value of these crops. A further perusal of Table 1.13 indicates that vegetable oil trade is dominated by palm oil accounting for 60–70% of total exports and imports. It is followed by soybean for its contribution in trade. The net import of oilseeds was 54.29 mmt and net export being 55.32 mmt during 2009–2009.

Among the major importers of oilseeds, China ranks first with net import of 44.14 mmt accounting for 47% of the total imports during 2008–2009 (Table 1.14). The other major importers of oilseeds include EU, Japan, Mexico, Taiwan, Turkey, Indonesia, Thailand, Egypt and South Korea. They along with China account for 85–90% of the total world imports of oilseeds. On export front, USA is the dominant player having share of 35–38% in total world exports. The other major exporters of oil seeds are Brazil, Canada, Argentina, Paraguay, Ukraine and Uruguay.

With respect to trade of vegetable oils (Table 1.15), China is again the net importer while Indonesia is the net exporter due to surplus production of palm oil in

Table 1.12 Major oilseeds: trade

	Quantities traded							
	Imports (mmt)				Exports (mmt)			
Crop	2005–2006	2006–2007	2007–2008	2008–2009	2005–2006	2006–2007	2007–2008	2008–2009
Copra	0.07	0.09	0.11	0.10	0.10	0.13	0.13	0.11
Cotton	1.10	0.84	0.75	0.55	0.96	0.84	0.84	0.48
Palm	0.15	0.13	0.14	0.13	0.18	0.15	0.10	0.15
Peanut	1.94	1.98	2.07	1.93	2.25	2.43	2.37	2.25
Rapeseed	6.68	7.01	7.57	12.2	6.98	6.63	8.13	12.06
Soybean	64.13	69.06	78.12	77.17	63.8	71.31	79.53	76.79
Sunflower	1.39	1.75	1.25	1.80	1.52	1.88	1.41	2.2
Total	75.45	80.86	90.00	93.87	75.79	83.36	92.51	94.04

Source: USDA (2010)

Table 1.13 Major vegetable oils: trade

	Quantities traded (mmt)							
	Imports				Exports			
Crop	2005–2006	2006–2007	2007–2008	2008–2009	2005–2006	2006–2007	2007–2008	2008–2009
Coconut	1.99	1.88	1.93	1.61	2.08	1.74	1.93	1.52
Cotton	0.07	0.08	0.09	0.07	0.18	0.19	0.21	0.19
Olive	0.58	0.65	0.59	0.55	0.61	0.71	0.65	0.68
Palm	28.15	29.70	32.90	36.63	29.08	29.62	34.79	36.79
Peanut	0.16	0.18	0.15	0.14	0.20	0.16	0.15	0.20
Rapeseed	1.47	2.2	2.02	2.44	1.67	2.00	1.93	2.38
Soybean	9.09	9.93	10.4	8.85	9.84	10.57	10.87	9.06
Sunflower	3.23	3.39	2.66	3.99	3.95	3.96	3.36	4.50
Total	44.74	48.01	50.73	54.29	47.60	48.94	53.88	55.32

Source: USDA (2010)

Table 1.14 Oilseeds: major trading partners

Imports (mmt)				Exports (mmt)			
Country	2006–2007	2007–2008	2008–2009	Country	2006–2007	2007–2008	2008–2009
China	29.7	38.64	44.14	USA	31.65	33.05	35.80
EU-27	17.16	17.03	18.01	Brazil	23.54	25.44	30.06
Japan	6.55	6.52	5.77	Canada	7.26	7.64	10.00
Mexico	5.43	5.29	4.77	Argentina	10.23	14.40	6.14
Taiwan	2.44	2.16	2.22	Paraguay	4.40	5.53	2.54
Turkey	1.94	2.14	1.65	Ukraine	1.24	1.17	3.68
Indonesia	1.51	1.42	1.62	Uruguay	0.78	0.84	1.00
Thailand	1.58	1.82	1.57				
Egypt	1.34	1.08	1.59				
South Korea	1.41	1.38	1.31				
Others	11.79	12.54	11.22	Others	4.27	4.44	4.81
Total	80.86	90.00	93.87	Total	83.36	92.51	94.04

Source: USDA (2010)

Table 1.15 Vegetable oils: major trading partners

Imports				Exports			
Countries	2006–2007	2007–2008	2008–2009	Countries	2006–2007	2007–2008	2008–2009
China	8.50	8.76	9.77	Indonesia	13.39	16.07	16.78
EU-27	9.11	8.80	8.84	Malaysia	13.73	15.73	17.02
India	5.44	5.93	8.76	Argentina	6.87	7.05	5.76
USA	2.53	3.11	3.23	Ukraine	1.89	1.35	2.16
Pakistan	2.25	2.28	2.24	USA	1.33	1.68	1.46
Egypt	1.20	1.27	1.58	Canada	1.30	1.36	1.57
Malaysia	0.85	1.17	1.27	Brazil	2.50	2.44	1.96
Iran	1.21	1.28	1.10	–	–	–	–
Bangladesh	1.23	1.13	1.00	–	–	–	–
Turkey	0.61	0.84	0.82	–	–	–	–
Others	15.1	16.16	15.67	Others	7.94	8.21	8.62
Total	48.01	50.73	54.29	Total	48.94	53.88	55.32

Source: USDA (2010)

the country. China along with India and EU accounted for 50% of total imports while Indonesia, Malaysia and Argentina accounted for 40% of total world exports of vegetable oils in 2008.

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Chapter 2
Breeding Major Oil Crops: Present Status and Future Research Needs

D.K. Yadava, Sujata Vasudev, Naveen Singh, T. Mohapatra, and K.V. Prabhu

Abstract Oils extracted from plants have been used predominantly as edible oil. Soybean, peanut, rapeseed mustard, sunflower, safflower, *Sesamum*, linseed, castor and cotton seed are predominant oil crops. Global status of nine major and minor oil crops has been discussed which includes their classification, contribution, major growing countries and objectives. Major objectives in oil crop improvement are enhancement of seed and oil yield, quality of oil according to its use, i.e. edible or industrial uses, breeding of varieties which fit in different cropping systems and breeding biotic and abiotic stress resistant/tolerant varieties. Achievements in varietal development programme of nine oil crops in India have also been discussed and future research needs to meet the increasing demand have also been highlighted. This review describes developments in use of biotechnological tools in seven edible oil crops, namely, *Brassica*, soybean, sunflower, groundnut, *Sesamum*, linseed and safflower and also highlights the prospects of using markers in genetic improvement of these crops. Molecular markers reported for genetic diversity assessment, mapping and tagging genes/QTLs for different qualitative and quantitative traits and their use in marker-assisted selection have been presented.

Keywords Oil crops • Breeding objectives • Research needs • Gene mapping • Genome maps • Molecular markers

1 Introduction

Oils extracted from plants have been used since ancient times and have been exploited in many ways. Predominantly, it is used as edible oil. It is also used in medicines and pharmaceuticals, industries, biodiesel, pet foods and component of

D.K. Yadava (✉)
Division of Genetics, Indian Agricultural Research Institute, New Delhi-110 012, India
e-mail: dkygenet@gmail.com

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many other products. Dietary fat, a concentrated source of energy, supplies about half of the calories and carries fat soluble vitamins. Its by-products are being used as feed, manures and find uses in many other industrial and domestic uses. There is large number of oil crops grown worldwide. Amongst them soybean, peanut, rapeseed mustard, sunflower, safflower, *Sesamum*, linseed, castor, cotton seed are predominant. Total world's oilseed production from major oil crops was 423.55 mt from 205.08 million hectares (mha) area during 2009–2010 (http://www.fas.usda.gov/psdonline). The leading countries in oilseed production are USA, Brazil, Argentina, China and India. The crop wise pretext is discussed here.

2 Soybean

Soybean (*Glycine max* L. (Merrill), $2n=40$) a *Papilionaceae* family plant is the most important grain legume in terms of production and international trade. In addition to high protein content (40%), the soybean seeds contain 18–23% oil and thus add to the importance of the species as an edible oil yielding crop. Soybean accounted for 57% of the world's oilseed production. Soybean has the longest recorded history of cultivation among crop plants dating back to Chou dynasty in 664 BC and northern China is considered as the centre of its domestication. In India, this crop was grown in isolated areas since ancient times. The feasibility trial conducted during 1963–1964 with the introduced material from the USA marks its modern cultivation in this country. Systematic breeding programme was initiated at Pantnagar and Jabalpur and later at many other centres, which led to the development of a large number of improved varieties. Introduction of this temperate crop to sub-tropical climatic conditions made it more vulnerable to problems like seed longevity, poor growth rate due to changed photoperiod, various biotic and abiotic stresses, etc (Hegde 2009a).

2.1 Objectives

The most important breeding objective is yield enhancement. Since this crop is mainly grown under rainfed condition, genetic enhancement of yield under rainfed situation has been a major challenge. Tailoring of high yielding plant type includes the desirable features like determinate to semi-determinate growth habit, erect and non-lodging with 100–105 days maturity to escape moisture stress. On the other hand, some hybrids are reporting around 20% heterosis and the male sterile systems for seed production are available; however, the efficient pollen transfer mechanism is a major obstacle in exploitation of hybrid vigour in soybean. Genetic variability for oil quantity and quality is available. Indirect selection for seed density and specific gravity would lead to high oil yields. Beside quantity, varieties with high oleic acid and low linolenic acid need to be developed.

Other important objective is to enhance seed longevity. Soybean is known for its poor storability. Due to vulnerable position of its embryo, it is highly sensitive to injury. Deterioration in seed quality is very fast particularly under tropical climate during storage. Many small seeded varieties have better germination than bold seeded ones. This undesirable association needs to be broken and is possible through breeding. Although bold seeded genotypes with high seed longevity have also been reported. Varieties which are resistant to mechanical damage and maintain more than 70% germination after 8–9 months of ambient storage need to be developed. Accelerated aging, electrical conductivity and vigour tests are generally used for screening and identification of promising lines from the breeding material.

Stability of performance is of utmost importance in soybean. Early maturity and photoperiod insensitivity are prerequisites for better adaptability and its suitability to different cropping systems. Early maturity would further help in combating the terminal drought through escape mechanism. Host plant resistance against diseases like rust, root rot, stem canker, bacterial blight, yellow mosaic virus and insects like stem borers, gram pod borers and sting bug would further help in stabilizing the yields.

3 Brassicas

The *brassicas* commonly known as rapeseed mustard are important group of edible oils and vegetables crops belonging to *Brassicaceae* or *Cruciferae* family. This group comprises of six cultivated species, namely, *Brassica campestris*/*rapa* ($2n = 20$, AA), *Brassica nigra* ($2n = 16$, BB) and *Brassica oleracea* ($2n = 18$, CC) are diploids; *Brassica juncea* ($2n = 36$, AABB), *Brassica napus* ($2n = 38$, AACC) and *Brassica carinata* ($2n = 34$, BBCC) are digenomic tetraploids, which evolved in nature following hybridization between the constituent diploid species. Rapeseed mustard is the third most important source of vegetable oil in the world and is grown in more than 50 countries across the globe. China, Canada, India, Germany, France, UK, Australia, Poland and USA are the major cultivators of different species. The estimated area, production and yield of rapeseed mustard in the world during 2009–2010 was 30.74 mha, 59.93 mt and 1.95 t/ha, respectively. Globally, India account for 21.7% area and 10.7% production (USDA 2010). During the last 7 years, there has been a considerable increase globally in productivity from 1.54 t/ha in 2003–2004 to 1.95 t/ha in 2009–2010 and production from 39.42 mt in 2003–2004 to 59.93 mt in 2009–2010. *Brassica rapa, B. napus* and *B. juncea* are grown predominantly for oil and seed meal. India is the second largest country in rapeseed mustard production and more than 85% of its area under rapeseed mustard is occupied by Indian mustard *B. juncea* (L.) alone. At present, the maximum average productivity in our country is around 1.19 t/ha (2008–2009) which is much below than that of the other *Brassica* growing countries. In UK, France and Germany, the average productivity of rapeseed mustard is two to threefold higher than India and the world average is also more than

50% higher than that of India. A reason for this low productivity is mainly the poor stability of performance despite availability of improved varieties with high yield potential. Production and productivity statistics of past decade and their relationship with weather parameters and disease outbreak indicates that the main reasons of fluctuation are unpredicted rainfall (drought or untimely rains), high temperature at different growth stages and infestation of diseases and insect pests like white rust, *Alternaria* blight, *Sclerotinea* stem rot, powdery mildew, downy mildew and aphid.

3.1 Objectives

For breaking the yield barrier, population improvement programme was followed involving diverse parents. Through the intervention of biotechnological tools, yield QTLs are to be identified and can be introgressed in improved backgrounds using marker-assisted selection (MAS). Poor plant stand is one of the factor for non-realization of actual yield potential in timely sown crop, which is mainly because of high temperature at seedling stage. If late sowings are done, high temperature at reproductive stage leads to forced maturity resulting in reduced yield with low oil content. Hence, genotypes having inbuilt tolerance to high temperature at seedling stage as well as terminal heat tolerance are the need of hour. Mustard being a crop of marginal lands, genotypes with inbuilt mechanism to yield higher under scanty moisture conditions are required. Hence, genotypes with high water use efficiency can be exploited. Fertilizers applied are not used efficiently; hence development of high fertilizer use efficient lines is also required. Salinity is becoming one of the limiting factors in *Brassica* production which needs attention. Genotypes tolerant to heavy metals and enhanced CO_2 utilization also need attention in the times to come.

There is no resistant sources available for *Alternaria* blight, *Sclerotinea* stem rot, aphid and painted bug, and trans genes are the option for development of transgenic *Brassica* having inbuilt resistance for these biotic stresses. Pyramiding the genes/QTLs for various biotic and abiotic stresses using the plant biotechnology tools has to be explored.

It is realized that the improvement through the use of conventional breeding approaches is tending to level off, since these breeding approaches do not mobilize sufficient amount of genetic variation, whereas hybrids offer an opportunity for mobilizing greater amount of genetic variability and available high heterotic response in Indian mustard. In order to increase the yield potential of *Brassica,* hybrids are one of the most viable options for breaking the yield barriers. Presently, there is about 15% yield increase in case of hybrids. Diverse cytoplasmic sources may give high heterotic hybrids under three line hybrid development programme. For saving the time, the introgression of CMS/restorer system to the identified combiners should be taken up through marker-assisted backcross breeding. Genotypes with high harvest index, basal branching from ground level, long and higher primary and secondary branches with synchronous higher number of siliquae are desirable

for yield enhancement. Quality is an important concern in the times to come. Breeding of Canola types Indian mustard varieties is the need of the hour to make this crop globally competent. Oil content also needs to be enhanced from the average 38–39% to that of >45%.

4 Sunflower

Sunflower (*Helianthus annuus* L., $2n = 34$) an *Asteraceae* family plant is native to the temperate North America, which is the centre of diversity for this important edible oil-yielding species. Sunflower is grown in all continents. Europe and America account for nearly 70% of total area and 80% of total production (Damodaran and Hegde 2007). Its cultivation in Asian countries is comparatively recent. Asia accounts for nearly 20–22% of the global sunflower and contributes to about 18% of the production. The productivity of sunflower in Asia is about 1.0 t/ha which is lower than the world average. India is the largest grower of sunflower in the Asian continent. This is a short duration crop which is adaptable to a wide range of agroclimatic situations, having high yield potential, suitable for cultivation in all seasons due to its day neutral nature and can fit well in various inter and sequence cropping systems. However, the average yield of this crop in India is lowest; it is less than half the world average, and static hovering around 0.5–0.6 t/ha. Emergence of new diseases and large climatic variations, particularly recurrence of drought stress during critical growth stages, has affected stability and yield on a regular basis. Therefore, there is a need to reorient the breeding objectives considering the adverse agroecosystems or target population of environments where the crop is grown.

4.1 Objectives

Hybrids in sunflower have recorded two times higher seed yield than the open pollinated varieties. Narrow genetic base is the major bottleneck in further improving the yields. Release of large number of hybrids in the past has broadened the base of hybrids in the country. Still there is need to improve the diversity of the parental lines to achieve the higher level of heterosis in sunflower. Diversification of male sterility source may also help in improving stability of the hybrids. Beside seed yield, oil content, which is hovering between 35 and 40% in hybrids, is also equally important and needs to be improved up to 45% so that this crop may be made more profitable.

For stabilizing the yields, host plant resistance against major diseases like downy mildew, *Alternaria* leaf spot, rust and viral necrosis and insects like capitulum borer, tobacco caterpillar, Bihar hairy caterpillar, green semilooper, cabbage semilooper, cut worms, leaf hoppers and thrips is required in the parental stocks for their exploitation through hybrids. Introgression of resistance against major insects like *Heliothis*

and *Spodoptera, Bt* sunflower needs to be developed. Identification of resistance/tolerance sources for drought related traits and their subsequent transfer in the improved genetic background would help in achieving the stability of production in diverse rainfed areas.

Being a crop of all seasons this crop is grown continuously after harvest of the earlier one which has led to micronutrient deficiency, toxicity and complex of diseases and insect pests leading to low yields. Breeding/management input is required to address this issue.

5 Groundnut

Groundnut (*Arachis hypogaea* L., $2n=40$), a *Papilionaceae* family plant, is an allotetraploid having South American origin. Recent studies have indicated that it originated in northern Argentina or southern Bolivia from hybridization between the diploid wild species *Arachis duranensis* and *Arachis ipaensis*. It is the fourth most important oilseed crop in the world, grown mainly in tropical, subtropical and warm temperate climates. It is presently cultivated in 108 countries of the world. Asia with 63.4% area produces 71.7% of world groundnut production followed by Africa with 31.3% area and 18.6% production, and North-Central America with 3.7% area and 7.5% production. Important groundnut producing countries are China, India, Indonesia, Myanmar, Thailand and Vietnam in Asia; Nigeria, Senegal, Sudan, Zaire, Chad, Uganda, Republic of Ivory Coast, Mali, Burkina Faso, Guinea, Mozambique and Cameroon in Africa; Argentina and Brazil in South America and USA and Mexico in North America (Hegde 2009a). Its seeds are a rich source of edible oil (43–55%) and protein (25–28%). About two-thirds of world production is crushed for oil and the remaining one-third is consumed as food. Its cake is used as feed or for making other food products and haulms provide quality fodder. In India, it ranks third after soybean and *Brassica*s. Domesticated groundnut exhibits a considerable amount of genetic variation for morphological traits such as growth habit, seed colour and size, number of seeds per pod and patterns of flower production on the stems. Besides, variation exists for the nature of reaction against pathogens and insects.

5.1 Objectives

The major breeding objectives in this crop are development of high yielding cultivars of suitable duration to escape moisture stress with resistance to various biotic stresses (foliar diseases like rust and early and late leaf spots and aflatoxin contamination by *Aspergillus flavans*, pod and stem rot, etc.) and tolerance to different abiotic stresses (moisture stress). Continuous efforts have yielded genetic resistance for these diseases. Short and medium duration and confectionery type varieties with multiple tolerance/resistance have been developed by ICARISAT as well as NARS

in India. Significant progress has been seen in understanding and underlying the mechanism of drought tolerance in groundnut. As it has been established that yield under water limited conditions is a function of transpiration (T), transpiration use efficiency (TE) and harvest index (HI), large exploitable genetic variation has been observed in germplasm of groundnut for these traits (Rachaputi and Wright 2003). There is a need to develop a selection index integrating T, TE and HI with appropriate weights for use as selection criteria in a breeding programme (Chandra et al. 2003). In addition to resistance/tolerance to the prevailing biotic and abiotic stresses, a variety for becoming successful should be in harmony with the edaphic and climate factors of ecosystem. The duration of the variety, irrespective of its growth habit should match with the period of soil moisture availability particularly under rainfed situations. Novel techniques such as genetic transformation, molecular markers added selection and gene transfer from alien sources need to be exploited more for making an impact on groundnut research.

6 Sesamum

Sesamum (*Sesamum indicum* L. $2n=26$) belongs to the family *Pedaliaceae* which has a wide distribution, covering tropical Africa, Madagascar, Arabia, India, Sri Lanka, tropical Australia and a few of the eastern islands of the Malayan Archipelago. It is an ancient oil yielding crop. Due to the presence of diverse wild species, Africa is considered the primary centre of origin, while India and Japan are considered as the two secondary centres of origin of this crop. India, China, Sudan, Mexico, Turkey, Burma and Pakistan are the important *Sesamum* producing countries. India ranks first, both in the area and production of this crop in the world. The annual area put under it in India is about 2.5 mha (45% of the world hectarage) and the total production is nearly 52,000 t. Its seeds contain 45–52% of edible oil (Hegde 2009b).

6.1 Objectives

Higher yields, improved plant architecture, adapted crop duration, resistance to diseases and pests and indehiscent capsules are the major objectives in this crop. The degree of dehiscence is a cultivar characteristic and is of great importance for mechanized harvesting. The leaf eating caterpillar (*Antigastra catalaulmlis* Dup.) and the gallfly (*Asphondylia sesalili* Felt) are the serious pests of *Sesamum*. Stem and root rot (*Macrophominia phaseoli* Maubl.), phyllody (virus, mycoplasina), bacterial leaf spot (*Pseudomonas sesami* Matkoff) and leaf curl are the important diseases of this crop which needs genetic interventions. Among the various options available for increasing the productivity, heterosis breeding is perhaps the most important way for the vertical yield increase in this crop. China has the distinction of successful exploitation of heterosis in this crop at commercial level with hybrid developed

through hand emasculation, GMS/CMS systems and exhibited the yield potential up to 3.0 t/ha. The programme on development of CMS lines through interspecific hybridization needs to be strengthened for exploiting some workable CMS system in this crop for hybrid development programme. This crop has been ignored for value addition to its oil. The development of varieties with low or zero anti-nutritional factors like oxalic and phytic acids needs attention for its value addition. In addition, the efforts should also be made to develop low free fatty acid (<2%) varieties of *Sesamum*. Increase in oil content is also one of the important components in varietal improvement of this crop.

7 Linseed

Linseed (*Linum usitatissimum* L., $2n = 30$) is a diploid, self-pollinated and homozygous species of *Linaceae* family. This genus comprises mostly herbs and shrubs in tropical and subtropical region. It is an important oilseed crop grown both for seed and fibre. It is an industrial oilseed crop and its each and every part has commercial and medicinal importance. India ranks second after Canada in terms of area and is at the fourth position in production after Canada, China and USA. The productivity of this crop is very low as it is grown under input starved and moisture stress conditions. The major diseases of this crop are wilt, rust, powdery mildew and *Alternaria* blight. Amongst the insects, bud fly is causing lot of losses to this crop.

7.1 Objectives

The average productivity of this crop at national (0.4 t/ha) as well as at global level (0.85 t/ha) is low in comparison to other oil crops like soybean, rapeseed mustard and groundnut. Hence, the breeding strategies for yield enhancement need immediate attention. Oil content is one of the important components in oil crops and it is around 28–30% in linseed varieties which has ample scopes for enhancement. Linseed oil has more than 50% linolenic acid which is fit for its industrial application but where linseed oil is being used as edible oil, the linolenic acid needs to be reduced. Efforts in this direction have already been successful with the development of low linolenic acid varieties LINOLA in Australia in 1984 and SOLIN in Canada in 1990. In India too national linseed programme in collaboration with BARC, Mumbai has developed some genotypes with less than 1% linolenic acid. Hence, the breeding efforts are needed further for development of low linolenic acid varieties, the oil of which can be widely used as cooking oil. As linseed is highly nutritious, efforts are needed to reduce its anti-nutrient components and also bio-convert its less acceptable omega-3 ALA into acceptable SDA. For achieving this objective, in addition to the conventional breeding, the biotechnological tools like marker-assisted breeding and genetic engineering may also be employed. Moisture stress being one

of the major constraints, the varieties with inbuilt water stress tolerance may be given more emphasis to enhance and stabilize the productivity for making this crop more remunerative. More concerted efforts for development of varieties resistant to different diseases like wilt, rust, powdery mildew and *Alternaria* blight are also required by using the different resistant donors already available in this crop.

8 Safflower

Safflower (*Carthamus tinctorius* L., $2n=24$) is a member of the family *Compositae* or *Asteraceae*, cultivated mainly for its seed, which is used as edible oil and as birdseed. Traditionally, the crop was grown for its flowers, used for colouring and flavouring foods and making dyes, especially before cheaper aniline dyes became available, and in medicines. Oil has been produced commercially and for export for about 50 years, first as an oil source for the paint industry, now for its edible oil for cooking, margarine and salad oil. Over 60 countries grow safflower, but over half is produced in India (mainly for the domestic vegetable oil market). Production in the USA, Mexico, Ethiopia, Argentina and Australia comprises most of the remainder. China has also significant area under safflower (Li and Hans-Henning 1996). Varietal improvement programme on safflower was initiated during 1935 in India which resulted in release of some varieties specific for limited areas. The All India Coordinated Research Project on Safflower was established in 1972 which led to the development of 29 varieties and hybrids for different safflower growing areas of the country (Hegde 2009a).

8.1 Objectives

The average productivity of safflower is still low (0.65 t/ha) in comparison to 1.4–2.3 t/ha in other parts of the world. This necessitates in breeding varieties with enhanced yield potential. With the availability of GMS systems in this crop, hybrid development has become reality in 1997. Now the cytoplasmic genetic male sterility system is a new hope to develop the high yielding publicly acceptable hybrids surpassing the problems associated with GMS-based hybrids. Safflower hybrids can offer greater stability in less favoured environments subject to biotic and abiotic stresses. It is evident from high yield performance of safflower hybrid DSH 129, which yielded about 18% higher seed yield than varieties under wilt, moisture and P stress conditions under large-scale field demonstrations (Reddy et al. 2004). There is a possibility of development of hybrids with high oil content, disease/insect resistance and abiotic tolerance by choosing the appropriate parental lines.

Varieties tolerant to drought will also definitely help in enhancing the productivity of this crop as about 80% reduction in the yield of safflower has been reported due to prolonged moisture stress. For immediate future need, the exploration of germplasm for moisture stress tolerance is required by designing appropriate

screening techniques. Germination under saline soils followed by seedling survival and establishment is very important for appropriate plant stand leading to higher economic yields. For the precise selection of parental lines under abiotic stress resistance breeding programme, the physiological, biochemical and morphological traits responsible for resistance, their relation with economic yield and the genetic diversity in these traits need to be determined (Sinclair et al. 2004).

The oil content in the released varieties is ranging from 28 to 30% which needs an increase of 5–8% in this crop. In addition to different conventional breeding methods, mutation breeding and genetic engineering can also be the options for developing the high oil safflower lines. Fatty acid profile of this oil crop also needs alteration for its best commercial value. Due to high proportion of linoleic acid (78%) in its oil, it is considered as a healthy oil as it reduces blood cholesterol but makes shelf life of this oil very short and less suitable for frying purpose for its use in food industry. Appropriate reduction in linoleic acid and increase in oleic acid will eliminate this problem and will maintain the tag of healthy oil of this crop. Another aspect of safflower oil quality is increasing the gamma tocopherol content, which is antioxidant in nature. Concerted efforts should be made to assay the vast collection of germplasm for tocopherol diversity for initiating the breeding programme for this component.

9 Niger

Niger (*Guizotia abyssinica* (L.f.) Cass., $2n=30$) is an *Asteraceae* family oil crop cultivated in Indian subcontinent and East African countries (Getinet and Sharma 1996). Its cultivation originated in the Ethiopian highlands and has spread to other parts of Ethiopia. Both Ethiopia and India are excellent sources of germplasm for varietal development. In 2002, the variety Early Bird Niger was developed and adapted to the United States by Glenn Page. Niger seeds contain about 40% edible oil with fatty acid composition of 75–80% linoleic acid, 7–8% palmitic and steric acids and 5–8% oleic acid (Dutta et al. 1994). The meal remaining after the oil extraction is free from any toxic substances but contains more crude fibre than most oilseed meal. Niger is a completely outcrossing species with self-incompatibility mechanism. Variability exists for morphological characters (Pradhan et al. 1995); however, these characters are not discrete and hence complicate the niger improvement programmes. Niger seed populations in Ethiopia and India are very heterogeneous, indicating the great potential for yield enhancement through breeding.

9.1 Objectives

Breeding objectives for niger seed are to increase seed yield and oil content and reduce shattering. With the development of single-headed plant types in sunflower and safflower, it has been postulated that single-headed dwarf types with uniform maturity must be developed for yield enhancement in this crop too. An increase in

oil content appears feasible because of existing genetic variability, which can be used in breeding research. As niger seed is self-incompatible, breeders in India and Ethiopia have adopted population improvement programmes such as mass selection and sibbing. Recently, a protocol for *Agrobacterium tumefaciens* mediated genetic modification was developed. This crop falls under minor oilseed crop in India and a lot of progress has been made after 1985. Fifteen improved varieties have been developed for general cultivation among the farmers. Well-known improved cultivars in India are Ootacamund, Deomali, Paiyur 1, IPG 76 and JNC 6.

10 Castor

Castor (*Ricinus communis* L., $2n = 20$) an *Euphorbiaceae* family plant is an important non-edible oil crop of the arid and semi-arid regions of the world. India, Brazil, China, Russia and Thailand are the major castor growing countries of the world. Castor is grown on about 1.26 mha area with about 1.14 mt production and world average productivity is about 0.90 t/ha. India's share in total castor area and production is 59.1 and 64%, respectively, with 1.5 t/ha average productivity which is much higher than the world average productivity. Castor seeds contain 40–55% oil, the highest among all cultivated oil crops. The kernels contain 64–71% oil. Its oil is world's most useful and economically important natural oil. Its oil contains 84–90% ricinoleic acid of total fatty acids which makes it as a unique vegetable oil. Castor oil is highly stable and variation in fatty acid is very minimal making it the best raw industrial oil. Castor cake is a very useful organic manure which contains 6.0% N, 2.5% P_2O_5 and 1.25% K_2O. It is a rich source of protein (25–40%), sugar (25%) and minerals (10%). But the presence of toxic constituents like ricin/*Ricinus communis* agglutin (RCA) makes it non-edible. There is a real breakthrough in the varietal improvement of this crop which is evident from the transformation of perennial types to annual types. A large number of high yielding hybrids and varieties have been developed. Although castor is a monoecious plant, the proportion of male and female flowers is greatly influenced by both genetic and non-genetic factors (temperature, humidity, plant age, nutritional factors, etc.). Identification of completely pistillate plants and presence of exploitable levels of heterosis paved the way for development of castor hybrids resulting in quantum jump in productivity of this crop (Shifriss 1961; Moshkin 1967; Zimmerman and Smith 1966). Some of the objectives which need interventions are as follows.

10.1 Objectives

Although good breakthrough has been made in the varietal development of this crop yet there is need to develop short duration varieties/hybrids suitable for specific situations like rainfed areas, semi-winter conditions, intercropping, mechanical harvesting, saline conditions and poor management conditions. Pistillate lines being used for hybrid development are highly sensitive to environment giving large number of ISF

under high temperature and water/nutritional stress and reversion in any order in S type. Therefore, breeding programme on development and diversification of stable and superior combining pistillate lines with disease resistance needs acceleration. Under these circumstances, there is an urgent need to develop CMS lines which is not yet achieved because castor is mono generic and no wild species exist. Due to long duration and monoculture this crop is exposed to many insect pests (semi looper, castor capsule borer, jassids, white fly and thrips) and diseases (*Fusarium*, *Macrophomina* root rot, reniform nematode and *Botrytis* grey rot) which causes 30–40% of yield losses. Drought under rainfed areas and salinity in major castor cultivation areas are the abiotic stresses which limit castor production. Concerted breeding efforts are required to incorporate the resistance/tolerance against these biotic and abiotic stresses. Hybrids and varieties with medium to bold light coloured seeds with high oil content (>50%) and high ricinoleic acid (>90%) are the millers' choice, hence efforts on breeding such genotypes should be concentrated. Ricin and RCA are two highly toxic endosperm proteins present in the deoiled castor cake which makes its cake unsuitable for animal and human consumption as a protein supplement. Utilizing the enormous variability available for protein content, breeding efforts are required to address these problems to make this crop more competitive and remunerative. Biotechnological approaches like efforts towards development of transgenics for insect resistance (Sujatha and Sailaja 2007) and silencing ricin and RCA genes are likely to deliver good returns in the times to come.

11 Varietal Improvement in India

Indian subcontinent is the natural repository of the oilseed crops, yet is importing about 40% of the total edible oil in the country. Its vegetable oil imports further raise by 14% and a sum of Rs. 32,000 crore was spent on this import during the oil year 2009–2010. This makes India the world's largest oil importer. Oilseed crops research in India got a boost in 1967, when the Indian Council of Agricultural Research sanctioned a multi-disciplinary and multi-location "All India Coordinated Research Project on Oilseeds" including five crops, namely, groundnut, rapeseed mustard, sesame, linseed and castor and subsequently niger, safflower and sunflower were also included under this project. To focus research on individual crops, Government of India started separate National Research Centres and AICRPs on groundnut, rapeseed mustard, soybean and linseed and elevated these centres later to directorates. This has led to the development of good number of improved varieties in these crops and their production and protection technologies. In the mean time, Government of India has launched the Technology Mission on Oilseeds and Pulses (TMOP) in 1986, which took a number of innovative and integrated measures to harness the best production, processing and marketing technologies. After the implementation of TMOP, area under oilseed crops increased from 19.0 to 26.1 mha, production from 10.83 to 24.94 mt and productivity from 0.57 to 0.955 t/ha between 1985–1986 and 2009–2010 (Table 2.1).

Table 2.1 Oilseed production (million tons) in India during 2001–2002 to 2009–2010

Crops	1985–2086	2001–2002	2002–2003	2003–2004	2004–2005	2005–2006	2006–2007	2007–2008	2008–2009	2009–2010
Groundnut	5.10	7.0	4.1	8.1	6.8	8.0	4.9	9.2	7.2	5.51
Rapeseed mustard	2.68	5.1	3.9	6.3	7.6	8.1	6.7	5.8	7.2	6.41
Soybean	1.02	5.6	4.6	7.8	6.9	8.3	8.7	9.4	9.1	10.05
Other six	2.03	3.0	2.2	3.0	3.1	3.5	2.9	5.4	4.2	2.97
Total	10.83	20.7	14.8	25.2	24.4	27.9	23.2	29.8	27.7	24.94

Table 2.2 Crop wise varieties developed in eight oilseed crops in India

	No. of varieties		
Name of the crop	Before TMOP	After TMOP	Total
Soybean	20	65	85
Groundnut	42	115	157
Rapeseed mustard	29	108	137
Sunflower	7	42	49
Sesame	28	48	76
Linseed	21	29	50
Safflower	9	20	29
Niger	1	15	16
Castor	16	19	35

There is tremendous increase in productivity of these crops but it is still lower than the world's average. Furthermore, stability of production is always a cause of worry in India.

The impact of technology mission on oilseeds and pulses is visualized with the development of a large number of area specific high yielding varieties of all edible oilseed crops (Shanmugham and Gunasekaran 2003, 2008) (Table 2.2). As far as improved technology is concerned, we have the improved varieties with very high yield potential in all the crops. From the comparison of present yield levels, and area and production with that of pre-TMOP period, very clear picture comes out and shows that we have attained much success to reach to the self-sufficiency, but still a lot has to be achieved. The crop wise achievements in terms of varietal development are given later.

11.1 Soybean

Twenty varieties were released from 1969 to 1985, whereas 65 new high yielding varieties have been recommended for general cultivation by the farmers in a period of about 25 years, i.e. 1986–1987. The impact of these varieties can be observed by consistent increase in area, production and productivity of this crop. The most popular varieties of this crop are JS 335, JS 93 05 and MAUS 71 covering more than 85% area and are contributed significantly in the better production of this crop. Some new varieties are also covering the area gradually which will also help in increasing the production of this crop in the times to come.

11.2 Rapeseed Mustard

This group of crop is a very complex one with four species, namely, *B. juncea, B. napus, B. rapa* (cvs toria, yellow sarson, brown sarson) and *B. carinata* grown for edible oils in India. The major area is under *B. juncea* and it contributes more than

85% of the total rapeseed mustard production. There are more than 137 varieties released for all the four oliferous species of *Brassica*. Presently, there are varieties with 2.5 t/ha of yield potential. In addition to varieties, hybrid development programme in *B. juncea* is also very strong and three hybrids have already been released for general cultivation. The dominating varieties of Indian mustard are Pusa Bold, Pusa Jai Kisan, Varuna, RH 30, Laxmi, Maya, Kanti, Rohini and Benoy (B 9) of *B. rapa* cv. Yellow sarson.

11.3 Groundnut

Groundnut is also one of the three most important oilseed crops in India. A total of 157 varieties have been developed since 1969 of which 42 varieties were developed up to 1985 and 86 improved varieties have been released for general cultivation since 1986–2007 in this crop. The highest production has gone up to 8.1 mt during 2003–2004 but again due to weather vulnerability, the production has drastically come down to 4.9 mt during 2006–2007. There are very high yielding varieties in this crop and the major contributor in the production of this crop is varieties like M 335, TAG 24, ICGS 76, TG 7A, AK 12 24, HNG 10, etc.

11.4 Sunflower

Sunflower is also one of the important oilseed crops contributing towards the national oil pool. Although this crop is covering about 10% of the country's total area but it fits well in all cropping systems due to its photo and thermo-insensitivity. This crop is of late introduction in the country and the first variety was developed during 1978. Since then 49 varieties and hybrids have been developed and released for general cultivation by the various public and private sector organizations. This is the only crop which has more than 80% sunflower growing area under the hybrids. From a negligible area during 1980–1981, now this crop has shown its presence in the Indian oil economy. The widely grown high yielding hybrids in sunflower are KBSH 44, KBSH 1, Poineer 6460, Poineer 3322. The most popular stable variety Morden which was released in 1982 is still having about 20% area of sunflower under cultivation.

11.5 Sesame

Although sesame is grown in almost all the states of the country but the major states where sesame cultivation is being done are Rajasthan, Gujarat, Madhya Pradesh, Orissa and Maharashtra. As breeding for high yielding varieties is concerned a lot

of efforts have been made for genetic enhancement of yield in this crop and as a result about 76 varieties have been developed of which 48 were developed after the implementation of TMOP. The varieties with about 1.0 t/ha yield potential have been developed for rainfed conditions too.

11.6 Linseed

Linseed is one of the minor oilseed crop grown in India. In this crop also a lot of breeding work has been done and 50 varieties have been developed. The seed yield potential of the improved varieties under irrigated conditions is 1.2–1.5 t/ha.

11.7 Safflower

It is another minor oilseed crop. A lot of efforts have been made in genetic enhancement of seed yield and 29 improved varieties have been developed in safflower.

11.8 Niger

This crop also falls under minor oilseed crop in India and a lot of progress has been made after 1985. Fifteen improved varieties have been developed for general cultivation.

11.9 Castor

India has made a big breakthrough in castor breeding programme. The first castor hybrid GCH 3 based on an exotic pistillate line was released for general cultivation in 1968. It is non-edible oil crop where 35 varieties and hybrids have been developed and are contributing in making India a global leader in castor production.

12 Research Needs for Yield Improvement and Its Stabilization

Development of varieties resistant to biotic stresses: In all the nine oilseed field crops grown in the country, the biotic stresses like insect pests and diseases effect the crops adversely in one or the other years due to which the production and

productivity fluctuates to unexpected levels. In almost all the crops, barring few area specific examples, the insect pests and diseases cause havoc to these crops in the lack of resistant/tolerant varieties. Hence efforts are needed to develop such varieties for yield enhancement as well as stabilizing the production of these crops. Use of modern biotechnological tools will definitely help in development of varieties for biotic and abiotic stresses.

Drought tolerant varieties with enhanced water use efficiency: Water stress at various stages of crop growth in all the edible oilseed crops is another major limiting factor for realizing the potential yield of present day varieties. Specific efforts are required to breed the varieties having high degree of tolerance to moisture stress along with high water use efficiency to utilize the available moisture in under field conditions to minimize the losses to the crop.

Development of varieties resistant to other abiotic stresses: Other than water stress other abiotic stresses are frost in mustard, salinity in almost all oilseed crops, high temperatures at the time of sowing and maturity in the rabi oilseeds like rapeseed mustard and linseed. Efforts are needed to overcome these stresses by tailoring genotypes tolerant to these stresses in respective crops.

Development of hybrids: For breaking the yield ceiling, exploration of various possibilities which can help in increasing the yield potential of the different oilseed crops is required. It is realized that the improvement through the use of conventional breeding approaches is tending to level off, since these breeding approaches do not mobilize sufficient amount of genetic variation, whereas hybrids offer an opportunity for mobilizing greater amount of genetic variability and available high heterotic response in different crops. In order to increase the yield potential of soybean, *Brassica*, safflower, sesame, linseed and niger hybrid development programme needs to be intensified. With the encouraging results of hybrids in sunflower, a special network programme may be launched for development of hybrids in oilseed crops like rapeseed mustard, soybean, niger, safflower, sesame and linseed.

Improvement of quality of oil and seed meal: Specifically in rapeseed mustard, the emphasis should be made to develop double zero varieties (erucic acid <2% and glucosinolate <30 μmoles/g of defatted seed meal cake). For improving the keeping quality of soybean oil, efforts should be made to reduce the linoleic acid content. In the crops like *Sesamum* and linseed, also improvement in quality may be taken at priority for value addition to their oil. Ricin and RCA are the two toxic proteins present in castor deoiled cake which also need genetic interventions.

Development of varieties with improved water use efficiency: Water is the most precious natural resource in the times to come. Oilseeds are already grown on marginal lands with limited irrigation facility. Therefore, efforts are needed to develop oil crop varieties with high water use efficiency.

Development of varieties with improved nutrient use efficiency: The agronomical experiments show that the nitrogen requirement of almost all the oilseed crops is 60–80 kg/ha. The analysis of soils and plant samples shows a gap between nitrogen

utilized by the plant and its availability in the soil. The higher dozes, i.e. more than 80 kg/ha of nitrogen does not yield good results. Hence, there is an urgent need to develop varieties which have high nutrient use efficiency.

13 Use of Molecular Tools for Oil Crop Improvement

For improvement of some of the biotic and abiotic stresses, there is problem either due to complex genetic control of that trait or non-availability of resistant source or non-availability of screening techniques or environmental effect on the traits under improvement. In such conditions, the possibility of using molecular tools like MAS helps in improvement of a trait. These techniques will not only help in the transfer of desirable trait but it will reduce the time taken for introgression of a particular trait. Where no source of resistance is available in the germplasm of particular crop, the transgenic approach may be explored for introgression of the resistance from other species.

Conventional methods of improving crops use the genetic variation available within the crossable limits. The germplasm provides the required parental lines for recombination breeding and making heterotic hybrids. Selection of right kind of parental genotypes, therefore, is the key to the success of a breeding programme. Those involved in genetic enhancement of crops heavily depend on the available passport data or results of limited evaluation of a sub-set of germplasm. A majority of the germplasm, although constitute the primary gene pool remain unutilized. Once the parental lines are chosen, they are inter-mated to generate segregating populations from which the desirable recombinants carrying the required gene combinations are selected. This process of selection is mainly based on phenotype in conventional schemes. Skilful eyes of the conventional breeder therefore play a vital role in selecting the desirable types from the pool of mostly undesirable segregants. Phenotype, however, is the product of interaction of genotype and environment. Particularly in respect to complex quantitative traits such as seed and oil yield, disease resistance, drought tolerance, etc., phenotype may not always reflect the actual genetic worth of the genotype. Even in case of Mendelian traits, selection of desirable segregants requires creation of selection environments, which may not be possible for routine screening of large populations. In contrast, selection based directly on genotype itself is more precise and efficient. Use of molecular markers to identify desirable recombinants, which is commonly known as MAS, makes gains from selection more predictive. The resources available in different oilseed crops with emphasis on the recent developments for carrying out marker-assisted breeding highlight the successful use of markers in selection and provide the prospect of MAS in oilseed improvement is summarized (Table 2.3).

A good progress has been made in soybean, *Brassica* and groundnut in development of molecular markers and genome maps, mapping and tagging QTLs and their application in MAS. The other oil crops need further biotechnological interventions for improvement of some of the specific traits which has been summarized as strength, weakness, opportunity and thrust for these crops (Table 2.4).

Table 2.3 Molecular tools for oil crop improvement

Crop	Marker/ trait	References
Development of molecular markers and genome maps in oilseed crops		
Soybean	RFLP	Lark et al. (1993); Skorupska et al. (1993); Shoemaker and Specht (1995); Lorenzen et al. (1995); Xia et al. (2007)
	AFLP	Kiem et al. (1997); Xia et al. (2007)
	SSR	Cregan et al. (1999); Song et al. (2004); Xia et al. (2007)
	SNP	Yoon et al. (2007); Hyten et al. (2008)
Brassica spp.	RFLP	Figdore et al. (1988)
	Isozyme markers	Arus and Orton (1983); Chen et al. (1989)
	EST Mining	Bhati et al. (2010)
B. oleracea	RFLP and RAPD	Slocum et al. (1990); Kianian and Quiros (1992); Landry et al. (1992); Kearsay et al. (1996); Ramsay et al. (1996); Voorrips et al. (1997); Li and Quiros (2001); Saal et al. (2001); Gao et al. (2007)
B. rapa	RFLP and RAPD	Song et al. (1991) Chyi et al. (1992); Kole et al. (1997)
	SSR, IP	Li et al. (2010a)
B. nigra	RFLP and RAPD	Truco and Quiros (1994); Lagercrantz and Lydiate (1995)
B. napus	RFLP and RAPD	Hoenecke and Chyi (1991); Landry et al. (1991); Ferreira et al. (1994); Uzunova et al. (1995); Foisset et al. (1996)
	SSR	Lydiate and Sharpe (2003)
	SRAP	Sun et al. (2007)
B. juncea	RFLP, AFLP, RAPD	Sharma et al. (1994); Cheung et al. (1997); Axelson et al. (2000); Mohapatra et al. (2002); Sharma et al. (2002); Pradhan et al. (2003); Mahmood et al. (2005), Kalita et al. (2007)
	SSR	Koundal et al. (2008); Parida et al. (2010); Yadava et al. (2009); Pradhan et al. (2011)
	IP	Panjabi et al. (2008)
Groundnut	RAPD	Halward et al. (1992); Garcia et al. (1995)
	RFLP	Halward et al. (1991); Kochert et al. (1991); Paik-Ro et al. (1992); Halward et al. (1993)
	SSR	Cuc et al. (2008); Jayashree et al. (2005); Moretzsohn et al. (2005); Wang et al. (2007); Varshney et al. (2009)
	EST	Luo et al. (2005)
Sunflower	RFLP	Gentzbittel et al. (1994); Berry et al. (1995); Jan et al. (1998)
	AFLP	Gentzbittel et al. (1995); Langer et al. (2003); Tamborindeguy et al. (2004)
	SSR	Paniego et al. (2002); Yu et al. (2003); Tang et al. (2002); Heesacker et al. (2008)
	SNP	Kolkman et al. (2007); Fusari et al. (2008)

(continued)

Table 2.3 (continued)

Crop	Marker/ trait	References
Sesamum	RAPD	Bhat et al. (1999); Davila et al. (2003)
	SSR	Dixit et al. (2005)
	AFLP	Laurentin and Karlovasky (2006); Laurentin and Karlovsky (2007)
	RAPD and ISSR	Sharma et al. (2009)
Linseed	Isozymes, RAPD, AFLP, RFLP	Spielmeyer et al. (1998); Oh et al. (2000); Fu et al. (2002), 2003; Krulickova et al. (2002); Adugna et al. (2006); Diederichsen and Fu (2006); Roose et al. (2006); Diederichsen (2007)
Safflower	RAPD	Amiri et al. (2001)
	AFLP	Johnson et al. (2007)
	RAPD, ISSR, AFLP	Sehgal and Raina (2005)
	ISSR	Yang et al. (2007)
Mapping and tagging QTLs		
Soybean	*Phytophthora infestans*	Diers et al. (1991); Polzin et al. (1994)
	Corn earworm (*Helicoverpa zea* Boddie)	Rector et al. (1998); Li et al. (1998)
	Soybean aphid (*Aphis glycines*)	Rouf-Mian et al. (2008)
	Super-nodulation	Landau Ellis et al. (1991)
	Cyst nematode resistance	Concibido et al. (1994) Mudge et al. (1997); Schuster et al. (2001); Guo et al. (2005)
	Hard seededness	Kiem et al. (1990a)
	Seed shape traits	Salas et al. (2006)
	Sprout-related traits	Lee et al. (2001)
	Seed longevity	Singh et al. (2008)
	Height and maturity	Mansur et al. (1993a)
	Seed oil and protein content	Diers et al. (1992); Lark et al. (1994)
	Reproductive and morphological traits	Kiem et al. (1990b); Mansur et al. (1993b)
	Salt tolerance	Lee et al. (2004)
	Oil quality	Bachlava et al. (2008); Li et al. (2008)
B. oleracea	*Plasmodiophora brassicae*	Figdore et al. (1993); Voorrips et al. (1997)
	Xanthomonas campestris	Camarago et al. (1995)
B. rapa	Club root	Saito et al. (2006); Werner et al. (2008)
	Xanthomonas campestris	Soengas et al. (2007)
	Albugo candida	Kole et al. (1996)
	Fatty acids	Teutonico and Osborn (1994); Tanhuanpaa et al. (1996, 1998)
	Seed coat colour	Teutonico and Osborn (1994); Chen et al. (1997); Rahman et al. (2007)
B. nigra	Flowering time	Lagercrantz et al. (1996)
B. napus	*Leptosphaeria maculans*	Dion et al. (1995); Ferreira et al. (1995a); Leflon et al. (2007)
	Turnip mosaic virus	Walsh et al. (1999)

(continued)

Table 2.3 (continued)

Crop	Marker/ trait	References
	Sclerotinia sclerotiorum	Zhao and Meng (2003)
	Verticillium wilt	Happstadius et al. (2003)
	Albugo candida	Ferreira et al. (1995c)
	Vernalization requirement	Ferreira et al. (1995b); Teutonico and Osborn (1995); Camarago and Osborn (1996)
	Oil content, protein, fatty acid	Arondel et al. (1992); Ecke et al. (1995); Hu et al. (1995); Tanhuanpaa et al. (1995); Jourden et al. (1996a, b); Jourden et al. (1996c); Thormann et al. (1996); Barret et al.(1998b); Fourmann et al. (1998); Hu et al. (1999); Schierholt et al. (2000); Zhao et al. (2006); Delourme et al. (2006); Qiu et al. (2006); Rahman et al. (2008); Nath and Goswami (2009)
	Glucosinolates	Uzunova et al. (1995); Toroser et al. (1995); De Quiroz and Mithen (1996); Hasan et al. (2008)
	Seed coat colour	Van Deynze et al. (1995)
	Male sterility/fertility restorer genes	Delourme et al. (1994); Jean et al. (1997); Delourme et al. (1998); Yi et al. (2006); Huang et al. (2007); He et al. (2008)
	Yield	Shi et al. (2009)
B. juncea	*Albugo candida*	Cheung et al. (1998); Prabhu et al. (1998); Mukherjee et al. (2001); Varshney et al. (2004); Panjabi et al. (2010)
	Seed coat colour	Upadhyay et al. (1996); Negi et al. (2000); Li et al. (2010b)
	Oil content	Sharma et al. (1999); Sharma et al. (2002)
	Erucic acid	Gupta et al. (2004)
	Glucosinolates	Stringam and Thiagarajah (1995); Good et al., (2003); Mahmood et al. (2003); Ripley and Roslinsky (2005); Ramchiary et al. (2007); Bisht et al. (2009)
	Moricandia arvensis	Ashutosh et al. (2007)
Groundnut	Nematode resistance	Garcia et al. (1995)
	Aphid resistance	Herselman et al. (2004)
	Rust resistance	Varma et al. (2005); Mondal et al. (2007)
	Drought tolerance	Varshney et al. (2009)
Sunflower	Fertility restoration and nuclear male sterility	Gentzbittel et al. (1995); Kusterer et al. (2002); Perez et al. (2005); Chen et al. (2006); Feng and Jan (2008)
	Branching	Gentzbittel et al. (1995); Rojas-Barros et al. (2008)
	Downy mildew	Mouzeyar et al. (1995); Slabaugh et al. (2003); Brahm et al. (2000)
	Orobanche	Tang et al. (2002)
	Rust (*Puccinia helianthi*)	Lawson et al. (1998)
	Chlorotic mottle virus	Lenardon et al. (2005)
	Oil quantity and quality	Perez et al. (2004)

(continued)

Table 2.3 (continued)

Crop	Marker/ trait	References
	High stearic acid content	Perez et al. (2006)
	Tph1 gene controlling beta - tocopherol accumulation	Vera-Ruiz et al. (2006)
	Pollen sterility and morphological traits	Kim and Rieseberg (1999)
	Restoring pollen fertility	Horn et al. (2002)
	Seed morphological traits	Yue et al. (2008b)
	Flowering	Leon et al. (2000)
	Lemon ray flower colour	Yue et al. (2008a)
	In vitro regeneration efficiency	Berrios et al. (2000)
	Drought tolerance	Jamaux et al. (1997); Herve et al. (2001); Kiani et al. (2007)
	Chlorophyll deficiency	Yue et al. (2009)
	Nutrient uptake	Lexer et al. (2003)
Sesamum	Closed capsule	Uzun et al. (2003)
Linseed	Flax rust (*Melampsora lini*)	Chen et al. (2001)
	Fibre quality	Roach and Deyholos (2007, 2008)
Marker assisted selection (MAS)		
Soybean	Corn earworm resistance in soybean	Walker et al. (2002)
	Pyramiding of soybean mosaic virus resistance genes	Saghai-maroof et al. (2008)
Sunflower	Identification of maintainer	Yue et al. (2007)

Table 2.4 SWOT analysis of some oil crops

Crop	Strength	Weakness	Opportunity	Thrust
Sunflower	Saturated maps, international, characterized gene pool	Still MAS not much adopted for QTLs	MAS could be adopted for several traits	*Alternaria*, yield plateau
Safflower	Skeletal map, markers (recently) germplasm	Very small group working on markers	Saturated maps, use of MAS, germplasm	*Alternaria* wilt
Sesame	Germplasm genomic resources	No map, very small group working on markers	Saturated maps, use of MAS, germplasm	Capsule shattering
Linseed	Germplasm genomic resources	No map, small group	MAPS and MAS	Bud fly

14 Future Prospects of Marker-Assisted Selection in Improvement of Oilseed Crops

Since last 25 years after the publication of the first paper in 1986 on the development and use of RFLP markers for construction of linkage maps in tomato and maize, considerable progress has been made in the application of molecular techniques in oil crops. Now the focus has shifted to the use of sequence-based STS and SSR markers to generate very high density genome maps and tag gene/QTLs in *Brassica*, soybean, sunflower and groundnut. In some of the oilseed crops, SNPs are also being discovered and used to understand genetic diversity pattern. The first requirement for successful use of markers in breeding has been fulfilled at least for some of the traits with the availability of tightly linked markers. Besides, MAS with the use of other molecular markers has been demonstrated for both qualitative and quantitative traits.

Use of the markers was limited by the factors like recombination between the marker and the target gene, low level of polymorphism between parents with contrasting traits and lower resolution of QTLs due to interaction with the environment. With the recent developments in the design of genome-wide sequence based SSR and SNP markers, it would not be difficult to find solutions to these problems particularly in crops like soybean, sunflower, *Brassica* and in the near future in groundnut. Availability of high-density genetic and physical maps will enable finding markers physically closer to the target gene that would not allow failure of MAS due to genetic recombination in these crops. Development of allele-specific markers, markers based on the sequences of the genes, polymorphic SNP markers would eliminate the possibilities of breakdown of the marker-trait linkage, low level of polymorphism in narrow crosses, etc. Construction of high density genome maps using SSR markers is the desirable, which would allow map-based characterization of genomes and rapid tagging of useful genes.

With the available tightly linked markers as in case of nematode and virus resistance in soybean, MAS for qualitative traits seems immediately feasible. Pyramiding of a number of genes against different races of a particular pathogen and also against different pathogens, nematodes and insects should now be aimed at, which would allow sustaining the gains in productivity of the oilseed crops. Enhancing productivity further and stabilizing production particularly under abiotic stresses would require strategic use of markers in these crops. Many QTLs for seed and oil yield as well as for salt and drought tolerance in crops like soybean, sunflower and *Brassica* have been mapped. There is a need to validate and fine map these QTLs to identify tightly linked markers. Detection of QTL and its validation has to be carried out using a large population (>200 individuals) across several locations. Their expression needs to be confirmed in the target/new genetic backgrounds. More than one population may be used in parallel to understand the effect of different genetic backgrounds. It would be essential to understand the kind and the extent of epistatic interactions to identify desirable QTL combinations to be used in different situations. All these

demand greater amount of research efforts, liberal funding, creation of additional infrastructure for precise phenotyping and high throughput genotyping, and newer experimental strategies.

The potential application of MAS in genetic improvement of the oilseed crops is quite high. More efforts are required in the coming years for realization of potential of MAS under field conditions in the form of commercial release of new varieties. Optimization of the cost of genotyping is required for routinely handling large samples as demanded by plant breeding experiments. Fortunately, due to significant reduction in cost, the genotyping technology is developing very fast, however, the investment in designing robust sequence based validated markers for important traits in oilseed crops should be viewed in the context of advantages in terms of saving time, effort and cost in the long run. While pursuing MAS particularly for difficult-to-phenotype traits, it should be kept in mind that use of markers is no substitute for conventional breeding. Conscious and strategic integration of MAS with traditional breeding of oilseed crops is desirable to harvest the benefits it offers.

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Chapter 3
Brassicas

S.K. Gupta

Abstract Oleiferous brassicas are interesting breeding material since they have a complete range of breeding systems ranging from complete range of cross-pollination to self-pollination. Besides improvement in production and productivity of various economically important brassicas, improvement in the nutritional profile of their oil and defatted meal, and development of traits like herbicide tolerance, male sterility, disease and insect-pest resistance, and development of hybrid cultivars remain the prime objectives for their genetic improvement. To achieve these goals, conventional breeding efforts in conjunction with modern biotechnological tools such as molecular marker-assisted selection, doubled haploidy breeding, in vitro mutagenesis, and transgenic technology offer a great promise. The doubled haploidy (DH) technology in combination with other biotechnological and conventional breeding tools has resulted in improvements in many yield and quality attributes in Brassicaceae. Interspecific and even intergeneric hybridizations have greatly helped in generating additional variability through the recovery of distant hybrids. Further, in vitro technologies such as microspore culture, and embryo and ovary rescue coupled with in vitro mutagenesis can also generate additional selection avenues by creating variability through gemetoclonal and somaclonal variation. This review focuses on breeding methods, which individually or in combination could be deployed for solving the pressing problems of male sterility and fertility restoration mechanisms for hybrid seed production in crop brassicas, their crossability improvement and generation of variability and quality improvement.

Keywords Brassicaceae • Origin and evolution • Double haploidy • Molecular-assisted selection • Transgenic technology • Male sterility and fertility restoration • In vitro mutagenesis

S.K. Gupta (✉)
Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus, Chatha, Jammu, India
e-mail: guptaskpbg@rediffmail.com

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1 Introduction

Rapeseed–mustard is the most important source of vegetable oils after palm and soybean (Beckman 2005). The rapeseed production has witnessed a steady upward movement during the past 25 years. More recently, the introduction of low erucic acid varieties enhanced its value as edible oil, particularly among the health conscious consumers and varieties with low glucosinolates increased the value of its defatted meal for use as a feed for livestock. The development of double low varieties (canola) (Downey and Rakow 1987) has made rapeseed one of the major plant oil sources at the global level, and now there is a constant tendency to increase its share in the production of oilseeds (Bartkowiak-Broda et al. 2005). Oleiferous Brassicas are generally derived from two species, *Brassica napus* L. and *B. campestris* L. syn. *B. rapa* L. *B. campestris* is also referred to as toria, sarson, summer turnip rape, Polish rape, and so on. Similarly, different names are also given to *B. napus* such as Argentine rape, Swede rape, and colza. The name rape is derived from the Latin word "rapum," which means turnip. All the rapeseed contributing cultivated *Brassica* species are highly polymorphic including oilseed crops, root crops, and vegetables such as Chinese cabbage, broccoli, and Brussels sprouts. However, a few of them are cultivated as salad, vegetable, and condiment crops as well.

B. juncea is of much importance in Asia and *B. napus* in Europe and Canada. Under European and Canadian conditions, both winter and summer (spring planted) forms of *B. campestris* (syn. *B. rapa*) and *B. napus* are being grown but in *B. juncea*, only spring form has evolved. Winter types of *B. napus* are largely grown under north-European, Chinese, and Canadian conditions (Rai et al. 2007). However, spring types of *B. campestris* are usually preferred and are largely grown in Sweden, Finland and some parts of Canada and north-west China. In the Indian subcontinent, genetic improvement of seed yield is the prime breeding objective while in Western world, breeding for quality receives greater attention (Jonsson 1973).

2 History

Early history suggests that rapeseed has been cultivated for several thousand years with its origins in Asia. Sanskrit writings of 2000–1500 BC directly refer to oleiferous *B. napus* forms (sarson types) and mustard. Seeds of *B. juncea* have been found in the archaeological sites in India dating back to ca 2300 BC (Prakash 1980; Weiss 1983). Two species, *B. napus* and *B. campestris*, having a range of morphotypes, are the crops of antiquity in India where much before the Christian era, they were used for many purposes including oil for cooking and frying, spice for seasoning food articles, vegetables, and for religious ceremonies (Mehra 1966). Since time immemorial, the Brassica crops have been a part and parcel of human agriculture system, and at present also they occupy a predominant place in the world's agrarian economy. The Chinese word for rapeseed was first recorded ca 2500 years ago and the

oldest archaeological discoveries may date back as far as to ca 5000 BC (Yan 1990). The Greek, Roman, and Chinese writings of 500–200 BC refer to rapiferous forms of *B. rapa* and also describe their medicinal values (Downey and Robellen 1989). Seeds of *B. juncea* have been excavated from Chanhudaro, a site of Indus Valley civilization that existed in the plains of Punjab along the river of Indus ca 2300–1750 (Piggot 1950). Species from the genus Brassica were cultivated in ancient Rome and also in Gallia (Fussel 1955), and seeds of these species had also been found in the old German graves and Swiss constructions from the Bronze Age (Neuweiller 1905; Schiemann 1932; Witmack 1904). In Dodoneus's "Herbalist" (1578), a mention has been made regarding the growing of *B. rapa* var. *rapifera* in 1470 as a winter crop. In his "Herball," Gerarde (1597) had very clearly differentiated between turnips (*B. rapa*) and nevews (*B. napus*). Rape has been recorded as an oilseed crop in Europe at least since the Middle Ages, but it is still uncertain which species was cultivated (Appelquist and Ohlson 1972).

Domestication of rapeseed in Europe appears to have started in the early Middle Ages, although the true turnip was probably introduced by Romans. Since many other oil-yielding plants, particularly olive tree, were available in southern Europe, *B. rapa* initially spread mainly as turnip rape crop within Europe. However, in more prosperous countries like the Netherlands, the farmers used almost all Brassica seeds to produce vegetable oils. Oil was extracted from "raepsaet, koolaet, and mostaert saet" according to a Dutch reference of fourteenth century, which means "the seeds of *B. rapa*, *B. oleracea*, and *B. nigra* (or *Sinapis alba*)" (Reiner et al. 1995). As *B. rapa* was most intensively grown at that time, it can be concluded that this crop was the major source of producing large quantities of vegetable oils. Seeds of *B. rapa* were first recorded in Europe in 1620 by the Swiss botanist Casper Banhin. However, Boswell (1949) was of the view that these existed much earlier than this. As per some anonymous authors, rapeseed was grown in Europe as early as in the thirteenth century.

In the Netherlands, the commercial plantings of rapeseed were recorded in the early sixteenth century. It had limited industrial use at that time until the development of steam power, when it was discovered that rape oil was an excellent lubricant for steam engines. *B. rapa* was the dominant species in the western Canada in the early 1970s. It is comparatively a recent introduction in Canada and the United States and is found as an occasional weed or volunteer in the cultivated fields (Muenscher 1980; Munz 1968). In late 1980s, large acreages *of B. rapa and B. napus* were grown in the Prairie Provinces and these crops gradually started getting established. However, the production area sown to *B. rapa* decreased to about 15–20% in 1990s (The Biology of Brassica rapa 1999). In Austria, the annual wild-type *B. rapa* is found as a weed in rye and potato crops situated in relatively cool and high areas with an altitude of about 1,000 m (Holzner 1981). In the 1970s, the information on its distribution had been very uncertain due to incross and possibility of its escape from culture (Reiner et al. 1995). Canola is a modern, high-quality form of rapeseed and it has originated in Canada through genetic modification and emerged in the 1970s as a viable oilseed, equipped with the appropriate genetics to

transform the oil and meal from unacceptable to highly desired products for both human as well as livestock consumption (Shahidi 1990). Today, the fatty acid profile of canola is considered as the most desirable of all vegetable oil profiles by nutritionists (Stringam et al. 2003). Although superior edible oils had been developed by 1971, the presence of high amount of glucosinolates in the meal still remained a major concern in the expansion of market of the vegetable oil derived from Brassicas. In 1974, Dr. Baldur Stefansson from the University of Manitoba successfully developed the first "double low" variety with reduced levels of both erucic acid and glucosinolates (www.canola-council.org). This led to the evolution of a greatly improved crop, which met specific quality requirements of an oilseed fit for human as well as livestock consumption. As a result of these improvements, the FDA gave GRAS (generally recognized as safe) status to rapeseed oil in 1985 for use in the U.S. food products. The word "canola" was coined and trademarked for such type of rapeseed products, low in both glucosinolates and erucic acid to distinguish them from traditional rapeseed. The name canola was initially registered by the Western Canadian Oilseed Crushers' Association for reference to oil, meal, protein extractions, seed, and seed hulls from or of varieties with 5% or less erucic acid in the oil and 3 mg/g of glucosinolates (www.canola-council.org). Later, the control of the term was transferred to the Rapeseed Association of Canada in 1980, which subsequently changed its name to Canola Council of Canada (2006). The new target of achieving the ideal glucosinolate level at 15 μmol is underway. Keeping the above facts in view, it may be conveniently inferred that all canola is rapeseed but all rapeseed is not canola.

3 Origin and Evolution

The *Brassica* genus is a very complex member of the Cruciferae family, and as such it contains many cultivated plants and wild species. It, therefore, possesses several taxonomic and classification problems. Also, there is a lack of consistency in the names of different oil-yielding Brassicas throughout the globe, which aggravates the problem further. The scientific nomenclature is highly confusing, which makes it difficult for many to decide as to what particular scientific name should be used for a particular plant. Bailey (1922) has listed many reasons responsible for the chaotic nomenclature of Brassicas.

The cytogenetic relationships between the rapeseed species as well as their closest allies were first explained systematically by U (1935) about 70 years ago. These relationships show that *B. campestris* (2n = 20, AA), *B. nigra* (2n = 16, BB) and *B. oleracea* (2n = 18, CC) are the primary species and *B. napus* (2n = 38, AACC), *B. carinata* (2n = 34, BBCC) and *B. juncea* (2n = 36, AABB) are the amphidiploids resulting from paired crossings between the primary species. Morinaga (1928, 1929a, b, 1934a, b) discussed that crop Brassicas include six cotydeme three elementary ones with 16, 18, and 20 chromosomes as diploid and three with higher

Table 3.1 Genus Brassica and their ecotypes

Species	Subspecies	2n Chromosome number	Common name	Use
B. nigra Koch	–	16	Black mustard	Condiment
B. oleracea	*aephala*	18	Kale	Vegetable/fodder
	aboglabra	18	Chinese kale	Vegetable
	botrytis	18	Cauliflower	Vegetable
	capitata	18	Cabbage	Vegetable/fodder
	gemmifera	18	Brussels sprouts	Vegetable
	gongylodes	18	Khol rabi	Vegetable
	italica	18	Broccoli	Vegetable
B. campestris				
	chinensis	20	Pak-choi	Vegetable
	japonica	20		Vegetable
	narinosa	20		Vegetable
	oleifera	20	Turnip rape	Oilseed
	pekinensis	20	Chinese cabbage	Vegetable
	rapa	20	Turnip	Vegetable/fodder
B. napus				
	oleifera	38	Rape	Oilseed
	rapifera	38	Rutabaga	Vegetable
B. juncea				
	rugosa	36	Chinese mustard	Vegetable
	oleifera	36	Indian mustard	Oilseed
B. carinata				
		34	Ethiopian mustard	Vegetable/ oilseed

Source: Kalia and Gupta (1997)

chromosome numbers of 34, 36, and 38 as tetraploid, the latter having evolved through interspecific hybridization in nature between any two of the elementary taxa (Table 3.1). Morinaga and his associates carried extensive cytogenetic studies in oilseed Brassicas and clarified the relationships between them (Prakash and Hinata 1980). According to the hypothesis of Morinaga (1934a, b), the three species with the higher chromosome number, *B. napus* L., *B. juncea* L. Czern. and Coss., and *B. carinata* A. Braun, are amphidiploids combining in pairs the chromosome sets of the low chromosome number species *B. nigra*, *B. oleracea*, and *B. rapa*. U (1935) verified the hypothesis with successful resynthesis of *B. napus*. Resynthesis of *B. juncea* and *B. carinata* was accomplished by Frandsen (1943, 1947). Robellen (1960) suggested that the low chromosome number species might have developed from the ancestral species, which could have even lower chromosome numbers. Also the chromosome analysis of the monogenomic species revealed that only six chromosomes were distinctly different, the remaining being homologous with one or another of the basic set of six.

4 Breeding Objectives for Varietal Development

Oilseed Brassicas includes number of crop species which have an amalgam of breeding systems ranging from complete cross-pollination to a high level of self-pollination. Therefore, they are quite interesting material from the breeding point of view. The different crop species of this group of crop, *B campestris* var. toria, lotni brown sarson, Banarasi rai (*B. nigra*), taramira (*Eruca sativa*), and so on, are highly cross-pollinated (because of the presence of self-incompatibility, presence of bright yellow opened petals, entomophily, high sucrose content ranging from 40 to 60% in their nectaries to attract honeybees and the extrorse anther condition which turns away from the stigmatic surface at the time of dehiscence), whereas *B. juncea*, gobhi sarson (*B. napus*), karan rai (*B. carinata*), tora brown sarson (*B. campestris*), and so on, are predominantly self-pollinated (because of the absence of self-incompatibility), light pale yellow petal color, low sucrose content (5–11) in the nectaries and introrse anther condition. However, even in the self-pollinated group, due to stray pollen contamination and visit by honeybees, bumble bees, and so on, the extent of out-crossing varies from 14 to 30% (Rai and Singh 1976; Rakow and Woods 1987; Rambhajan Chauhan and Kumar 1991; Singh 1958). The self-incompatibility is of homomorphic sporophytic type (Bateman 1955) and is genetically controlled by a series of "S" alleles. The presence of same allele in the pollen and stigma will inhibit the germination of the pollen grains or will prevent the pollen tube from penetrating the stigmatic surface of the style and effecting fertilization. The evolution of mating system in genus *Brassica* is also very interesting. There is strong intergenomic interaction affecting the mode of pollination. The three primary, monogenomic species are highly cross-pollinated, whereas their amphidiploid products are predominantly self-pollinated. The commercially cultivated species *B. campestris*, however, contains both self-compatible (yellow sarson, torabrown sarson) and self-incompatible forms (toria, lotni brown sarson). In this crop species, Iotni brown sarson appears to be the logical progenitor of its different cultivated forms. The evolution in this crop species has followed two independent pathways. On one hand, toria type has evolved as an escape from the onslaught of the biotic and abiotic stresses but retained its self-incompatibility gene complex. The early maturity (75–100 days) makes it a better material to survive the stresses imposed by frost injury, aphid infestation, and the threat from *Alternaria* leaf blight disease in comparison to its parental form lotni brown sarson, which usually takes 125–140 days for crop maturity and suffers heavily on account of these stresses. There is very good morphological similarity, chromosomal homology, and cross-compatibility between toria and Iotni brown sarson. The only visible difference between them is their relative number of days taken to crop maturity. On the other hand, tora brown sarson has evolved from lotni types, this is primarily because of the cultivator's preferences for the bold seeds, uniform types, and tall growing plants, which are considered very suitable for the mixed or intercropping systems being followed by the farmers. However, in the long process of human selection for uniformity, the self-incompatibility gene complex has been lost. Later, as a result of

macromutation(s) in tora brown sarson, the yellow sarson types have evolved and have been retained by the farmers for better seed and quality values. However, in India these types are being replaced by the *B. juncea* types because of their better yield performance, stability of production, and comparatively better tolerance to various biotic and abiotic stresses. Under European and Canadian conditions, both winter and summer (spring planted) forms of *B. campestris* and *B. napus* are being grown. But in *B. juncea*, only the spring form has evolved. Winter types of Gobhi sarson (*B. napus*) are largely grown under north European, Chinese, and Canadian conditions. But because of the short crop growing period and comparatively better winter hardiness, spring types of *B. campestris* are usually preferred and are largely grown in Sweden, Finland, and some parts of Canada and northwest China. In Indian subcontinent, the spring types of *B. juncea* and *B. campestris* cultivars are largely grown. Serious attempts are now being made to introduce *B. napus* for cultivation in northwest India but on the whole, *B. napus* is the dominant commercial species and covers nearly 75% of the total cropped area under oilseed Brassicas. It is, thus, clear that toria, Iotni brown sarson, taramira, *B. nigra*, and *B. tournefortii* are highly cross-pollinated crops, and maintain very high degree of heterozygosity. Panmixis generation after generation in nature eventually frustrates the efforts of enforced inbreeding or the fixation of genotypes. Hence, in such outbreeding population, breeding superior performing cultivars with high yield would obviously require adoption of a breeding procedure which maintains the balanced heterozygosity for the optimum plant productivity. This could be accomplished through selection (mass selection, recurrent selection, disruptive selection, and so on), breeding of synthetic and composite varieties, and ultimately by developing superior performing hybrids. On the other hand, for breeding purpose, the predominantly self-pollinated crops, such as yellow sarson, mustard (*B. juncea*), gobhi sarson (*B. napus*), *B. carinata*, and so on, should be treated as often cross-pollinated crops. The breeding objectives and appropriate breeding procedures for this group of crops are discussed in this chapter.

In the Indian subcontinent, genetic improvement of seed yield is the prime breeding objective, while in the western world, breeding for quality receives greater attention. In the Asian countries, centuries of rapeseed and mustard cultivation have led to the development of local land races of *B. juncea* and *B. campestris*, and these now form the basic raw material for the breeders.

In these crops, high number of siliquae/plant and more number of seeds/siliquae have been observed to be important yield attributes associated with its higher yield expression and could form suitable criteria to breed for high seed yield (Anand et al. 1975; Nagalakshmi 1992; Ramanujam and Rai 1963; Shabana et al. 1990). Dry matter accumulation at rosette stage and leaf area index (LAI) has also been observed to be positively associated with seed yield (Olsson 1990). Early maturing varieties (80–90 days) are usually required in the Indian subcontinent for fitting in the relay, multiple, and intercropping systems. These are suitable for escaping frost injury and for growing in the drought-prone or dryland areas with scanty rainfall.

Development of high yielding, early maturing varieties is also a major breeding objective in central China and in western Canada where frost-free days in growing

season are usually less than 100 days. The early maturing varieties complete their life cycle during this period and escape the frost injury. All over the world, breeding for resistance to diseases and insect pests has become as important breeding objective. In the Indian subcontinent, *Alternaria* leaf blight, white rust, downy, and powdery mildews are the major diseases, while in the western countries, blackleg (*Leptosphaeria maculans* Desm.) is important in Canada and Australia. Some other diseases which could cause considerable economic losses to these crops are clubroot (*Plasmodiophora brassicae*), root rot (*Rhizoctonia solani* Kuhn), stem root, and so on. In some areas, *Sclerotinia sclerotiorum* (Lib.) could pose an equal or even greater threat to cultivation of Brassicas than the blackleg disease. Races of white rust (*Albugo candida*) that could attack *B. campestris* (Race 7) and *B. juncea* (Race 2) have been identified (Pidskalny and Rimmer 1985).

European and Canadian *B. napus* cultivars are resistant to all known races of white rust, but many Chinese varieties are susceptible to Race 7 (Fan et al. 1983a, b). *B. juncea* varieties possess comparatively better field tolerance to leaf blight caused by *Alternaria brassicae* than that of the *B. campestris* selection (Rai et al. 1976). *B. carinata* selections have also been observed to show comparatively better tolerance to leaf blight than other *B. campestris* or *B. juncea* selections (Bansal et al. 1990). In the Indian subcontinent, mustard aphid (*Lipaphis erysimi* Kalt.), mustard sawfly (*Athalia proxima*), and leaf miner (*Bagrada cruciferarum*) are the important insect pests that cause considerable economic losses. *B. juncea* selections are reported to possess better tolerance to mustard aphid than *B. campestris* selection (Rai and Sehgal 1975; Rai et al. 1987). In *B. campestris*, two potential sources of dwarfing genes have been reported, and it has been suggested that they could be utilized meaningfully in developing semidwarf cultivars of toria and sarson for cultivation under high population densities for obtaining high seed yield (Rai and Kumar 1978; Rai and Singh 1993; Tyagi et al. 1983). The comparatively better salt tolerance of *B. juncea* than of *B. campestris* has made it a better choice for its cultivation under the salt-affected soils of north-western Indian states. The relative ability of the spring rapeseed cultivars to withstand the onslaught of frost at flowering time is considered important in northwest India and Sweden. There is a variable reaction of the Indian and Swedish cultivars for the frost tolerance (Aberg 1984). Several biotechnology groups are now working to transfer genes for tolerance to glyphosate, chlorosulfuron, and other herbicides into the agronomic background of the various oilseed *Brassica* varieties.

In Europe and Canada, breeding for oil and cake better suited to human nutrition, and livestock feeding has received higher research priority than anywhere in the Asian countries. While a high erucic acid rape oil is liked by industry, zero or low glucosinolate (00) oil is usually required for the human consumption. The rapeseed oil with zero erucic acid content is more or less parallel to groundnut or sesamum oil in its fatty acid composition. Consumers in east Indian states usually prefer mustard oil with pungency for frying fish or preparing pickles, while those in the west Indian states prefer oil with low pungency (Rai 1976). Now the development of “00” or canola quality varieties has been developed in different parts of the world.

4.1 Genetic Resources

The success of any crop breeding program normally depends on the extent of favorable genes available in the genetic stocks handled by the breeders. At international level, IBPGR collects, maintains, and handles the genetic diversity of a number of agrihorticultural crops. In India, National Bureau of Plant Genetic Resources (NBPGR) conserves about 19,600 accessions of different oilseed crops including 4,584 of the oilseed Brassicas and its wild allies. These are now being conserved under long-term storagein gene banks for its possible use in future (Singh and Rana 1994).

All these genetic stocks are being maintained by either sibmating or selfing. Under field conditions, an isolation distance of 400 m is required to be maintained. In the insect proof cages, glass house chambers, sibmating of the culture is affected by introducing honeybee. Pure stocks of the self-incompatible inbred lines have to be maintained by bud pollination and by selfing under muslin cloth bags in self-compatible lines. Over years, a number of genetic stocks have been identified for desirable agronomical attributes like earliness, tolerance or resistance to diseases and insect pests, shattering, frost tolerance, and so on. Some of these genetic stocks are now being utilized in crossing programs in India in intervarietal and interspecific crosses to create new genetic variability, and some are being utilized as base population for selection work. The exotic cultivars have so far not been used for direct commercial cultivation in India because of their late maturity and low yield.

4.2 Sources of Creating New Genetic Variability

In rapeseed, hybridization is accomplished by emasculating the flower buds that are due to open the following day. Next day, stigma of emasculated buds are dusted with the freshly dehisced pollen from the stamen of selected plant. Under storage conditions, pollen viability has been observed to last up to 35 days (Chiang 1974). In oilseed Brassicas, because of the cross-pollinating nature of the primary species, enough variability is available, but for searching new desired genes or gene complexes for resistance to diseases, insect pests, male sterility, fertility restoration, and so on, it requires to resort to purposeful intervarietal or distant hybridizations. Intraspecific crosses (i.e., in case of *B. campestris*; crosses between tora, Iotni brown sarson, tora brown sarson, and yellow sarson cultivars) are much successful and the success rate for such crosses, if carefully made, is greater than 90% and a single emasculated and pollinated bud may yield 10–20 crossed seeds per siliquae. However, the success rate of interspecific hybridization depends much on the genetic relationship, genomic constitution of parental species used, and also on the direction of cross. In general, the interspecific hybridization is more successful, if an amphidiploids species (*B. juncea*, *B. napus*, or *B. carinata*) is used as the female parent, which has one genome in common with the pollen parent. Hybrid between monogenomic primary species are rather more difficult to be obtained with success rate of 0.002 and 0.03 hybrids per pollinated flower (Downey et al. 1980; Mahapatra

and Bajaj 1987; Quazi 1988). The basic understanding of crossability relationship among the oilseed *Brassica* species is important to the breeders of these crops because there are good possibilities of transferring agronomically important attributes like diseases and insect pest resistance, cytoplasmic male sterility (CMS), fertility restoration, desirable quality attributes, and so on. Rao (1990) observed that out of six possible combinations between *B. juncea*, *B. napus*, and *B. carinata* including reciprocals, the *B. juncea* × *B. napus* cross was easier to be made. *B. juncea* × *B. napus* hybrid plants were observed to be more vigorous than their reciprocal. Good success was also obtained in *B. napus* × *B. carinata*, but the hybridization between *B. carinata* × *B. juncea* in either direction was rather difficult, primarily because of their nonsynchrony of flowering rather than any of its crossability barriers. In elaborate fraction I protein analysis and restriction pattern of chloroplast DNA studies, *B. nigra* and *B. campestris* have been identified as the female parental genomes in *B. carinata* and *B. juncea*, respectively (Uchimiya and Wildman 1978), and *B. oleracea* as the female parent of *B. napus* (Ichikawa and Hirai 1983; Prakash and Chopra 1991; Raut and Prakash 1985). It has now been possible to transfer blackleg-resistant genes from *B. juncea* to *B. napus* because of possible recombination between A and C genomes in *B. juncea* crosses and A and B genomes in *B. carinata* crosses (Sacristan and Gerdemann 1986). A line completely resistant to blackleg disease caused by *L. maculans* was selected from the F3 progenies of cross *B. juncea* × *B. napus* (Roy 1984). Resistance to *P. brassicae* has also been transferred from *B. napus* to *B. oleracea* (Chiang and Crete 1983). Same resistance has also been transferred to *B. napus* from *B. campestris* by subsequent backcrossing with *B. napus*. When a white rust-resistant line of *B. carinata* was crossed with that of *B. juncea*, the F1 was observed to be resistant to white rust with some additional resistance to *A. brassicae*. This has shown the possibility of transferring white rust resistance from *B. carinata* to *B. juncea* (Singh and Singh 1987, 1988). The Swedish rapeseed cultivar 821 has been developed from the cross *B. napus* × *B. chinensis* (He et al. 1987). The triazine resistance has been transferred from *B. napus* to *B. oleracea* (Ayotte et al. 1986, 1987). CMS has been transferred from *B. juncea* to *B. napus* through interspecific hybridization followed by four generations of backcrossing (Mathias 1985). The CMS was transferred from radish to *B. oleracea* (Bannerot et al. 1974; Mc Collum 1988). The fertility restorer genes for Polima-type CMS system of *B. napus* has been found in the *B. napus* var. Zem (Fan and Tai 1985). Genes for earliness have also been introgressed from *B. napus* and *B. carinata* to *B. napus* varieties. The genes for high linoleic acid have been transferred from *B. napus* to *B. napus* through selection in F2 generation (Roy and Tarr 1985; 1986). There are good possibilities of incorporating shattering resistance from *B. napus* and *B. carinata* to *B. napus* cultivars (Prakash and Chopra 1988; Rao 1990). Wide hybridization has been reported with some degree of success in the crosses of *B. spinescens* (2n = 16) × *B. campestris* (2n = 20) and for the production of *B. napus* × Raphanobrassica hybrids (Agnihotri et al. 1990a, b, c) by embryo rescue and ovary culture techniques. Protoplast fusion has helped in obtaining somatic hybrids of *B. oleracea* with Moricandia arvensis which possess intermediates C3–C4 photosynthesis carbon metabolism (Toriyama et al. 1987). From the

above-mentioned examples, it is clear that both interspecific and intergeneric hybridizations have much potential for creating new variability for rapeseed improvement. The fact is that the available natural variability of oilseed Brassica Landraces/germplasm has not yet been fully tapped and exploited with a few exceptions. If we search systematically, the needed characteristics could be found within the species of interest from close relatives. Few examples include spotting of the needed early maturing selections of *B. napus* in India and Canada, vary widely in seed size, oil content in *B. napus* in India, resistance to white rust, and blackleg in *B. napus* and *B. campestris* in France and Australia. But wherever usable variability is not available in the working germplasm, the induced mutagenesis could as well be explored and utilized.

4.3 Induced Mutagenesis for Creating New Variability

Induced mutagenesis is a useful tool for creating new variability hitherto not available, and a number of studies utilizing ionizing radiations (X-rays and cobalt 60) and chemicals such as ethylemethane sulfonate (EMS) have been used. Usually 60–80 Kr doses of rays are quite effective. Induced mutagenesis has been used to obtain mutant lines with 3% linolenic acid in *B. napus* (Rakow 1973; Robbelen and Nitsch 1974), for spotting seed color mutant in mustard (Verma and Rai 1980a), and for tolerance to leaf spot disease (Verma and Rai 1980b). It has also been very helpful in developing a number of rapeseed varieties in Sweden. Induced mutagenesis has also been used to create new variability for earliness, compact plant type, and yellow seed color in mustard at Bhaba Atomic Research Centre, Trombay, India. Two high-yielding lines of mustard (TM2 and T4) have emanated from this program.

5 Breeding Methods

Crucifers includes number of cultivated crops and wild species that have a breeding system ranging from complete cross-pollination to a high level of self-pollination. Therefore, these are quite interesting material from the breeding point of view. Selection procedure in cross-pollinated species vary from mass selection to recurrent selection and in predominantly self-pollinated ones, the desirable plants are usually selected from broad base population such as land races, segregated population, germplasm complexes, gene pools, etc., and are bulked. This bulked seed is repeatedly grown cycle after cycle. One cycle of mass selection in toria is reported to have given a yield improvement of 8.2% (Chaubey 1979).

Segregating populations or the progenies from the crosses could also make good base population for initiating recurrent selection program. In this method, the desirable individual open-pollinated plants (around 3,000) are harvested and threshed separately. A part of this seed is saved and other part of it is planted in a progeny rows,

evaluated visually and superior rows are selected, tagged and harvested separately. After harvesting and threshing, the seeds are analyzed for their 1,000-seed weight, oil content, glucosinolates and protein content, etc. Thereafter, equal quantities of the reserved seed from the selected plants are composited. This way, the first of recurrent selection cycle is completed and this composited seed is grown again in field in isolation, where intercrossing takes place among the plants within the composited populations. The second cycle of recurrent selection starts with the harvesting of the single plants (around 1,000) from this population. A bulk seed sample is harvested from the remaining plants of the population for use in replicated yield trials to determine response to selection in each recurrent cycle for character under improvement viz., oil content, seed yield or tolerance to a disease. Recurrent cycle selection is continued till reasonable level of improvement is achieved.

In self-pollinated crucifers, pure line selection is usually followed in India, which involves the isolation of superior performing lines from a genetically broad base population based upon their progeny performance. Various improved varieties like Varuna, Krishna, Kranti, Shekhar, Sita, RH-30 and Durgamani have all been developed from such simple breeding efforts (Rai 1983a, b).

6 Pedigree Method

This method may be effectively utilized for concentrating favorable genes for various economic traits and has been used to produce many cultivars in *B. napus* and *B. juncea.* In India, various high yielding varieties were developed following the pedigree selection. In this method, 5–10 F_1 plants are grown to obtain F_2 seed and 1,000–3,000 F_2 plants are grown and harvested individually from which F_3 progenies are secured. In F_4 generation, the selection is practiced. The variation among F_4 families is a good indication for the effectiveness of further selection. This method has been utilized to develop a low erucic acid high yielding and winter hardy *B. napus* variety from a cross between high erucic winter *B. napus* variety "Rapol" and the low erucic acid spring *B. napus* variety "Oro."

7 Backcross Breeding

When the desirable gene is available from unadapted or wild population, backcrossing would be the right choice, but if the favorable gene is available in an adapted or cultivated material's background, then pedigree method of selection would be the most appropriate procedure. The spring *B. napus* variety "Wester" had been developed by a combination of backcross and pedigree breeding. Backcross breeding has been used to transfer the low glucosinolate content of *B. napus* variety Bronowski, into a number of commercial cultivars of Gobhi Sarson (*B. napus*) in

various parts of the world. This method is also used to transfer new traits such as fatty acid composition, seed color, herbicide, and insect-pest resistance.

8 Development of Synthetics and Composites

In Indian subcontinent, development of composite varieties is being viewed as a possible way out for increasing the average yield production of Brassicas as these are largely grown under the rainfed conditions. These are subjected to all sorts of biotic and abiotic stresses (Rai 1979). Although synthetic in *B. napus* cultivars were also marketed in Europe, they were often not uniform and therefore this method of breeding is no longer used in *B. napus*. In Canada, efforts to develop synthetic in spring *B. napus* were not very encouraging for successful commercial cultivation. The composite breeding program in *B. rapa* and other Brassicas usually involves the production of number of intervarietal hybrid or by making their blends. This is followed by evaluation of inbreeding depression in seed yield from F_2 and later generation and the evaluation of the performance of the experimental checks against the ruling checks (Rai 1982).

Development of synthetic varieties requires the development of inbred lines, their testing for general combining ability (GCA) by making all possible cross-combinations, predicting F_2 performance constituting a number of experimental synthetic, testing the yield in trials over location and finally releasing those which excel the standard check.

9 Development of Hybrids

The basic requirement for developing commercial hybrids in crops like rapeseed is the availability of proven experimental hybrids (preferably with more than 20% standard yield heterosis), stable performing male sterile (A), maintainer (B), and fertility restoring (R) lines, good synchrony of flowering in seed and pollen parent, and adequate seed setting on male sterile seed parent through natural cross-pollination. High level of heterosis for seed yield in both spring and winter forms of *B. napus*, that is, quantitatively, 40% heterosis for yield has been reported in summer rape and 60–70% for winter form (Grant and Beversdorf 1985; Lefort-Buson and Datte 1982; Sernyk and Stefansson 1983).

A number of initial studies have demonstrated that there is considerable heterosis for yield in brassicas (Schuster and Michael 1976; Lefort-Buson and Dattee 1982), *B. rapa* (Sernyk and Stefansson 1983; Schuler et al. 1992) and *B. juncea* (Singh 1973; Larik and Hussain 1990; Pradhan et al. 1993). In India, 11–82% check parent yield heterosis has been reported in mustard (*B. juncea*), 10–72% in Gobhi Sarson, and 20–107% in *B. campestris* (Das and Rai 1972; Labana et al. 1975;

Yadava et al. 1974; Doloi 1977; Srivastava and Rai 1993), which is sufficiently high for its exploitation in hybrid cultivars. A range of 14–30% natural outcrossing is usually observed in these crops. So, this is sufficient to justify the efforts to develop cytoplasmic male sterile (CMS) lines and search for usable fertility restorer lines for producing the hybrids.

In oilseed Brassicas, a number of CMS sources viz., *Brassica carinata* CMS, *B. juncea* CMS, *B. oryrhina* CMS, *B. tournefortii* CMS, *Raphanus*-based ogura CMS, *B. napus*-based Polima CMS, *Siettiana* CMS, *Siifolia* CMS, etc., are now well known and some of them are being worked with rather intensively. Out of these CMS sources, fertility restoration has been identified in *Raphanus*-based Ogura CMS, Polima CMS in the western countries and it has been detected in the CMS-based crosses in *B. tournefortii, B. juncea* CMS, Polima CMS, and *Siifolia* CMS in India. Fortunately, Punjab Agricultural University, Ludhiana in India has recommended to release first CMS-based Gobhi Sarson hybrid PGSH-51 for cultivation in Punjab state in India.

9.1 Commercial Hybrids in B. campestris

High level of exploitable yield heterosis has been reported in *B. campestris* hybrids (Das and Rai 1972; Hutchenson et al. 1981). In this species, CMS system has been developed by backcrossing *B. campestris* cultivar "Yukina" into the *Diplotaxis muralis* cytoplasm (Hinata and Konno 1979). The *B. campestris* "yukina" CMS was stable and restorer genes have been identified for this CMS source but the genes for maintenance of CMS will have to be transferred into the background of the adapted commercial cultivars of *B. campestris* before the hybrids could be put to test in *B. campestris*. In China, where adequate labor is available, gobhi sarson hybrids have been produced utilizing this type of male sterility (Lee and Zhang 1983).

9.2 Self-Incompatibility and Hybrid Seed Production

On the basis of sporophytic type of self-incompatibility, a theoretical model of triple cross $[(A \times B) \times C] \times [(D \times E) \times F]$ technique has been suggested by Thompson (1964) to exploit heterosis and produce commercial hybrid in these crops. However, practically it has not been put to commercial use. The difficulties of producing commercial quantities of selfed seeds of self-incompatible parental lines of the hybrid and also difficulties in detecting the breakdown of self-incompatibility in the production plots during flowering duration make it rather economically unviable and unprofitable. Self-incompatibility is a good outbreeding mechanism in nature, but unfortunately, due to very high self-incompatibility, heterozygosity, and as a result of high inbreeding depression, it frustrates the efforts to produce and maintain the homozygous lines which could produce the hybrid cultivars. It is a difficult task to maintain the inbred lines through continued selfing, primarily because of big loss of vigor of the inbred population to grow and produce seeds.

10 Artificial Synthesis of Amphidiploids for Commercial Cultivation

The commonest choice for direct interspecific hybridization is to double the chromosome number in the sterile hybrids and to establish fertile amphidiploids. This provides stability and could help in the preservation of gene complexes of both the componential species by enhancing the preferential pairing of the homologous chromosomes. Artificial synthesis of some of the commercially cultivated amphidiploid species, viz., *B. napus*, *B. napus*, and *B. carinata* has been reported long back (Frandsen 1943; Ramanujam and Srinivasachar 1943; UN 1935). Artificial synthesis of *B. napus* and *B. carinata* is comparatively easier than *B. napus*. Synthesis of *B. napus* has added to the usable variability for use in India. Undoubtedly, the derivatives of synthetic *B. napus*, *B. napus*, and *B. carinata* could provide additional material for widening the range of genetic variability. It is now possible to use some of these resynthesized digenomic strains in interspecific hybridization via backcross to obtain useful genotypes for commercial production programs. At IARI, New Delhi, Raut and Kaul (1982), Raut and Prakash (1985), and Prakash and Raut (1983) have synthesized early maturing and productive amphidiploids of *B. napus* by crossing early indigenous strain of *B. campestris* var. Iotni brown sarson with that of *B. oleracea* var. *botrytis*. The selections obtained are now being field tested for their comparative yield performance with commercial check varieties. The efforts to produce and improve tetroploids of toria variety T22 (Rajan 1955; Sikka and Rajan 1957) and various yellow sarson strains for genetic improvement of seed and oil yield has not met with much of success, though the induced tetraploidy has been of some use in developing high yielding fodder varieties of Brassicas in west European countries.

11 Development of Herbicide Tolerant Cultivars

Herbicides provide an inexpensive and effective means of control of weeds in crop Brassica. Development of herbicide-resistant cultivar in Brassica was started in 1960. The tolerance was cytoplasmically controlled and effective against triazine family of herbicide. Identification of triazine-tolerant biotype of bird's rape mustard led to the development of triazine-tolerant *B. napus* oilseed cultivars through introgression of the tolerant weed biotype cytoplasm in oilseed rape. Through an interspecific cross and backcross program, the tolerant cytoplasm of *B. campestris* was combined with the nucleus of *B. napus* to produce the first triazine-tolerant cultivar, OAC Trinton (Beversdorf et al. 1980). Triazine-tolerant *B. napus* cultivars are very useful and indeed essential in fields, where highly competitive weeds such as wild mustard (*S. arvensis* L.),stickweed (*Thlaspi arvense* L.), and quack grass (*Agropyron repens* L.) are found, suggesting that the growth rate and yield of triazine-tolerant cultivars will always be significant than that of recurrent parent (Downey and Rimmer 1993).

12 Development of *Alternaria* Blight and Aphid-Resistant Cultivars

Breeding for resistance to diseases and insect pests has now become an important breeding objective. In the Indian subcontinent, Alternaria leaf blight, white rust, and downy and powdery mildews are the major diseases in rapeseed, while in the Western countries, blackleg (*L. maculans* dasm.) is important in Canada and Australia. Yield losses may range up to 70% varying from location to location and year to year. No resistance has been reported against this disease in oilseed Brassicas. However, some of the interspecific crosses have been attempted between *B. napus* and *B. alba* with an objective to transfer Alternaria resistance from *B. alba* to *B. napus* (Brim et al. 1987; Chevre et al. 1991; Dueck and Degenhardt 1975; Rai 1976). Tewari (1991) have shown that more distantly related Crucifereae species may be very resistant to black spot. Some other diseases, which could cause considerable economic losses to these crops, are clubroot (*P. brassicae*), root rot (R. I Kuhn), stem rot, and so on. In some areas, *S. sclerotiorum* (Lib.) could pose an equal or even greater threat to the cultivation of Brassicas than blackleg disease. Races of white rust (*A. candida*) that could attack *B. campestris* (Race 7) and *B. napus* (Race 2) have been identified (Pidskalny and Rimmer 1985). European and Canadian *B. napus* cultivars are resistant to all known races of white rust but many Chinese varieties are susceptible to Race 7 (Fan et al. 1983). *B. napus* varieties possess comparatively better field tolerance to A. brassiceae-caused leaf blight than that of the *B. campestris* selections (Rai 1976). *B. carinata* selections have also been observed to show comparatively better tolerance than other *B. campestris* or *B. napus* selections (Bansal et al. 1990). In the Indian subcontinent, mustard aphid (*L. erysimi* Kalt.), mustard sawfly (*Sathalia proxima*), and leaf minor (*B. cruciferarum*) are the important insect pests that affect and cause economic losses. *B. napus* selections possess better tolerance to mustard aphid than *B. campestris* selections (Rai and Sehgal 1975; Rai et al. 1987).

13 DH Breeding and In Vitro Mutagenesis

Doubled haploidy (DH) breeding through microspore culture is very well developed in Brassicas (Maluszynski et al. 2003; Xu et al. 2007). The DH technology in Brassicas aims at developing fully homozygous plants in a single generation, which could be further used in mutation breeding, genetic engineering, in vitro screening for complex traits like drought, cold and salinity tolerance, and for developing mapping populations for linkage maps using molecular markers (Pratap et al. 2007). Several methods are available for DH production in Brassicas such as microspore culture, anther culture, and ovary/ovule culture. The possibility to produce haploids in *B. napus* from anther culture (Keller and Armstrong 1978) and microspore culture (Lichter 1982) has provided the breeders with a new tool for breeding improved cultivars of rapeseed and mustard (Zhou et al. 2002a, b).

The initiation of microspore culture experiments was followed by extensive investigations on various aspects of embryogenesis in anther and microspore culture and as a result, DH technology has been developed to its present form in Brassicas (Charne and Beversdorf 1988; Yu and Liu 1995; Wang et al. 1999, 2002; Shi et al. 2002). Microspore culture technique has widespread applications in *Brassica* breeding due to its relative simplicity, efficiency in haploid and doubled haploid production, mutation and germplasm regeneration, and gene transformation (Xu et al. 2007). Also microspore cultures provide the best material for mutation induction in haploid cells (Szarejko and Forster 2007). Microspore embryogenesis is affected by a number of factors such as donor plant genotype and conditions, pretreatment, growth stage of the anther/microspore to be cultured, culture media and environment, and diploidization process, etc. (Dunwell 1996; Gu et al. 2003, 2004; Zhang et al. 2006; Pratap et al. 2007).

Mutagenic treatments may have significant effects on the efficiency of DH breeding. McDonald et al. (1991) reported that UV light had harmful effects on embryo formation in rapeseed though regeneration remained unaffected and at the same time, gamma irradiation decreased the frequency of embryos and plants. The induction of mutation in haploid cells involves isolating the developing microspores at the late uninucleate stage followed by pretreatment and their culturing on specialized media, which lead to direct embryogenesis rather than formation of pollen (Szarejko and Forster 2007). Mutagenic treatment is given shortly after the isolation of microspores or after pretreatment, before the first nuclear division. Due to direct embryogenesis, uninucleate microspore is the ideal target for in vitro mutagenesis. Also, microspores are far more sensitive to mutagenic treatments than other explants and therefore yielded better results.

DHs also provide an efficient screening material for the desired mutants and other material for complex traits. Since through microspore-derived DHs we can obtain a very large number of synchronously developing embryos, we can modify the system to screen them in vitro for various desirable traits. For example, for development of herbicide-resistant Brassicas, the active chemical is introduced in the culture medium after mutation treatment (Beversdorf and Kott 1987) and the surviving plants after chromosome doubling could be raised under controlled conditions and later screened for this trait. Similarly, effective selection could also be done for drought, cold, and salinity tolerance. By using this technique, several herbicide-resistant mutants have been developed in rapeseed (Kott 1995, 1998; Swanson et al. 1988, 1989).

Though embryogenic microspores are the prime targets for mutagenic treatment, other haploid tissues and cells have also been treated with mutagens in Brassicas. In *B. napus*, isolated microspores have been treated with chemicals such as EMS (Beversdorf and Kott 1987), NaN_3 (Polsoni et al. 1988), MNU (Cegielska-Taras et al. (1999) and ENU (Swanson et al. 1988, 1989) and physical mutagens such as gamma rays (Beversdorf and Kott 1987; McDonald et al. 1991), X-rays (McDonald et al. 1991) and UV rays (Ahmad et al. 1991; McDonald et al. 1991). *B. napus* anthers have also been treated with gamma rays and fast neutrons by Jedrzejaszek et al. (1997). Similarly, microspores of *B. carinata* have been treated with EMS and UV rays (Barro et al. 2001, 2002) and *B. campestris* with UV rays (Zhang and

Takahata 1999; Ferrie and Keller 2002). In *B. juncea* also, isolated microspores as well as haploid embryos have been treated with chemical mutagens.

Despite great promise, the use of DH technology as a routine breeding tool for *Brassica* improvement is yet to be seen, mainly due to problems associated with anther/microspore culture (Pratap et al. 2007). These include low regeneration rate, highly genotype-specific response and high frequency of callogenesis but low recovery of DH plants. The focus of rapeseed breeders has lately shifted toward more specific and practical goals such as development of herbicide-tolerant varieties, development of male sterile lines for hybrid seed production, oil and meal quality improvement, and also drug production (Gupta and Pratap 2008). For this, DH breeding has to be adopted in conjugation with newer ideas such as directed in vitro mutagenesis, in vitro screening for desirable traits, and incorporation of molecular markers.

14 Genetic Transformation

Biotechnology has opened up new horizon for novel agronomic and quality traits in responsive crops such as Brassicas by providing access to novel molecules, ability to change the level and pattern of gene expression and development of transgenics with insecticidal genes. With the development of genetic transformation techniques, it has become possible to bring about quick and dramatic improvements in the tolerance to many Lepidopteran and other insect pests, and herbicides, improvement in oil quality for industrial and domestic use and development of pharmaceuticals and industrial products. Much emphasis is now placed on the transgenic technology toward the improvement of cultivated Brassicas. As a result, the global area of biotech canola has reached to an estimated 5.5 million ha in 2007 (James 2007), majority of it being under herbicide-resistant canola.

Successful genetic transformation systems have been developed in many economically important Brassicas such as *B. napus* (Moloney et al. 1989), *B. oleracea* (De Block et al. 1989), *B. juncea* (Barfield and Pua 1991), *B. carinata* (Narasimhulu et al. 1992), *B. rapa* (Radke et al. 1992) and *B. nigra* (Gupta et al. 1993). However, among all the systems, *Agrobacterium tumefaciens*-mediated gene transfer is most widely used in *Brassica* and it is also quite efficient and practical in most of the species in the genus (Cardoza and Stewart 2004).

Rapeseed cultivars tolerant to herbicides such as imidazoline, glyphosate, and glufosinate are available commercially in USA and Canada (Cardoza and Stewart 2004). For insect resistance, the gene from *Bacillus thuriengiensis* has been introduced in canola cultivars (Stewart et al. 1996; Halfhill et al. 2001), which leads to overproduction of δ-endotoxins in the insects feeding on transgenic canola. This crystalline prototoxin gets inserted into the midgut plasma membrane of the insect, leading to lesion formulation and production of pores that disturb the osmotic balance. These cause swelling and lysis of the cells and as a result, the larvae stop feeding and die (Hofte and Whiteley 1989; Schnepf et al. 1998; Shelton et al. 2002).

Canola varieties with increased linolenic acid (Liu et al. 2001), stearate (Hawkins and Kridl 1998), laurate (Knutzon et al. 1999), and increased enzyme activity (Facciotti et al. 1999) have been developed through genetic transformation. Further, Brassicas have been transformed to develop various industrial and pharmacological products also. For example, *B. carinata* has been transformed for the production of hirudin, a blood anticoagulant protein (Chaudhary et al. 1998) while *B. napus* has been used for the production of carotenoids (Shewmaker et al. 1999). Development of male sterile lines and fertility restoration systems has also been achieved through genetic transformation in *B. napus* (Jagannath et al. 2001, 2002), which could be of tremendous potential for development of commercial hybrid cultivars. Similarly, salt- and cold-tolerant tolerant lines have also been developed in *B. juncea* by engineering of the bacterial *codA* gene (Prasad et al. 2000). Transgenic lines of "Wester" having high palmitic and stearic acids have been developed by Hitz et al. (1995) High oleic acid containing *B. napus* and *B. juncea* lines with better shelf life have also been obtained through the transgenic technology (Stoutjesdijk et al. 2000).

15 Quality Improvement

Brassica oil is nutritionally superior to most of the other edible oils due to the lowest amounts of harmful saturated fatty acids and a good proportion of mono- and polyunsaturated fatty acids in it (Agnihotri et al. 2007). However, the value of its oil and meal gets restricted due to the presence of two major antinutritional substances, erucic acid – a long carbon chain unsaturated fatty acid, and glucosinolate – the sulfur-containing compounds.

Oil quality mainly relates to fatty acid composition of the seed. High contents of erucic and eicosenoic acids in *Brassica* oils decrease the profile of oleic, linoleic and linolenic acids, rendering them inferior in quality to those from other oilseeds (Gupta and Pratap 2007). Therefore, one of the most important breeding objectives in *Brassica* breeding has been the genetic modification of the seed quality by changing the proportion of fatty acids suitable for nutritional as well as industrial purposes. Modifications in the compositions in fatty acids have been achieved in past through various conventional breeding methods coupled with biotechnological techniques such as induced mutation, in vitro embryo rescue, DH technique and genetic engineering, especially posttranscriptional gene-silencing (Agnihotri et al. 2007).

Dietary recommendations in many countries focus attention on limiting total fat intake to 30% of energy and saturated fat intake to 10% of energy. Breeding approaches in reducing the saturates include interspecific crosses followed by selection, reconstitution of *B. napus* from *B. rapa* and *B. oleracea* strains with reduced saturate levels, and mutagenesis in both *B. rapa* and *B. napus*. For reduction in linolenic acid, both mutagenic source and genetic transformation can be used. Gas liquid chromatography (GLC) (Craig and Murphy 1959) and the technique of using only half of the cotyledon to test the erucic acid content together provide quick means to screen very large populations necessary to identify genetically changed

Brassica strains with low or zero erucic acids. With this technique, the desirable strains with half cotyledon intact have been grown and carried forward and with this, low erucic acid strains of *B. napus* (Stefansson et al. 1961; Downey and Harvey 1963) and *B. campestris* (Downey 1964) had been developed in early 1960s. Later, such strains were developed in *B. juncea* (Kirk and Oram 1981) and *B. carinata* (Alono et al. 1991). Gupta et al. (1994, 1998) identified low erucic acid genetic stocks among the Indian accessions of *B. juncea.* Several low erucic acid *B. juncea* genotypes have been developed in India through interspecific hybridization (Khalatkar et al. 1991; Malode et al. 1995), and transgressive segregation through interspecific/intergeneric hybridization, followed by pedigree method (Agnihotri et al. 1995; Agnihotri and Kaushik 1998, 1999a, b). Similarly, other fatty acids have also been modified in oleiferous Brassicas and high oleic and low linolenic acid *B. juncea* genotypes have been developed (Oram et al. 1999; Potts et al. 1999).

Brassica seed meal is an important source of nutrition for animals. However, undesirable components in the meal such as glucosinolates render them unfit for animal and human consumption. In high concentrations, in nonruminants like swine and poultry, it hydrolyzes to form thiocynates, isothiocynates or nitriles and can adversely affect iodine uptake by the thyroid gland and can reduce their weight gains (Fenwick et al. 1983). The high-performance quantitative GLC technique (McGregor et al. 1983a, b, Spinks et al. 1984; Brazezinski et al. 1986) has made it possible to obtain the profiles of glucosinolates and also measure their absolute levels. Besides glucosinolates, other antinutritional factors such as sinapine, phenolic acid, tannins and phytic acid also interfere with the digestive enzymes, especially those affecting protein hydrolysis. To improve the quality of *Brassica* seed meal, the glucosinolate contents should either be decreased or altogether eliminated from the meal through appropriate breeding techniques. However, unfortunately, the genes controlling glucosinolate content in rapeseed are either pleiotropic or in linkage with the seed filling stage and have a positive correlation with 1,000-seed weight (Oliveri and Parrini 1986). This renders the strict selection difficult for quality traits in early segregating generation, lest genotypes for high seed yield could be lost. Therefore, it is advocated to keep the population heterozygous for quality characters and select the plants for these characters in advanced generation.

Till date, the "Bronowski" gene is the only known source for low glucosinolates content and no natural germplasm source for stable low glucosinolates genes has been reported (Agnihotri et al. 2007). "Bronowski" is a Polish *B. napus* cultivar, which has a glucosinolate content of about 12 μmol/g oil free meal and 7–10% of erucic acid in the oil. Considerable success has been achieved in Australia in the development of low glucosinolate genotypes using mutagenesis, interspecific hybridization, and tissue culture coupled with pedigrees election (Oram et al. 1999). In India, two transgressive segregants (TERI 5 and TERI 6) with low glucosinolate and a Canadian accession BJ-1058 have been used to develop low-glucosinolate genotypes in the background of *B. juncea* var. Pusa Bold (Agnihotri and Kaushik 2003a, b; Agnihotri et al. 2007).

Breeding of "canola" type of cultivars in Brassica with less than 2% erucic acid in the oil and less than 30 μmol/g of glucosinolate in defatted meal (commonly

known as "00") has been done and several such varieties viz., Cyclone (Denmark), Shiralee (Australia) and AC Excel (Canada) are now available (Rakow 1995). In Australia, several double low cultivars of *B. juncea* have shown promising yield potential (Burton et al. 2003b). In India, Agnihotri and Kaushik (2003a, b) have reported successful introgression of double low traits in *B. juncea* cultivar "Varuna" using low erucic acid donors TERI (OE) M21 and Zem-1, and low glucosinolate line BJ-1058. The double low *B. napus* varieties GSC-865 and TERI-Uttam-Jawahar have been released for commercial cultivation in the states of Punjab and Madhya Pradesh, respectively (Agnihotri et al. 2007).

Introduction of double low (low erucic acid–low glucosinolates) genotypes of *B. napus* has followed their extensive cultivation in many countries of the world and experimental work toward development and improvement of low erucic acid germplasm for other species is being pursued at global level (Rakow and Raney 2003). At present, the breeding efforts in the development of canola quality double low *B. napus* cultivars in improving the oil composition and enhancing vitamin levels are underway in many countries of the world including Germany (Luhs et al. 2003), Canada (Raney et al. 2003a,b), United States (Corbett and Sernyk 2003), Australia (Gororo et al. 2003), France (Carre et al. 2003) and Poland (Spasibionek et al. 2003). Yellow seed coat color also adds to high oil content and therefore this could also be another breeding objective for improved Brassicas.

16 Conclusion

The current trends in the rapeseed breeding research indicate that to maintain the tempo of progress in quality and yield improvement work in these crops, much expanded research efforts would be needed to solve the emerging and challenging problems ahead. Now, there would be far greater need for the collection, computerization, and creation of new usable genetic variability, greater application of cytoplasmic male-sterility techniques, chromosomal mapping studies, and assimilation of many new and novel ideas for tackling new problems. It would also be desirable to make broad-based genepools of different cross-pollinated species of oilseed Brassicas and maintain at least one gene pool having all the available collection of the group. Such a population is likely to have more natural recombination hitherto not available in nature and provide an opportunity to break some of the existing undesirable genetic linkage, and provide good base population for future recurrent selection programs. Exploitation of heterosis in oilseed Brassicas would require more intensive and concerted efforts for effective utilization of cytoplasmic male sterility. In years to come, meaningful basic work would also be needed on the stability of sterility in CMS lines, understanding of the mechanism of fertility restoration, extent of cytoplasmic penality that would normally be expected on using CMS lines from a very distant wild types/species of genera and on perfectization of hybrid seed production techniques. In the Indian subcontinent, some of recently introduced materials of the disease-resistant or canola quality lines from outside may not be

high yielders per se under the local condition, but they could possibly make good parental lines for production of hybrids and if that possibility exists, it should be explored. The production of doubled haploid lines, either by microspore or anther culture techniques, is now possible to rapidly produce homozygous inbred lines from the promising *B. napus* and *B. campestris* genotypes as well. Such inbreds could be produced and used to develop more productive hybrid cultivars. The inputs available from biotechnology may more purposefully be utilized in solving the pressing problems of male sterility, fertility restoration, crossability, and oil and seed meal quality. An exciting and challenging area of rapeseed breeding research would be to develop cultivars with built-in genetic resistance to devastating insect pests like mustard aphids and *Alternaria* leaf blight disease in the Indian subcontinent and for the important diseases like blackleg (*L. maculans* Desm.), clubroot (*P. brassicae*), *S. sclerotiorum*-caused damage, and white rust diseases in some of the western countries where these diseases threaten production. Development of fertilizer responsive, nonlodging, compact plant types with high population densities would be more rewarding breeding preposition in the years to come. Incorporation of dwarfing genes and development of semidwarf varieties of *B. campestris* could pay rich dividends. Presently, in the Indian subcontinent, much emphasis is being laid on the genetic improvement of yield but the future will see much expanded genetical and breeding investigations to improve quality characteristics of the commercial cultivars to meet the export needs. The search for genes governing thermo- and photoinsensitivity and also better photosynthetic activity would receive far greater attention than what is at present. The incorporation of such genes in rice and wheat has proved useful in expanding the areas of their production. So, why not in rapeseed? The cultivars with these genes could be grown over a wide range of crop growing conditions and this would help in increasing the overall production of these important oil crops.

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Chapter 4
Sunflower

Yalcin Kaya, Sinisa Jocic, and Dragana Miladinovic

Abstract Sunflower (*Helianthus annuus* L.) is one of the main oil crops in the world. Sunflower is a native crop in North America. It was first domesticated by the Indians who used it as food and medicine as well as body painting in ceremonies. *Helianthus* genus comprises 51 species, 14 annual and 37 perennial. Interspecific hybridization plays an important role in sunflower breeding, especially when the variability of the cultivated form has been exhausted and it becomes necessary to look for desirable genes from wild types. During its historical development, sunflower breeding has gone through three phases characterized by the breeding method dominantly employed: (1) mass selection, (2) method of individual selection for developing open pollinated cultivars, and (3) method of sunflower hybrid development. The development of variation in initial breeding material is a primary task in the genetic and breeding programs of sunflower. Methods of molecular breeding are already used in sunflower breeding as tool for acceleration of breeding process. A great number of molecular markers have been developed during last three decades. Their convenience for the use in sunflower breeding depends on the type and goal of research. Major goals in sunflower breeding remain high seed and oil yield, improved oil quality, as well as resistance to different stresses. Broomrape has been the most serious problem in sunflower production in Southern and Eastern Europe leading to considerable yield losses up to 100% and reducing sunflower seed quality. Although genetic resistance is the most effective and feasible control against broomrape, application imidazolinone (IMI) herbicide as post emergence application offers an efficient control to broomrape too. Weed control with transgenic herbicide-resistant genes have been used widely in some crops in the world, but in sunflower only IMI and SU herbicide resistance which is transferred to cultivated sunflower from wild types utilizing backcross breeding is commonly used. Non-oilseed sunflower seeds

Y. Kaya (✉)
Trakya Agricultural Research Institute, Edirne, Turkey
e-mail: Yalcinkaya22@gmail.com

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are used mostly for confectionery as snack but also for feeding birds and small pets. Other direction of non-oil sunflower breeding is creation of ornamental varieties.

Keywords Sunflower (*Helianthus annuus* L.) breeding • MAS • Interspecific hybridization • Breeding methods • Molecular breeding • Herbicide resistance

1 Introduction

Sunflower (*Helianthus annuus* L.) is one of the main oil crops in the world. Although it originated from North America, nowadays, sunflower grows mainly in the Black Sea region having more than a half percent of the world production and plant area (Kaya et al. 2008a) since the first breeding efforts and higher seed and oil yielding varieties were developed in Russia. Today, the world sunflower areas are about 23 million ha and the production is about 30 millions MT. Sunflower produces healthy oil, well accepted by the consumers because of its high content of mono- and poly-unsaturated fatty acids as well as vitamin E. In the recent years, new sunflower oil types have been developed through conventional breeding approaching for specific applications, mainly in the food industry. Such specialty oils will play an important role in a further development of the sunflower crop.

2 History, Origin and Domestication

2.1 Sunflower History

Sunflower is a native crop in North America. It was first domesticated by the Indians who used it as food and medicine as well as body painting in ceremonies. Archaeological evidence indicates that the Indians started to cultivate and use sunflower as early as 2300 BC and it means that sunflower domestication could be before that of corn, beans and squash. Sunflower seeds were usually mixed as flour in soups and meals or even used as coffee at earlier times. On the other hand, sunflower hulls and petals were utilized for preparing dyes, petals and pollens for face painting, while sunflower oil was used in cooking and hair treatment (Heiser et al. 1969). In addition to medicinal purposes such as treating warts and snake bites, expelling worms, improving eyesight, etc., sunflower was also a symbol of the solar deity for some American tribes.

Sunflower was taken to Europe by Spanish explorers in 1500s and was utilized widespread for ornamental and medicinal purposes (Heiser et al. 1969). After great breeding efforts to increase the oil content in Russia in the mid-twentieth century, sunflower turned into essential plant identity in the world. Sunflower is largely produced in many parts of the world, after discovering cytoplasmic male sterility

(CMS) system (Leclercq 1969) combined with fertility restoration by nuclear genes (Kinman 1970) with enabling commercial production of hybrid seed.

2.2 *Origin and Domestication*

Sunflower (*H. annuus* L.) belongs to Compositae (Asteraceae) family and its chromosome number is 17. Helianthus is derived from the Greek words "helios", meaning sun, and "anthus", meaning flower. Sunflower has the same meaning in many languages such as "Sonnenblume" in German, "Girasol" in Spanish and "Tournesol" in French. *Helianthus* genus has diploid, tetraploid and hexaploid species but the cultivated sunflower (*H. annuus*) is the most important species largely grown. *Helianthus* genus has 51 species (14 annual and 37 perennial) (Heiser et al. 1969; Schilling and Heiser 1981; Jan and Seiler 2007; Fernandez et al. 2010).

Recent archaeological evidences indicated that sunflower was domesticated first in central USA (Hayes Site in Tennessee) 4,300 years ago (Crites 1993). The origin of cultivated sunflower has been also investigated applying molecular techniques. Based on their study in many wild and cultivated sunflower lines, Rieseberg and Seiler (1990) indicated that sunflower cultivated lines had a single origin of domestication, because these lines exhibited reduced allozyme variability and all of them were characterized by a single cpDNA, using RFLP haplotype. Harter et al. (2004) found that domesticated sunflowers arose from wild populations in the central part of the USA, based on patterns of nuclear *s*imple *s*equence *r*epeats (SSR) diversity. On the other hand, Burke et al. (2002) utilizing QTL analysis, concluded that strong direct selection on especially increasing seed size have played an important role in sunflower domestication.

3 Genetic Resources

Sunflower germplasm are very important sources for plant breeding, consisting of genetic variability from cultivated ones to wild species with keeping ex situ (accessions preserved in seed banks) and in situ resources (wild populations and land races). They carry relevant traits such as disease resistance (downy mildew, Phomopsis, Rust, Sclerotinia), CMS, male fertility restoration (Rf), abiotic stress tolerance (drought, salinity), plant architecture (petiole-less type), etc.

The wild and cultivated sunflower germplasms preserved in seed banks supply very useful and valuable genes to sunflower breeders. N.I. Vavilov All-Union Scientific Research Institute (VIR) at St. Petersburg, Russia and U.S. National Plant Germplasm System (NPGS) Ames, Iowa, USA have the largest collections in the world having 2,811 and 3,860 accessions, respectively, including wild species and cultivated origin collected in many places from the world (Fernandez et al. 2009).

Table 4.1 The classification of annual *Helianthus* species ($n = 17$)

Section	Species
Helianthus	*H. annuus* L.
	H. anomalus S.F. Blake
	H. argophyllus Torr. & A. Gray
	H. bolanderi A. Gray
	H. debilis Nutt.
	Subsp. *debilis*
	Subsp. *cucumerifolius* (Torr. & A. Gray) Heiser
	Subsp. *silvestris* Heiser
	Subsp. *tardiflorus* Heiser
	Subsp. *vestitus* (E. Watson) Heiser
	H. deserticola Heiser
	H. exilis A. Gray
	H. neglectus Heiser
	H. niveus (Benth.) Brandegee
	Subsp. *canescens* (A. Gray) Heiser
	Subsp. *niveus*
	Subsp. *tephrodes* (A. Gray) Heiser
	H. paradoxus Heiser
	H. petiolaris Nutt.
	Subsp. *fallax* Heiser
	Subsp. *petiolaris*
	H. praecox Engelm. & A. Gray
	Subsp. *hirtus* (Heiser) Heiser
	Subsp. *praecox*
	Subsp. *runyonii* (Heiser) Heiser
Agrestes	*H. agrestis* Pollard
Porteri	*H. porteri* (A. Gray) Pruski

Due to the lack of possibility of genetic resources to be preserved in seed banks, especially wild species, a significant proportion of the wild sunflower populations exist in their natural habitats as in situ especially in USA and some places in North America. However, it is not possible to keep them safe for long time and some species are endangered or even extinct because of urbanizations, hybridizing with common sunflower and their lower genetic diversity (Seiler and Rieseberg 1997).

3.1 Wild Sources

Helianthus genus has 51 species consisting 14 annual and 37 perennial species (Schilling and Heiser 1981; Jan and Seiler 2007; Fernandez et al. 2009). All annual species of *Helianthus* includes cultivated sunflower *H. annuus* L. are diploid ($2n = 34$) (Table 4.1).

3.2 Interspecific Hybrids

The wild *Helianthus* species is a very valuable source in sunflower breeding programs having desirable genes to obtain introgressed sunflower hybrids with improved disease resistance, oil yield and quality, etc., interspecific crosses. Interspecific hybridization plays an important role in sunflower breeding, especially when the variability of the cultivated form has been exhausted and it becomes necessary to look for desirable genes from wild types (Table 4.2). The interspecific hybridization has been successfully applied for sunflower to produce new cultivars with useful traits of both parents and incorporate desirable trait of one specific to another. Advanced breeding techniques such as embryo rescue, polyploidization, protoplast fusion, and other molecular methods are used as well (Jan 1997).

Although relevant results on interspecific crosses have been obtained by far, there are still some difficulties on wide crosses between different species of *Helianthus*, especially in perennial species. Crosses between cultivated sunflower and diploid annual species have been easily performed, but the resulting progeny is more or less female sterile due to translocations (Chandler et al. 1986). In such crosses, performed for breeding purposes, cultivated sunflower is usually used as the female parent to avoid cytoplasm loss, unless the cytoplasm from the wild species is desired (Serieys 2002).

Wide crosses between annual and perennial *Helianthus* species (diploid ones, $2n = 34$) were generally obtained either by pollination and natural achene development or by in vitro embryo rescue methods application (Christov 1991; Jan 1996; Sukno et al. 1998; Faure et al. 2000). However, crosses between sunflower and perennial species, e.g. pollinating sunflower with hexaploid (*H. tuberosus*) or diploid species (*H. mollis* or *H. maximiliani*) of Section Atrorubentes, usually failed (Faure et al. 2002).

The level of hybridization in progeny could be determined by molecular markers in the interspecific crosses. Hybridization was performed by leaving embryos to develop normally on the head (classical crossing) or using embryo rescue. F_1 sister progeny shared different sets of molecular markers representing a few of those of the wild species used as the pollen donor (Jan 1997) .

3.3 Other Sources (Public Released Materials, Open Pollinated Varieties, Landraces, Inbred Lines, etc.)

Public lines, land races and open pollinated varieties are also very important resources for sunflower breeding that comprise unique morphological and physiological traits and specific characteristics such as high oil and fatty acid content (Vick et al. 2007), disease (Gulya et al. 1997) and herbicide resistance (Miller and Al-Khatib 2002). Additionally, these lines have suitable plant design and stabile genes, and exhibit characteristics fixed to the environments so that they could be used as a main plant in the crossings as well as tester lines.

Table 4.2 The classification of perennial *Helianthus* species

Section	Series	Species	Chromosome no. (n)
Ciliares	Ciliares	*H. arizonensis* R.C. Jacks.	17
		H. ciliaris DC.	34, 51
		H. laciniatus A. Gray	17
Ciliares	Pumili	*H. cusickii* A. Gray	17
		H. gracilentus A. Gray	17
		H. pumilus Nutt.	17
Atrorubens	Coronasolis	*H. californicus* DC.	51
		H. decapetalus L.	17, 34
		H. divaricatus L.	17
		H. eggertii Small	51
		H. giganteus L.	17
		H. grosseserratus M. Martens	17
		H. hirsutus Raf.	34
		H. maximiliani Schrad.	17
		H. mollis Lam.	17
		H. nuttallii Torr. & A. Gray	
		Subsp. *nuttallii*	17
		Subsp. *parishii* (A. Gray) Heiser	17
		Subsp. *rydbergii* (Britton) R. Long	17
		H. resinosus Small	51
		H. salicifolius A. Dietr.	17
		H. schweinitzii Torr. & A. Gray	51
		H. strumosus L.	34, 51
		H. tuberosus L.	51
Atrorubens	Microcephali	*H. glaucophyllus* D.M. Sm.	17
		H. laevigatus Torr. & A. Gray	34
		H. microcephalus Torr. & A. Gray	17
		H. smithii Heiser	17, 34
Atrorubens	Atrorubentes	*H. atrorubens* L.	17
		H. occidentalis Riddell	
		Subsp. *occidentalis*	17
		Subsp. *plantagineus* Heiser	17
		H. pauciflorus Nutt.	
		Subsp. *pauciflorus*	51
		Subsp. *subrhomboideus* Rydb.	51
		H. silphioides Nutt.	17
Atrorubens	Angustifolii	*H. angustifolius* L.	17
		H. carnosus Small	17
		H. floridanus A. Gray ex Chapm.	17
		H. heterophyllus Nutt.	17
		H. longifolius Pursh	17
		H. radula (Pursh) Torr. & A. Gray	17
		H. simulons E. Watson	17
		H. verticillatus Small	17

4 Major Breeding Goals

4.1 Seed Yield

Seed yield is the main goal not only in sunflower breeding but in all crop improvement programs. In sunflower it is a quantitatively inherited component, highly influenced by environmental factors, that also depends on the genetic potential of the cultivar and contributions of other yield components, such as seed weight, head diameter and plant height (Dagustu 2002; Kaya et al. 2003, 2005, 2007c; Joksimovic et al. 2004; Goksoy and Turan 2007). Kaya et al. (2009a) also indicated that the earliness of hybrids also played an important role in determination of the seed yield in sunflower with earlier flowering period and physiological maturity duration than shorter than 107 days. They also mentioned that in order to get higher yield performance, oil-type sunflower hybrids should have higher seed volume, higher oil content, taller plant height, larger heads and lower husk contents.

On the other hand, hybrids have higher seed yield potential due to the heterosis in sunflower. Fernandez et al. (2009) indicated that the major achievements in improving grain yield in sunflower are related to the improved combining ability of the hybrid parents and selection for adaptation to specific conditions such as durable plant stem, high self-fertility and pronounced head inclination to resist the influence of extremely hot temperatures, sun lights and bird damages.

4.2 Oil Content

Since sunflower is cultivated mainly for oil production, the oil content and yield are the main issues in sunflower breeding. In most parts of the world, crushing factories give extra premium to each increase of oil content over 40%. After great breeding efforts of Pustovoit in the first part of twentieth century, sunflower is turned from ornamental plant in the gardens into an oil crop not only in the USSR, but also throughout the world. The local varieties produced in Russia contained only 30–33% of oil and increased up to 43–46% in 1950s especially when the Pustovoit Method of Reserves started to be applied (Fick and Miller 1997).

The oil content and yield are also influenced by environmental factors and other yield traits. Kaya et al. (2009b) indicated that over 70 g seed weight per thousand, 53% oil content, 24 cm head diameter, 73 day on flowering, 105 day at physiological maturity and 45 day at seed filling periods tended to reduce the oil yield of sunflower hybrids. The oil content is also negatively correlated with husk content but Kaya et al. (2007c) observed a negative correlation between yield and oil content up to 40–45%, but both values increased equivalently after this point.

4.3 Oil Quality

Oil quality in sunflower is determined by the fatty acid composition and the levels of tocopherols, sterols, carotenoids and other compounds. Normal sunflower oil is composed of 55–65% linoleic acid (C18:2) and 20–30% of oleic acid (C18:1). The remaining 5–10% comprise of palmitic and stearic acids (C16:0 and C18:0, respectively). The standard sunflower oil contains high proportion of linoleic acid which is a polyunsaturated fatty acid and also a good source of calcium, phosphorus, nicotinic acid and vitamin E (Friedt et al. 1994; Joksimovic et al. 2006). There exists a negative correlation between the contents of oleic and linoleic acid and their contents are genetically controlled (Fick and Miller 1997).

The first oleic type sunflower was developed by Soldatov (1976) from induced mutation (treated with a 2.5% solution of dimethyl sulphate) then it spread out worldwide using this source of genes developing high oleic (HO) hybrids having over 90%. Fernandez-Martinez et al. (2009) mentioned that the inheritance of HO acid content determined partial dominance of at least single gene, Ol_1 dominance of one or modified single recessive gene.

Sunflower oil has higher E vitamin content as a source of antioxidant, having α, β and γ-tocopherol. Increased β-tocopherol is controlled by a single recessive gene called tph1 and a recessive gene tph2 controls increased γ-tocopherol (Demurin 1993). Due to the achievements of sunflower breeding for different oil quality, sunflower oil from high-oleic hybrids with altered tocopherol profile (Ol + tph1; Ol + tph2Ol + tph1tph2) will have much longer shelf-life than the standard sunflower oil (Skoric et al. 2008).

4.4 Seed Quality

Sunflower seeds, comprising kernel and hull contain significant amounts of amino acids, proteins and other compounds forming the nutritional value of the sunflower meal used in animal nutrition. Hull content of sunflower seed is one of the essential quality features because of the negative relationships between it and the oil content. The husk percentage in sunflower should be about 20–25% in order to get higher oil content but in confectionery types it is about 70%. However, the oil mostly extracted from seeds during the crushing process by chemically to obtain crude oil and the rest part of oil with protein comprises rich meal for animals.

High-quality sunflower meal should have lower fibre, higher lysine and protein content, and lower phenolic compounds such as chlorogenic, caffeic and also phytic acids that reduce the nutritive value of sunflower meal. Therefore, the main goal in the breeding program, especially for the meal nutritive quality of sunflower is to increase the protein content (known to be around 17% in current cultivars) and lysine which is deficient in sunflower, as well as to reduce the fibre content to improve meal digestibility. The protein content is quantitatively inherited by the genotype of the plant with predominance of additive gene effects and medium to high heritability (Alza and Fernandez-Martinez 1997; Fernandez-Martinez et al. 2009). On the other

hand, the existence of variability for higher lysine content in sunflower seeds have been reported in cultivated germplasm and wild sunflower accessions and it could be increased by selection (Ivanov 1975; Christov et al. 1993).

4.5 Morphological Traits

4.5.1 Plant Height

Sunflower is normally a tall plant. Some wild types could reach 4–5 m, while cultivated ones are usually about 150–200 cm high. The height of the plants is very dependant to climatic and soil conditions and while drought or poor nutrition soil drastically reduce it, irrigating and less water stress affect the plant height very positively. In addition to the standard-height (150–180 cm) hybrids, both semi-dwarf (100–150 cm) and dwarf (50–100 cm) ones have been also produced in the world. Although reduced plant height has many advantages such as resistance to lodging and some diseases, higher plant density, etc., plant height also influences the yield positively and is indicated as a very valuable yield trait in many studies (Dagustu 2002; Kaya and Atakisi 2003; Kaya et al. 2003, 2005; Hladni et al. 2004; Dusanic et al. 2004). Several genetic sources of plant dwarfness have been identified in wild types transferred by sunflower breeders to the cultivated ones. The plant height is a quantitatively inherited trait and reduced plant height which means reduced internodes length and number of leaves is controlled by a single recessive gene (Miller and Fick 1997) or by two recessive genes (Velasco et al. 2003).

4.5.2 Head Size, Shape and Inclination

The head diameter which is one of the main yield traits is greatly influenced by the environmental conditions similar to the plant height. The head size may change 5–50 cm (even the largest one is 82 cm) and normal size of the head is about 18–25 cm. The sunflower head shape reveals from concave to convex, and the inclination may vary horizontal to completely turning down to soil. However, ideal type of sunflower head could describe as medium size (20–25 cm), not much thick and weakly convex head for sunflower because larger heads would increase husk content and loosing seed so seeds could fall down easily.

Specific head shape and head inclination types could get advantages under certain conditions such as facing down heads that are tolerant to sun-burning and birds, half-turned down heads that are tolerant to Sclerotinia and Botrytis head rots. Head size, shape and inclination are quantitative traits (Miller and Fick 1997) and sunflower breeders should consider optimal head size and head shape with optimum plant density to increase sunflower yield. Thus, head diameter indicated as one of the most important yield traits influenced greatly the seed yield by many researchers (Kaya and Atakisi 2003; Kaya et al. 2003; Joksimovic et al. 2004; Sridhar et al. 2005; Goksoy and Turan 2007).

4.6 Phenological Traits

The cultivated sunflower reaches normally about 60–70 days to flowering and 80–100 to physiological maturity. Flowering period has high heritability (Miller and Fick 1997) and plays more important role than physiological maturity to comprise seed yield in sunflower (Kaya et al. 2007c). Kaya et al. (2009a) indicated that sunflower hybrids should have also earlier flowering period and physiological maturity duration shorter than 107 days to get higher yield, because earlier plants avoided drought stress, later hybrids were influenced greatly by dry conditions due to limited rain and very hot seasons. On the other hand, longer grain-filling period described between flowering and physiological maturity give the chance to the plants to accumulate more dry matter during this time, so sunflower breeders should consider it as a selection criterion too (Miller and Fick 1997; Kaya et al. 2005, 2007c).

4.7 Male Sterility and Restoration System

The CMS and fertility restoration are vital traits in the commercial hybrids seed production. While CMS prevents producing pollen in female plants, crossing fertility restorer male plants with them turn into normal plants so this system is used to generate F_1 hybrid seed. CMS which is a maternally inherited trait occurs as a result of mutation changes in the cytoplasm or the incompatibility between nucleus and cytoplasm in sunflower (Fernandez-Martinez et al. 2009). Although many CMS sources have been identified from wild types, PET1 cytoplasm (generated from interspecific cross between *H. petiolaris* and *H. annuus*) which was reported by Leclercq (1969) is used commonly in commercial sunflower hybrid production.

Many CMS sources need at least two genes for fertility restoration, but some of them show single-gene restoration (Jan and Vick 2007). Fertility restoration genes derived from wild *Helianthus* species (Kinman 1970) are also available for sunflower breeding to restore CMS. On the other hand, nuclear male sterility (NMS) which is generally controlled by a recessive gene (Ms) in sunflower is not used in the commercial hybrid production and only gives the opportunity to sunflower breeders to make hybrids in early generations (Miller and Fick 1997).

4.8 Non-Oilseed and Utilizing as Energy Crops

Non-oilseed sunflower seeds are used mostly for confectionery as snack but also for feeding birds and small pets. Confectionery types have lower oil content, and mostly larger and longer seed size and white-grey colour. However, if it has small seeds, it is mostly used for birds. Breeding goals for confectionery seeds are lower cadmium

rate, higher protein and vitamin E (tocopherol) content to increase the nutritional value and the shelf life (Lofgren 1997a, b).

Oleic-type sunflower production and consumption started rapidly both for healthy frying oil and also non-food purposes like biodiesel in recent years, but there is not enough production yet for biodiesel due to higher demand for frying oil in Europe. The lower iodine value, higher stability and suitable oxidative (Vanozzi 2006; Kaya et al. 2007a,b) rate of mid-oleic and high-oleic sunflower oil compared to the currently dominant linoleic sunflower oil, will turn the oleic-type sunflower oil into an alternative biodiesel source (Table 4.3). Sunflower HO oil may be used in biocarburants in the form of methyl esters. Oleic sunflower oil conforms to both EU Biodiesel Standard of EN 14214 (Grompone 2005). This means oleic sunflower oil may be easily used as biodiesel source.

4.9 Resistance to Diseases

Although some sunflower diseases affect only locally or in specific environments, some of them result in great important yield losses in sunflower production. The most serious ones are downy mildew (*Plasmopara halstedii*), Phomopsis (*Diaporthe helianthi*), Sclerotinia stalk and head rot (*Sclerotinia sclerotiorum*), charcoal rot (*Macrophomina phaseolina*) Verticillium wilt (*Verticillium dahliae*), sunflower rust (*Puccinia helianthi*), Phoma black stem (*Phoma macdonaldii*), Alternaria (*Alternaria* spp.) and Rhizopus head rot (*Rhizopus* spp.). Chemical application is effective in the control of some diseases, but developing resistance genes is considered the most effective and sustainable control in sunflower.

Both vertical and horizontal genetic resistance mechanisms have been identified in wild sunflower species and determined resistance genes are transferred successfully to cultivated ones. Especially in downy mildew, Phomopsis, Phoma black stem and Verticilium wilt, resistance breeding overcame these diseases and resistant cultivars are planted greatly in the market. However, pyramiding of resistance genes with combination of both vertical and horizontal resistance mechanisms is very efficient strategy to obtain durable resistance especially in appearance of new races or complex and polygenic control in some diseases. On the other hand, very successful breeding efforts continue on Sclerotinia wilt and stem rot, rust and some viruses, resistant genes will be available to use in near future (Gulya 2009; Liu et al. 2010).

4.10 Resistance to Insects

Insects are generally not considerable problem when compared with diseases and broomrape parasite but few of them have economically damage in some sunflower production areas. However, grasshoppers could attack periodically sometimes, European sunflower moth (*Homoeosoma nebulellum*) resulted great yield reduction

Table 4.3 Physical and chemical properties of vegetable oils

Oil type	Iodine value	Cetane number	Lower heating value (kJ/kg)	Viscosity (mm^2/sn)	Cloud point (°C)	Pour point (°C)	Flashing point (°C)
Normal diesel	115–120	40–55	43–45,000	1.3–4.1	−15 to 5	−35 to 15	120–130
Biodiesel US ASTM standard	93	45	–	1.9–6.0	–	–	>130
EU biodiesel standard	115	49	–	3.5–5.0	–	−10	100
Canola oil	94–120	37.6	39,709	3.7	−3.9	−31.7	246
Mid oleic sunflower oil	94–122		–	4.1	–	−33	250
High oleic sunflower oil	88–115	49–53	–	4.8	−10	−27	270
Linoleic type sunflower oil	110–143	37.1	39,575	3.7	7.2	−15	274
High oleic safflower oil	90–100	49.1	39,516	4.1	−12.2	−20.6	293
Safflower oil	126–152	41.3	39,519	3.1	18.3	−6.7	260
Sesame oil	104–120	40.2	39,349	3.5	−3.9	−9.4	260
Cottonseed oil	90–119	41.8	39,468	3.35	1.7	−15	234
Palm oil	36–61	42.0	–	–	–	–	–
Soybean oil	117–143	37.9	39,623	3.3	−4.9	−12.2	254

Grompone (2005) and Kaya et al. (2008c)

in nineteenth century in Europe, and especially sunflower seed weevil (*Smicronyx fulvus*), sunflower stem weevil (*Cylindrocopturus adspersus*), banded sunflower moth (*Cochylis hospes*) and sunflower midge (*Contarinia schulzi*) are the major insects in USA that cause economic damages to sunflower (Charlet et al. 2009; Knodel et al. 2010).

Selected sunflower accessions, interspecific crosses and sunflower lines were evaluated in field for reduced seed damage from larval feeding by the sunflower moth, red sunflower seed weevil, or banded sunflower moth and some sunflower plants have revealed the existence of variability for resistance (Charlet et al. 2009). Although effective insecticide control is possible for sunflower insects resulting in crop loses, resistant and tolerant cultivars were developed by utilizing from interspecific hybridization into cultivated sunflower for some insects. Host-plant resistance can provide a long-term solution to managing these pests with lower input costs for producers and with less environmental impact instead of focused primarily on insecticidal control.

4.11 Resistance to Broomrape

Broomrape (Orobanche cernua Loeffl.) has been the most serious problem in sunflower production in Southern and Eastern Europe leading to considerable yield losses up to 100% and reducing sunflower seed quality. Furthermore, this parasite is developing new and more virulent races year by year which overcome the resistance of the varieties and hybrids commonly used in production. In the past, broomrape races A, B, C, D and E overcoming resistance provided by Or1, Or2, Or3, Or4 and Or5 genes, influenced severely the sunflower production areas in Turkey and some European countries from 1958 to 1985 (Gagne et al. 1998). The widespread use of resistant cultivars usually leads to the appearance of new races of the parasite that overcome the resistance genes each 20 years (Skoric 1988; Kaya 2003).

After a broomrape immune period, at the end of the twentieth century, a new Orobanche race called F was determined in Turkey (Kaya 2003), in Romania (Pacureanu-Joita et al. 1998) and in some areas of Spain (Alonso 1996; Sukno et al. 1999; Fernandez-Martinez et al. 2000). Although known races exhibited a monogenic and dominant inheritance, this new F race which was differentiated by LC-1093 sunflower line was determined by additive dominant allelic reaction and two loci with two types of epistasis (Fernandez-Martinez et al. 2004). However, new broomrape race other than F were observed also in Spain and Turkey in the recent years. However, Turkish F race is more virulent than Spanish and additionally there could be another one or two more races than the known in the region (Kaya et al. 2004). Recent studies showed that new races appeared in Russia (Gontcharov 2009), Bulgaria (Shindrova 2006) and Ukraine (personal communication). Hence, highly tolerant hybrids against these new races are planted in these countries with not affecting seed yield, but a few broomrapes still emerge in these hybrid plants in the field without any knowledge on being a new race or not yet.

The flowers of broomrape produce a large number of very small seeds which fall to the surface of the soil so the parasite is spread easily and quickly by wind and not controlled efficiently with cultural methods such as rotation, later planting, etc. Although genetic resistance is the most effective and feasible control against broomrape, application IMIs (imidazolinones) herbicide as post emergence application offers an efficient control to broomrape too (Demirci et al. 2003).

4.12 Resistance to Herbicides

Although weed control with transgenic herbicide-resistant genes have been used widely in some crops in the world, only IMI and sulfonylurea (SU) herbicide resistance which is transferred to cultivated sunflower from wild types utilizing backcross breeding is used commonly in sunflower. Herbicide resistance appearing in sunflower inhibited by acetolactate synthase (ALS) or called as acetohydroxyacid synthase (AHAS) (Kaya and Evci 2007) and IMI and SU herbicide resistance genes were identified in weed populations of *H. annuus* in Kansas, USA (Al-Khatib et al. 1998; Miller and Al-Khatib 2002). While herbicide resistance populations are caused by mutations in AHAS, the specific mutations have not been identified at this time. Kolkman et al. (2004) identified two mutations in the sunflower AHAS1 gene that likely provided resistance to AHAS, inhibiting herbicides and they discovered an Ala205Val mutation in sunflower lines developed by introgressing into USDA elite inbred lines. R gene identified from ANN-PUR populations showed partial dominance and a second gene in some genetic backgrounds affected the degree of resistance (Bruniard and Miller 2001). Furthermore, a new IMI-resistant gene was developed through ethyl methane sulfonate mutagenesis called CLHA-PLUS which is inherited as a single, partially dominant nuclear gene (Sala et al. 2008). This mutant line possessed higher levels of tolerance to imazapyr and imazamox that was observed in sunflower lines carrying the already described gene Imr1 which traced back to wild populations.

First, SU-resistant lines were developed in similar way with IMI resistance using classical backcrossing method from wild types in Kansas (Miller and Al-Khatib 2004). On the other hand, genetic diversity of SU herbicide resistance was also found in native *H. annuus* and *H. petiolaris* populations collected in some states from USA and the 57% of these accessions exhibited resistance to tribenuron (belong to SU herbicide group). More resistance to tribenuron was found in populations collected in Colorado, Kansas, Nebraska and South Dakota (Olson et al. 2004). Another SU-resistant gene was also developed using chemical mutagenesis and commercial sunflower hybrids were released and planted widely in some countries (Kaya and Evci 2007).

On the other hand, cross-resistance among the common mutations of ALS genes has been reported as early as 1992 (Guttieri et al. 1992). The mutation of ALA205 to VAL at the conserved region AFQEPT of the ALS gene provided higher resistance to the IMI herbicide, but only moderately low resistance to SU herbicide

(Bruniard and Miller 2001). However, Fabie and Miller (2002) mentioned that USDA source of SU resistance (USDA GH274-1) gave moderately high cross-resistance to the IMI herbicide, as well as complete resistance to the Express SU herbicide. They also indicated that the conserved region of the ALS gene involved in the USDA SU-resistant germplasm would be the AITGQVPRRMIGT region, or a mutation of the PRO197.

IMI post emergence herbicide (Imazamox + Imazapyr) with genetic resistant to IMI herbicide hybrids called CLEARFIELD System controls many of the broadleaf weeds causing yield losses in sunflower such as *Xanthium strumarium*, *Sinapis arvensis*, *Chenopodium album*, *Cirsium arvense*, *Convolvulus arvensis*, *Avena* spp., *Datura stramonium*, *Amaranthus* spp. successfully in sunflower production in the world (Demirci and Kaya 2009). IMI-resistant sunflower hybrids are more common in some countries such as Turkey due to the effective control on both broomrape and key weeds like Xanthium, etc. However, SU-resistant hybrids are preferred widely in Hungary, Romania, etc., because less expensive than IMI herbicide and broadening control spectrum of weeds especially in non-broomrape problem areas.

4.13 Tolerance to Stress Conditions

Wild sunflower species and relatives provided many gene sources for plant breeding leading to tolerance for biotic and abiotic stresses such as drought tolerance, salinity and poor soil conditions, etc. The sunflower is one of the most drought-tolerant plants in summer crops comparable to cotton, corn, sugar beet, etc. because of its extensive root system. Improved drought tolerance is one of the first objectives of breeders. Sunflower germplasm screened to identify putative traits such as stay green trait, delayed leaf senescence, transpiration efficiency and canopy morphology as well as yield performance under stress (Kiani et al. 2007). *H. anomalus* and *H. deserticola* are excellent candidates for drought tolerance genes based on their adaptation to desert environments (Seiler 2004). Similarly, *H. argophyllus* which has silver leaves and tomentose which reduces transpiration rates reflects sunbeams and reduces transpiration and is controlled by a single dominant gene have been suggested as a source of useful traits to improve water-use efficiency such as higher stomatal densities and leaf pubescence (Tavoljanskiy et al. 2004; Fernandez-Martinez et al. 2009).

H. paradoxus which inhabits sporadic salt marshes in USA has three times more stable salt (up to 1,300 mM) than cultivated sunflower and also exhibiting high salt tolerance with having higher leaf succulence and leaf sodium sequestration (Karrenberg et al. 2006; Edelist et al. 2006). Being able to sow early to maximize the growing season and to escape drought stress has increased the importance of low-temperature tolerance in sunflower. Transcriptome activity of sunflower is related to resistance chilling and frost tolerance that observed wild species under suboptimal temperatures (Hewezi et al. 2006). On the other hand, the tolerance to boron and Molibden deficiency and the reducing of accumulation of Cadmium in

the seed were determined in wild types and transferred into cultivated sunflower (Miller and Fick 1997).

Seed yield is a trait that is exploited by cumulative effects of a large number of yield-contributing traits. For drought tolerance breeding, sunflower breeders continue to develop cultivars which have higher yield potential under stress condition with analysing of plant characteristics with significant effects on drought tolerance mostly focusing on lower leaf canopy and reduced transpiration. However, selection of suitable genotypes for drought tolerance, seedling recovery %, root weight, higher harvest index, drought susceptibility index, root system, leaf water status, proline, abscisic acid and dehydrin content of plant were main useful traits for it (Rauf 2008).

4.14 Tolerance to Bird Depredation

Bird damage which commonly appears 3 weeks before or at the time of seed ripening is a serious problem and a limiting factor in sunflower production. Although many methods are used in sunflower to get away from birds such as netting, irritating with noisy or flashing devices even some chemical repellents, there is no efficient control to scare the birds. However, some morphological traits as long involucral bracts, horizontally oriented heads facing downwards, higher anthocyanin content in the seeds, concave heads, and long head-to-stem distances reduce bird attacks so sunflower breeders should consider these traits in the selection for bird depredation (Gross and Hanzel 1991).

5 Breeding Methods

During its historical development, sunflower breeding has gone through three phases characterized by the breeding method dominantly employed: (1) mass selection, (2) method of individual selection for developing open pollinated cultivars and (3) method of sunflower hybrid development.

5.1 Mass Selection

Mass selection of sunflower as a method of improving this plant species undoubtedly has its origin in early domestication of the sunflower plant. Archaeological results show that American Indians were the first to domesticate sunflower in 4625 BC (Crites 1993). Sunflower was utilized as food (roasted kernel and meal), as oil resource (skin protection from sunrays and for hair beautification) and for decorative purposes (religious ceremonies). Harvest of each particular sunflower head being an individual operation, and each variation in kernel size being obviously noticeable, it is no wonder that plants boasting largest kernels were the ones chosen

for planting. Applying QLT analysis, Burke et al. (2002) established that direct selection for kernel size increase played the crucial role in sunflower domestication. Mass selection has most probably created the cultivated sunflower as we know it today from wild *H. annuus* that featured small kernel and arborescent stem. Using several molecular techniques, many researchers have confirmed this hypothesis (Arias and Rieseberg 1995; Cronn et al. 1997; Harter et al. 2004).

Following its introduction into Europe in 1510 (Putt 1997), sunflower was used exclusively as a decorative plant for more than two centuries, only to become an industrial plant when it reached Russia. Towards the end of the nineteenth century, sunflower spread rapidly and a large number of local cultivars were created, grown mostly in gardens and under various environmental conditions. These early sunflower cultivars featured wide variability, especially concerning the length of the growing period and seed characteristics. Regarding the latter, there were two basic types: (1) cultivars with full round seed, thin hull and oil content 20–30% used for oil processing, and (2) cultivars with large long seed, thicker hull and oil content 15–20% used for food (Gundaev 1971). Such local cultivars were created by mass selection, i.e. by selecting plants from a population based on their phenotype, followed by planting seeds of the selected plants in bulk so as to create new cultivars, or sustaining cultivar purity of the existing cultivars. At the turn of the twentieth century, the most significant contribution of mass selection (selection by farmers) was attained: creation of cultivars resistant to sunflower moth (Homoeosoma nebulella) and sunflower broomrape (Orobanche cumana), both of which had posed serious threats to sunflower growing itself (Marinkovic et al. 2003).

It can be said that the onset of scientific sunflower breeding was in 1912, when research station Kruglik was established in Kuban region of Russia (Skoric 1988). Additional two research stations were established the same year in the provinces of Saratov and Kharkov. A large number of cultivars were created in these stations, the most important one being Saratovski 169 grown at over one million hectares at that time. The improved method of mass selection was applied here, based on selection of phenotypically desirable plants, their isolation and assessment of their value based on progeny. The selected plants would be isolated and their S_o progeny would be planted separately. The selected progeny would then be classified in groups according to the analysed traits, and groups created in such manner would be bulk planted in space isolation. This mode of sunflower mass selection is basically similar to ear-to-row method of maize selection, thus it could be termed head-to-row method. It was utilized in other breeding centres worldwide resulting in cultivars in Argentina (Luciano and Davreux 1967), Serbia and Mexico (Robles 1982).

Mass selection has become obsolete in contemporary sunflower breeding, even though it is still being used in some less-developed breeding centres. The main advantage of this method is its simplicity and cost-effectiveness. Its efficiency depends on gene effect on a trait for which selection is performed, trait heritability, interaction between genotype and environment, and sample size. Higher efficiency is achieved with highly heritable traits controlled by additive genes. Mass selection did not improve sunflower yield, but significant results were accomplished regarding early maturity, oil content and resistance to diseases and insects (Morozov 1947; Vranceanu 1974).

5.2 Method of Individual Selection for Developing Open Pollinated Cultivars

Method of individual selection with seed reserves was introduced into sunflower breeding in 1920s by Pustovoit (1967). Named after its author Pustovoit's method of reserves, this is the most widespread and most successful method of creating sunflower cultivars.

This method consists of individual selection of the best plants from the initial population which are harvested individually, whereas seed from each plant is divided in two groups – one for planting and another for reserves. Super-elites of the best cultivars, inter-cultivar hybrids and best progenies from the previous selection cycles serve as initial populations, out of which at least 15,000–20,000 plants are chosen with at least 1,500–2,000 seeds per plant. During growing period there are phenotypic observations concerning desirable plant architecture, while yield and seed characteristics are assessed after harvest, primarily regarding hull and oil content. The following phase sees 1,200–1,500 best progenies chosen, but seed from that year is not planted because plants were open-pollinated with discarded progenies as well. Seed from reserves is taken from chosen plants and planted in the following year. Planting is performed in one row in two replicates, while each third row is planted with elite seed of the best cultivar for those particular agro-environmental conditions. During growing period the same observations are performed as would be when choosing elite plants, with most attention being paid to disease and pest resistance, seed yield, hull and oil content. It is advisable to choose circa 200 best progenies for the next generation. In the third year the same procedure is repeated; additionally the chosen progenies are planted with control on one more plot infected with a sunflower disease, depending on the set outcome of selection, such as broomrape, downy mildew, etc. Several best progenies are multiplied in the following cycle, in bulk or in groups, depending on variability. Reserve seed from the beginning of the cycle is used for planting. Planting is performed as randomized block design in 5–6 replicates so as to assure that each progeny is crossed with each other in open pollination. Space isolation of 2–3 km is necessary. In this way it is assured that the best progenies pollinate each other, i.e. heterozygotes increase in number within the newly created population. During growing period individual plants are phenologically assessed. Each plant is individually harvested and analysed for seed yield, hull and oil content. Seed from selected plants within one progeny is mixed and serves for preliminary tests in the following year. Besides this, seed gained in this way can be used as initial material in a new selection cycle. Preliminary tests are set up in one location and this is a comparative trial among best selected progenies and best cultivars in one particular region. Based on the achieved results in productivity, the best progenies are chosen for pre-varietal and varietal trials which are set up for 3 years in several locations, providing us with information on adaptability and stability of the best newly created cultivars.

A significant breakthrough in cultivar creation was accomplished by Pustovoit (1963, 1974) when she introduced interspecies hybrids as initial material. Sunflower

wild relative *Helianthus tuberosus* was included in the breeding process. Cultivated sunflower is diploid ($2n=34$) and *H. tuberosus* is hexaploid ($2n=102$) which results in sterile interspecies hybrids ($2n=68$). This problem was overcome by employing temperature shocks during meiosis. Temperature shock is applied for 7–10 days by exposing interspecies hybrids day temperature of 25–30°C and night temperature of 3–5°C. The outcome of this temperature shock are plants with $2n=34$ chromosomes, which in turn enables back-crossing with cultivated sunflower. The following generation is back-crossed once again, and such progenies are tested in field for resistance to dominant diseases. The best progenies are then mutually crossed and the procedure is repeated in the following generation. After this, the best progenies are manually multiplied under isolators. In the following six generations, these progenies are tested for dominant diseases in field and artificially infected in glass-house, while additional laboratory tests are performed to analyse seed yield, hull content, oil content, 1,000-seed weight, etc. At the end of this cycle, the best progenies are manually multiplied under isolators and included as initial material for Pustovoit's method of reserves.

Creation of cultivars with increased seed oil content is the basic contribution of this selection method to development of sunflower production. Leading sunflower cultivars grown before this method was introduced had seed oil content of 30–33%. Employment of Pustovoit's method of reserves increased oil content to 43% in 1935, 46% in 1953 and 51% in 1958, when cultivar Peredovik (Panachenco 1966) was created. Owing to this characteristic, cultivar Peredovik began to be grown in Northern America and Western Europe during 1960s, i.e. sunflower began to be grown as a world oil crop (Fick and Miller 1997). Moreover, significant results were gained regarding early maturity and resistance to diseases and sunflower moth (Gundaev 1971). Cultivars created by this method were grown at circa five million hectares in the former USSR in 1973 (Pustovoit and Gubin 1974), while the method itself was successfully used to create cultivars in other countries such as Romania (Vranceanu 1974) and Serbia (Skoric 1988). Additionally, genetic variability of sunflower was significantly increased by introduction of interspecies hybrids in sunflower selection by Pustovoit (1963). Cultivars created by employing this method served as source of genes for resistance to downy mildew, broomrape, rust, verticillium and other sunflower diseases, as well as initial populations for creation of inbred lines in the process of hybrid development. This method is still being employed in some breeding centres within the former USSR and some developing countries as well.

5.3 Method Sunflower Hybrid Development

The basic goal of this sunflower breeding method is utilization of heterosis, which is a phenomenon of increased vigour in F_1 generation relative to parents, which results in higher yields. Genetically speaking, heterosis is a result of intra-allelic interaction (dominance and superdominance) to a greater extent, and a result of inter-allelic interaction (epistasis) to a lesser extent. This is in fact a state of maximum heterozygosity which is most successfully attained by crossing genetically

unrelated self-pollinated homozygous lines (inbred lines). The first research on utilization of sunflower heterosis was carried out during 1940s (Morozov 1947; Unrau and White 1944). Inter-cultivar hybrids were used in these early phases of sunflower heterosis utilization, but it was soon discovered that heterotic effect is greater in inter-line hybrids (Kloczowsii 1967). However, the practical application of sunflower hybridization method commenced much later due to the lack of corresponding system of male sterility. The first attempts of commercial use of hybrids were made during 1950s in Canada where inbred lines with high level of self-incompatibility were used as maternal component of hybrids (Putt 1962). Nonetheless, practical production most often gained circa 50% hybrid seed, rendering hybrid seed production using self-incompatibility unable to meet legislation requirements needed for release of this seed category. The next significant step in commercial use of sunflower hybrids was the discovery of NMS (Kuptok 1935; Leclercq 1966; Putt and Heiser 1966). In most cases, this feature is controlled by one recessive gene. Owing to the discovery of male sterility gene by Leclercq (1966) due to this gene's relation to anthocyanin gene, sunflower hybrids began to be commercially used in France and Romania during 1970s. This system provided almost 100% hybrid seed and such hybrids yielded up to 24% more than cultivars (Vranceanu 1974). The basic drawback of this production system is low cost-effectiveness of seed production which required high labour input to discard anthocyanin-fertile plants from maternal rows and green male-sterile plants from paternal rows. True commercial use of sunflower heterosis phenomenon became possible only after the discovery of CMS (Leclercq 1969) and corresponding fertility-restoring gene (Kinman 1970). The process of hybrid development based on CMS is a complex process consisting of two phases: (1) creation of inbred lines and (2) testing combining abilities of the newly created inbred lines.

5.3.1 Creation of Inbred Lines

Appropriate choice of initial material used for creation of inbred lines is of crucial importance for the successful outcome of sunflower breeding. The following can serve as initial material for creation of inbred lines: local populations, newly created and commercial cultivars; inter-cultivar, inter-line and interspecies hybrids; populations created by planned crossing and population improved by recurrent selection. What is important is that the initial material contains high genetic variability, which is a prerequisite for gaining a larger number of genetically unrelated inbred lines. Apart from this, the size of initial population is important – it is not to be less than a hundred plants under self-pollination conditions (Skoric and Marinkovic 1981), in fact it is desirable to be larger than that.

There are three basic concepts when choosing initial population: cultivar concept, trait concept and gene concept. Cultivar concept was used in the early years of sunflower hybrid utilization and comprises choice of a large number of cultivars and local populations to be used as initial material from which a large number of inbred lines are created under the assumption that a certain number of those lines

will express desirable traits. Even though this concept gave satisfactory results in developing first sunflower hybrids in advance breeding programmes, it is no longer being applied due to its accidental nature. Much more successful is the trait concept, which is based on preliminary testing of cultivars and inbred lines used to create initial populations. Crossing lines and cultivars of known traits provides higher genetic divergence of the initial population and decreased participation of undesirable traits, which assures higher success in creating perspective lines. Previous genetic research enabled insight into genetic basis for series of sunflower traits, which in turn enabled introduction of gene concept based on insight into genetic constitution of the selected trait. This concept was exceptionally important in sunflower breeding for disease resistance, especially regarding downy mildew (Jocic et al. 2010), broomrape, etc., breeding for oil quality (Skoric et al. 2007) and breeding for tolerance to herbicides (Jocic et al. 2008). The better insight into genetic constitution of a particular trait, the more adequate is the choice of lines and cultivars for planned creation of initial populations. Consequently, the success in creating new inbred lines of desirable traits is much larger.

Sunflower is an open-pollinated plant which allows self-pollination, so that inbred lines are created in the process of self-pollination through six or more generations. Early research on sunflower inbreeding was carried out in 1920s by Corden who created the first inbred lines by self-pollinating the cultivar Mammoth Russian. Hamilton (1926) determined that self-pollination in sunflower decreases yield by 15–50% in relation to open-pollination. Putt (1941) determined that the percent of self-pollination in sunflower inbreeding varies greatly depending on the origin of the initial material. Inbreeding was used to create lines of increased oil content and resistance to diseases and insects (Jagodkin 1937; Voskoboinik and Soldatov 1974). Methods used to create inbred lines are pedigree method, bulk method and single-seed descent method (Fernandez-Martinez et al. 2009). Due to its highest efficiency, pedigree method is the most often used method to create sunflower inbred lines. Pedigree method is the method of individual selection of plants in segregation generations and monitored origin or pedigree of the selected plants all the way to homozygous lines. During growing period it is necessary to test the initial populations by methods of artificial infection for resistance to dominant diseases and to perform phenological observations. Based on the achieved results, the best plants from the initial population are placed under self-pollinating conditions by isolating them with cloths or paper bags just before flowering. In the first self-pollination year it is especially important to discard genotypes of distinct self-incompatibility, since this feature hinders creation, growth and maintenance of self-fertilized sunflower lines.

Seed from plants of the first self-pollinated generation (S_0 or F_2 depending on the initial population) is planted employing pedigree method using head-to-row principle. Planting all subsequent generations is performed using the same principle. Plants of S_1 generation are very different from each other, since traits were segregated as the result of self-pollination and plants of the initial population were heterozygous for most traits. Special attention is paid to the following traits: length of growing period, plant height, seed yield per plant, head position, 1,000 seed

weight, hull content, oil content, resistance to diseases and other specific traits determined to be breeding goals. Best plants from best progenies are selected for further planting, while extremely weak progenies are discarded from the creation process. In S_2 generation more uniformity arises within each progeny, and differences between different progenies increase. The effect of self-pollination is more and more reflected even in some progenies or individual plants; there are some degenerative issues of general stunting, leaf yellowing, albinism, partial sterility, etc. Progenies of S_3 generation (inbred lines) are largely uniform, while differences between the lines increase. Inbreeding depression is even more expressed for traits such as plant height, seed yield, etc. After 6–8 generations of self-pollination and selection, the negative effect of inbreeding ceases, resulting in lines uniform for most traits, i.e. homozygosis is over 96%.

Inbred lines created in this way can directly be used in sunflower production via synthetic cultivars. High-yielding synthetic cultivars can be created by mixing 3–5 inbred lines (Putt 1966; Voskoboinik and Soldatov 1974). However, hybrids have a higher genetic potential, so that inbred lines are mostly used to develop hybrids, except in some countries where sunflower breeding has not reached that level (Ado et al. 1991; Shabana 1990).

5.3.2 Testing Combining Abilities of the Newly Created Inbred Lines

Newly created inbred lines should be tested so as to determine which ones will provide heterosis in F_1 generation. Heterosis being the state of maximum heterozygosity, crossing genetically distant inbred lines in F_1 generation achieves heterozygosity for the most number of alleles resulting in whole organism's fitness. Nonetheless, crossing any two lines does not necessarily cause heterosis, since lines can be genetically related. Due to this, it is needed to test combining abilities of the newly created lines, since the value of any line mirrors heterosis it would provide when combined with other lines. Final assessment of the value of even most carefully selected inbred lines is performed based on their results in hybrid combinations. Good combining ability means the ability of one inbred line to provide superior progeny when combined with another line. Combining abilities can be general and specific. General combining ability (GCA) is a mean value of an inbred line based on its performance when crossed with any other line. Specific combining ability (SCA) is a value of an inbred line when crossed with a specific other line.

Combining abilities of inbred sunflower lines are mostly tested in S_4 generation, though the method of early testing of combining abilities after the first generation of self-pollination proved to be successful in identifying lines of good combining abilities (Shein 1978). GCA is mostly estimated by polycross and topcross methods, while SCA is estimated by diallel cross method.

Polycross method – Lines in GCA testing are planted in space isolation of at least 3 km and in four replications, with ten plants per replication set up as randomized blocks, so as to provide open-pollinating conditions for each line to fertilize every other plant. Progeny of each polycrossed line is tested in comparative trials, while

GCA is estimated based on the productivity. This method is based upon the assumption that each line will be fertilized by each other line. However, in real conditions this is hardly possible due to different lengths of growing period of different lines, different length of flowering period, different attractiveness to pollinators, different pollen production, etc. These are the reasons why this method is seldom used in testing combining abilities of sunflower.

Topcross method – GCA estimate of new lines is performed based on testers. These can be cultivars or lines of known good combining abilities, but inbred lines which serve as parents for the best commercial hybrids are most often used as testers. Miller et al. (1980) and Dominquez and Fernandez-Martinez (1987) determined that lines of the best combining abilities can be successfully identified in this way. There are two versions of this method:

Lines in the process of testing are planted in space isolation on a plot together with tester designed so that there is one line row and one tester row. Artificial male sterility is induced in tested lines by applying solution of gibberellic acid. Pollination with tester is provided by insects, which necessitates beehives to be placed on the plot, since bees are the main sunflower pollinators. Developed hybrids are tested in comparative trials with commercial hybrids. Their GCA is estimated according to productivity results, i.e. seed yield and oil yield. The basic drawback of this method is the fact that treatment with gibberellic acid does not provide 100% male sterility in plants. Applying solution of gibberellic acid in 50–100 ppm when bud size is 1–1.5 cm gives good results in most cases (Miller and Fick 1978). However, various genotypes react differently so that some genotypes give better results with higher or lower concentration, or with earlier or later application. Besides this, it has to be assured that even in genotypes which respond to determined concentration and time of application, all plants must be in the same developmental phase (Piquemal 1970). Additionally, application of gibberellic acid can have adverse effects, such as decreased level of pollination, head diameter, number of flowers, hampered head growth and deformations of inflorescence, etc. depending on concentration and time of application of gibberellic acid (Miller 1987). Owing to these problems, this method was not widely used in testing combining abilities of inbred sunflower lines.

Since inbred lines which are parents to the best hybrids are used as testers most often, sterile forms of such lines have been created which have good combining abilities, so that these can be used as testers. Sterile form is manually crossed with a pollen mixture from at least five isolated plants of a newly created line. Hybrids developed in such a way are tested in comparative trials with commercial hybrids on a plot with enough sunflower plants to ensure pollination, since most such hybrids are sterile. Advantage of this method is parallel testing for presence of fertility-restoring genes in newly created lines. According to the productivity, GCA is estimated and inbred lines which yield most with tester are chosen for further processes, while those who performed poorly are discarded. This eliminates a large number of lines, and chosen ones are submitted to diallel cross to test specific combining abilities.

Diallel cross method – it can be used to estimate both general and specific combining abilities, as well as to determine the effects of reciprocal crosses. Even though

the employment of this method gains most reliable data on combining abilities of inbred lines, being based on crossing each line with each other (including reciprocal crosses), it can however not be used on a large number of lines due to practical constraints. This is why diallel crosses are used only on chosen lines of good GCA and other agronomic traits, so that SCA could be estimated. Apart from this, diallel cross method is often used in genetic research to determine the mode of inheritance for a specific trait, number of genes that control it and gene effects.

Heterosis in sunflower is mostly utilized through two-way (single-cross) hybrids developed by crossing maternal inbred lines possessing CMS and paternal inbred lines possessing fertility-restoring genes. Consequently, self-pollinated lines of the best combining abilities are transformed into a sterile form or fertility-restoring genes are inserted into them by the back-cross method. Three-way and four-way (double-cross) hybrids are used much less, regardless of the fact that they are more adaptable and stable than two-way hybrids owing to their heterogeneity (Vulpe 1974; Fick and Zimmer 1976; Schuster and Friedt 1988). The basic advantage of two-way hybrids is their uniformity and higher yields (Miller 1987; Skoric 1988). Hybrids achieve seed yields 25–30% higher than cultivars. Besides higher genetic potential for seed yield, hybrids also have other advantages over cultivars. They are genetically homogenous and uniform in plant height and growing period, resulting in decreased harvest losses and seed of the same moisture appropriate for storing. Another important advantage of hybrids over cultivars is easier insertion of genes for resistance to dominant sunflower diseases, rendering hybrids more resistant than cultivars.

To develop new cultivars in sunflower; it firstly need to create genetic variation, then improve the populations such as landraces, village populations, etc., intensive selection process for developing open-pollinated varieties and finally inbred lines need to develop to obtain hybrid cultivars.

5.4 Creating Genetic Variability

The development of variation initial breeding material is a primary task in the genetic and breeding programs of sunflower. Seed yield is the main goal in sunflower breeding programmes but sunflower yield reach near almost maximum level by the use of a same CMS and fertility restorer sources for sunflower hybrid production due to reducing heterosis. Therefore, wild sunflower is likely to provide broader genetic base and the needed new genes to increase yield supplying higher photosynthesis rate, water and fertilizer-use efficiency and crops biomass. Furthermore, wild sunflower species and relatives also provided many gene sources for plant breeding leading to quality improvement, disease resistance and tolerance for biotic and abiotic stresses such as drought tolerance, salinity and poor soil conditions, etc. These useful genes, which have obtained from the wild species broadened narrow genetic base of cultivated sunflower with supplying remaining source of desirable agronomic traits for improving cultivated sunflower. To broaden genetic capacity, to increase

heterosis and to integrate new useful genes such as resistance, better quality and higher yield performance into developed inbred lines from wild types and derived interspecific hybrids from them in breeding programmes, wild species should be certainly existed in sunflower breeding nurseries.

However, there are some obstacles to utilize from wild types such as cross-incompatibility, embryo abortiveness, sterility and reduced fertility so tissue culture methods used commonly to overcome them in sunflower. Standard tissue culture variables such as methods of staging and preparation of explants, composition of culture media, cultural conditions, timing of the regeneration process, plant establishment, and maintenance of fertility have all been described for sunflower to widen the genetic variability. The most favoured explants for culture initiation and plant regeneration are mature cotyledons, immature embryos, hypocotyls and excised meristems (Ivanov et al. 2002). Therefore, tissue culture is one the most common methods in sunflower breeding programmes to assist new genes into initial materials in the nurseries. However, some methods of somatic hybridization, "in vitro" embryo culture, chromosome doubling, etc., are frequently used also for interspecific crossing in wild types to be associated with the utilizing from interspecific hybridization to expand the genetic variability in the sunflower breeding (Atlagic 2004; Drumeva et al. 2005).

Another method to generate genetic variability the breeding program is mutagenesis which gives opportunity to breeders to get new traits which are not found in their germplasm collections. Many successful results were obtained in sunflower utilizing from mutation such as HO content (Soldatov 1976), higher gamma tocopherol (Velasco et al. 2004), IMI and SU herbicide resistance (Kaya and Evci 2007; Sala et al. 2008), shorter plant height, higher oil and protein content and lower husk content (Fernandez-Martinez et al. 2009), etc.

On the other hand, selected and developed superior genotype as called great success in plant breeding is result of reduction in genetic variability for the crop undergoing selection. Therefore, breeders should consider carefully their genetic material to maintain sufficient genetic variation for future needs and also should manage germplasm regularly to introduce new sources as well as developing new recombinants to broaden genetic variability.

5.5 Population Improvement

Population improvement gives opportunity usually to consider several yield traits with varying degrees of agronomic and economic importance at the same time due to genetic correlations existing among these traits and not be considered separately. The primary objective is to improve genetically divergent populations through recurrent selection, permitting the extraction of lines with yield and other agronomic traits superior to current cultivars used by farmers with expanding their genetic base of populations mostly on seed yield and minimizing risks of insect pest and disease in the crop. Recurrent selection which is a cyclic and gradual procedure

means continuously re-selection, generation after generation, with crosses of the selected families and with the goal of promoting gene recombination to increase the frequency of favourable alleles within a population. Recurrent selection conducts in three phases; in a repetitive manner, development of progenies, evaluation of progenies in replicated trials and recombination of the superior progenies based on the evaluation trials (Fehr 1987).

Although this method was previously used to improve maize populations, some great success was obtained in sunflower utilizing from this procedure too. Both phenotypic in which the phenotype of the individual plant serves as the basis for selection, and genotypic recurrent selection in which a system of matings is used to develop relatives or identified as some type of progeny tests constitute of the basis of selection use commonly in sunflower. However, the important difference between phenotypic and genotypic selection is that in the latter additional information from phenotypic values of relatives often provides a more reliable guide to the breeding value of an individual than the phenotypic values alone.

In phenotypic recurrent selection in sunflower; firstly the parents selected from initial populations constituting by combining high-performing lines are determined and then they are crossed randomly by emasculating by hand or gibberellic acid to each other. S_0 material evaluated based on their phenotypes are shelved in first year and then these selected materials planted in separate rows and mated each other randomly to compose C_1 plants for following year. Phenotypic selection is conducted for favourable traits over C_1 plants and they are evaluated also against diseases and pests, and then they are shelved and bulked after harvest. Utilizing from phenotypic recurrent selection, many great success were obtained in sunflower such as grain yield, increasing oil content, resistance to diseases and insects, improving plant type, etc. (Fick and Miller 1997; Fernandez-Martinez et al. 2009). Vear et al. (2007) improved significantly of Sclerotinia head rot resistance after over 15 cycles. Similarly, Charlet et al. (2006) developed some quantitatively tolerant lines which are obtained from interspecific hybrids to red sunflower seed weevil, sunflower moth, banded sunflower moth and sunflower stem weevil utilizing from phenotypic recurrent selection.

In genotypic recurrent selection in sunflower, many S_0 progenies are selecting from initial populations and self-pollinated. In the second generation, part of the seed is grown and evaluated for the traits of interest in replicated trials. Selected S_1 progenies are recombined to form the C_1 population, which is accomplished by random mating plants obtained from reserve S_1 seed. In the test cross or half-sib progeny recurrent selection, test crosses instead of S_1 progenies are evaluated. Selected plants in the C_0 initial source population are shelved and simultaneously crossed with a tester the first year. The type of tester used depends on the objectives of selection. If the objective is selection for GCA a broad base heterogeneous unrelated population is used as tester or if the objective is selection for SCA a stabile inbred line is used as a tester. Genotypic recurrent selection method utilizing S_1 progeny or testcross evaluation have been effectively used in sunflower hybrid breeding to improve yield and combining ability (Fick 1978) and drought resistance (Fernandez-Martinez et al. 1990).

6 Molecular Breeding

The future of the sunflower as the crop depends on introduction of useful genetic diversity from wild species and use of information on genomes of wild and cultivated sunflower in breeding. Availability of DNA markers facilitated studies of sunflower genome and enabled identification of agronomically important genes (Burke et al. 2005).

Methods of molecular breeding are already used in sunflower breeding as tool for acceleration of breeding process. Among these methods is determination of foreign genes in back-cross progenies (Dimitrijevic et al. 2010), as well as uniformity check during inbreeding and hybrid seed production. Molecular breeding methods are also used to confirm the success of interspecific crosses and somatic hybridization (Taski-Ajdukovic et al. 2006) and to detect genetic relationships within the genus *Helianthus* (Sossey-Alaoui et al. 1998) and between different sunflower populations during hybridization (Pankovic et al. 2000). Finally, it is possible to perform early identification of agronomically important traits, quality traits, disease resistance or stress tolerance by marker-assisted selection (MAS) and isolation of specific genes to be used in genetic transformations (Marinkovic et al. 2003).

6.1 Molecular Markers and Linkage Maps

In sunflower, as in other plant species, genetic markers were originally used in genetic mapping to determine the order of the genes along chromosomes, and evolved from morphological markers through isozyme markers to DNA markers. A great number of molecular markers have been developed during last three decades. Their convenience for the use in sunflower breeding depends on the type and goal of research.

The first molecular genetics linkage maps of cultivated sunflower were developed by means of RFLP (Berry et al. 1995, 1996, 1997; Genzbittel et al. 1995, 1999; Jan et al. 1998) and random amplified polymorphic DNA (RAPD) (Rieseberg et al. 1993; Rieseberg 1998) markers. Subsequently, several genetic linkage maps were constructed by means of amplified fragment length polymorphisms (AFLPs) (Peerbolte and Peleman 1996; Gedil et al. 2001b).

RFLP markers (*r*estriction *l*ength *p*olymorphism) enabled for the first time determination of differences between genotypes at the molecular level. Berry et al. (1995) used 234 markers and identified 17 LG that correspond to sunflower chromosomes. The most complete RFLP map was produced by Jan et al. (1998), with 271 loci detected with 232 probes. Markers were grouped into 20 LG that cover 1,164 cm of genome. Out of 271 loci, 202 were co-dominant, and the others were dominant. Although they are very useful, RFLP markers are not frequently used today. Their use is limited due to the lack of public bank of RFLP probes and low resolution of the maps (Yu et al. 2003).

AFLP (*a*mplification *f*ragment *l*ength *p*olymorphism) markers have been used to fingerprint elite sunflower inbred lines (Hongtrakul et al. 1997), to construct new genetic maps, and to increase the density and to fill gaps of already developed genetic maps.

RAPD (*r*andom *a*mplification of *p*olymorphic *D*NA) markers have been used for mapping in sunflower, particularly in wild species. Rieseberg et al. (1993) constructed a *Helianthus anomalus* map based on 161 RAPD markers and one isozyme locus. RAPD maps were also developed for wild *H. annuus* and *H. petiolaris* (Rieseberg et al. 1995), based on 212 and 400 RAPD loci, respectively. Gedil et al. (2001a) added 296 AFLP loci to a 104 RFLP loci map based on markers from Berry et al. (1996) and Jan et al. (1998), and constructed an AFLP-RFLP map that comprised 17 linkage groups, had a mean density of 3.3 cm, and was 1,326 cm long.

Microsatellites or SSR were used for the construction of genetic map that was developed on F_2 and RIL sunflower populations. Today, 2,040 SSR markers are available for the use in the breeding (Paniego et al. 2007). Yu et al. (2002) were among the first researchers that constructed SSR map with 131 markers, while Tang et al. (2002) determined 1,093 unique SSR sequences. Tang et al. (2003) showed that screening of complete sunflower genome could be done with the use of 459 SSR markers, with average distance of 3.1 cm. In the same work the authors completed RFLP map constructed by Berry et al. (1997) with 120 SSR markers. That map with 657 loci at 1,432 cm and mean density of 2.2 cm per loci is the most complete sunflower SSR map up today (Tang et al. 2003). SSR markers are rarely dominant in field crops, but in sunflower there are 9% of zero alleles (Yu et al. 2002). SSR marker resources developed for sunflower create the basis for rapidly, efficiently and fully integrating first generation genetic linkage maps developed by use of RFLP markers (Berry et al. 1995, 1996, 1997; Genzbittel et al. 1995, 1999; Jan et al. 1998).

According to Kolkman et al. (2007), frequency of SNP (*s*ingle *n*ucleotide *p*olymorphism) in their study was 1/32 bp non-coding i 1/63 bp in coding region of sunflower inbred lines. Expected number of SNP in the whole sunflower genome is at least 76.4 millions, which leads to density of 54,571 SNP/cm. For this reason, there is increased tendency of use of these markers in the sunflower genome studies.

Eighteen genetic maps with different completeness and density of wild and cultivated sunflower were created with the use of almost 1,100 RFLP markers, several hundred RAPD and AFLP markers. Despite the great number of DNA markers, there is no unique, publicly available sunflower genetic map (Tang et al. 2002). There are four LG nomenclatures which makes comparison of the results of different researchers more difficult (Knapp et al. 2001).

6.2 Marker-Assisted Selection

MAS is practical application of molecular markers in plant breeding, and is being used in sunflower breeding as well. MAS is indirect selection for certain trait, where molecular marker that is inherited with the studied trait is used as selection criterion. Molecular markers have several advantages compared to classical morphological markers and enable increased efficiency of conventional breeding (Vasic 2001).

Molecular markers do not depend on the environment and could be detected in all stages of plant development (Mohan et al. 1997).

The most common application of MAS in sunflower breeding is marker-assisted backcross breeding for gene introgression. In general, marker assistance is expected to provide higher efficiency, reduced cost and shorter duration of the backcross breeding scheme, compared with conventional methods. Co-dominant markers are the most useful for marker-assisted backcrossing because selection in backcross progeny involves selection for heterozygous progeny. Marker-assisted backcross breeding is also very effective in transferring genes or QTLs determining valuable traits from wild donor genotypes into elite breeding lines, reducing both the time required and the risk of undesirable linkage drag with unfavourable donor attributes (Perez-Vich and Berry 2010). Gene pyramiding is a useful approach to enhance the durability and degree of pest and disease resistance, or to increase the level of abiotic stress tolerance where resistance or tolerance-related traits can be pyramided together to maximize the benefit of MAS through simultaneous improvement of several traits in an improved genetic background. Vear (2004) suggested that major genes need to be backed up by quantitative, non-race-specific resistance QTL for increasing disease resistance durability.

MAS and molecular markers are used in sunflower breeding for introduction of many desirable traits, but only their use in introduction of several most important traits will be discussed and described here.

6.2.1 Oleic Acid Content

Marker studies related to HO acid content in sunflower began with the identification of two RAPD makers linked to the Ol1 gene (Dehmer and Friedt 1998). Subsequent studies demonstrated that the Ol1 gene cosegregates with a seed-specific oleoyl phosphatidyl-choline desaturase gene (FAD2-1) that is strongly expressed in normal-type (low oleic) and weakly expressed in mutant (HO) lines (Hongtrakul et al. 1998; Lacombe and Berville 2001; Martinez-Rivas et al. 2001). Hongtrakul et al. (1998) and then Lacombe et al. (2002) showed that HO sunflower lines derived from Pervenets mutant carry specific RFLPs revealed using a Δ12-desaturase cDNA as a probe. These RFLPs determine the Δ12 HO specific allele, Δ12HOS. The normal LO lines do not carry the Δ12HOS allele but another allele named Δ12LOR at this locus (named Δ12HL locus) (Lacombe et al. 2002). The Ol1-FAD2-1 locus mapped to LG 14 (Perez-Vich et al. 2002) of the public sunflower genetic map, and was found to underlie a major oleic acid QTL explaining 56% of the phenotypic variance for this character (Perez-Vich et al. 2002).

6.2.2 Downy Mildew Resistance

There are up to ten downy mildew resistance genes described, denoted Pl, carrying resistance to various downy mildew races and mapped to genetic maps (Vear 2004). Markers useful for indirect selection of downy mildew resistance genes – Pl_2, Pl_6 i Pl_{arg}

were isolated with the combination of RAPD and AFLP methods (Brahm et al. 2000), while AFLP map was used for localization of QTLs for the resistance to the same disease (Al-Chaarani et al. 2001). Brahm et al. (1998a, b) used RAPD markers for mapping of downy mildew resistance genes while Pankovic et al. (2001) used RAPD, SCAR and SSR markers in order to develop new PCR markers for the Plasmopara resistance. Pankovic et al. (2007) proposed increasing MAS efficiency in backcross programmes to introgress the Pl6 gene conferring resistance to downy mildew race 730 by using a combination of closely linked co-dominant cleaved amplified polymorphic sequence (CAPS) markers with dominant markers developed from resistance candidate genes.

6.2.3 Sclerotinia Resistance

Resistance to other diseases such as Sclerotinia is complex, involving several loci with different effects and highly dependent on environmental conditions. For this quantitative resistance, there are no specific genes and races described, although lists of QTL are becoming available (Perez-Vich and Berry 2010). Mestries et al. (1998) identified loci for resistance to Sclerotinia of leaf and capitulum with the use of RFLP markers. Bert et al. (2000) used AFLP and RFLP for mapping of genes responsible for resistance to Sclerotinia on leaf and capitulum.

QTLs for resistance to Sclerotinia concerning the capitulum reaction to the ascospore test have been identified on 14 of the 17 sunflower linkage groups in different crosses, explaining individually less than 20% of the phenotypic variance (Bert et al. 2002, 2004; Yue et al. 2007).

QTLs for reaction to mycelium tests on leaves and capitula and for natural attack on terminal buds have also been reported (Mestries et al. 1998; Bert et al. 2002, 2004), which often appear to co-localize with the QTLs for resistance to the ascospore test (Vear 2004).

QTL studies on Sclerotinia midstalk rot resistance reported six to nine QTL for each of the three resistance traits evaluated (leaf lesion, stem lesion and speed of fungal growth), each with a small effect (Perez-Vich and Berry 2010). In total, between 24.4 and 33.7% of the genotypic variance for resistance against Sclerotinia could be accounted for by these QTL (Micic et al. 2004). Despite the complex genetic architecture of Sclerotinia resistance, QTLs consistent across environments (Bert et al. 2002), generations (Micic et al. 2005a) and mapping populations (Micic et al. 2005b) have been identified, which constitute valuable tools for the establishment of MAS programmes aimed at improving Sclerotinia resistance (Perez-Vich and Berry 2010).

6.2.4 Orobanche Resistance

Resistance to the parasitic weed *Orobanche cumana* appears to follow a similar pattern to that of downy mildew. Dominant resistance genes Or1 through Or5, conferring

resistance to races A through E, respectively, have been described by (Vranceanu et al. 1980). Many researchers tried to locate Or genes and to find markers close to them. RAPD (Atanasova et al. 2004; Lu et al. 2000), SCAR (Lu et al. 2000) and SSR markers (Tang et al. 2003; Iuoras et al. 2004) were used for this purpose.

Tang et al. (2003) tried to identify SSR marker closely connected to Or5 gene, and to position Or5 on public genetic map of sunflower. Seventy-eight SSR markers were tested by multiplex PCR. Three SSR markers (ORS 1222, ORS 1036 and ORS 1114) were polymorphic between resistant and susceptible population. The probable reason why such a great number of markers is needed to obtain the results and why the closest SSR marker is located 6.2 cm from the gene is that the gene is located near telomeres or in the telomere region which is susceptible to recombination. None of the markers is located upstream from OR5 gene, so the MAS is limited to centromere side of the locus. Recent genetic and molecular studies have revealed a more complex genetic control of broomrape resistance. Perez-Vich et al. (2004) reported that phenotypic variance for race E resistance was mainly explained by a major QTL on LG 3 (Or5 gene) associated to the resistance or susceptibility character, while race F resistance was explained by QTL with small to moderate effects, mainly associated with the number of broomrapes per plant (Perez-Vich and Berry 2010).

6.2.5 Resistance to Herbicides

Sunflower biotypes resistant to two classes of AHAS-inhibiting herbicides such as IMIs or SUs have been discovered. Kolkman et al. (2004) identified, cloned and sequenced three AHAS sunflower genes: AHAS1, AHAS2 and AHAS3, which were mapped to LG 9, 6, and 2, respectively. In addition, these authors identified mutations in codons 197 and 205 in AHAS1 that conferred resistance to IMI and SU herbicides, respectively, and developed a SNP genotyping assay diagnostic for the codon 205 mutation (Perez-Vich and Berry 2010).

Tribenurone-methyl is herbicide that inhibits enzyme ALS. Tolerance to SUs and other herbicides that inhibit ALS could be divided into two main groups: "target-site based" and "non-target site-based" (Preston and Mallory-Smith 2001). Kolkman et al. (2004) identified three genes (AHAS1, AHAS2 and AHAS3) in resistant (mutant) and susceptible (wild type) genotypes. In AHAS1, almost 48 SNPs were detected, on indel of 6 bp in AHAS2 gene and one SNP in AHAS3 gene. Each of these changes confers resistance to ALS inhibitors, but they have different effect on specific herbicide, and, in some cases, there is unique binding site of specific substrate for certain protein.

Although there is a significant improvement in the use of molecular markers and MAS in sunflower breeding, there are still problems that hamper their wider use. One of the greatest problems in the use of molecular markers in breeding is high cost. This problem could be partially overcome by simplifying DNA extraction procedure which represents half of the costs of PCR analysis, as well as with the use of specific PCR markers (Mohan et al. 1997). Another problem is lack of libraries of

sequences and markers specific for certain loci (Knapp et al. 2000), as well as lack of cooperation and coordination between different research groups and between public institutions and private companies.

7 Confectionery Breeding

Sunflower is growing also for confectionery other than oil type in many countries like China, USA, Turkey, Spain, Russia, etc. However, sunflower consumes in shell mainly or no shell in the world. Seed colour is one of the main characteristics for confectionery. While white with grey stripe seeds prefer mostly in Turkey, grey colour with stripes is popular in USA, Spain and China but black seed is more preferable in Balkan countries and Russia (Kaya et al. 2008b). Low selfing rate, transpiration efficiency and seed size, broomrape, rust, poor adaptation capability are the main problems in confectionery sunflower production in many countries of the world (Kaya 2004; Sun 2009).

Confectionery sunflower has an abundance of genetic variation due to that cultivars mostly are open pollinated. Liu et al. (2003) observed very larger diversity and lower degree of genetic similarity utilizing from AFLP and RAPD markers. They detected an abundant genetic diversity among local varieties of confectionery sunflower in China because of longer years artificial and natural selection gradually formed local varieties having specific biological characteristics and well adapted in different environmental conditions. Similarly, Dong et al. (2007) could not notice any genetic resemblance among 70 germplasm representing 12 provinces of China characterized by AFLP.

Confectionery sunflower seed should be ideally at least over 80 g 1,000 seed weight, have less than 30% oil content, higher seed size, lower cadmium rate, higher protein, oleic acid and vitamin E (Tocopherol) content (Jovanovic et al. 1998; Lofgren 1997a, b). The seed size is the main criteria for the quality of confectionery sunflower. While larger sizes (>15 mm) type goes into the in-shell market to be used as snack, medium-size seeds are hulled for the kernel market both for consuming as snack or bakery and smaller sizes go for bird and pet feeding market (Hofland and Kadrmas 1989; Chikkadevaiah et al. 1998).

To produce larger seeds; of course firstly plants should have a genetic potential, then larger seeds could be obtained by irrigating (or enough rain during the vegetation period) in normal row planting (70×40 cm) or decreasing plant population per ha especially in normal rain-fed areas. For instance, confectionery sunflower grows at 1 m×50 cm as only 20,000 plant per ha to obtain larger seed size in fallow areas of Middle Anatolia region in Turkey (Kaya 2004). Therefore to increase seed length one of the main goals in confectionery sunflower breeding and it could be increased with selection. Sun (2009) indicated that polygenic system control seed length in sunflower but 1–2 major genes play important roles based on performed QTL analysis. He also mentioned that large seed length was linked closely with rust resistance in the same area based on DNA markers and linkage map.

Therefore, newly developed cultivars should have higher yield capacity, self fertility rate and larger seeds with combining higher oleic acid and vitamin E (Tocopherol) content to increase in the nutritional value of seed and in shelf life of them.

8 Ornamental Breeding

Archaeological finds show that American Indians were first to domesticate sunflowers and used their flowers, among other things, for decoration in various religious ceremonies. After the introduction of sunflowers to Europe by the Spaniards, the flower of the sun or the New World flower, as it was called at the time, quickly gained popularity as an ornamental plant. For almost two centuries, sunflowers were grown in Europe exclusively as an ornamental plant. After the oil content in sunflower seeds was increased by selection, the production of this new industrial crop started to spread all over the world. Today, the sunflower is a major oil crop worldwide. Nevertheless, its use as an ornamental plant has never ceased. First, ornamental sunflower varieties were quite tall (over 2 m), with yellow flowers. Some of these varieties can still be found in some seed companies in America, which offer them under the names Mammoth Russian, Russian Giant, Tall Russian and Mammoth. These varieties are a curiosity for themselves as they are on the market for over 130 years and they are still popular among customers.

In addition to these old varieties of ornamental sunflower, some wild relatives of the sunflower can also be found on the market. This is the first place the silver leaf sunflower or *Helianthus agrophyllus.* It originates from sandy coastal parts of southern Texas. It is an annual, branching plant. Its leaves and stem are covered with long silky hairs, which make it attractive even when not in bloom. It blooms in the period July–October. Although in nature it grows only on sandy soils, it tolerates all soil types and it is widely cultivated as an ornamental plant. It appeared in catalogues of seed companies already in 1889. Another popular ornamental sunflower is *Helianthus petiolaris* or prairie sunflower, which has extremely long flower stems suitable for use as cut flower. It is a branched annual species with dark green leaves and stem. It blooms in the period June–November. *Helianthus debilis* is also used as ornamental plant, primarily due to a long blooming period. It is a branched form, which blooms successively from May to October. It is present on the American flower market for about a century, under the name of Italian White. In addition to these annual wild species, perennial species such as *Helianthus occidentalis*, *Helianthus grosseserratus* and *Helianthus rigidus* are grown in gardens as ornamental plants.

Discovery of varieties with chrysanthemum-type flowers and varieties with red-coloured ray flowers has been important for current breeding of ornamental sunflowers. Among the most attractive ornamental sunflowers there is the Chrysanthemum type also so-called Chrysanthemoides or the double sunflowers or Florepleno (Fick 1976; Heiser 1976; Knowles 1978). The Chrysanthemum type owes its unusual appearance to the fact that the corolla of disc flowers has become elongated, somewhat assuming a ligulate-like aspect. This mutant, which looks like

a giant chrysanthemum, is illustrated in old herbals, and the mutation that caused it apparently occurred in the first 100 years after the sunflower reached Europe. Two Chrysanthemum cultivars, Sun Gold and Teddy Bear, which are still present on the market, were developed on the beginning of the twentieth century. First, clear description of this trait was done by Cockerell (1915a, b), but although this genotype can be considered one of the first known morphological mutants in plants, studies on its inheritance pattern are scarce and contradictory. One completely dominant gene (Luczkiewicz 1975; Secerov-Fiser and Skoric 1991) and a minimum of two genes (Fick 1976) have been reported to control the chrysanthemum type. Fambrini et al. (2003) support a genetic model involving one semi-dominant major locus and an unknown number of modifiers.

The variety with red ray flowers was found by Cockerell (1915a, b) near his home in Colorado. Realizing the importance of this discovery, especially for horticulture, while simultaneously being acquainted with the basic genetic laws, he made series of crossings and succeeded in selecting plants with red ray flowers. He sold the seed of these plants to the English company Sutton & Sons, and so the red sunflowers quickly spread around the world. As a result we now have a variety of decorative sunflowers differing in ray flower colour, from the typical yellow colour to various shades of red, orange, lemon yellow and combinations of these colours.

The current ornamental sunflower breeding proceeds goes into several major directions depending on breeding purpose (Miklic et al. 2008). In the first place, there is the production of ornamental sunflower as cut flowers. Genotypes for this purpose must have a strong but not thick stem, to support the length of at least 80 cm, short vegetation period, resistance to low temperatures, foliar diseases and long transport, and they should last long in a vase. For this purpose, non-branched genotypes are used, with a large flower and resistance to lodging because they are grown in dense stands. This group also includes genotypes with branching on the top of stem. The main central flower is small and a few short branches remain with the main stem when cut. The third type of genotypes used for this purpose has branches along the entire stem and they all bear a flower. The length of the lateral branches that are cut must be 70 cm. The main objective in the production of ornamental sunflower as cut flowers is to obtain as many useable flowers per unit area as possible. Two concepts are applied in order to achieve this objective. The first one includes the development of non-branched genotypes that tolerate dense planting (50 cm between rows and 15 cm in the row). The second includes the development of branched genotypes which are planted in a stand normal for the sunflower, but the branching feature results in the production of 4–5 first-class flowers and 4–5 second-class flowers.

The second direction of ornamental sunflower breeding is that intended for garden production. It has been designed for flower lovers who wish to decorate their gardens with ornamental sunflower. Genotypes for this purpose are characterized by resistance to low temperatures and foliar pathogens, strong plant habit and branching. The height of these genotypes ranges from 50 to 170 cm, depending on whether they are intended for use as a hedge or to be combined with other flowers.

The third direction of ornamental sunflower breeding is that intended for growing in pots. Genotypes for this purpose have a stem height of 30–40 cm, small leaves,

a short period to blooming and two types of branching – along the entire stem or basal. Ornamental sunflowers of this type are produced in greenhouses and are transported to flower shops just before blooming. They must be adapted to conditions of production in the greenhouse and their leaves should not wither during transport.

The genotypes for all three directions of breeding must met certain common criteria in terms of flower appearance (Cvejic and Jocic 2010). The flower head consists of ray flowers, which are sterile and arranged along the edge of the head, and disc flowers located in the central part of the head. The disc flowers are fertile and they produce pollen. To extend the life span of blooms in a vase, the disc flowers should be sterile too. This is a desirable characteristic first of all because an increasing portion of the human population is sensitive to allergies among which the allergy to pollen is a major one. The colour of disc flowers may be yellow or dark red (anthocyanin). The ray flowers should be rounded and they should completely encircle the head, with no space between them. They should be short, not longer than the radius of the head. The outline of the ray flower should not be spiky or jagged but rather straight. The colour of ray flowers should be lemon yellow, yellow, orange, gold, red or variegated.

Knowledge about inheritance of floral colour and production of new combinations should provide larger genetic variations and success in ornamental sunflower breeding. When crossing red-flowered sunflower lines with the yellow, orange and lemon yellow-flowered lines, all F_1 plants showed a "gaillardia" pattern in which a band of red pigment occurred near the centre of the ray flower petals, whereas the peripheral parts of the petals were the same colour as that of the non-red parent. Two genes are required for the expression of red colour of ray flowers (Fick 1976; Secerov-Fiser and Skoric 1991). The ray flower colour of F_1 plants from crosses involving lemon yellow and yellow lines has indicated that yellow is dominant to the lemon yellow colour. Results of Skaloud and Kovacik (1975) and Secerov-Fiser (1985) suggested that a single dominant gene was involved in the inheritance of this characteristic. Conversely, Fick (1976) suggested that two genes control the inheritance of yellow and lemon yellow colour. The ray flower colouration that is of non-red type is encountered in different variants, beginning from pale yellow and concluding with apricot, with a wide range of intermediate types. The yellow ray flower colouration is most common, which is controlled by complementarily interacting dominant alleles of different genes (Tolmachev 2006). In the homozygous recessive state, these genes control other types of colouration, for example, the gene o governs the orange colour; the gene l the lemon colour; and the genes ly and ap the light yellow and apricot colours, respectively. According to the results of Sharypina et al. (2008) those genotype combinations may be written out as follows: lines with yellow flower colouration – LLOOLyLyApAAp; lemon yellow colouration – llOOLyLyApAp; orange colouration – LlooLyLyApAp; apricot colouration – LLOOLyLyapap; and light yellow colouration – LLOOlylyApAp. From these data it may be concluded that recessive alleles of the genes ap, ly, o, and l correspond to the occurrence of apricot, light-yellow, orange and lemon yellow ray flower colouration, respectively. It has been found that the lemon yellow ray flower colouration is recessively epistatic to the orange and light yellow colouration.

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Chapter 5
Sesame

U. Najeeb, M.Y. Mirza, G. Jilani, A.K. Mubashir, and W.J. Zhou

Abstract Sesame (*Sesamum indicum* L.) is one of the oldest domesticated oilseed crops. Due to the presence of high oil, protein and other nutritional elements, its seed has become an important ingredient of food and feed. However, lack of information about sesame yield structure has restricted the process of crop improvement through breeding. Sesame breeding methods vary from plant selection and hybridization to molecular breeding. Genetic variability in a species is the basic requirement of any breeding program. Available genetic diversity is either directly used for evaluation and selection or desired traits are combined into a single plant via hybridization and backcrossing. Sesame germplasm evaluation and selection for high-yielding varieties are based on genetic heritability estimates of yield-related traits including higher number of capsules, branches and plant biomass, etc. Mutational techniques are employed for broadening genetic diversity of sesame breeding material. Concentrations and application time of any mutagen were found critical for mutation-breeding program. Large number of sesame varieties possessing desirable traits for higher yield and better quality has been developed through mutagenesis. Application of innovative breeding methods helps to reduce our dependence on existence of genetic variability within a species and overcome the limitations of conventional breeding. For this purpose biotechnological techniques have been introduced to sesame breeding programs. Protocols for sesame in vitro culturing and genetic transformation are optimized by using appropriate concentration of hormones and nutrients. Various marker-assisted selection (MAS) techniques such as isozymes, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR), etc. are also used in sesame breeding to study genetic variability of sesame to increase selection efficiency.

U. Najeeb (✉)
Crop Sciences Institute, National Agriculture Research Centre, Islamabad, Pakistan

Institute of Crop Science, Zhejiang University, Hangzhou, 310029, China
e-mail: najeeb_ullah@yahoo.com

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1 Introduction

Sesame (*Sesamum indicum* L.) is pioneer among the domesticated oilseed crops being still cultivated throughout the world in about 70 countries, out of which 26 are located in Africa and 24 in Asia. It is grown worldwide over an area of 75 million hectares producing 60,000 t seed (FAOSTAT 2008). Myanmar, Sudan, China and India are the leading sesame-producing countries of the world. The crop is generally adapted to tropical regions of world, where it is mainly grown for edible seeds and oil (Weiss 2000). Despite its ideal adaptation to dry climates, sesame can also be grown in humid, tropical and subtropical regions. Traditionally, it is cultivated between latitudes 6 and 10°N (Agboola 1979) with the highest yield-producing countries in Asia and Africa (FAOSTAT 2008). *Sesamum orientale* and *Sesamum indicum* are the alternatively used scientific names of sesame (Bedigian 2003). However, Nicolson and Wieserma (2004) proposed *S. indicum* name against *S. orientale*, which was conserved against *S. orientale* and is in use since 2005.

1.1 Botany

Sesame plant is an annual in habit, with indeterminate growth and possesses diploid chromosome number of $2n = 26$. Plants are erect to semi-erect depending on branching types; ovate to lanceolate leaves with pointed apices, the leaf margins are entire to serrate, and stem is round or square type. Flowers range in size containing small-sized tubular calyx and five-lobed corollas and color, e.g., white, violet, red or maroon. Corolla is campanulate having lower corolla lobe longer than the upper one with one sterile and four functional epipetalous stamens.

1.2 Nutritional Composition

Sesame seeds are rich in oil and protein with high dietary energy value of 6,355 kcal kg^{-1}. Chemical composition of seed shows that it contains 57–63% oil, 23–25% protein, 13.5% carbohydrate and 5% ash (Tunde-Akintunde and Akintunde 2004; Elleuch et al. 2007). It is rich source of various nutritional elements including iron, magnesium, manganese, copper and calcium, and important vitamins B_1 (thiamine) and E (tocopherol). Due to its flavor and stability as well as high-quality cooking value, sesame fat is of great significance in the food industry. The presence of sesamin and sesaminol lignans in its nonglycerol fraction contributed to oxidative stability and antioxidative activity of sesame oil (Wu 2007). These antioxidative

agents terminate the process of oxidative damage in cells by capturing oxidative radicals. Antioxidants are also used as dietary supplements to cure cancer and heart diseases. The most abundant fatty acids present in sesame oil include: oleic acid (43%), linoleic acid (35%), palmitic acid (11%) and stearic acid (7%) contributing toward 96% of total fatty acids (Elleuch et al. 2007).

1.3 Origin and History

Most of sesame wild relatives are found in sub-Saharan Africa (Bedigian 2003), but these are also present in India in small numbers (Desai 2004). Its archeological evidences are documented in Pakistan (2250 and 1750 BC) at Harappa in the Indus valley. Major development and differentiation of genus *Sesamum* has been documented in Africa (about 30 species) although most of them are synonyms of just 20 species (Ihlenfeldt and Grabow-Seidensticker 1979). These species have spread from African to Asian and South American countries.

There have been contradictions about the exact origin of sesame for a long time. In the beginning, it was believed to be domesticated in Africa; later evidence from inter-specific hybridization and molecular analysis confirmed its domestication in the Indian subcontinent (Bedigian 2004). Domesticated sesame showed morphological and cytogenetic affinities with the south Indian native *Sesamum mulayanum* (Bedigian 2003), which also contains same number of chromosomes (Annapurna-Kishore-Kumar and Hiremath 2008). Further, Nanthakumar et al. (2000) confirmed proximity between cultivated sesame and its progenitor through RAPD markers.

1.4 Classification

Sesame also known as Till or Gingelly belongs to genus *Sesamum* and family *Pedaliaceae*. More than 38 species have been described in this genus, which are classified into different groups on the basis of their geographic distribution, morphologic and cytogenetic information (Kobayashi 1991).

1.5 Culinary Aspects, Processing and Medicinal Use

Sesame seed is used as an ingredient in several food products and animal feed, whereas its oil is medicinally important for application in pharmaceutical industry. Williamson et al. (2007) reported high sesamin (0.67–6.35 mg g^{-1}) and gamma tocopherol (56.9–99.3 μg g^{-1}) levels among various sesame accessions, which help in the prevention of hypertension and stroke (Noguchi et al. 2004). The antioxidant lignans in sesame seed viz., sesamolin and sesamin are active ingredients of various antiseptics, bactericides, vermicides, disinfectants, moth repellants, anti-tubercular

agents, etc., and proved beneficial for animal and human health (Ashakumary et al. 1999). In addition, neuroprotective effects of sesamin cause hypoxia-induced cell death of cortical cells present in culture (Hou et al. 2003). Sesame oil can also be used as alternative diesel fuel by mixing with methanol and sodium hydroxide (Saydut et al. 2008).

1.6 Genetic Variability

Broad-based plant germplasm resources are imperative for sole and successful crop improvement. Genetic diversity has become more important as cropping intensity and monoculture continue to increase in all the major crop-producing regions of the world. A complete array of sesame germplasm consists of

1. wild relatives, weed races and local races
2. obsolete lines and cultivars
3. improved varieties.

Proper understanding of genetic variability, heritability and correlation studies of plant traits is vital for effective use of germplasm in any breeding program (Ganesh and Thangavelu 1995). Germplasm banks are source of genetic variability and are essential for improvement of crop species. Crop variability is characterized by genetic and phenotypic parameters used for identification and selection of desirable parents for breeding program. Despite the high nutritional value, historic and cultural significance of sesame, there has been little focus on sesame research. No international agency (CGIAR, Consultative Group on International Agricultural Research) is assigned work on sesame crop (Bedigian 2003). Similarly, limited information regarding its genetic diversity is available. Centers for sesame genetic diversity are found in India, China, Central Asia and Abyssinia (Hawkes 1983). Large genetic diversity of sesame should be considered, while planning conservation strategies or exploiting it for breeding programs. Presently, molecular techniques including isozymes (Isshiki and Umezaki 1997a), random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) are being employed to study genetic variability in sesame (Abdellatef et al. 2008; Kim et al. 2002).

1.7 Breeding Objectives

There are various objectives for sesame breeding.

- High seed yields
- Superior plant architecture (ideotype)
- Indehiscent capsules
- Improved oil quality
- Resistance to diseases and pests

Crop improvement has resulted in rapid replacement of old races, wild and weedy species and cultivars. These materials are excellent source of genes for adoptability and resistance to biotic and abiotic stresses. The genetic resource management includes collections, conservation, evaluation characterization, classification and cataloging of germplasm. Lack of specific research and understanding of yield-related attributes limited production and extension process of sesame (Ashri 1989). Yield is an important but complex parameter of crop that is affected by various factors. Development of high-yielding varieties is the ultimate goal of any plant breeder. For efficient crop breeding and improvement, it is of utmost importance to ascertain the contribution of each yield-related trait toward yield, and to select components maximizing yield. Such studies are helpful in determining the model plant type for species.

Indeterminate plant growth habit of sesame and seed shattering at maturity resulted in poor adaptation of plant architecture to modern farming techniques (mechanized harvesting) (Çağırgan 2006). Due to indeterminate sesame growth habit, flowering continues for long time, this heterogeneous capsule maturation causes harvesting problem and yield losses. Development of sesame varieties with improved architecture and determinate habit can assist sesame yield improvement programs. Sesame yield potential is negatively affected by its early senescence and susceptibility to biotic and abiotic stresses (Rao et al. 2002). Sesame is susceptible to phyllody disease caused by phytoplasma, resulting stunted plant growth and yield losses (Singh et al. 2007). Development of phyllody-resistant varieties is one of the important objectives in sesame breeding program. Sesame wild species possess genes for resistance to biotic and abiotic stresses, which can be introduced into cultivated varieties either through backcrossing or genetic engineering.

2 Breeding Methods

Plant breeding is a combination of both science and art for effective management of available genetic variability and creation of new ones to attain desired goals. It is the process to identify and select plants possessing desirable traits, and/or to develop an ideal type plant by combining these desired traits into single plant. Breeding methods used for sesame genetic improvement are simple varying from plant selection to hybrid development and molecular breeding. Application of biotechnology and molecular breeding methods can boost the breeding process for development of superior sesame varieties.

2.1 Conventional Breeding

Conventional breeding is under the control of human for choice of parental lines, and selection of their offspring to direct the evolution process for crop production

according to their desires. Although low percentage of cross pollination is reported, the sesame is predominantly regarded as a self-pollinated plant (Ashri 2007). Development of sesame types with desirable characters is achieved through pedigree selection from segregating generations of different crosses. In conventional plant breeding these traits are manipulated to get desired genetic combination through various procedures.

There are several advantages of conventional breeding, it is technically simple, convenient and need no sophisticated tools. It is suitable for improvement of many traits or polygenic or traits with unidentified genes at one time. However, there are certain disadvantages of conventional methods including incompatibility in crosses, limitation of genetic variation within crop gene-pool and time consuming. Selection of plants with desirable traits from segregating generations is a time-consuming process, and sexual breeding methods are not useful for improving sexually sterile crops.

2.1.1 Pure Line and Mass Selection

Evaluation and consequent selection of improved lines are the first step in breeding process that largely depends on the knowledge of plant genetic diversity and heritability. Selection is regarded as the most ancient and basic procedure in plant breeding in which desired plants are selected from genetically variable population. These lines are evaluated against existing commercial varieties for yield and other traits for making justified plant selection.

Information about relationship between yield and yield-contributing attributes is very important for a successful breeding program (Ganesh and Sakila 1999). Plant selection with appropriate type sesame is essential for increasing seed yield and developing novel sesame varieties. It is considered that breeding based on additively controlled characters helps improving sesame yield (Mubashir et al. 2007). Since seed yield is a polygenic character, it is essential to identify yield-contributing attributes for selecting high-yielding sesame cultivars. Various physiological traits are useful for determining selection criteria including higher number of capsules, branching and biomass, harvest index, which exhibit significantly positive correlation with seed yield in sesame (Sarwar and Hussain 2010). Large numbers of sesame cultivars and lines have been classified on the basis of diagnostic morphological and genetic traits such as flower characters including phyllotaxis, number of nectar, flower or capsule per axil and carpel number per capsule (Sarwar et al. 2005). These classifications provide foundation for development of high-yielding sesame varieties.

High genetic advance and heritability for yield-related parameters including seed yield, capsule number and branches per plant were documented by Sarwar and Haq (2006), who evaluated 106 sesame genotypes from different parts of the world. They concluded that selection of sesame elite genotypes for seed yield is possible on the basis of these characters. On the basis of these phenotypic and genotypic marker traits, various high-yielding sesame varieties have been selected, and a positive correlation of these traits with seed yield was confirmed (Sarwar et al. 2005). Plant characteristics such as bicarpels, monocapsule, branch and tricapsules

have been used as marker in pedigree selection method by Baydar (2005) to obtain high-yielding sesame varieties.

High heritability estimates of disease infestation are under additive gene action control, and consequently help in the selection of disease-free sesame plants. El-Bramawy and Abd Al-Wahid (2009) screened 28 sesame genotypes for resistance to *Fusarium oxysporum* under field conditions for two successive seasons. Two genotypes "S2" originated from a selection and "H4" from hybridization demonstrated stable resistant to *Fusarium* wilt throughout the evaluation. Some other genotypes including Mutants-8, A-130, H-1 and S-1 also maintained their resistance classes during the two successive seasons. In another study, Arslan et al. (2007) evaluated 29 gamma rays (γ-rays) induced mutants and selected sesame plants exhibiting high level of resistance to *Fusarium* blight.

2.1.2 Hybridization

In conventional plant breeding, hybridization is the most frequently used technique. It helps to combine the desirable traits from different plant lines into a single plant through cross pollination. Desired traits such as disease resistance and improved oil quality can be transferred from wild relatives of a crop species to the cultivated forms. Heritability estimates and combining ability studies assist in predicting genetic improvement of different types and are useful in hybrid selection program.

In sesame, emasculation is the simplest and most commonly used technique for producing F_1 hybrids through cross pollination. Additively control characters can be effectively transferred through hybridization process. In sesame high heritability for yield-related parameters, i.e., the number of branches per plant, the number of capsules per plant, seed yield per plant and seed yield per square meter, shows that additive gene action governs these characters (Sarwar and Haq 2006). Bisht et al. (2004) made crosses among 24 diverse and un-adapted parental lines in various combinations and selected high-yielding sesame plants from a progeny of 103 crosses. Phyllody-resistant sesame cultivars were developed through intra- and inter-specific crosses among different sesame cultivated and wild species, and it was revealed that disease resistance is governed by one dominant (wild species) and one recessive (cultivated species) gene (Singh et al. 2007).

Production of male sterile lines provides an opportunity to facilitate cross pollination process for hybrid seed production, and to exploit sesame heterotic vigor. Sesame cytoplasmic male sterile (CMS) lines were developed by hybridizing *S. indicum* with its wild relative *S. malabaricum* (Bhuyan et al. 1997). Later using CMS system, Bhuyan and Sarma (2003) obtained 36 hybrid combinations of diverse origin. Out of which many hybrids exhibited high heterosis for seed yield, oil content and capsules number per plant. Heterosis, a phenomenon of increased vigor, is obtained by hybridization of inbred lines. Heterosis breeding is a common technique for developing high-yielding sesame varieties that may exhibit 77–540% heterotic effect (Yadav et al. 2005). Mubashir et al. (2009) conducted an experiment comprising of five parental lines and their ten crosses, recording 40.35–255.12% heterosis in yield-contributing components.

2.2 Mutation Breeding

Mutation breeding involves induction of new genetic variability through spontaneous or artificial mutagens (chemicals or physical). It minimizes our dependence on the use of wild species or species from other cultivars. Induced mutants are evaluated and selected for desired traits. However, development of large number of mutants with undesirable traits limits its wide application in the breeding programs.

Mutagenic techniques are successfully employed in sesame to induce genetic variability. Applications of appropriate doses of physical mutagen or concentration of chemical mutagen are important to get adequate mutations that could benefit sesame breeding program. Researchers at FAO/IAEA have initiated coordinated research project for genetic improvement in sesame, and developed 142 mutants having agronomically useful characters by using both physical and chemical mutagens and devised method for mutation breeding for sesame (Van Zanten 2001). Following were the recommendations for mutagen treatment.

Well-adapted, homozygous and uniform varieties should be selected for mutation induction for improvement of one or two characters at a time. Lower dose ranges of mutagens are more suitable for inducing desirable mutations, i.e., γ-rays 150–800 Gy, fast neutrons' irradiation 30–80 Gy. For chemical mutagenesis, first seeds are pre-soaked in water for 24 h (4°C). Then soaking into chemical mutagen, e.g., in ethyl methane sulfonate (EMS) solution (0.4–1.0% v/v) with phosphate buffer (pH = 7) for 2–4 h or in sodium azide (NaN_3) solution (4–6 mM) with Sörenson phosphate buffer (pH = 3) for 4–6 h at 18–24°C.

Sesame mutants have been selected for desirable traits of higher yield and quality (Wongyai et al. 2001), improved plant architecture (Çağırgan 2006), seed retention, larger seed size and seed color (Hoballah 2001). A research program on radiation-induced mutagenesis has been initiated to induce genetic variations and to screen desirable "plant type" (Chowdhury and Datta 2008). Sengupta and Datta (2005) identified a narrow leaf mutant in sesame through nitrous acid and hydrogen peroxide treatments in different doses, and the mutant yielded higher number of capsule per plant on the main axis than control.

Early maturing and high-yielding sesame mutants have been developed by using NaN_3 and colchicines, and Mensah et al. (2007) found that 0.0625% NaN_3 and 0.125% colchicine were the most efficient concentration for inducing mutations in sesame. The γ-ray-induced mutants with improved plant architecture were developed having closed capsule, determinate growth habit, resistance to *Fusarium* blight, etc. These mutants had improved oil quality with considerably higher oleic acid and low linoleic acid contents (Arslan et al. 2007).

Indeterminate sesame habit is a challenge for sesame breeders, and mutagenic breeding approach is applied to solve this problem (Çağırgan 2006). A spontaneous indehiscent mutant "id" was discovered in 1942 in Venezuela by Langham (1946). However, due to its low yield and other undesirable side effects it was not used in commercial varieties. The first determinate sesame mutant (dt-45) was selected by Ashri (1981) from an M2 population by irradiating Israeli variety "No-45" with

γ-rays (500 Gy). Çağırgan (2006) irradiated seeds of four sesame cultivars with γ-rays (150–750 Gy) and found three true botanical determinate mutants (dt-1, dt-2 and dt-3) of cultivar Muganlı-57 and dt-4, dt-5 and dt-6 of cultivar Çamdibi. They also proved that selection of determinate growth habit mutants depends upon population size, cultivar response to mutagenic treatment and careful screening.

Marker traits are always useful in genetics and breeding as they are easily scorable and selectable in field conditions. Cytogenetical and agronomical aspects of some morphological (leaf and pollen related) marker mutants were induced following different doses of X-rays and γ-rays (Chowdhury et al. 2009). These morphological sesame mutants exhibited distinctive traits viz., narrow, elongated, thick leaf types, ovate, ternate elongated petiole type and white, pigmented flower type. Out of different mutants, thick leaf mutants were the most desirable plant types possessing superior agronomic traits such as plant height, primary and total branches per plant, capsule on main axis, distance from base to first branching, total capsule per plant, seed yield and seed protein content than control. Mary and Jayabalan (1995) induced mutation-affecting leaf morphology in sesame at M2 following EMS treatments to seeds.

2.3 Innovative Breeding

Shortcomings in the conventional breeding (sexual reproduction) are overcome by genetic engineering techniques that introduces desirable genes directly into the target crop making gene pool unbounded. Only desirable traits are improved in this method, therefore, large populations and multiple generations are not required for selection of plants. In addition, there are no limitations for application of this technique to sterile and vegetatively propagated crops.

Likewise, these techniques also have certain drawbacks; only simple and monogenic traits are transferred most of the time, they are relatively expensive and technically demanding and they are controlled by government organizations. Various innovative approaches are used for sesame breeding viz., in vitro culture, genetic transformation and molecular breeding as described below.

2.3.1 In Vitro Culture and Screening

Somatic plant cells are used for in vitro culturing on nutrient media and new plants are generated from these explants. Plant regeneration through tissue culture is a source of creating genetic variations, heritable variants with desirable agronomic traits are selected, and used in further breeding programs. Plants can also be selected for resistance traits at early stage by exposing cells of calli to pathogens, or isolated pathotoxins by eliminating unwanted plants from the large population. Three factors affect plant regeneration process, viz., genotype, explant source and culture conditions.

Tissue culture and regeneration through in vitro culturing can speed up breeding process by producing a number of stable regenerants via callus or somatic embryogenesis in a short span of time. In sesame in vitro culturing, cotyledon (Yadav et al. 2010) hypocotyl and shoot tips (Baskaran and Jayabalan 2006) have been reported to be more responsive to callus induction and plant regeneration. Appropriate concentrations of plant growth regulators and their combinations are very important to achieve successful plant regeneration from cultured cells and tissues, and were optimized in different studies. Application of BAP (benzylaminopurine) in the nutrient media was reported essential and the most effective cytokinin for shoot induction and plant regeneration in *S. indicum* (Yadav et al. 2010). Baskaran and Jayabalan (2006) studied the effects of plant growth regulators on callus induction in hypocotyls and cotyledon explants of sesame, and reported callus induction on media containing 2.2–22.6 μM 2, 4-D and 2.6–26.8-μM NAA (α-naphthalene acetic acid), increased shoot proliferation on BAP and Kn (kinetin), whereas rooting took place on NAA (8.0 μM).

In another study, Saravanan and Nadarajan (2005) investigated in vitro response of four sesame varieties on different media components for callus induction, multiplication, shooting and rooting. The highest callusing frequency was recorded at 2, 4-D (3 mg L^{-1}) with 100 mL of coconut milk followed by 2, 4-D (3 mg L^{-1}) with casein hydrosylate (0.1 mg L^{-1}). Significantly higher shoot multiplication ratio was achieved in MS media (Murashige and Skoog 1962) supplemented with 1-mg L^{-1} indole acetic acid (IAA), 1–1.5-mg L^{-1} BAP and 1.25-mg L^{-1} Kn. Tissue culture and plant regeneration protocol for wild species of genus *Sesamum* was also optimized by Dasharath et al. (2007a, b). They used *Sesamum occidentale* and *Sesamum radiatum* and found 8-mg L^{-1} Kn along with BAP as the best combination among different levels of BAP and Kn applied.

2.3.2 Somatic Hybridization

Sesame is a self-pollinated crop; however, conventional crosses between cultivated sesame and its wild relatives have been attempted, the hybrids were difficult to produce. Use of wild relatives in hybridization program is restricted due to cross incompatibility and low hybrid frequency through embryo culture. Hybrid plants can also be developed through fusion of somatic plant cells. Protoplast fusion is helpful to overcome sexual incompatibility as distantly related species can be fused. In vitro culturing system can help to multiply F_1 plants in the lab first and then to transfer them into the field (Dasharath et al. 2007a).

In sesame, Dasharath et al. (2007b) successfully developed inter-specific hybrids between cultivated *S. indicum* and its wild relatives *S. radiatum* and *S. occidentale* through ovary and ovule culture. In another study, a simple and efficient protocol for production of hybrids of a cross between *Sesamum alatum* and *S. indicum* were optimized through ovule culture (Rajeswari et al. 2010). For this purpose, capsule retention without embryo abortion was delayed by spraying mixture of growth regulators 289-μM gibberellic acid (GA_3), 80.6-μM NAA and 23.3-μM Kn. The plants

were regenerated through direct organogenesis of 7-day-old capsules by culturing them on MS medium containing 8.8-μM BAP, 2.8-μM IAA and 1,712.3-μM glutamine. The developed hybrids were screened for phyllody resistance which exhibited moderate resistance.

2.3.3 Genetic Manipulation

Sexual incompatibility among plants limits the application of conventional breeding. In genetic engineering techniques, specific genes from any organism (plants, bacteria, fungi, animals and viruses) coding for desired traits are introduced into the genome of any plant. Various techniques are used to obtain transgenic plants viz., DNA transfer through *Agrobacterium* or direct DNA transfer via bombardment, electroporation and polyethylenglycol permeabilization.

The *Agrobacterium*-mediated DNA transformation is the most commonly used techniques in plants (Xu et al. 2009). Desired genes are first transferred to plasmid DNA of *Agrobacterium* and then allowed to transmit into individual plant cells for their expression. This method is suitable for *Agrobacterium* susceptible plants. However, it cannot be used for many economically important plants including cereals; therefore, direct DNA uptake method is applied.

Sesame yield is limited due to different biotic and abiotic stresses (Rao et al. 2002). Some wild sesame species possess resistance genes, but post-fertilization barriers restrict their transfer to cultivated crops through conventional breeding. Establishment of in vitro plant regeneration is a prerequisite of any genetic transformation system that is already optimized (Were et al. 2006). Sesame has been reported as susceptible to *Agrobactarium tumefaciens* infection (Taskin et al. 1999). Protocol for genetic transformation and plant regeneration of sesame were optimized by Were et al. (2006). A significant interaction between hormonal concentration and macronutrients for plant regeneration was recorded, and application of 20-μM TDZ along with 2.5-μM IAA was found the best for successful plant regeneration. Yadav et al. (2010) optimized an *A. tumefaciens*-mediated transformation protocol to generate fertile transgenic sesame plants. In this method, cotyledon explants were used for plant regeneration via multiple shoot organogenesis. They recovered plants on MS basal medium containing 25.0-μM BAP, 25.0-mg L^{-1} kanamycin and 400.0-mg L^{-1} cefotaxime.

2.3.4 Marker-Assisted Selection

Marker-assisted selection (MAS) process has revolutionized plant breeding disciplines by increasing selection efficiency at early stages of development and characterization in later generations (Cahill and Schmidt 2004). The MAS program has been widely applied tool in commercial crop breeding and product development in a variety of agriculturally important economic crops, including cereal, oilseeds, vegetables and ornamentals.

Various morphological plant traits, their geographical origins and genotype-specific bands developed through molecular markers provide useful information about economic importance of crop, and help in further classification (Ali et al. 2007). Molecular markers have been applied for studying genetic diversity by using various *S. indicum* accessions (Abdellatef et al. 2008) and suggested the usefulness of RAPD technique in sesame breeding and conservation programs, for proper maintenance of germplasm banks and efficient parental line selection.

However, in spite of high economic value, a limited number of reports are available regarding the application of molecular markers for sesame improvement and studying genetic variability viz., isozymes (Isshiki and Umezaki 1997b), ISSR (Kim et al. 2002), amplified fragment length polymorphism (AFLP) (Ali et al. 2007) and simple sequence repeat (SSR) markers (Dixit et al. 2005). The application of MAS is generally limited to exploration of genetic variability and germplasm evaluation. Only few studies are conducted for tagging desired genes to facilitate the process of plant selection for genetic improvement. Construction of genetic linkage maps is a useful technique for tagging of the desired traits in sesame molecular breeding (Wei et al. 2009). Using MAS, Uzun and Çağırgan (2009) tagged *dt* gene, which regulates determinate growth habit in sesame. Development of molecular markers could assist sesame plant identification and selection for breeding programs, and facilitate integration of these genes into improved cultivars.

3 Conclusion

Conventional and innovative breeding methods are complementary to each other for improving crops. Depending on breeding objectives, their application can be appropriate or inappropriate; however, none of the improvement strategies alone is totally perfect. Sesame breeding objectives can be achieved by devising breeding programs with specific targets for crop improvement. Application of biotechnology along with conventional breeding methods is a useful approach for breeding superior varieties in a short time. In addition, the construction of molecular genetics maps, tagging-desired traits for marker-assisted selection and positional cloning could be more reliable tools in genetic studies than morphological traits. Similarly, physiological and pathological studies offer deeper understanding and effective ways to identify plant traits useful for solving particular problems. Co-ordination of breeding, physiology, pathology and biotechnology will be very helpful for increasing the productivity and production of sesame, and will provide a model for other crops.

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Chapter 6
Safflower

Theodore J. Kisha and Richard C. Johnson

Abstract Safflower (*Carthamus tinctorius* L.) is an ancient crop with numerous past and present uses. Traditionally safflower was grown for its flowers, which were used as a fabric dye and for food coloring, flavoring, and medicinal purposes. Today, as a result of manipulation of well-characterized germplasm resources, it has become an important oil seed crop, bred for specialty niches through the development of healthier or more heat stable oil constituents, winter hardiness, and disease resistance. Molecular methodology has facilitated characterization of the world-wide diversity of safflower and identified geographical regions of similarity to assist breeders in the exploitation of available diversity. The development of molecular markers from expressed sequences should aid researchers in mapping genes of importance and reducing population size and generations required for the development of new varieties by using marker-assisted selection. Sequencing technology has established relationships among species of *Carthamus*, further aiding in the exploitation of diversity within the secondary gene pool. A coordinated, collaborative effort among safflower researchers in the development of marker-assisted characterization of global diversity would further increase the utility of available germplasm resources.

Keywords Safflower (*Carthamus tinctorius* L.) • Germplasm resources • Molecular methodology molecular markers • Sequencing technology • Global diversity • Germplasm resources

T.J. Kisha (✉)
USDA-ARS, Western Regional Plant Introduction Station, Washington State University, Box 646402, Pullman, Washington, DC 99164, USA
e-mail: theodore.kisha@ars.usda.gov

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1 Introduction

Safflower (*Carthamus tinctorius* L.) is an ancient crop with numerous past and present uses (Li and Mündel 1996). The Food and Agriculture Organization (FAO 2010) of the United Nations estimated the world safflower production at approximately 600,000 t with production in India being foremost, exceeding more than double that of any other country (Table 6.1). Traditionally safflower was grown for its flowers, which were used as a fabric dye and for food coloring, flavoring, and medicinal purposes. A brief and very interesting description of the spread of safflower throughout the ages is given by Weiss (2000). Weiss mentions references to safflower dating back almost four millenia, from florets in the tomb of Amenophis I (Scweinfurth 1887) in 1600 BC to a revenue-papyrus of Ptolemy II from around 260 BC indicating his monopoly of vegetable oils, including that of safflower (Keimer 1924). Its use as a dye is well known, but perhaps not so well known was the export of the dye from Egypt for the coloring of cheese in Italy, France, and England as early as the eighteenth century (Hasselquist 1762). Its use in Gloucestershire for coloring sausages and cheese was in such quantities as to have a purgative effect (Hanelt 1961). While synthetic dyes are now more common, the trend toward natural products may increase the value of crops such as safflower to accommodate food and textile industries.

Today, seeds are the major plant part used, resulting in a high-quality edible and industrial oil and bird feed (Knowles 1989; Bergman et al. 2007). Newer uses include specialty oil types to improve human diet (Velasco and Fernández-Maryinez 2004), biofuel (Bergman and Flynn 2009), and, because of the ease with which oleosin proteins are isolated from safflower seed (Lacey et al. 1998), production of transgenic pharmaceuticals (McPherson et al. 2004; Mündel and Bergman 2009). Singh and Nimbkar (2006) have provided an excellent review of safflower, including its history, cytogenetics, tissue culture, and breeding methodologies. At the time of their publication, however, little information was available on the molecular genetic diversity. More recently, Mündel and Bergman (2009) have published a review of safflower that covers genetic resources, major breeding achievements, crossing techniques, and new breeding technologies. This chapter discusses the present and future breeding objectives and focuses on the genetic diversity within safflower and molecular information that has become available in the past few years.

Vavilov (1951) proposed three centers of origin for safflower, which included India, Central Asia (Northwest India, Afghanistan, Tadjikistan, Uzbekistan, and Western Tian-Shan), and Abyssinia (Ethiopia and Eritrea). More important to modern plant breeding, however, may be Knowles' (1969) reference to "centers of similarity," which may be more indicative of types available for enhancement of specific traits. Furthermore, modern genetic techniques have placed some doubt on Vavilov's original proposals. The wild species of safflower native to Ethiopia has 32 pairs of chromosomes, as opposed to the 12 pairs in cultivated varieties, and thus, is not considered a center of origin as proposed by Vavilov (Knowles 1969). Ashri and Knowles (1960) included *C. tinctorius*, *Carthamus oxyacanthus*, and *Carthamus palaestinus* in their Sect. I based on chromosome number 12 and their ability to cross readily and produce fertile hybrids. *Carthamus arborescens*

Table 6.1 Annual production of safflower, the number of researchers listed, and genetic resources held by country

	Metric tons produced				
Country	2006	2007	2008	Researchers	Genetic resources (accessions)
India	228,600	240,000	225,000	24	9918
Mexico	73,536	113,334	96,413	3	1504
US	86,820	94,798	140,810	10	2484
Argentina	17,800	58,000	33,480	–	
Ethiopia	5,957	11,176	8,075	1	197
Kazakhstan	45,700	43,940	45,740	–	
China	30,000	32,000	32,500	3	7683
Tanzania	5,000	5,000	5,000	1	
Uzbekistan	3,257	3,500	3,500	–	
Canada	2,000	2,000	2,000	2	456
Australia	13,942	2,040	2,040	5	425
Iran, Islamic Republic of	500	500	500	3	
Spain	67	70	70	1	6
Russian Federation	130	40	90	–	429
Occupied Palestinian Territory	6	6	6	–	
Hungary	239	240	N/A	1	
Israel	–	–	–	2	
Morocco	–	–	–	–	
Pakistan	38	49	60	2	
Bulgaria	–	–	–	1	9
Germany	–	–	–	1	166
Romania	–	–	–	1	24
Slovenia	–	–	–	1	14
Switzerland	–	–	–	1	1
Bangladesh	–	–	–	1	
Egypt	–	–	–	5	
France	–	–	–	1	
Iraq	–	–	–	1	
Italy	–	–	–	3	
Kenya	–	–	–	1	
Korea	–	–	–	2	
Myanmar	–	–	–	2	
Nepal	–	–	–	1	
New Zealand	–	–	–	1	
Philippines	–	–	–	1	
Portugal	–	–	–		
Sudan	–	–	–	3	
Tajikistan	1,570	1,036	562		
Tekirda	–	–	–	1	
Kyrgyzstan	13,045	12,039	12,300		
Turkey	395	2,280	7,068	3	
UK	–	–	–	1	

Estimates of production are from the Food and Agriculture organization of the United Nations (http://faostat.foa.org/site/339/default.aspx). The number of researchers within country is based on Zhang and Johnson (1999) (IPGRI is now Bioversity http://www.bioversityinternational.org/)

and *Carthamus caeruleus* were also in Sect. I, but did not produce fertile hybrids. Ashri (1974) noted natural interspecific hybridization between *C. tinctorius* and *Carthamus tenuis* to occur when late planted cultivated safflower existed among the wild, unrelated wild species, but hybrids were sterile. He purports, however, that introgression probably occurred between the two species because, although the hybrids were sterile, they gave a greater mean number of bivalents than crosses of *C. tenuis* with either *C. oxyacanthus* or *C. palaestinus,* which are more closely related to cultivated safflower. Although *C. oxyacanthus* has been considered as the wild ancestor of cultivated safflower, Ashri and Knowles (1960), Garnatje et al. (2006), Bassiri (1977), and Chapman and Burke (2007) examined the phylogenetic relationships among 23 individuals of *C. tinctorius*, *C. oxyacanthus*, *C. palaestinus*, and *Carthamus gypsicola* using DNA sequence data from seven nuclear genes and found *C. palaestinus* to be more closely related to *C. tinctorius*. They thus propose *C. palaestinus*, which is native to the deserts of southern Israel and Western Iraq, as the wild progenitor of cultivated safflower. Sasanuma et al. (2008) examined 13 taxa of *Carthamus* using DNA sequence data from a nuclear gene and from an intergenic spacer region in the chloroplast. They also found *C. palaestinus* to be more closely related to *C. tinctorius* than any of the other species tasted, including *C. oxyacanthus*. Sehgal et al. (2009) using a multi-pronged DNA assay of RAPD, ribosomal DNA repeat unit length polymorphism, internal transcribed sequence (ITS) restriction fragments, and comparative sequence analysis of internal (ITS) and external (ETS) transcribed sequences, and Bowles et al. (2010) combining sequence and microsatellite data, also reached the same conclusion.

2 Breeding

Safflower, a diploid with 12 chromosome pairs (Ashri and Knowles 1960), is a predominately self-pollinating species, but has the potential for considerable outcrossing with pollen transfer by a variety of insects (Butler et al. 1966; Rudolphi et al. 2008). Moreover, the degree of outcrossing depends on genotype and environment. The thin-hulled trait has a pleitropic effect on anther dehiscence which deters pollen collectors, which prefer lines with normal hull morphology or anatomy (Rubis et al. 1966; Weiss 2000). High temperatures during pollination can reduce the time that pollinators spend collecting, which can decrease the amount of outcrossing (Ahmadi and Omidi 1997). Time to flowering is genetically controlled, but genotype and environment interact with day length, and flowering can be accelerated by high temperatures (Weiss 2000). Staggered planting of crossing blocks will ensure a timely source of pollen when stigma and pollen are ready among all desired genotypes. Cross pollination procedures are described in detail by Mündel and Bergman (2009) with excellent color images.

There are about 25 species of wild safflower divided by Ashri and Knowles (1960) into different sections based on chromosome number. Many of these are weedy, such as *C. oxyacanthus*, a noxious weed in the USA, complicating its

regeneration at the USDA Western regional Plant Introduction Station (WRPIS). Species with 12 chromosome pairs tend to cross readily. These include safflower (*C. tinctorius*), *C. persicus Desf. Ex Willd, C. oxyacanthus, and C. palaestinus*. The WRPIS has no *C. persicus Desf. Ex Willd* available, only 40 *C. oxyacanthus*, and just one *C. palaestinus*. *C. flavescens*, from areas of Turkey, Syria, and Lebanon, is entirely self-incompatible. *C. oxyacanthus*, indigenous from northwestern India to central Iraq, is a mixture of self-incompatible and self-compatible types. *C. palaestinus*, found in the desert areas of western Iraq, Jordan, and southern Israel, is a self-compatible species. Additional details concerning crossing safflower with wild *Carthamus* species can be found in Knowles (1989).

Historically, breeding objectives have included increased yield, increased or improved oil content, increased or improved protein, winter hardiness, disease and insect resistance, and the development of characteristics to facilitate hybrid production. Among the Crop Science registrations are materials representing some of the most significant advances in safflower germplasm (http://www.ars-grin.gov/cgi-bin/npgs/html/csr.pl?SAFFLOWER). The first registration was for Nebraska 10 (PI 572428) by J. Williams in 1964. It was described as an "early maturing, high-yielding variety," developed as a single selection from 852 to 895 by C.E. Classen at Alliance, Nebraska, USA in 1946. Knowles (1968) registered UC-1 (PI 572434), the first safflower with a fatty acid profile similar to olive oil; that is, 78% oleic and 15% linoleic. This was essentially the reverse of traditional, high linoleic safflower. Other notable contributors include germplasm registrations for rust, verticilium, fusarium, rhizoctonia, and phytophthora root rot resistance by C. Thomas, D. Zimmer, and L. Urie. H.H. Mündel and cooperators released three early developing cultivars and four germplasms for the Canadian Prairie. J. Bergman and cooperators have registered 13 cultivars, the most of any contributor. These include those developed for disease resistance, high oleic acid content, high linoleic content, and for bird and livestock feed. Oil and meal evaluations by Johnson et al. (1999) lead to work by Velasco and Fernández-Maryinez (2004) to register CR34 and CR-81, high alpha-tocopherol germplasm (Vitamin E). The release CR34 was derived from PI 304597 and CR81 from PI 406001.

A cooperative germplasm exchange with Li Dajue at the Beijing Botanical Garden in China in the late 1980s and early 1990s led to the first registrations of three winter hardy safflower lines, PI 651878, 651879, and 651880 (Johnson and Li 2008). These were developed by overwintering PIs 543995, 544006, and 544017 identified with overwintering capability; surviving plants were selected at Pullman, WA, over two cycles of selection.

Although unsaturated vegetable oils are considered most healthy, *trans*-fats resulting from partial hydrogenation of vegetable oils are widely considered detrimental to human health (Mozaffarian et al. 2006). The partial hydrogenation makes liquid vegetable oil solid at room temperature to increase shelf life and make vegetable fats for spreads and baking. Increased saturated fatty acid content, resulting in more viscosity, could reduce or eliminate the need for hydrogenation of vegetable oils for solidification. Hamdan et al. (2009) selected accessions based on their fatty acid profiles available in the Germplasm Resources Information Network

(GRIN: http://www.ars-grin.gov/npgs/) (Johnson et al. 1999) and developed safflower oil with high saturated fatty acid for potential applications in the food industry. Line CR-50 with high palmitic acid was developed from PI 306686 and CR-13 with high stearic acid was developed from PI 198990.

3 Disease

Mortensen et al. (1983) found both *Alternaria carthami* and *Alternaria alternata* to be problems in Montana, resulting in seed with inferior germination and seedling vigor. Patil et al. (1993) indicated diseases of safflower to be one of the most important constraints to production in both drought-prone areas and assured rainfall zones of India, with *Alternaria* spp. being the most damaging with losses recorded up to 50%. They conducted a 5-year study of 1,500 accessions from the world safflower germplasm collection under a grant from the US Department of Agriculture. They found accessions resistant to *A. carthami* Chowdhuri, *Cercospora carthami* Sund. and Ramak., *Ramularia carthami* Zaprom., *Erysiphe cichoriacearum* D.C., wilt caused by a complex of *Fusarium oxysporum* Sehl. Ex Fries and *Rhizoctonia bataticola* Bult. or *Rhizoctonia solani* Kuhn. The hybrids produced from crosses with a susceptible safflower indicated that resistance to all but the mildew from *E. cichoriacearum* D.C. was dominant. F_2 progeny were not tested because of the sheer numbers of plants involved. Singh et al. (2001) also found resistance to *F. oxysporum* to be dominant. However, F_2 progeny segregated in a ratio of 13:3, resistant to susceptible, suggesting the role of an inhibitory gene.

Urie and Knowles (1972) tested approximately 2,400 plant introductions and entries from both the USDA and commercial breeders for resistance to verticillium wilt (*Verticillium albo-atrum* Reinke and Berth.). They found 48 of those tested to have resistance. A search of GRIN of the National Plant Germplasm System (NPGS) found 33 accessions resistant to Fusarium, 30 resistant to *Verticillium*, 18 resistant to *Alternaria*, four resistant to *Sclerotinia*, and nine resistant to rust.

Thomas and Zimmer (1971) developed a safflower composite resistant to phytopthera root rot (*Phytopthera dreschsleri* Tucker) from selections from PI 250724 and PI 253538, from Portugal and Iran, respectively. Both PIs were segregating for resistance. Resistant greenhouse tested seed from homozygous-resistant plants were bulked. This accession (CSR-210) also shows high level of resistance to verticilium wilt, all known races of *F. oxysporum*, and rhizoctonia blight. Unfortunately, it is no longer available from the NPGS. Rubis (1981) developed the Arizona Wild Composite (AWC, PI 537682) by open pollinating the thin-hulled line A4138 with 12 Carthanus species (*Carthamus alexandrines, C. arborescens, Carthamus baeticus, C. caeruleus, Carthamus dentatus, Carthamus flavenscens, Carthamus glaucus, Carthamus lanatus, C. oxyacanthus, C. palaestinus, Carthamus syriacus*, and *C. tenuis*). The exact pedigree of the composite is unknown, but plant and seed characteristics indicate that most of the introgressive germplasm came from *C. flavescens* and *C. oxyacanthus*. Leaf, flower, and spine characteristics of the F_1

population also evidenced crosses to many of the other species. This accession is highly heterozygous and heterogeneous and varies in rosetteness, earliness, spininess, flower color, seed size, seed shape, seed color, hull type, hull percentage, and other characteristics. Thin-hull facilitated recurrent selection from this population with flood treatment resulted in several lines with resistance to root rot. PI 537690 exhibited 95% survival in a nursery that showed an overall 95% kill. These accessions and others developed from the AWC are available in GRIN.

Heaton and Klisiewicz (1981) developed a disease-resistant alloploid from a cross between *C. tinctorius* L. and *C. lanatus* L. The alloploid had 34 chromosomes, presumably 22 from *C. lanatus* and 12 from *C. tinctorius*, and the doubled haploid had $2n = 64$ chromosomes, the morphology of *C. lanatus*, and showed resistance to important safflower pathogens, including *Alternaria*, *Fusarium*, *Verticillium*, and bacterial blight. The alloploid is fertile and self-pollinates, but the sterility associated with non-homology of the majority of chromosomes prevents backcrossing to *C. tinctorius*. A breeding scheme effecting a translocation in an alien addition line of *C. tinctorius* needs to be achieved to introduce genes from *C. lanatus* into the cultivated *C. tinctorius*. To date, these authors could not find attempts to map genes responsible for any of the diseases affecting safflower.

4 Biofuels

Emphasis on renewable energy sources has kindled an interest in the role for oilseed crops in the production of biodiesel. A study begun in 2006 at Montana State University (Bergman and Flynn 2009) evaluated biodiesel prepared from sunflower, flax, soybean, canola, camelina, crambe, and both high-linoleic and high-oleic safflower oils. Safflower and sunflower oilseed crops produced the most gallons of oil and the most biodiesel per acre. They also had the lowest clod filter plugging point of the oilseed crops, and high-oleic safflower, along with soybean and high-erucic rapeseed biodiesel had the highest oxidative stability. Results of the study documented that safflower and sunflower grown in Eastern Montana could produce more biodiesel per acre than soybeans in the Corn Belt states.

5 Germplasm

Despite some useful breakthroughs in biotechnology allowing the tapping of tertiary gene pools (distant taxa) for genes with specific purposes, primary and secondary gene pools (same and related species, respectively) are still the most important sources of genetic variation for plant breeders. Germplasm collections worldwide provide genes for today's breeding efforts, while preserving other genes for future needs. Availability of genetic diversity is of limited use, however, without the identification and characterization of that diversity, so it can be exploited and applied in

an efficient manner. Transgressive segregation for quantitative traits, such as yield, in crop plants relies on the recombination of many different genes positively affecting that trait. Given the potential number of genetically distinct progeny from a single cross and the number of parents available for crossing, knowledge of parental characteristics and their relationship with one another is imperative. This is especially true when searching collections for useful traits such as pathogen resistance. The preservation of diversity of crop genetic resources remains as important today and for the future as it was in the past, as resources continue to be needed to meet future challenges associated with climate change, disease evolution, and the increasing needs of a growing population.

Germplasm collections remain a critical resource for development and improving safflower (*C. tinctorius* L.) cultivars and germplasm. Genetic resources are the essential raw materials needed for improving crops and for developing new, value-added uses. Safflower (*C. tinctorius* L.), with its numerous and varied uses (Li and Mündel 1996), has benefited from the diversity of genetic resources conserved and distributed by genebanks. A germplasm directory for safflower was compiled by Zhang and Johnson (1999) which documented 18 different collections in 14 countries. This publication can be found on the safflower web page (http://safflower.wsu.edu/). India reported the largest collections with nearly 10,000 total accessions held at both the National Bureau of Plant Genetic Resources in New Delhi (2,393 accessions) and the Project Coordinating Unit for Safflower in Solapur (7,525 accessions). Other significant collections are in China, Mexico, and the USA. The US safflower collection was developed starting in the late 1940s and is located at the WRPIS at Pullman, WA (http://www.ars.usda.gov/main/site_main.htm?modecode=53481500). It now includes more than 2,400 *C. tinctorius* accessions. The WRPIS is part of a national network of germplasm repositories that collectively make up the USDA-ARS NPGS. The US collection is represented by germplasm from more than 50 countries, and accessions are available to scientists worldwide. Table 6.1 lists world production by country (FAO 2010), an estimate of the safflower genetic resources held in that country (Zhang and Johnson 1999), and gives the number of researchers studying safflower.

6 Diversity

Numerous studies have been undertaken to assess the genetic diversity of global safflower germplasm. Most of these studies, prior to the 1990s were analyses of morphological and agronomic traits. The first large-scale evaluation of the world collection was initiated under a USDA PL 480 project at the Volcani Center, Beit-Dagan, Israel in 1966. Ashri (1971) evaluated nearly 2,000 lines for variation in reaction to *Erysuphe cichoracearum* D.C. (powdery mildew), *Puccinia carthami* Cda. (Safflower rust), the leaf spot diseases *R. carthami* Zaprom. and *C. carthami* Sund. and Ramak., and phyllody, which causes a reversion of florets to miniature branches with leaves and is caused by a mycoplasma. Ashri et al. found disease reactions to be associated with

geographic origin and speculated that this may be a result of selection pressure. There were also correlations with morphological characteristics.

Ashri et al. (1974) also studied variation in yield components from 903 lines from regions within the world collection. Of the three major yield components, heads per plant, seeds per head, and seed weight, heads per plant were found to be the most important and to range from an average of 14.8 in Iraq to 54 in Romania. Overall, there were significant differences in yield components of lines from different regions. However, because of mutual compensation among components, there were no significant differences among regions for yield.

Another large-scale study (Ashri et al. 1977) evaluated variation in oil content, iodine value, and their associations with morphological characters at three sites in the USA and one in Israel over a span of 12 years. More than 1,000 lines were evaluated, but not all of the lines were represented at each location. Oil content ranged from 16 to 38%, and high oil among local varieties was an indication of the progress from selection and breeding efforts. This early study showed divergence among regions for oil content, with lines from the Indian subcontinent, Iran, Afghanistan, and Egypt having the highest oil content, whereas those from Portugal, Spain, France, and Morocco the lowest. There was, however, extensive variability among local cultivars of various origins. Associations of morphological characters with oil content were evaluated to determine whether field identifiable traits could be used in breeding efforts for increased oil. Correlations differed within gene pools. The length of the outer involucral bracts (OIBs) was significantly and positively correlated with oil content in the Indian gene pool, but significantly and negatively correlated with Iranian lines. Yield per plant and yield components showed inconsistent correlation with iodine value. Correlation does not necessarily imply cause and effect and regional divergence of these characters may be a result of random association.

Regional evaluations, even on a smaller scale, are important to breeding efforts as genotype by environment interactions requires breeding for local conditions. Elfadl et al. (2010) examined 467 accessions from 11 geographical regions grown under organic farming conditions in Germany. Accessions were acquired from the USDA collection, the Vavilov Institute, and three other collections in Germany and exhibited considerable variability for all traits studied except lodging. Principal component and cluster analyses grouped accessions according to geographical regions. Accessions from the Americas, Africa, the Mediterranean, and West Central Europe formed one cluster, accessions from Central and South-Eastern Europe and Germany formed another, and those from Central Asia, South Asia, and East Asia each clustered distinctly. A study in Spain (Pascual-Villalobos and Alburquerque 1996) examined the suitability of 23 accessions for use as a dryland winter crop on the Mediterranean. They concluded that enough diversity existed among the accessions tested to provide an opportunity for selection in a breeding program for local conditions. Jaradat and Shalid (2006) examined phenotypic diversity in a subset of 591 salt-tolerant safflower accessions from the USDA collection. Their objective was to quantify phenotypic diversity among the accessions and identify salt-tolerant, high-yielding germplasm adapted to a short growing season, with a long rosette period and a high potential for biomass, seed, and dye production. They estimated 79

and 21% of the total diversity of the Middle East accessions was partitioned within and among populations, respectively, and were able to identify 87 accessions with traits adaptable to the growing conditions of Middle East.

Core collections from germplasm repositories attempt to represent the bulk of the genetic diversity in a manageable number of accessions. These cores are invaluable to breeders for initial screening for novel characteristics or disease resistance, where evaluation of the entire collection is impractical or prohibitively expensive. They can be based on geographical, morphological, and, more recently, molecular genetic diversity, or a combination of these characteristics. The USDA core collection of safflower consists of 210 accessions and represents about 10% of the total accessions held at the WRPIS (Johnson et al. 1993). An evaluation of oil and meal characteristics of 203 core and 797 non-core accessions (Johnson et al. 1999) revealed that the core was not fully representative of the non-core accessions, but they did capture a large fraction of the diversity in oil and meal factors present. The mean oil content of the non-core accessions was significantly higher ($P<0.05$) and was likely because of the presence of the numerous improved lines in the non-core accessions. The core had higher mean palmititc acid, stearic acid, and cathartic phenolic glucosides, but lower α-tocopherols and bitter phenolic glucosides. The range in oil content between the core and non-core accessions was similar. Analysis of variance of regional means resulted in highly significant F-ratios, but the variance within regions was also significantly different, which may have complicated results. The highest mean percentage oil was from accessions from the Americas, which, again, was likely due to the improved lines included in that region. This also resulted in low linoleic acid and high oleic acid means from the Americas. In some, but not all cases, oil and meal factors were differentiated between regions.

The USDA core collection was also evaluated for seven quantitative traits (Johnson et al. 2001). The results showed that for each factor measured, there was a considerable variation among accessions, indicating that the core collection was highly diverse. Comparison among regions were not significant for either OIB length or yield, but were significant for OIB width, head diameter, days to flower, plant height, and weight per seed. Accessions from SW Asia were the most distant from other regions, but S. Central Asia and East Africa grouped together.

Dwivedi et al. (2005) developed a core collection of 570 accessions based on geographic information and 12 morphological descriptors on 5,522 accessions held in India. Approximately 10% of the accessions were randomly selected from each of 25 clusters derived from the analysis of the morphological characters. Accessions from South Asia and Southeast Asia accounted for almost 80% of the accessions in the core, reflecting their predominance in the collection as a whole. The remaining accessions were from the Americas, Mediterranean, Europe, West Asia, Australia, the former USSR, and Africa. Mean comparisons and frequency distributions indicated that the variation of the entire collection had been preserved in the core subset.

The abundance of genetic variability held in world collections, and the regional divergence within them can yet be exploited to produce even more variability through recombination.

Molecular markers can be used for identifying duplicate accessions, developing and testing special groups within collections (such as core collections), estimating

and comparing diversity among countries or regions, and identifying acquisition needs and in genetic mapping. Bassiri (1977) was able to uniquely identify 14 cultivars of safflower and nine ecotypes of the wild *C. oxyacanthus* using isozyme analysis of the acid phosphatase and the cathodal peroxidase systems. Carapetian and Estilai (1997) examined 20 safflower cultivars with nine enzymatic systems. Five of the enzymes were monomorphic and four were polymorphic. Selfed progeny revealed a three-banded marker system for menadione reductase, indicating that this was a dimeric enzyme with more than one homozygous locus. Zhang (2001) characterized 89 safflower accessions from 17 countries with isozymes. Seven polymorphic loci revealing 15 polymorphic alleles classified the accessions into four major groups, but there were no clear regional associations among the groupings.

Methods using markers revealed by the polymerase chain reaction (PCR) have more recently been reported. Sehgal and Raina (2005) characterized 14 Indian safflower cultivars using RAPD, simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP). AFLP markers were found to be the most efficient system in their study, with two primer pairs sufficient to genotype the cultivars. Yang et al. (2007) examined genetic relationships among 48 safflower accessions from 32 countries using inter-simple sequence repeat (ISSR) markers. Twenty-two primers revealed 355 polymorphic bands and uniquely distinguished all accessions. Relationships were closer among accessions from the same continent.

Johnson et al. (2007) used AFLP markers to characterize 96 accessions from the USDA collection representing seven world regions (the Americas, China, East Africa, East Europe, the Mediterranean, South Central Asia, and Southwest Asia). Regions differed in all pair-wise comparisons using a bootstrap procedure comparing distances within and among populations. There was a weak but significant correlation of the AFLP matrix with a phenotypic data matrix with 16 attributes consisting of oil, meal, and growth characteristics ($r=0.12$, $P= 0.05$). This weak correspondence between molecular and phenotypic data underscores the need for both types of characterization to enhance management and utilization of germplasm.

Chapman et al. (2010) also conducted an analysis of accessions representing geographic centers of similarity using a suite of 24 microsatellite markers developed from expressed sequence tags (EST) and a pair of chloroplast markers. They analyzed 70 accessions with 4–8 accessions belonging to each of ten putative centers of similarity (Ashri 1975). The centers are (1) the Far East, (2) the Indian subcontinent, (3) Iran/Afghanistan, (4) Israel/Jordan/Iraq/Syria, (5) Turkey, (6) Egypt, (7) Sudan, (8) Kenya, (9) Ethiopia, and (10) Morocco/Spain/Portugal/France. American accessions were not included in their primary analysis, as these are considered secondary introductions. A posteriori analysis of the molecular data using the software STRUCTURE (Pritchard et al. 2000) actually placed the accessions into five well-defined groups: (1) Europe, (2) Turkey/Iran/Iraq/Afghanistan, (3) Israel/Jordan/Syria, (4) Egypt/Ethiopia, and (5) the Far East/India/Pakistan/Sudan.

Many of these accessions were also represented in the AFLP analysis of Johnson et al. (2007). Re-analysis of their data excluding the American accessions revealed strikingly similar results, but with several differences. STRUCTURE analysis using the technique of Evanno et al. (2005) placed the 80 accessions into eight likely groups (Table 6.2, Figs. 6.1 and 6.2). Afghanistan accessions formed a unique

Table 6.2 A list of the accessions associated with the eight groups designated by analysis using the software STRUCTURE. The country of origin is followed by the plant introduction (PI) number

Afghanistan	Afghan220647	Middle East	Kenya209296
	Iraq253759		Syria181866
	Afghan253908		Kazakhstan262444
	Iran380800		Iran250833
	Kuwait286199		Iran405984
	Afghan304595		Turkey251984
	Afghan268374		Italy253523
			Turkey301048
Europe	Romania209287		Turkey407624
	Bulgaria253531		India306974
	Hungary312275		Ukraine369848
	Hungary253541		RussianFed369849
	Poland253544		Israel226993
	Spain239226		Pakistan304408
	Poland311738		Africa262437
	Poland253543		
	Spain613465		
Egypt/Sudan	China506427	India	Syria386174
	India260637		India279051
	Kazakhstan314650		Kazakhstan305540
	China544041		Africa209289
	Sudan237547		Sudan237548
	Sudan237549		India283764
	Sudan305531		India248808
	China544052		India451956
	Sudan305529		India199889
	Egypt306613		India307055
	Egypt250537		Bangladesh401479
	Kenya209295		Kenya209297
	Sudan305534		Kenya209300
	Africa262438		India562638
	China514630		
	Turkey304503		
Pakistan	Pakistan259992	Ethiopia	Ethiopia193473
	Greece254976		Ethiopia262433
	Sudan271070		Ethiopia257582
	Hungary253540		Eritrea273876
	Iran406015		Syria262430
	Tajikistan369847		
	Pakistan248625	China	China543995
	Pakistan250202		China544006
	Pakistan426523		China544028
	Iran251398		China544033

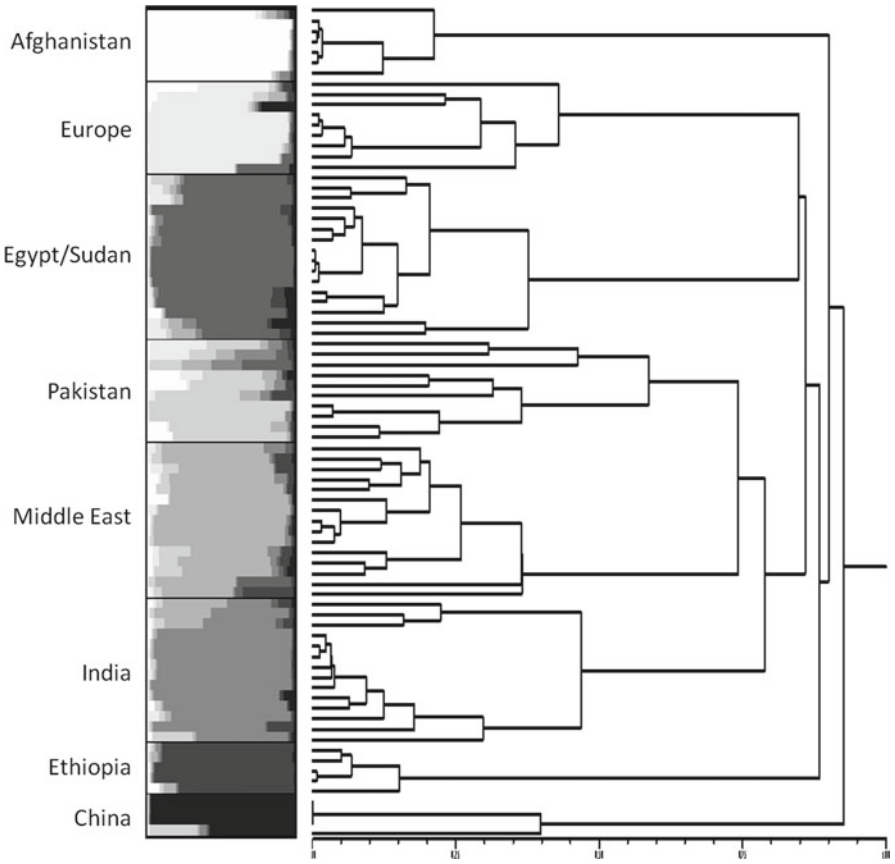

Fig. 6.1 Dendrogram of 80 safflower accessions from Johnson et al. (2007) showing eight distinct groups as evidenced by analysis using STRUCTURE. The tree was constructed based on the proportions of an individual's alleles belonging to a particular group

group, as did China and Ethiopia. Although there was some mixture, which would be inevitable given the amount of germplasm exchange that must have taken place in the past, the eight groups could be relatively distinguished as (1) Middle East, (2) Egypt/Sudan, (3) Ethiopia, (4) Afghanistan, (5) Europe, (6) India, (7) Pakistan/Iran, and (8) China.

In contrast to SSR markers, AFLP markers are biallelic and dominant. Although less informative at a locus, they allow for the efficient sampling of many loci (Powell et al. 1996; Gaudeul et al. 2004; Greene et al. 2008). Thus, AFLPs lend themselves to studies in which more loci are needed to estimate diversity because genomic heterogeneity is high (Mariette et al. 2002). Despite being dominant markers, AFLPs have shown themselves effective in discriminating among populations and correctly assigning individuals to populations, compared with SSRs (Gaudeul et al. 2004; Woodhead et al. 2005). Recently, Chapman et al. (2009)

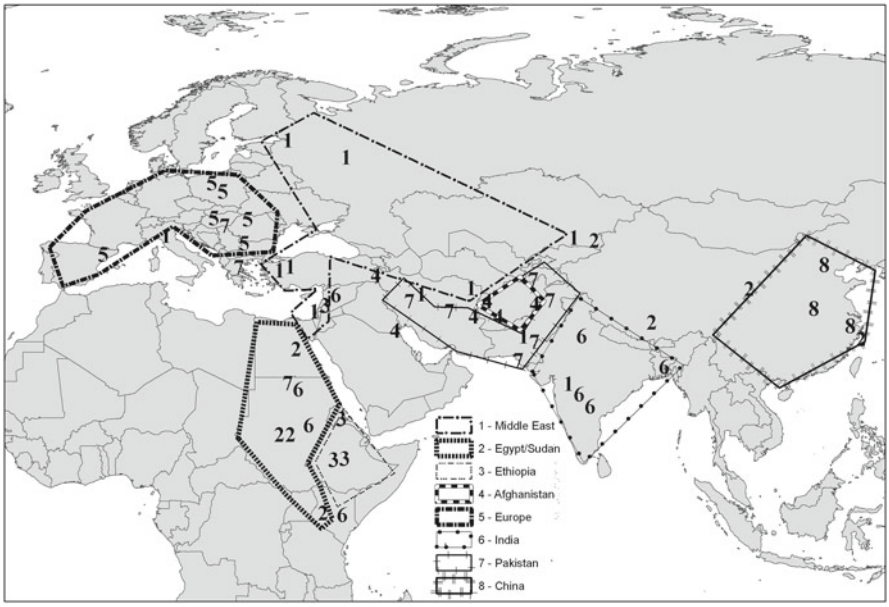

Fig. 6.2 Geographic representation of the 80 safflower accessions from Johnson et al. (2007) showing eight distinct groups as evidenced by analysis using STRUCTURE

developed a set of microsatellite primers from EST, some of which were used in their analysis described above. Microsatellites derived from ESTs have the unique characteristic of being associated with expressed genes, and may be more indicative of actual genetic differences than random markers. It is important to remember that, although random markers are effective at defining divergence, the association of markers through random drift and adaptation are separate processes (Holdregger et al. 2006). Another molecular marker with characteristics of both EST-SSRs and AFLP is the Target Region Amplification Polymorphism (TRAP) (Hu and Vick 2003). Although producing semi-random markers at multiple loci, TRAP markers can be designed to explore specific types of genes (Miklas et al. 2006). Regardless of the type of molecular marker used, more characterization of safflower with molecular markers from diverse world sources is needed to enhance germplasm management and utilization.

Although there have been numerous studies of genetic diversity in safflower using molecular markers, they share a common feature. Few, if any, studies of genetic diversity can be directly compared or compiled. One of the reasons may be due to the fact that most studies are limited to a few accessions or to accessions from a limited area of interest. As a consequence, after publication, the marker data may be lost or forgotten. Because only a small percentage of the world safflower collection is ever analyzed at a given time, and with different marker systems, or with different marker loci within a given system, collation on a world-wide scale is not possible. Kisha and Ryder (2006) make a case for organized development of common markers for diversity analyses within a given species. A subset of microsatellite

primers, AFLP primers, or designated set of other types of markers for universal use would allow data to be stored for posterity and used to generate comparisons for future marker studies. Virtual cluster analyses based on the comparison of new accessions to a complete database of accrued marker information would result in savings of both time and money. Relationship queries can be adjusted to filter data based on geographical regions, environments, latitude, etc., much as descriptor data are available through germplasm banks. Because of the somewhat imprecise nature of naming markers based on fragment size, the database would need to be curated, by a center or collaborating centers within a network responsible for a particular species. Collaborators need to define a core set of primers for each marker type, covering the genome randomly and uniformly and provide a number of "reference" accessions with defined markers so that virtual analysis could be anchored, and images defined of the expected marker pattern with monomorphic and polymorphic bands. The benefits for the conservation and use of genetic resources that can be drawn from available molecular data are almost limitless. The construction of a universal molecular database as a common platform for storage and analysis of genetic resources marker data could greatly enhance the utility of germplasm on a global scale. Its development may seem like a daunting task, but it can come to fruition by the construction of locally created databases developed through collaborative efforts among members of germplasm conservation centers and researchers.

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Chapter 7
Oil Palm

G.F. Ngando-Ebongue, W.N. Ajambang, P. Koona, B. Lalu Firman, and V. Arondel

Abstract The oil palm is a perennial monocot belonging to the genus *Elaeis* with two main species, *E. oleifera* found in Central and South America and *E. guineensis* originating from the coastal regions of West and Central Africa from where it was later introduced in South-east Asia. Due to its higher oil yields, *E. guineensis* is the widely used species in oil palm plantations throughout the world. Since 2006, the oil palm has overtaken soybean to become the most important oil crop in the world, as it contributes palm oil (95%) and palm kernel oil to about one-fourth of the world's total oils and fats production. The spectacular boom of the oil palm during the past 60 years is mainly supported by continuous breeding, allowing oil yields to reach an average of 3.5 tons of palm oil/ha/year (up to 10 tons palm oil/ha/year for the best genotypes in the most suitable conditions of South-east Asia) for the present Tenera hybrids and thus, making the oil palm the highest oil-producing crop. The bulk of oil palm plantation and palm oil production is provided by Indonesia and Malaysia, as both countries contribute about 44 and 41.5%, respectively, to the world palm oil production.

The application of new techniques such as molecular breeding, tissue culture and genetic engineering to oil palm breeding will undoubtedly provide a real breakthrough toward the oil palm production, as it has been the case for other oil crops. Efforts to increase yields should also be supported by the view to broaden the genetic base of the material currently available in breeding programs through collection exercises and appropriate management of the collected natural oil palm resources. Despite savings of considerable time and space and numerous other advantages expected from the implementation of the above-mentioned new disciplines to oil palm breeding, field experimentation will remain the unique way to indisputably identify promising outliers.

G.F. Ngando-Ebongue (✉)
Specialised Centre for Oil Palm Research of La Dibamba, PO Box 243, Douala, Cameroon
e-mail: caiman2307@yahoo.com

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1 Introduction

Since 2006, the oil palm has overtaken soybean to become the most important oil crop in the world, as it contributes to about one-fourth of the world's total oils and fats production (Oil world annual report 2008). Two types of oils are produced from its fruits, palm oil extracted from the mesocarp and internationally referred as CPO or crude palm oil (95% of total oil production of the crop) and palm kernel oil (PKO) extracted from the kernel. The spectacular boom of the oil palm during the past 60 years is mainly supported by continuous breeding, allowing oil yields to improve by +36% after two selection cycles with an average of 3.5 tons of palm oil/ha/year for the present Tenera hybrids and thus making the oil palm the highest oil-producing crop (Gascon et al. 1988; Cochard et al. 1993). The bulk of oil palm plantation and palm oil production is provided by Indonesia and Malaysia, as both countries contribute about 44 and 41.5%, respectively, to the world palm oil production (Oil world annual report 2008). Palm oil is used for cooking, margarine, vanaspati, shortenings, detergents, and cosmetics. It is also used in pharmaceutical industry as a source of carotenes (pro-vitamin A) health supplement and more recently as a source of biofuel. Due to rise in petroleum prices, new nonfossil, "green" and renewable energies are called upon to sustain world economic growth. Since then, there has been a tremendous demand for biofuels such as biodiesel. However, considering the increasing world population and the concomitant increase of the demand for dietary oils, it will appear difficult for the oil palm to meet this requirement without a significant increase in palm oil production. As ecological constraints are becoming a major limiting factor in the extension activities of oil palm plantations which is totally dependent on natural forests, the search for additional yield increase appears as the prime goal for oil palm breeders in the near future. Another constraint limiting continuous oil palm extension is the high labor demand and poor mechanization of this crop. Hence, cultivation of oil palm is profitable only in those developing countries where labor is cheap and easy available. With the continuous increase of living standards in these countries, it will become difficult for the oil palm to remain competitive without a significant reduction of labor needs and the relevant costs. As about 15–25 years are needed to carry out a selection cycle on this perennial crop, it will be difficult for breeders to meet requirements in time without developing new strategies. With the advent of molecular breeding and genetic engineering techniques, a real breakthrough may be achieved in increasing the yield potential of this crop. Moreover, these new techniques can also be used to modify the oil palm to produce high value products with potential applications in oleochemical, nutraceutical and

pharmaceutical industries or to improve the profitability of the crop through the development of new low oil acidity or nonshedding fruit lines with reduced labor needs. An overview of the breeding of oil palm and insights into the recent techniques has been presented in the following sections.

2 Origin

The origin of the oil palm is believed to be in Africa, more precisely along the gulf of Guinea, as many historical (Crone 1937; Opsomer 1956; Surre and Ziller 1963; Rees 1965a; Zeven 1965) and fossil (Raymond 1961; Zeven 1964; Elenga et al. 1994; Raynaud et al. 1996; Ergo 1997; Sowunmi 1999) evidences are provided to support this fact. Wild and semi-wild palm groves are found in a coastal belt running from the northernmost Senegal region through Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, The People's Republic of Congo, Angola up to the southernmost Democratic Republic of Congo. The center of origin and diversity of the oil palm appear to be concentrated in the tropical forests of Nigeria, Cameroon, Congo, and Angola.

The oil palm was introduced in South-east Asia in 1848, when four seedlings were planted in the Buitenzorg (now Bogor) Botanic Gardens in Java (now Indonesia). The palms that sprang from these four seedlings were similar and all Dura, and were used as decorative plants along avenues. All the South-east Asian palm groves are believed to descend from these four palms.

3 Biology

The oil palm (*Elaeis guineensis* Jacq.) is a monocot of the order of Arecales, and belongs to family Palmae and subfamily Cocosideae. It is included in the tribe Cocoideae together with the genus *Cocos* (coconut). *Elaeis* is derived from the Greek word "elaion" meaning oil and "elaia" meaning olive. In addition to the African oil palm the genus *Elaeis* includes two other species of American Origin namely *E. oleifera* sometimes also referred to as *E. melanococca*, which is easily crossed with *E. guineensis* and *E. odora* Trail, a less-known species of secondary importance.

3.1 Elaeis guineensis

E. guineensis is the African oil palm, a perennial tree crop with indeterminate growth. At maturity, the African oil palm presents a large crown of 30–45 green palms measuring 5–9 m long, topping a unique cylindrical pseudo-trunk or stem (Fig. 7.1a). Depending on environmental and hereditary factors, the stem can extend at a rate of

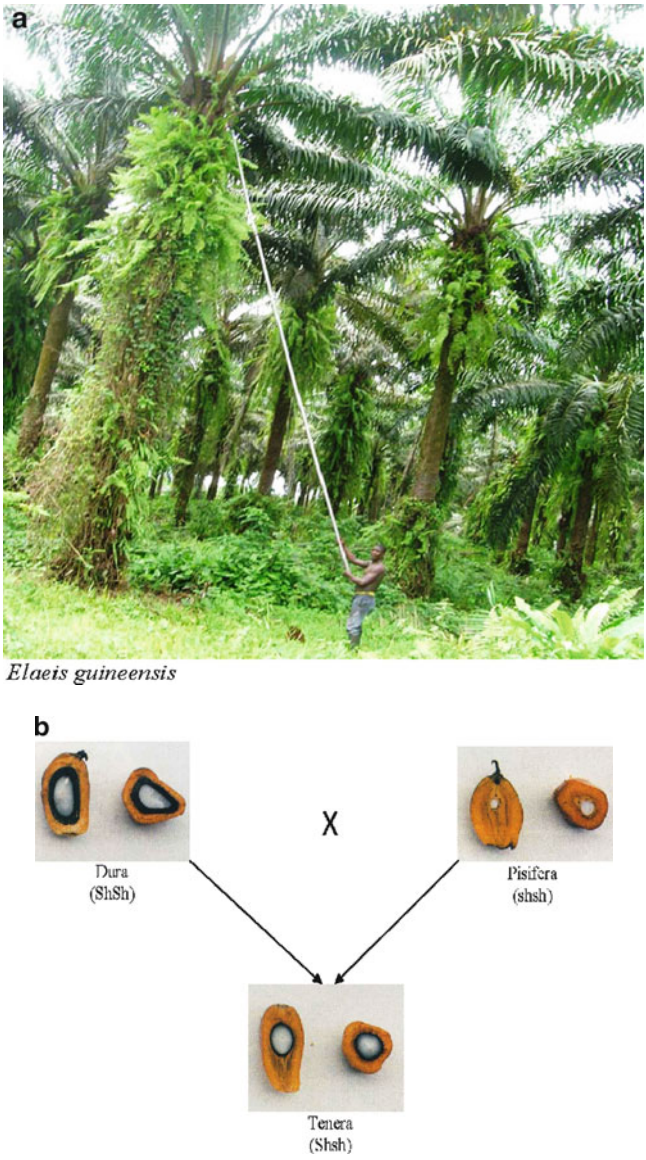

Fig. 7.1 (**a**) *Elaeis guineensis*, the African oil palm. (**b**) Dura, Pisifera and Tenera fruit forms and the monogenic inheritance of the shell thickness gene (sh)

30–60 cm/year between the ages of 6 and 15. Generally palm plantations are exploited up to 25–30 years when the height of palms is between 12 and 15 m. Above this limit, it becomes difficult (Fig. 7.1a) to harvest the fresh fruit bunches (FFBs) and the palms are cut and replanted. As a monoecious crop, male and female flowers occur alternatively on the same plant, usually in distinct male and female inflorescences.

An inflorescence is initiated in the axil of every leaf. Upon pollination at the anthesis stage, the female inflorescence may develop and give rise to a fruit bunch 22–26 weeks later. Fruit bunches can be found on the African oil palm 2–3 years after field planting, and appear at the axil of leaves as compact and ovoid masses spiked with many spines. The fruit bunches become heavier as the palms get older, with weights ranging from 10 to 50 kg for a total of 500–4,000 fruits/bunch on 10-year-old palms. The oil palm fruit is a 2–5 cm length sessile drupe generally of ovoid shape weighing from 3 to 30 g. The fruit consists of two main parts, the seed commonly named kernel and the pericarp which includes the hard endocarp or shell protecting the seed, the fibrous and fleshy mesocarp or pulp rich in palm oil and the outer and smooth exocarp. The oil palm is a unique oil crop, as it produces two main vegetable oils, namely palm oil extracted from the mesocarp of the fruit and PKO extracted from the seed or kernel. The main characteristic of the fruit is the thickness of the shell, as three types of oil palms can be identified based on this criterion (Fig. 7.1b):

- The Dura palm characterized by a thick shell (2–8 mm), and a mesocarp to fruit ratio between 35 and 75%. The frequency of occurrence of the Dura fruit form in wild palm groves is about 97%.
- The Pisifera palm is a shell-less fruit type with mesocarp to fruit ratio between 90 and 99%. Very rare in wild palm groves, the Pisifera form is above all characterized by a pronounced female sterility, as most of the female inflorescences dry and die before the mature stage.
- The Tenera palm has a thin shell (0.5–2 mm) and a thick and fibrous mesocarp. The mesocarp to fruit ratio ranges between 55 and 96%. Beirnaert and Vanderweyen (1941) showed the monogenic inheritance of the shell gene, as the Tenera form was proven to be a hybrid resulting from the cross between Dura and Pisifera. Since then, the Tenera hybrid has been used as commercial material in industrial plantations throughout the world. Only specialized units with full mastering of artificial pollination techniques and a seed garden planted with selected Dura and Pisifera parent palms can produce 100% certified Tenera seeds. The seed production activity is generally linked to ongoing oil palm breeding programs in research units, thus, allowing a rapid transfer of the genetic improvement to the farmers. Sometimes, unaware small holders use seeds picked up in 100% Tenera plantings to set up their own plots. This $T \times T$ ($F_1 \times F_1$) cross will give rise to segregating F_2 progenies in the classical Mendelian ratios of ¼ Dura, ¼ Pisifera and only ½ of the suitable Tenera. The decreased yield resulting from the use of such planting material is about 30–40% than that of a 100% Tenera planted plot, hence demonstrating the importance of the choice of the planting material while setting up an oil palm plantation.

3.2 Elaeis oleifera

The American oil palm *E. oleifera* (Fig. 7.2) is found in tropical countries of Central and South America, namely Brazil, Colombia, Venezuela, Panama, Costa Rica,

Fig. 7.2 *Elaeis oleifera*, the American oil palm

Nicaragua, Honduras, French Guiana, and Surinam (De Blank 1952; Ferrand 1960; Meunier 1975; Rajanaidu 1986b). In comparison with *E. guineensis*, the stem of *E. oleifera* is characterized by a slow growth rate (5–10 cm/year, that is about one-fifth of that of *E. guineensis*), and often become procumbent as the palm reaches the age of about 15 years, with the crown remaining in an erect position (Hartley 1988). Another characteristic feature of *E. oleifera* is the leaf shape, with leaflets lying in one plane as in the case of coconut. The pollen is of foul-smell, and the fruit bunches are conical, spiked with shorter spines and are partly covered by spathes at maturity. The fruit bunches are smaller than those of *E. guineensis*, with an average weight of 8–12 kg and hardly 30 kg. Fruits are smaller (1.5–5 g in Colombia, 5–13 g in Brazil), with parthenocarpic fruits constituting up to 90% of the total (Ooi et al. 1981). The shell thickness varies between 1 and 3 mm, and the mesocarp/fruit ratio is between 29 and 50% for normal fruits and up to 80% for parthenocarpic fruits (Corley and Tinker 2003). Fruits are mostly orange in color at maturity. Unlike *E. guineensis*, no

Dura, Pisifera or Tenera fruit types have been reported for *E. oleifera*. The oil extracted from the mesocarp of the fruit is characterized by a high unsaturated fatty acid content (59–91% against 25–72% for *E. guineensis*). However, the low bunch yield and poor oil extraction rate of *E. oleifera* limits its industrial exploitation.

3.3 E. guineensis × E. oleifera

Many interspecific *E. guineensis* × *E. oleifera* crosses have been made with an aim to develop hybrids having the characteristics of both the parents. The main difficulty encountered with the hybrid is the poor fertility. Pollen production from the hybrid is low and of poor quality, and also the hybrid's inflorescences appear to be less attractive to the pollinating weevil *Elaeidobius kamerunicus*. As a result of this, fruit set in the hybrid is usually poor, fruit/bunch ratio and oil content of the mesocarp are low (Hardon 1969; Meunier and Boutin 1975). Research works on the hybrid are currently directed toward introducing the interesting traits of *E. oleifera* through a series of back-crosses into the best known combinations of *E. guineensis* genotypes (Obasola et al. 1977; Tam et al. 1977; Sterling et al. 1988; Sharma and Tan 1990; Le Guen et al. 1993; Chin 1993; Oboh 1993; Din and Rajanaidu 2000). The hybrids are used for industrial plantation only in Latin American regions highly contaminated with basal stem rot disease and thus, unsuitable for economically viable *E. guineensis* plantations.

4 Climate and Soil Requirements

The oil palm can be grown on many types of soils. Though the most suitable conditions are found on sand to clay well-drained soils with good organic matter and cations exchange content, the oil palm can still be profitably cultivated in many soil conditions that appear suboptimal, provided that the general physical characteristics are not extreme and that climatic conditions are satisfactory (Quencez 1996). The main limitation for oil palm cultivation appears to be an adequate combination of rainfall and sunshine. Thus, a minimum requirement of 2,000 mm of rainfall/year evenly distributed throughout the year (at least 100 mm each month), minimum mean temperatures above 20°C and maximum between 28 and 34°C and at least 1,800 h of sunshine/year with solar radiation of about 15 MJ/m^2/day are needed to allow this crop to reach its potential (Hartley 1988; Jacquemard 1995). In addition to a higher vapor pressure deficit in the atmosphere due to the presence of an important cloud layer in Africa, differences in these climatic conditions are put forward by many authors to explain the differences in yields between West Africa and Southeast Asia for the same planting material (Quencez 1996; Nouy et al. 1999). In order to ensure an optimal capture of solar radiation, the recommended planting for the oil palm is an equilateral triangle system with a 9 m side (i.e., a 7.8 m spacing between lines) at a density of 143 trees/ha.

5 Products of the Oil Palm

As said earlier, the oil palm is a unique oil crop, as it produces two major vegetable oils: palm oil extracted from the mesocarp of the fruit and PKO extracted from the endosperm of the kernel. Due to their respective chemical compositions, palm oil is mainly used for food purposes, whereas PKO serves mostly to nonfood usages (cosmetics, soap factory).

5.1 Chemical Composition

As it is the case for many oils, palm oil is principally made up of triacylglycerols (95% w/w of total fats), a chemical component comprising a glycerol molecule esterified with three fatty acids. The length of this hydrocarbon chain may vary from 4 to 28 carbon atoms generally even-numbered for natural fatty acids, though the most common fatty acids contain 16 or 18 carbon atoms. Unlike the saturated fatty acids which are chemically more stable, the presence of double bonds confers a specific reactivity to unsaturated fatty acids (Table 7.1). Polyunsaturated fatty acids with double bonds located at positions omega-3 and -6 are the precursors of some essential components of human metabolism such as hormones and liposoluble vitamins. The nature of the fatty acids present in an oil is thus a key indicator of its nutritive value. Also, the fluidity of oils at room temperature is proportional to the amount of unsaturated fatty acids. Although the chemical composition of palm oil may vary considerably from one breeding population to another, saturated and unsaturated fatty acids are present in approximately equal amounts (Jacquemard 1995; Berger 1996; Tan et al. 2000). There are two major fatty acids, saturated palmitic acid (C16:0) and unsaturated oleic acid (C18:1). Unlike palm oil which contains almost exclusively 16 and 18 carbon atoms length fatty acids, PKO has fatty acids with shorter chains (mainly lauric acid C12:0) similar to those found in coconut oil, both being known as "lauric oils." Moreover, the amount of saturated fatty acids in PKO is close to 85%, thus restricting its usage to nonfood purposes. Like in other oils, fatty

Table 7.1 Fatty acid composition of palm oil

FA	Symbol	% Total FA
Myristic acid	C14:0	0.9–2
Palmitic acid	C16:0	39.2–45.8
Palmitoleic acid	C16:1	0–0.4
Stearic acid	C18:0	3.7–6
Oleic acid	C18:1	37.4–44.1
Linoleic acid	C18:2	8.7–12.5
Linolenic acid	C18:3	0–0.6
Arachidic acid	C20:0	0–0.4

acids are present in palm oil essentially as triacylglycerols. There is a preponderance of unsaturated fatty acids at the *sn*-2 position of the glycerol molecule, with 85% of them being located at this position (Sambanthamurthi et al. 2000). This may be important in relation to the bioavailability of these important nutrients and their protective effect on heart diseases (Goh 1998). The triglyceride composition of palm oil also partially determines most of the physical characteristics of the oil such as melting point and crystallization behavior (Hernqvist 1984).

5.2 Palm Oil and Human Health

There has been a long-term controversy about the adverse effects of palm oil-based diets on human health, as many studies have established the link between saturated fat-rich diets and high levels of LDL (low-density lipoprotein) "bad" cholesterol, with respect to coronary heart diseases. Hence, nutritionists generally advise the usage of unsaturated fatty acid-rich oils for a healthy and balanced diet to the detriment of palm oil. A review by Sambanthamurthi et al. (2000) presents an overview of research works postulating that oil palm-enriched diet increased the level of total and LDL cholesterol, with some discrepancies in the procedures. Recent studies have shown no significant increase of serum LDL and total cholesterol in palm oil diet fed human and animal models. On the other hand, a significant increase in serum "good" HDL (high-density lipoprotein) cholesterol and a decrease of the ratio LDL/HDL were noticed, together with a beneficial effect on apolipoproteins A1 and B distribution (Sambanthamurthi et al. 2000). Hornstra (1988) also showed an antithrombotic effect of palm oil-rich diets. This was combined to a positive effect on the modulation of the thromboxane/prostacycline ratio, hence reducing thrombosis and atherosclerosis risk factors (Hornstra et al. 1987; Rand et al. 1988; Abeywardena et al. 1991). The anti-tumoral effect of palm oil was also demonstrated, and it was suggested that this can be attributed to some minor components from the unsaponifiable matter such as tocotrienols and carotenoids (Sundram et al. 1989; Nesaretnam et al. 1992). Carotenoids and particularly β-carotenes are the precursors of vitamin A, and palm oil is the richest natural source of carotenoids. Palm oil is, therefore, recommended and broadly used in many supplementary feeding programs to combat vitamin A deficiency in children (Latham 1979; Gopalan et al. 1992; Seshadri 1996). Unfortunately, these compounds are very fragile and are almost completely destroyed by high temperature treatments during the refining process. Palm oil can be fractionated into a highly unsaturated triacylglycerol fraction fluid at room temperature called olein and a concrete stearin fraction where most of the saturated triacylglycerols partition. This olein fraction is an ideal compromise between crude and refined palm oil, as it contains almost three-fourth of the carotenoids present in the CPO and a greater proportion (approximately 70%) of unsaturated fatty acids. The stearin fraction can also be valorized in the food industry in place of hydrogenated fats for the manufacturing of margarine. Apart from tiny *trans* quantities found in meat and ruminant's milk products, unsaturated fatty acids

are naturally present in the *cis* chemical form. The industrial hydrogenation process is used to remove double bonds from unsaturated fatty acids to create a fully saturated "oil" that will be solid at room temperature. However, this also generates *trans* fatty acids which represent a significant health hazard pointed out recently, as they constitute a risk factor for heart diseases and are classified as toxic by the World Health Organization (Chardigny et al. 2008). In some countries such as Denmark and Austria, industrial hydrogenation is banned and the *trans* fatty acid content in food items is strictly regulated. Therefore, palm stearin may appear as a natural source of fat for the production of "bio"-labeled margarine.

6 Selection and Breeding

Like other oil crops, the aim of oil palm breeding is to maximize oil yield, and thus, increasing the profitability of the crop. Subsidiary objectives also exist and may become more or less important according to the breeding schemes involved and current issues.

6.1 History of Selection

Early works on the improvement of the oil palm began both in South-east Asia and Africa at the beginning of the twentieth century. In Asia, research works were undertaken by large plantation companies of Indonesia and Malaysia, and by the Algemene Vereniging van Rubberplanters ter Oostkust van Sumatra (AVROS) and the Department of Agriculture, Malaya. This was confined to the Deli Dura palm, a Dura of relatively high quality compared to its African counterpart.

In Africa, improvement work on oil palm began in the French colonies under the "Services de l'Agriculture" and was taken over as from 1946 by the IRHO (Institut pour la Recherche sur les Huiles et Oléagineux). In the Belgian colony of Congo (former Zaïre), selection was undertaken by the "Institut National pour l'Etude Agronomique du Congo Belge" (INEAC), and led to the elucidation of the monofactorial transmission of the shell thickness gene between Dura, Tenera, and Pisifera fruit types (Beirnaert and Vanderweyen 1941). Research work in Africa therefore emphasized on the development of Tenera crosses from different origins (Yangambi, La Mé, Ekona, Pobé, NIFOR). An important step on improvement work on oil palm was the organization in 1946 of the "Experience Internationale" by the IRHO, a large-scale exchange of materials involving the IRHO research stations in the Ivory Coast, Benin and Congo-Brazzaville, INEAC in the Belgian colony of Congo (former Zaïre, now Democratic Republic of Congo) and SOCFIN in Malaysia. Early observations on the planted crosses led to the following findings: (1) Important differences were observed between palms from different origins regarding vegetative characters, bunch yield components, and oil/mesocarp ratio. This led to the identification of two distinct groups: Group A with palms characterized by a small number

of large bunches (usually Deli, Angola also), and group B with palms having a large number of smaller bunches, generally from Africa (La Mé, Sibiti, Pobè, NIFOR, Ekona). (2) Crosses between Asian Deli and African origins yielded significantly more than crosses within origins (Gascon and de Berchoux 1964). This "inter-origin" effect was later recognized as resulting from heterosis or hybrid vigor in crosses between genetically distant materials (Durand-Gasselin et al. 2000a).

These findings led to the generalization of the use of Dura and Pisifera parent palms through D×P crosses for the production of Tenera seeds, and the choice of these parent palms in two distinct groups regarding their bunch characteristics: the Dura female parent ("mother") palms in group A and the Pisifera male parent ("father") palms in group B.

6.2 Breeding Methods

Three stages can be distinguished in the improvement of a plant: assembly or creation of a pool of variable germplasm, selection of superior individuals from the pool, and utilization of the selected individuals to create a superior variety (Dudley and Moll 1969). Based on these basic principles, two major breeding techniques have been used extensively on oil palm, the reciprocal recurrent selection (RRS) and the family and individual selection (FIS). There has long been a debate between advocates of FIS and RRS. Many breeding programs tend to use a combination of both for more efficiency.

6.2.1 Reciprocal Recurrent Selection

The RRS of Comstock et al. (1949) was adopted by the IRHO in 1957, and extensively used in French speaking countries (Ivory Coast, Benin, Cameroon) and Nigeria (NIFOR). This breeding method is designed to concentrate favourable genes scattered among a number of individuals by selecting in each generation among the progeny produced by matings inter se of the selected individuals (or their selfed progeny) of the previous generation (Allard 1960). In the case of the oil palm, the aim of the RRS is to test one with respect to the other individuals from two different origins with distinct and complementary yield components: group "A" comprising origins with a small number of large bunches (Deli, Angola) and group "B" which include origins with a large number of smaller bunches (La Mé, Pobé, Yangami, Nigeria, Cameroon). Palms from group A are Dura and are used as female parents, while palms from group B are Tenera or Pisifera and are used as male parents (Gascon and De Berchoux 1964; Meunier and Gascon 1972). Two types of crosses are carried out:

- Palms from group A are crossed with those from group B. The D×P or D×T hybrids obtained are planted in comparative field trials, the results of which enable the classification of strains and parents and the detection of specific combining abilities.

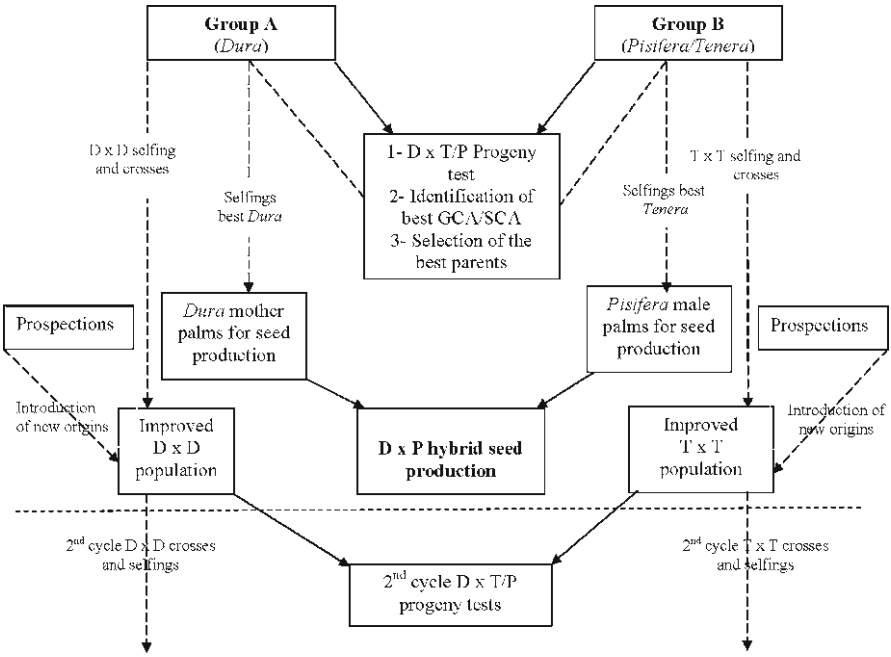

Fig. 7.3 The reciprocal recurrent selection (RRS) scheme applied to the oil palm

- Parents yielding the best progenies are selfed and crossed between themselves in D×D crosses for Dura female parents on one hand, and T×T or T×P crosses for the Tenera/Pisifera male parents on the other hand. These D×D and T×T/P selfs and crosses form the next generation from which D×T/P test crosses are made and a new cycle is started. In some cases, new origins are introduced simultaneously to enrich both groups. The RRS is summarized in Fig. 7.3. However, identifying elite D×P crosses is of practical interest only if they can be reproduced and supplied in sufficient quantities to farmers. The laws of quantitative genetics theorize that a cross can be exactly "reproduced" using selfs of its parents. This theory was verified by Jacquemard et al. (1981) who showed that the reproduction of a given cross had the same mean value, and is used since then for seeds production. For this purpose, Dura and Pisifera parents of the best D×T/P hybrids are selfed and used to set up seed gardens for commercial hybrid seed production. In reality, the selfings are carried out at the same time as the D×T/P hybrid crosses as well as a certain number of D×D and T×T/P crosses are made as a speculation. This procedure enables the gain of one generation, given that D×D and T×T/P crosses can be used for seed production as soon as the results of the D×T/P test crosses are known. Compared to other breeding methods, the main disadvantage of the RRS is the large program size, thus requiring a lot of time and space: up to 500 crosses and 180 selfs need to be planted over 600 ha and evaluated

during 15–25 years in order to select the top 15% crosses (Gascon et al. 1988; Nouy et al. 1991; Soh 1999). Another probable drawback is the limited number of parents from the two base populations that can be tested, thus making it impossible to avoid some inbreeding in the selected populations (Hardon 1970). On the other hand, the main advantage of the RRS is that it takes into account the general and above all the specific combining abilities. Also, this approach has the commercial advantage that very large number of seeds can be produced on single "reproductions" of the best progeny tested D×T/P crosses, thus generating more homogenous plots during the setup of plantations.

6.2.2 Family and Individual Selection

Selection of Deli Dura parents for further breeding and as mother palms for commercial hybrid seed production is based on family and individual palm performances (Rosenquist 1990). Individual selection emphasizes on characters with high heritability, while those with lower heritability are assessed in the family selection stage. Tenera parent selection for further Pisifera parent breeding is also based on family and individual palm performances. Being female sterile, Pisiferas cannot be selected on their phenotypic performance, and are chosen as male parents for D×P hybrid seed production based on their Tenera sibs performances in the T×T crosses followed in some cases by a D×P progeny test trial. As a result, programs based on FIS may come to bear a distinct resemblance to RRS programs, and some have been described as "Modified Recurrent Selection" (Soh 1987, 1999; Rajanaidu et al. 2000a). The main advantage of this method is that more recombinant crosses and genotypes can be turned over within shorter time and smaller space without the need of extensive progeny tests. Rosenquist (1990) estimated that with fixed resources for trials, two to three times more palms from a base population could be evaluated by FIS rather than RRS.

6.3 Issues of Oil Palm Breeding

The main challenge of oil palm breeding is to meet with the increasing world demand for oils and fats which has evolved from 103 million tons in 1998 to 155 million tons in 2007 (Oil world annual report 2008). The second issue is to limit the pressure generated by oil palm plantations on the forest in palm oil-producing countries. These two reasons urge oil palm breeders to develop planting materials with the highest possible yields per hectare.

Another issue is to secure the farmer's investment through the development of a planting material resistant to the major oil palm diseases. The oil palm is a perennial crop which can be exploited for up to 30 years, but the financial investment required to set up a palm plantation is considerable.

The last issue is to increase the productivity which is very low for the oil palm compared to other oil crops. The management of oil palm plantations requires much

labor, and the profitability of this crop is only due to the fact that labor costs are low in palm oil-producing countries. The profitability/labor ratio can be increased through the development of new planting materials with lower harvesting frequencies and thus reduced labor needs, as harvesting is highly labor-consuming.

6.4 Breeding Objectives

The main trait assessed by oil palm breeders is palm oil yield expressed in tons per hectare per year. This trait has two components, bunch yield (also made up of bunch number and mean bunch weight) and oil/bunch which depend on the fruit/bunch, mesocarp/fruit, and oil/mesocarp ratios. All these traits are polygenic, and although some of them may be highly heritable (mesocarp/fruit, bunch number, unsaturated fatty acid content, mean bunch weight), others are less heritable (bunch yield, oil/mesocarp, fruit/bunch) (Meunier et al. 1970).

Besides oil yield, some vegetative characters are also measured, among which the palm height increment. Reducing the height of palms is of interest to breeders, as harvesting costs increase with the height. Also, palms with reduced height are proven to be more resistant to wind during tornadoes (Tailliez and Valverde 1971).

Palms are also selected for resistance to some diseases, the main ones being the *Fusarium* wilt predominant in Africa, the *Ganoderma* basal stem rot widespread in South-east Asia and fatal yellowing disease found mainly in South America.

Many other additional characters are assessed in breeding programs such as tolerance to drought, fatty acids composition of palm oil with the objective of producing an oil with high unsaturated fatty acids content, carotene content for its pro-vitamin A nutritive value, longer bunch stalk for ease of harvesting, nonshedding fruits and more recently the activity of the lipase of the fruit mesocarp, both related to palm oil acidity and harvesting frequencies (Ngando Ebongue et al. 2006, 2008).

6.5 Major Breeding Achievements

According to Davidson (1993), 70% of the oil palm yield improvement in Malaysia for the previous 50 years was attributed to breeding improvement and 30% to improved agronomic practices. Breeding achievements are discussed below with regard to the two main breeding methods.

6.5.1 FIS Programs

Hybridization and selection among the progenies of the four original progenitors of the Bogor Botanical Garden gave rise to Dura Deli that were used as commercial planting material in Indonesia and Malaysia up to the early 1960s. Results from Hardon et al.

(1987) showed that progress in oil yields achieved through selection within Deli Dura families after three to four generations of breeding programs was about 60%.

The first plantations in South-east Asia using D×P Tenera hybrids were established in Malaysia in the late 1950s. It has been estimated that about 30% of further increase in oil yields of the Deli Dura populations was achieved after two generations of breeding programs for the Tenera hybrid seed production (Hardon et al. 1987; Davidson 1993). The estimated improvement for the Tenera hybrids was found to be about 10–15% per generation (Soh et al. 2003). Assuming a generation time of 10 years and a total improvement of about 90%, this is an equivalent to at least 1% oil yield improvement/year. Through the FIS program, some interesting breeding populations were developed such as the SP540 parent imported from the Eala garden in the then Belgian Congo in 1923 to Sungei Pantjur in Indonesia where it was further selected, giving rise (in part) to the well-known AVROS Tenera/Pisifera breeding population widely used for seed production in the whole South-east Asia. The main characteristics of this breeding population are a vigorous trunk growth, and high oil yield from big fruits with thick mesocarp. Of course, the most important breeding population in South-east Asia is the Deli Dura with bigger fruits and thicker mesocarp than Duras commonly found in Africa. It is generally used as female parent in commercial hybrid seed production units linked to breeding programs throughout the world. A number of subpopulations were developed from this breeding population such as Ulu Remis Deli or Dabou Deli. Other recombinant breeding populations can be listed here such as URT (Ulu Remis Tenera) and Dumpy AVROS partly developed from the well-known Dumpy Dura E206 sought-after for its dwarf genes and strong resistance to *Fusarium* wilt. They are used as Pisifera male parents for commercial hybrid seed production.

6.5.2 RRS Programs

The RRS scheme was introduced in the CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) related breeding programs in the 1950s, following the "Expérience Internationale." Of the 529 crosses of D×T/P planted in the first cycle, only 15 of them with an average yield above 18% of the mean were selected for release as commercial seeds (Gascon et al. 1981). A second cycle comprising about 500 ha of trials was started in 1975, and results showed that the yields from these second cycle crosses were 18% better than those from the first cycle (Gascon et al. 1988). The main characteristic of the RRS-based programs was the introduction of a common standard cross L2T×D10D present in most of the trials, whose average yield was about 8% above the average mean of the first selection crosses. The best second selection cycle crosses yielded 22% more than L2T×D10D, this makes an increment of about 30% after two selection cycles, indicating an overall progress of about 1% per year as stated by many authors (Gascon et al. 1988; Durand-Gasselin et al. 2000a). In the conditions of Côte d'Ivoire, oil yields increased from an average of 3.3 tons palm oil/ha/year at the end of the first selection cycle to

4.5 tons palm oil/ha/year for the second cycle palms. The latter were able to produce up to 6–7 tons palm oil/ha/year in more favourable agro-ecological conditions in Indonesia. These results are comparable to those obtained through the FIS programs, thus corroborating the assumption that there seems to be little choice between the two breeding schemes.

In addition to progress in yield, the growth rate of palms has been significantly reduced, and resistance to *Fusarium* wilt considerably improved (Renard et al. 1972; De Franqueville and Renard 1990; Durand-Gasselin et al. 2000b).

The RRS programs set up in Africa gave rise to interesting breeding populations such as La Mé (Côte d'Ivoire) characterized by a smaller palm structure, NIFOR (Nigeria), Yangambi (Zaïre) for which interest has declined because of its greater growth rate and susceptibility to *Fusarium* wilt, Angola, Widikum and Ekona (Cameroon). Except for the Angolan Dura with fruits characteristics close to Deli Dura, these African breeding populations are mainly used as a source of Pisifera male parents in commercial hybrid seed production units. Within the CIRAD network, the most produced commercial Tenera hybrid seeds are derived from crosses between Deli Duras and La Mé Pisiferas.

7 Commercial Hybrid Seed Production

In almost all breeding programs, seed production units of various capacities are set up in order to produce commercial Tenera hybrid seeds. As the Tenera hybrid results from the cross between Dura (D) and Pisifera (P), the main task during seed production is to ensure that no open pollination is done and only D × P crosses are effectively carried out through controlled pollination. Conventionally, the female parent is a Dura and Pisifera palms are used as male parents, due to the high propensity for abortion of their female inflorescences. As mentioned by many authors (Donough et al. 1992; Chin 1999; Rao and Kushairi 1999), numerous quality control procedures are needed to ensure the production of pure Tenera hybrid seeds.

Basically, pollen is produced from the male inflorescence of a Pisifera father palm, and this pollen is subsequently used to pollinate a previously isolated female inflorescence on a Dura mother palm at anthesis.

7.1 Pollen Production

This activity constitutes a key factor for a reliable commercial seed production unit, as no controlled pollination could be performed without pollen. Pollen is produced from a set of Pisifera father palms, generally fewer than the Dura mother palms. In some seed production units, about 5 million seeds are produced with only a dozen of Pisifera father palms. Normally, oil palm inflorescences can be either male or

female (or rarely mixed). The sex ratio which is defined as the ratio of females to total inflorescences is an important factor for pollen production. While the sex ratio is expected to be high for Dura mother palms or planted industrial Tenera plots, the opposite is essential for the Pisifera father palms. Numerous works have emphasized the importance of water stress in sex ratio determination, as the latter can be significantly increased through irrigation or reduced during drought (IRHO 1970; Turner 1977; Corley and Hong 1982; Mite et al. 2000). Thus, producing pollen in sufficient quantities may become a difficult task for seed production units generally located in suitable areas for oil palm concerning water deficit and rainfall. In such conditions, various techniques are used in order to induce stress conditions on the palm and influence the sex ratio so as to increase male inflorescence and pollen production, the most widespread being pruning. Durand-Gasselin et al. (1999b) showed an increase in male inflorescence and pollen production on pruned Pisifera palms. The response was observed 18–24 months after pruning according to the age of the palms. It is, therefore, essential to set up an accurate turnover for the pruning of the Pisifera father palms in order to ensure sustainable pollen production.

The male inflorescence is isolated with an air-permeable canvas/synthetic bag impermeable to external contaminating pollen tied on the base of the peduncle with a rubber strap about 7–10 days prior to anthesis for pollen production. Once anthesis is observed through a plastic window on the bag, the male inflorescence is harvested still in the isolation bag and dried for about 3 h in an oven (38–39°C) or air-conditioned room. The inflorescence is vigorously shaken to collect pollen, sieved and further dried in an oven at 38–39°C till 6% moisture content. The pollen is vacuum-packed in sealed ampoules and can be stored at −20°C for several months. Detailed information on pollen production and storage are provided by Bénard and Noiret (1970), IRHO (1979), and Donough et al. (1992).

7.2 Pollination of the Female Inflorescence

Female inflorescences are isolated on Dura mother palms with canvas/synthetic bags in the same manner as the Pisifera parent palms. Once the female inflorescence reaches anthesis, pollination is carried out by puffing a 1:10–1:20 w/w pollen:talcum powder mixture through a hole in the plastic window on the bag using a wash bottle or a sprayer, and the inflorescence is allowed to develop for 3–4 weeks before the bag is removed.

In order to check the reliability of the work of pollinators and ensure stringent quality control measures against illegitimate pollination, many precautions have been established, of which blank pollinations. In the latter, pollinators are given only talcum powder without their knowing and allowed to pollinate some female inflorescences. The resulting fruit bunches are later harvested and inspected for any sign of fruit set. Details about the pollination of female inflorescence and quality control are provided by Donough et al. (1992).

7.3 Seed Processing

After pollination, the resulting FFB is harvested 5–6 months later. Each bunch is identified by a label which will accompany the resulting seeds throughout the process. Upon arrival, the FFB is weighed, chopped and spikelets are allowed to ferment for few days in order to facilitate the removal of the fruits. The latter are also kept in conditions allowing the outer mesocarp to rotten, and a machine called "depericarper" is used to remove the rotten mesocarp from the fruit through friction on rough metal surfaces. New generation depericarpers can process fresh fruits without any previous fermentation step, thus allowing considerable time saving. The resulting seeds are further hand-washed with sand, air-dried, and the residual fibers are meticulously cleaned up with a knife. The seeds are counted, treated against fungus and insecticides and stored at 22°C and 65% relative humidity, conditions suitable for a long-term storage (up to 1 year) without rapid loss of germination abilities (Rees 1965b; Mok and Hor 1977; Mok 1982). Many quality control steps are also instituted in this process, and details are given by Periasamy et al. (2002) and Corley and Tinker (2003).

7.4 Seed Germination

The oil palm seed is dormant when harvested. Prior to germination, moisture content of the previously stored seeds is raised, and a heat treatment of 80 days (or 60 days for some seed production units) at 39–40°C is needed in order to break this dormancy and allow a flush of germination to occur. The commonly used technique is the "dry heat" method where the seeds are heated at moisture content between 17 and 19% for germination. These conditions are best achieved by packaging the seeds in polythene bags and temperature-controlled heat chambers. At the end of heating period, moisture content is raised to about 23–25%, and generally more than 80% of the seeds germinate within 3–4 weeks. The seeds are sorted out weekly to separate the germinated seeds. The germinated seeds need to be transplanted in a nursery. Details on seed germination are given by Hussey (1958, 1959), Rees (1962), and Corrado and Wuidart (1990).

8 Future Trends for Oil Palm Breeding

Tree cloning and molecular breeding techniques undoubtedly appear to be the core tools for the development of breeding programs on the oil palm in the future. The time and space savings generated by the use of these techniques could be of great interest for a perennial crop as the oil palm. Besides this, other newly arising breeding issues such as alteration of oil composition for industrial or health purposes, collection and management of natural germplasm resources, and the need for the maintenance of genetic diversity will be discussed in this section.

8.1 Marker-Assisted Selection

For a long time, selection of oil palm has been performed through conventional breeding by phenotypic evaluation of the traits of interest in the offspring of a cross. This implies to wait for the offspring to be old enough (12–15 years) to fully evaluate its agronomic value. Moreover, the latter could be significantly affected by the environment. Marker-assisted selection (MAS) is an approach that allows selecting DNA markers that are linked to the trait of interest. To be feasible, the MAS approach requires a previous identification of DNA markers tightly linked to the *locus* (*loci*) that carries the trait. This can be done by mapping the trait relative to DNA markers whose positions are known on the genetic map of the oil palm. An alternative approach is feasible when a candidate gene responsible for the trait of interest has been identified through fundamental research by assessing the co-segregation of the trait and the candidate gene in a segregating population. This last approach has already been used successfully on other oil crops (identification of the gene of desaturases for the control of the level of unsaturated fatty acids in rapeseed, sunflower and soybean; elongase gene for the synthesis of very long chain fatty acids in rapeseed oil).

The interest of MAS resides in the fact that it can be used to select individuals bearing desirable traits prior to the phenotypic expression of these traits. This may be very useful for a perennial crop such as the oil palm, as 0.5 ha of land and 12–15 years could be required to characterize a single cross in a trial through conventional breeding methods. The advent of MAS will allow selection at the nursery stage for specific monogenic traits such as shell thickness or resistance to diseases which are controlled by single genes. In the case of more complex traits controlled by multiple genes or linked to several *loci* (quantitative trait *loci* or QTL), it is possible to quantify the relative part of the trait brought by each *locus*. An example is the iodine value (an indication of the proportion of unsaturated fatty acids of an oil) which is linked to several *loci* with a single *locus* carrying 80% of the trait (Singh et al. 2009). Although several markers may be needed in such cases for an accurate selection, using MAS will allow a first screening stage and the selection of suitable candidates to be assessed through field trials, thus increasing the chances of selecting desirable extreme outliers for a given trait. The advent of oil palm genome maps makes it possible to identify the exact location of specific QTL in the genome using molecular markers. However, the accuracy of this location is directly related to the distance between the marker and the target gene, thus the density of the map, as they may not be close enough to allow them to co-segregate and hence prevent recombination.

In the oil palm, the inability to generate large genotypic and gene expression data sets that allow linkage analysis and trait dissection in a wide range of breeding material is currently a bottle-neck for trait analysis and MAS. The majority of data available relates to a limited number of crosses per breeding program and often to limited numbers of oil palms within each cross (Mayes et al. 1997; Rance et al. 2001; Chua et al. 2001). With the decreasing cost of sequencing and the increasing efficiency of sequencing techniques, the genetic map of the oil palm is getting

increasingly dense. A program to map the oil palm genome using RFLP, AFLP, SNPs, and microsatellite probes have been initiated at Malaysian Palm Oil Board (MPOB) over the last few years, and markers associated with traits such as fruit color and shell thickness have been identified. Another important step was made with the creation and mapping of 371 oil palm microsatellite markers in the EU INCO-DEV FP5 program, LINK2PALM (http://www.neiker.net/link2palm/OilP/DefOIL.htm) (Billotte et al 2005, 2010). Research programs set up by three different consortia had led to the sequencing of the entire oil palm genome. However, there is limited public availability of all these data.

8.2 DNA Markers

DNA markers can be used in practice for many purposes such as confirmation of pedigree of legitimacy, assessment of genetic diversity or for MAS. Different types of DNA markers have been developed and are used extensively for studies on oil palm. The choice of the appropriate marker is determined by technical criteria (high polymorphism, codominant inheritance, stability, abundance, and dispersion throughout the genome), simplicity and cost of the technique.

Restriction fragment length polymorphism (RFLP) is the well-known marker technique and has been used to establish the first oil palm genetic maps (Jack and Mayes 1993; Mayes et al. 1997). Although they provide reliable and repeatable results, yet have the disadvantage of being laborious and time-consuming. The introduction of polymerase chain reaction was undoubtedly one of the major innovations in DNA techniques. Many PCR-based molecular markers have been developed, namely rapid amplification of polymorphic DNA (RAPD) and "microsatellites" or simple sequence repeat (SSR) and many other variants. Microsatellites developed by Billotte et al. (2001, 2005) were used to assess the genetic diversity of MPOB oil palm collection (Bakoumé et al. 2007). Despite their lower reproducibility and resolution, PCR-based techniques can be easily automated and are more suitable for routine analyses, and they are therefore widely used on oil palm. Amplified fragment length polymorphism (AFLP) markers have also been used on oil palm (Barcelos 1998). They also use restriction enzymes as PCR, and thus combine the precision of RFLPs with the simplicity of PCR.

Currently, there is a great interest in the analysis of single nucleotide polymorphism (SNPs), as this will help further to saturate the oil palm genetic map and hence locate more precise markers (Rajinder and Cheah 2005).

8.3 DNA Sequence Information and Gene Expression

Another outstanding development in molecular biology techniques for the past years is the increasing interest in the study of expression marker systems for the detection of specific physiological effects. These markers can be used to monitor expression

level changes coinciding with a physiological event such as embryogenesis or lipid biosynthesis. Strategies to elucidate the function of plant genes have developed rapidly in the past two decades, and techniques such as overexpression and inactivation of genes in the model plant *Arabidopsis thaliana* have provided great insights into the fundamental principles of plant physiology as well as opening new avenues for crop improvement which should also benefit to the oil palm. The emergence of "omics" techniques such as proteomics, metabolomics and transcriptomics has provided new insights in the study of gene expression, as an entire "transcriptome" can be analyzed at a specific developmental point, under a particular stimulus or in a particular trait-related tissue. These techniques are under-led by the construction of very extensive expressed sequence tags (EST) collections and cDNA libraries. The recent development of very high throughput sequencing technologies such as "long fragment" Roche® 454 pyrosequencing and "short fragment" Solexa® and SOLID® was probably one of the most important technological developments in molecular biology techniques since the advent of PCR. Combined to microarray analysis, these methods are being used to produce and analyze enormous amounts of sequence data (Gigabases of sequence) by many consortia in order to ensure a comprehensive coverage of the oil palm genome and develop new sets of expression markers. Some consortia recently claimed to have fully sequenced the oil palm genome. Unfortunately, these data are not publicly available. Another interest brought by these new sequencing techniques is that it is no longer necessary to know the sequence of a genome to perform large-scale gene expression studies. Indeed, the number of ESTs for a given gene can be considered to reflect the abundance of the transcripts (Weber et al. 2007). Therefore, in addition to provide useful sequence information, ongoing large-scale cDNA sequencing projects will give useful insights on transcriptional regulation of oil accumulation in palm fruits, as attested by two recently published studies (Tranbarger et al. 2011; Bourgis et al. 2011).

8.4 Genetic Engineering and DNA Transformation

The most commonly used transformation methods in plants involve particle bombardment through "gene guns" or "biolistics," crown gall bacterium *Agrobacterium tumefaciens*-mediated transformation, electroporation by creating transient holes in cell membranes through electric shocks and viral transformations (transduction). Some of these methods have already been experienced successfully with the oil palm (Parveez et al. 1996, 1997, 1998a, b; Chaidamsari et al. 1998; Parveez 2000; Ghulam Kadir et al. 2005).

Target genes for transformation are generally genes coding for a new enzyme not initially present, in order to give the transformed plant a function that it did not have before. Gene expression can also be increased through overexpression or lowered by the antisense method. The latter involves the insertion of a synthetic gene with a DNA sequence that complements the target gene, and so blocks its expression and cancels out its activity. Currently, the guideline objective of transformation studies

on oil palm is probably the modification of oil composition, with the aim of increasing the proportion of unsaturated fatty acids. In palm oil, the level of saturated and unsaturated fatty acids is almost equal, with about 4.5% stearic and 44% palmitic acid (saturated), and 39% oleic and 10% linoleic (unsaturated) acids (Lin 2002). Due to its nutritional value, high oxidative stability and stability during heat treatment, the monounsaturated oleic acid appears as a suitable compromise between saturated and polyunsaturated fatty acids, and is therefore, more desirable than all the other fatty acids. In this regard, many programs have been designed in order to increase its content in palm oil. Sambanthamurthi et al. (2000) found correlations between iodine value which is an indication of the proportion of unsaturated fatty acids of an oil and KAS II (3-keto-acyl-ACP synthetase II) activity, an enzyme of the oil synthesis pathway in plants responsible for the conversion of C16-ACP, a precursor of palmitic acid (C16:0) to C18-ACP which is the precursor of C18 fatty acids. Positive correlations were also found between KAS II activity and the levels of oleic (C18:1) and linoleic (C18:2) acids individually and stronger when these two fatty acids were considered in combination. Also, Siti Nor Akmar et al. (2001) showed that δ-9 desaturase (the enzyme responsible for the conversion of the precursor of stearic acid C18-ACP into C18:1-ACP, the precursor of oleic acid) is very active compared to stearoyl-ACP thioesterase which converts C18:0-ACP into stearic acid C18:0, and that an increase in KAS II activity will lead to the increase of oleic acid (C18:1) and not stearic acid (C18:0) content. The increase in KAS II activity will lead undoubtedly to the increase of both oleic (C18:1) and linoleic (C18:2) acids contents. In order to increase the sole oleic acid content of palm oil without a concomitant increase in "contaminant" linoleic acid content, it will also be necessary to inhibit the oleate desaturase (the enzyme responsible for the conversion of oleic acid into linoleic acid) gene by the antisense method. Research works are currently being carried out on the oil palm about the transformation of KAS II and oleate desaturase, with the view of successful insertion and expression of the putative functional genes. Some cDNAs have already been identified and expressed (Siti Nor Akmar et al. 2001; Ramli et al. 2004; Syahanim et al. 2007).

Besides the modification of oil composition, other targets for transformation studies on oil palm have been identified such as resistance to diseases particularly *Ganoderma*, introduction of new specialty high value products such as PHB (poly-3-hydroxybutyrate) and PHBV (polyhydroxybutyrate-co-valerate) genes in oil palm for the production of biodegradable plastics (Parveez et al. 2008; Mat Yunus et al. 2008) and the modification of enzymes involved in fruit abscission (Henderson et al. 2001). The latter may be useful in relation to harvesting frequency and the proportion of loose fruits. Delaying ripe fruit abscission may help reduce considerable labor requirements generated by both parameters, and thus increase the profitability of the crop.

It is also important to identify appropriate promoters in order to control the expression of the target genes. When seeking for modification of oil composition in the fruit mesocarp, it will be important to ensure that the desired changes occur only in the mesocarp, as changes elsewhere such as in lipid cell membrane layers could be damaging by modifying their biological properties. Several promoters that drive

transcription specifically in the mesocarp or the kernel of the oil palm fruit have been reported by many authors (Siti Nor Akmar et al. 1995, 1996, 2001, 2003; Kemp and Stratford 2000; Shah and Cha 2000), with the identification of mesocarp and kernel-specific cDNAs. Light-harvesting chlorophyll A/B binding (LHCB) protein promoter for targeting specific expression in oil palm leaves was identified by Chan et al. (2008).

8.5 Tissue Culture and Production of Clonal Planting Material

Being a heterogeneous mixture of nonuniform progenies, the commercial oil palm planting material is very variable. Thus, individual palms within a commercial planting may yield considerably more than the field average. Reproducing such elite palms on a large scale will lead to considerable yield increase. This can be achieved through conventional breeding, but it will take more than 20 years. In the case of many perennial crops, genetically uniform material can be produced by the means of vegetative propagation using suckers, cuttings, or grafts. Given that the oil palm has a single growing point without any sucker, it cannot be reproduced by vegetative propagation using the latter. However, this can be done through tissue culture, i.e., growing small pieces of explants (generally immature leaves) on special nutrient solutions. The resulting plant shoots named clones can be grown in a nursery in the same manner as conventional seedlings. The history of oil palm tissue culture is reviewed by Corley (1993) and Jones (1995). Early experiences on oil palm tissue culture propagation started in the 1960s. The first clonal plantings took place in the late 1970s in Malaysia, and there was a rapid expansion in the 1980s until the discovery of floral abnormalities on clonal material.

As it is the case for many other crops, the main pitfall encountered by oil palm tissue culture is the somaclonal variation. As a result, a floral abnormality is observed, and leads to the production of "mantled" parthenocarpic fruits and severe bunch failure directly affecting oil yield. The current explanation of this abnormality is likely to be that of an epigenetic change involving methylation of the homeotic or flowering MADS box genes (Van der Linden et al. 2005). Susceptibility to this abnormality varies between and within clones. Recent advances have been made in the exploration of the epigenetic mechanism underlying this abnormality (Adam et al. 2006, 2007; Syed Alwee et al. 2006). Although controversial data were obtained by different authors (Corley et al. 1986; Duval et al. 1988; Besse et al. 1992; Jones et al. 1995; Durand-Gasselin et al. 1999a; Eeuwens et al. 2002), extended time in culture, the type of callus, re-cloning and culture media seemed to influence the frequency of occurrence of this flowering abnormality. Putative molecular markers were developed to detect these abnormalities at early stages, but they were later found to be clone specific and thus of poor efficiency. There is a recent renewed interest on clonal material by industrials, as difficulties associated with its production are progressively being overcome. Early studies estimated the cost of a plant obtained by tissue culture over five times the one of a conventional seedling, and a

financial model indicated that a yield increase of at least 20% was required for clonal planting to become profitable (Corley et al. 1988). In order to close this gap, significant efforts must be done to reduce production costs and the proportion of abnormal clones. The proportion of abnormal clones can be reduced significantly by limiting production of clones from each embryogenic callus. Also, cloning of seedlings has been proposed to limit the risk of mantling (Soh et al. 2003). New tissue culture techniques such as suspension culture in liquid medium with high capacity for uniform plantlet production, possibilities of automation and hand labor and costs reduction (Teixeira et al. 1995; Tahardi 1998; Zamzuri 1999), recloning of the best clones from ramets in the field (Soh et al. 2001) makes it possible to envisage profitable large-scale commercial production of clonal planting material in the near future.

Clonal hybrid seeds can also be produced by cloning Dura and Pisifera parent palms, with selection of parents for cloning based on specific combining ability. Bi-clonal seeds are produced when both parents are clones, and seeds are said to be semi-clonal when only a single parent is a clone. Due to the fact that crosses are confined to a limited number of parental D×P combinations, clonal hybrid seeds are more uniform and high-producing (the expected oil yield gain is about 15%) than conventional D×P seeds. They seem to represent a compromise between tissue culture plantlets and conventional D×P seeds, as the costs for clonal hybrid seed production are much lower and the proportion of somaclonal abnormality reduced because of the limited plantlet production from each clonal parent compared to tissue culture plantlets.

Another alternative to the production of large-scale homogeneous planting material is the use of true breeding completely homozygous doubled haploids to produce F_1 hybrid crosses. These F_1 hybrids generally exhibit some level of heterosis (hybrid vigor), are genetically uniform and have allowed spectacular yield increases in outbreeding crops such as maize (Forster et al. 2007; Dunwell 2009). Normally it requires at least six generations of selfing to produce highly inbred parental lines which can be considered as homozygous through conventional breeding. For a perennial crop such as the oil palm, this will need a minimum generation time of 4–5 years, making a total of 24–30 years. True breeding completely homozygous genotypes can also be obtained by doubling haploid plants containing the gametic (n) number of chromosomes in somatic cells. The doubling of the number of chromosomes of haploid cells is obtained during microspore culture of haploid pollen or (rarely) ovule mother cells by using cell division inhibitors such as colchicine or some herbicides. The resulting doubled haploids (2n) have two sets of identical chromosomes and are therefore completely homozygous and fertile (Corley and Tinker 2003). Attempts to produce oil palm homozygous doubled haploids through microspore culture have been mentioned by some authors (Odewale 1989; Latif 1991; Tirtoboma 1998), but there have been no reports of any doubled haploid production. A screening test involving visual detection of abnormal "off-type" germinated seeds, SSR markers and flow cytometry to identify candidate haploid genotypes and progress with production of homozygous doubled haploids have been mentioned by Nelson et al. (2008, 2009), but production of F_1 hybrids is yet to be reported.

However, there is also a drawback to the large-scale propagation of clonal planting material, as this may dramatically reduce genetic variability and thus enhance crop vulnerability with respect to epidemic pests or diseases. Additional updated information on advances in biotechnology and molecular breeding applications for the oil palm are provided by recent review articles by Price et al. (2007) and Rival (2007).

8.6 Mesocarp Lipase and Oil Acidity

Fatty acids are generally present in oils as part of triacylglycerol molecules. The presence of free fatty acid moieties is an indication of the impairment of oil quality, as dietary oils are proclaimed to be unfit for human consumption above the limit of 5% (w/w) free fatty acids (Codex Alimentarius commission/FAO/WHO food standards 2005). Triacylglycerol hydrolysis can be from microbial origin, caused by the microorganisms which enter the fruit mesocarp as a result of improper storage and/or delayed processing of fruits and liberate a lipase (Hiol et al 1999; Houria et al. 2002). Hydrolysis can also be autocatalytic without any enzymatic contribution, depending on initial moisture and free fatty acid contents (Loncin and Jacobsberg 1965). Below 0.1% moisture content, autocatalytic hydrolysis is highly unlikely to occur. The major cause of triacylglycerol hydrolysis is the presence of an endogenous lipase in the mesocarp of the oil palm fruit which is activated at maturity upon wounding and/or bruising of the fruit. According to Desassis (1957), 15 min is enough to hydrolyze 40% of the triacylglycerols of a bruised fruit. In order to limit the harmful action of this lipase, ripe fruit bunches must be harvested regularly (generally every 7–10 days) and processed rapidly. In fact, the oil palm is a poorly mechanized and very labor-demanding crop, and is profitable only because it is grown in developing countries where labor costs are low. The development of low lipase activity genotypes producing low acidity palm oil will contribute significantly to reduce labor costs and thus the profitability of the crop. Though initial studies concluded to the absence of an endogenous lipase in the palm fruit mesocarp (Oo 1981; Tombs and Morris 1982), many independent studies have shown undoubtedly that a lipase does indeed exist in the fruit mesocarp (Henderson and Osborne 1991; Sambanthamurthi et al. 1995; Ngando Ebongue et al. 2006). Lipase activity was assayed among a representative cross section of palms from the germplasm of the specialized centre for oil palm research of La Dibamba in Cameroon. Results showed a wide variation (about 1–100) in mesocarp lipase activity levels between palms from different *E. guineensis* genotypes, with the highest values reaching 850 IU/g dry mesocarp. However, some samples showed very low mesocarp lipase activity levels (below 10 IU/g dry mesocarp) comparable to values recorded for the *E. oleifera* samples. More interestingly, this low mesocarp lipase activity was correlated to low palm oil acidity level (Ngando Ebongue et al. 2008). It was demonstrated that palm oil acidity remained below 5% for the low mesocarp lipase activity genotypes when loose fruits were collected 18 days after the normal harvesting and processing, whereas acidities between 12 and 25% were recorded for high mesocarp

lipase activity genotypes in similar conditions (Ngando Ebongue et al., unpublished data). Based on these results, it is possible to reduce harvesting frequencies from once every 7–10 days to a 15–20 days periodicity without any subsequent impairment of palm oil quality. Additional works are being carried out at La Dibamba in collaboration with CIRAD in order to identify new low lipase genotypes and above all study the determinism of this trait.

8.7 Collection and Management of Natural Germplasm Resources

Genetic variability is the base for any breeding program and is the starting point for all improvement strategies. As it is the case for the Deli Dura breeding population which has its origin in the four palms introduced at the Bogor Botanical Garden in 1848 by Dutch colonists, most of the breeding populations used in oil palm breeding programs throughout the world came from a small number of ancestral palms, thus limiting genetic variability. This situation may even get worse when large-scale production of clonal planting material will become effective. Concern has grown that intensive breeding on this narrow genetic base is leading to limited variation within the elite material and potentially reducing the rate of future breeding progress (Rosenquist 1986). It therefore, rapidly became obvious for oil palm breeders that new *E. guineensis* and *E. oleifera* natural germplasms of sufficient genetic variability must be sought after in order to broaden the genetic base of existing breeding programs. Africa was naturally designated as the appropriate site for natural *E. guineensis* germplasm collection, as it was proven to be the continent where this crop originated from. The first prospection was carried out in the 1920s in the then Belgian colony of Congo (former Zaïre and now Democratic Republic of Congo). Since then, numerous prospection exercises have been undertaken throughout the wild and semi-wild populations existing in the palm belt across West and Central Africa (Vanderweyen 1952; Meunier 1969; Obasola et al. 1983; Rosenquist 1986; Hartley 1988; Ataga et al. 1999). Thanks to prospections undertaken by N. Rajanaidu (Rajanaidu et al. 1979, 1986a, 1991, 2000a; Rajanaidu and Rao 1988; Rajanaidu and Jalani 1994), MPOB has established a comprehensive collection of *E. guineensis* material covering the entire oil palm belt of West and Central Africa. Recent prospection exercises were carried out in the Cameroonian oil palm belt by the specialized Centre for oil palm research of La Dibamba (Cameroon), in association with the Colombian Oil Palm Research Centre (CENIPALMA) and the Indonesian Palm Oil Board (IPOB) in 2007 and 2008, respectively.

Most of these prospection exercises were proven to be scientific successes, as numerous desirable traits and other traits absent in existing breeding populations were discovered. However, with a few exceptions, introgression of the selected wild populations into improved populations of current breeding programs is yet to be effective, as the management of the impressive set of data derived from these natural germplasm collections may prove to be very tedious. There is little doubt that the advent of molecular markers and their intensive usage in oil palm breeding will help

to boost this process, as an accurate assessment of genetic variation through molecular markers will allow the conservation of a minimum number of the collected individuals while securing the maximum amount of genetic diversity. The MPOB natural oil palm collection was assessed using AFLP (Kularatne et al. 2000), RAPD (Shah et al. 1994), RFLP (Maizura et al. 2006), joint RAPD–RFLP (Rajanaidu et al. 2000b), and SSR markers (Bakoumé et al. 2007). These studies provided useful information on the amount of genetic diversity and relatedness among natural oil palm populations from different African areas.

One of the most important aspects of these prospection exercises is that they contribute to the conservation of the genetic variability present in wild palm groves, as the latter are more and more threatened by deforestation.

8.8 Oil Palm and Environmental Issues

The world's consumption of oils and fats is forecasted to increase from 166.5 million tons in 2006 to 232.4 million tons in 2020, that is a 40% increase within 14 years (Oil world 2008). In order to meet this demand, additional efforts must be contributed toward achieving further yields increment and/or continuous extension of planted areas. In many cases, the latter could only be achieved to the detrimental of wild forests or on less arable lands. Tropical forests host the richest fauna and flora biodiversity, but they are being cleared at an alarming rate in the past years as a result of human agricultural activities. It therefore becomes obvious that increasing yield per hectare is the most suitable approach toward sustainable and environment-friendly increase of the world's production of oils and fats in order to satisfy the rising demand. Present-day best oil palm genotypes can yield up to 10 tons oil/ha/year in ideal conditions. However, yields may vary considerably between industrial plantations and small holders who generally lack agronomic expertise and/or appropriate production tools. As the small holder sector may account for up to 40% of the total planted palm grove in some palm oil-producing countries such as Cameroon, increasing the low yields of these small holders will surely increase the average oil palm yield. This can be achieved through appropriate supervision of small holder's merged plantations by agronomic experts and above all the supply of suitable improved 100% Tenera planting material.

Breeders will also undoubtedly have to increase the yields per hectare of the current planting material in order to allow the oil palm to meet the world's rising demand for oils and fats. According to estimates by Corley (1983, 1998), potential yields of about 18 tons oil/ha/year could be achieved by the oil palm. Though GMOs (genetically modified organisms) and molecular breeding are considered to be opposed to more environment-friendly agricultural practices, there is increasing evidence for a key role of molecular breeding techniques and genetic engineering toward additional yield increment and ecological intensification of oil palm cultivation. Efforts toward environment-friendly oil palm cultivation led to the creation in 2004 of the RSPO (Roundtable on Sustainable Palm Oil), a not-for-profit association

involving stakeholders from many sectors of the oil palm industry with the objective to promote the growth and use of sustainable oil palm products through credible global standards and engagement of stakeholders.

9 Conclusion

The implementation of new molecular breeding techniques, tissue culture and genetic engineering to oil palm breeding will undoubtedly provide a real breakthrough toward the achievement of significant yield increases, as it has been the case for other oil crops. These new tools may also be useful to tailor the oil palm to produce high value products in order to reduce labor needs or satisfy specific oleochemical, nutraceutical, pharmaceutical, and biofuel industry requirements. Recent advances have considerably increased our understanding of the main processes involved in oil palm breeding through these new disciplines. Efforts to increase yields should also be supported by the view to broaden the genetic base of the material currently available in breeding programs through collection exercises and appropriate management of the collected natural oil palm resources. Despite considerable time and space savings and numerous other advantages expected from the implementation of the abovementioned new disciplines to oil palm breeding, appropriate field experimentation and the assessment of desirable phenotypic traits will remain the unique way to indisputably identify promising outliers. Definitely, the outstanding problems are numerous and offer many exciting challenges of sufficiently diverse nature to satisfy the interest of most oil palm breeders in the upcoming years.

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Chapter 8
Coconut

S.A.C.N. Perera

Abstract Coconut grows in the tropics mainly in coastal areas at low altitudes, in environments of high humidity and high temperatures. Its oil is characterized by a relatively high melting point, narrow melting range, absence of unpleasant odour and a certain resistance to oxidation and rancidity. Coconut oil is rich in short-chain fatty acids and exhibits very good digestibility. In addition to its food uses, coconut oil is widely used as an industrial vegetable oil. Because of its multitude uses it has been termed as "one of nature's greatest gifts to mankind" and also as "the Tree of Life."

Coconut (*Cocos nucifera* L.) is a monocot belonging to the family Arecaceae, subfamily Cocoideae and is the sole species of the genus *Cocos*. Coconut possesses a diploid genome with 16 pairs (2n = 2x = 32) of chromosomes. Classifications proposed identify two main varieties of coconut, tall or typica and dwarf or nana. Tall is the commercially viable variety while dwarf has been used extensively in genetic improvement for producing hybrid coconuts.

High nut yield has been the primary objective of coconut breeding followed by precocity, low stature and the tolerance to biotic and abiotic stresses. Coconut breeding is hindered by a number of factors such as long generation interval, cross pollination breeding behaviour of tall coconuts resulting in highly heterogenous populations, low number of seeds produced per palm and the lack of a viable vegetative propagation method. Mass selection and hybridization have been the widely used breeding methods of improvement and at present the majority of the genetically improved coconut plantations have been derived through mass selection. The hybrids between tall and dwarf types have become popular due to their higher nut yields, precocity and the lower stature compared with tall coconuts. Biotechnology offers prospects to overcome some of the inherent constraints in coconut breeding. Molecular marker systems such as RAPD, AFLP, SSRs and DArT have been used extensively for the genetic diversity studies and development of several genetic linkage maps.

S.A.C.N. Perera (✉)
Coconut Research Institute, Lunuwila, 61150, Sri Lanka
e-mail: chandrikaperera2003@yahoo.com

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1 Introduction

Coconut, *Cocos nucifera* L., is one of the major oil crops in the world growing in the tropics mainly in coastal areas between the latitudes 20° North and South of the equator and at altitudes from sea level to about 1,200 m in moderate to well-aerated, deep soil. The main coconut growing areas are located in Asia, Oceania, West Indies, Central and South America and West and East Africa. It has been the major perennial plantation oil crop in the humid tropics before the introduction of oil palm and main source of food and livelihood for about 10 million families in over 80 coconut growing countries.

2 Botany

Coconut (*C. nucifera* L.) belongs to the lower group of flowering plants known as monocots; family Arecaceae (formerly, Palmaceae) and the subfamily Cocoideae. Subfamily Cocoidae includes 27 genera and 600 species and is currently the sole species of the genus *Cocos*. Coconut possesses a diploid genome with 16 pairs ($2n = 2x = 32$) of chromosomes.

Inflorescences of the coconut are formed in the axils of every leaf of a bearing tree. Being a monoecious plant, produces male and female flowers separately on the same tree but on the same inflorescence. The coconut produces inflorescences in continuous succession bringing in chances of overlapping and consequent interspadix pollination. The inflorescence consists of many flower-bearing spikelets situated on a central axis or a peduncle. Its size varies from 0.75 to 2 m in length depending upon the variety and the individual palm.

The male flowers are the first to open, starting at the top of each spikelet and proceeding towards the base. The whole process of male flower opening, pollen shedding and abscising of the flower takes a day. However, the male phase lasts for about 20 days in many varieties of coconut due to the high number of male flowers produced in an inflorescence.

A female flower remains receptive for 1–3 days and the duration of the female phase varies with the variety and the type of the environment. The number of female flowers in the inflorescence is also variable averaging about 10–15.

The fruit of the coconut, botanically known as a fibrous drupe and popularly the "nut", consists internally of the endospermic kernel with the embryo embedded in it and externally protected by the fruit coat known as the pericarp. The pericarp consists of three distinct and well-defined regions, viz, the exocarp or epicarp, the mesocarp and the endocarp. The outermost region of the fruit coat is the exocarp (or epicarp) which consists of a tough smooth and hard fibrous material.

3 Varietal Classification

Various cultivars have been classified on the basis of phenotypic characters such as size, shape, number and composition of the nuts and bunch, and measurable components of the leaves and stem. To a certain extent, colour of the young nut and petioles of leaves have been used as a guide in differentiation. The consistency of the coconut fluid and meat, colour and thickness of the shell as well as the smell and taste of nut components have also been utilized to describe certain populations. In recent years, techniques of electrophoresis, oil and protein analysis of the meat and pollen morphology and DNA analysis have been used in varietal identification. Studies on floral biology and flowering habit are being done to augment the recorded information.

The first systematic classification of varieties and forms of coconut was done in 1949 by Narayana and John. They identified two groups, tall and dwarf (Figs. 8.1 and 8.2). The tall group composed of three botanical varieties: *typica*, with nine forms, *spicata* and *androgena*. The dwarf group consisted of two varieties: *nana* with two forms and *javanica*. Among these varieties the tall group was the most common cultivar that was extensively grown in plantation scale. Out of the dwarf group variety *nana* was identified as a delicate and early bearing (3 years) type while the variety *javanica* was identified to be vigorous and comparatively late bearing (4 years).

The survey of literature on coconut varieties by Gangolly et al. (1957) formed the basis for the classification of Menon and Pandalai (1958). This classification retained the tall and dwarf groups of the previous classification by Narayana and John (1949).

Fig. 8.1 Crown of a tall coconut palm

Fig. 8.2 Crown of a dwarf coconut palm

Liyanage (1958) in his classification of Sri Lankan coconut varieties reduced the number of coconut varieties to three, *typica* (tall), *nana* (dwarf) and a new variety named *aurantiaca* (intermediate). *Androgena* and *spicata* were omitted from this classification while *nana* was broadened to include *javanica*. The variety *aurantiaca* included semi-tall types which were distinct from either tall or the dwarf groups.

A fourth classification of coconut varieties was made by Fredmond et al. in 1966, based on pollination characteristics. Two coconut varieties – dwarf, distinguished by being autogamous or self-pollinating, and tall by being allogamous or cross pollinating – were identified as distinct varieties in this classification. However, the dwarf is easily cross pollinated, especially when surrounded by tall palms and tall palms possess the ability for self-pollination to a certain degree (Whitehead 1965; Rognon 1976).

A somewhat different classification, to the above was proposed by Harries (1978), in which two types of coconut have been distinguished. Accordingly, the "Niu kafa type" or wild coconut contains large, long, angular, thick husked and slow-germinating nuts with less free water content while the "Niu vai type" or selected and cultivated type has more spherical nuts with an increased proportion of endosperm, reduced husk content and early germinating nuts. Harries (1978) suggests that introgression of these two types and continued selection and dissemination by man has resulted in the wide range of varieties and pantropical distribution of coconuts found today.

The common feature in all the above classifications is the grouping of coconut as talls and dwarfs. Talls gained importance from the early days of plantation

Table 8.1 Contrasting characters of tall and dwarf coconuts

Character	Tall	Dwarf
Stature	Tall (≃ 20–30 m)	Short (≃ 10–15 m)
Stem circumference	Enlarged with a bulbous base	Thin and no bole formation
Life span	60–100 Years	40–50 Years
Time taken for flower initiation	5–8 Years	2.5–4 Years
Mode of pollination	Highly crossed	Highly selfed
Bearing nature	Continuous	Seasonal
Nuts/palm/year	Average 40–60	Average 80–100
Whole fruit size	Very small to large	Very small to medium
Copra amount and quality	200 g/nut; good	80–100 g/nut; inferior
Leaf and bunch attachment	Strong	Fragile
Pigmentation of nuts	Mixture of green, brown and yellow	Pure green, brown, yellow or red

Fig. 8.3 Colour, size and shape variations of coconuts

establishment as being the commercially more viable group of coconuts. However, later with the initiation of genetic improvement programmes of coconut, dwarfs were identified as highly potential parents in hybridization to incorporate precocity and high nut numbers into tall varieties. The contrasting features of tall and dwarf phenotypes of coconut are given in Table 8.1.

To make complete and comprehensive classification, a catalogue of local names and other important characteristics of each form/variety/accession need be prepared. Such an effort has recently been made by Coconut Genetic Resources Network (COGENT) by developing the International Coconut Genetic Resources Database (CGRD). Systematic morphological and molecular investigations are essential for identifying true genetic differences between varieties/forms/variants/populations to be efficiently used in coconut breeding (Fig. 8.3).

4 Oil Quality

Coconut oil is characterized by a relatively high melting point, narrow melting range, absence of unpleasant odour and a certain resistance to oxidation and rancidity (Philippines Coconut Authority 1979). In the coconut growing countries, oil is widely used for domestic consumption and is rich in short-chain fatty acids. It exhibits very good digestibility, serves as fat source in infant milk, used as ice cream fat and confectionery oil. Coconut oil can be converted to hard butter by hydrogenation and interesterification for use in a variety of confectionery formulations (Banzon 1977; Marcus and Puri 1978; Lansing 1985). Coconut oil is particularly rich (48.2%) in lauric acid (Jones 1991) and is used as a raw material in soap, glycerine and margarine. The lauric acid from coconut oil is used to manufacture detergents, cosmetics and pharmaceuticals. The newly emerging coconut oil product is virgin coconut oil (Bawalan and Chapman 2006) which is expelled under low heat from fresh coconut meat to preserve its natural vitamins and enzymes (Marikkar et al. 2007). Virgin coconut oil has now secured a significant international market in the pharmaceutical industry. Coconuts being the most prominent tropical oil crop in the twentieth century, studies on fatty acid composition in coconut oil have been conducted a few decades back. Such studies have revealed the fatty acid Lauric acid to be the biggest constituent exceeding 40% (ranging from 44–49%) out of the total fatty acid content followed by Myristic acid which ranges from 13 to 19 %. Apart from these major fatty acids Caprylic acid, Palmitic acid, Oleic acid and Capric acid were found to be present at percentages varying from 5 to 8 percent. In addition, the fatty acids, Stearic, Linoleic and Caproic have also been found to occur in lower percentages not exceeding 3% (Philippines Coconut Authority, 1979).

5 Goals

Yield: Despite the multitude of economically important yield parameters in the coconut palm, weight of meat or copra is the basic yield component that is targeted in many of the coconut breeding programmes. Number of nuts produced during a given time period and the weight of meat in each nut contribute for the final copra yield per palm. A complexity arises on many occasions due to the negative correlation of the number of nuts in a bunch and the weight of copra per nut. Increasing number of bunches per unit time period while maintaining a balance between nut number and the nut size will be the viable approaches to realize higher copra yields.

Precocity: The second major goal in coconut breeding is precocity. The long vegetative phase, which lasts for about 6–7 years in many of the commercially

viable tall cultivars, discourages coconut planting as a business venture because of its late returns for investments. The genetic potential of the dwarfs for early flowering and bearing provides the required genetic material for breeding for precocity.

Drought tolerance: Prolonged drought is one of the main limiting environment factors for growing coconut in many of the coconut growing countries. Coconut being a monocot possessing a fibrous root system which does not grow into deeper layers in the soil, suffers from moisture stress than many of the dicot perennials having a tap root. Hence, breeding for tolerance to moisture stress is another important goal to extend the cultivation of coconut to newer areas.

Tolerance to biotic stresses: Attempts are being made to identify tolerant genotypes to be used in breeding programmes for insect pests and diseases particularly for the microscopic coconut mite, *Aceria guerrerronis* for which the existing chemical control methods are only marginally effective (Perera et al. 2008). The most devastating groups of pathogens of coconut are the phytoplasmas and viroids which are intracellular, causing incurable deadly diseases such as lethal yellowing in Africa, Kalimantan wilt in Indonesia and Kerala Root wilt in India while Cadang cadang in Malaysia is reported to be caused by viroids. The countries plagued by phytoplasma diseases include tolerance breeding for such diseases as a primary objective in their coconut breeding programmes (Nair et al. 2009).

6 Principles of Coconut Breeding

6.1 Mass Selection

Coconut being the sole species of the genus *Cocos,* breeding is limited to intraspecific level only. The application of attractive breeding methods, which are widely used in several other crop plants, is hindered by many factors such as the long generation interval, cross pollination breeding behaviour of tall coconuts resulting in highly heterogenous populations, the low number of seeds produced per palm and the lack of a viable vegetative propagation method. Consequently, breeders face severe limitations in selecting breeding methods for coconut genetic improvement despite the availability of several attractive and easy to practice breeding techniques.

The majority of the coconut plantations in the world are derived from mass selection. This was informally done by the growers themselves at the end of the nineteenth century when large plantations were established. In many of these cases, seed nuts were selected mainly based on the size, weight and their shape. The genetic structure of resulting coconut populations was modified by successive selection for fruit characteristics. Later, mass selection was scientifically also recognized as the most fundamental method for coconut breeding (Liyanage 1955). By mid-twentieth century, the system of selection of the best trees within the best plots was applied and

breeders throughout the world adopted this system of mass selection. The common selection criteria were the yield of copra per palm or one of its components such as number of fruits produced or per nut copra content.

Three options of mass selection are available depending on the reproduction system used: mass selection using open pollination, selfing or intercrossing between parents. The advantage of mass selection using open pollination is its simplicity. In this method, seed nuts are collected from seed parents, selected for desirable characteristics at a certain time or over a certain period of time. In this method, the seed nuts arising from open pollination of the female parents form the improved population, which may or may not undergo further selection cycles. The efficiency of mass selection using open pollination suffers due to two aspects. Firstly, the tall coconuts are naturally cross pollinating and thus the pollen donor may contribute less desirable characteristics to the subsequent progeny, reducing the efficiency expected in selection. Secondly, although the talls are mainly allogamous, interspadix selfing is a possibility, especially due to high rate of inflorescence emission in high yielding palms (Bourdeix 1988). The resultant progeny may suffer from inbreeding depression reducing the expected benefits of selection. The speed of inflorescence emission varies between seasons, as a result the selection results may differ depending on the season during which the seed nuts are harvested. However, a positive response has been achieved for selection recording a maximum of 14.4% gain in the first generation from a selection of 5% best palms (Liyanage 1972). The severe selection required for achieving higher genetic gains limits the pool of selected seed parents thus reducing the seed nut production capacity.

The first method of mass selection with open pollination is practiced in certain coconut growing countries including Sri Lanka, as a stop-gap measure to meet the demand for improved cultivars in national coconut planting programmes. In Sri Lanka, the selection of seed parents by open pollination is done in two stages:

(a) Selection of high yielding blocks from suitable estates based on yield figures for the past 5 consecutive years
(b) Selection of individual palms within high yielding blocks based on nut yield, husked nut weights and good agronomic features

The second of the mass selection method, use of selfed progeny of selected palms, seems to be of limited effectiveness in certain cases. Bourdeix (1999) has reported a reduction of 15–25% in fruit production in a single generation selfing of a tall coconut population. Continuing with self-pollination to obtain pure lines remains a long-term prospect and it is impractical because four generations which are required to create 95% homozygosity require a period of 25–60 years depending on the method of parent evaluation.

The third method of mass selection, using intercrossing, is theoretically the most effective mass selection method in coconut. This method has been used in Sri Lanka to get a progeny for planting in seed gardens which later served as the parent stock in developing improved cultivar CRIC60 which is an intravarietal cross between Sri Lankan talls. Such intrapopulation breeding methods have been applied in Indonesia and India for developing improved cultivars.

6.2 *Hybridization*

6.2.1 Hybrids of Single Crosses

Coconut breeders normally practice straightforward parental selection based on combining abilities. The long term involved in purifying the parental lines and producing a generation of progenies makes it difficult to start hybridization programmes from purified parental stocks. The most popular scheme of producing improved coconut cultivars is through single cross hybridization using parents with good combining ability. Accordingly, a parental genotype is usually selected based on its proven performance, normally with respect to copra and or oil production. The second parent is selected in such a way as to complement the known weaknesses of the formerly chosen parent, such as precocity, stress tolerance, etc.

Dwarf×Tall (D×T) and Tall×Tall of unrelated tall populations (T×T) single cross hybrids are the most common commercially recognized hybrids in the world at present. Such single crosses are recorded to provide the greatest opportunity for extracting hybrid vigour and higher yields (Wright 1980).

Out of the single crosses, D×T hybrids are the most popular in order to achieve precocity and higher nut yields. The resultant progeny of such crosses normally inherit high productivity, broad adaptability and tolerance to certain pest and diseases from its tall parent, and precocity, higher growth and bunch emission rates and higher nut yields from the dwarf parents (Bourdeix et al. 1998). D×T crosses facilitated the transmission of much sought precocity from dwarf populations into the commercially viable improved cultivars. The use of dwarf as the female parent in such crosses grants the ease of pollinating the mother palms due to its short stature. However, T×D crosses also have been reported in Sri Lanka to overcome the limitation of the smaller number of the dwarf palm pool compared to tall. The experiments have shown no significant difference between T×D and D×T hybrids with respect to early growth parameters, precocity and nut and copra yields indicating the lack of maternal effects in the crosses (Perera et al. 2007). Resources such as male sterile lines and self-incompatible lines which reduce the difficulties associated with hybridization have so far not been recorded in coconut.

In many of the coconut growing countries, breeding schemes are in place to produce T×T hybrids within and between genetically distant tall populations, facilitated by the natural out crossing of tall populations. Open pollinated palms from selected tall parents with outcrossing behaviour would similarly exhibit hybrid vigour and could be naturally produced in isolated gardens (Batugal and Bourdeix 2005). Bourdeix (1988) have reported that in the long run certain T×T crosses can have a cumulative production equivalent to the yield of D×T, as demonstrated in the comparison between the West African Tall (WAT)×Rennel Island Tall (RIT) improved hybrid and PB121 (Malayan Yellow Dwarf×West African Tall).

The hybridization between D×D is not very popular due to the sensitivity of dwarfs to environmental stress such as drought and low fertility although the worlds first reported coconut hybrid was a D×D cross. However, there are breeding programmes involving dwarfs in Thailand, geared to improve Aromatic Green

dwarf varieties and in Sri Lanka to produce a shorter variety for home gardens (Meegahakumbura et al. 2008). An experiment conducted in Ivory Coast tested three possible hybrids between Malayan Yellow, Red dwarfs (MYD & MRD) and Brazilian Green Dwarf (BGD) and compared them with Malayan Yellow Dwarf (MYD) used as a control (Le Saint and de Nuce de Lamothe 1987). The hybrid MYD×MRD produced an average of 3.8 tonnes of copra per hectare which was comparable to the production levels of a D×T hybrid. A main advantage in D×D hybrids is their genetic homogenity. Generally, dwarf parents can be considered as pure lines due to their natural self-breeding nature and their resultant progenies are expected to be genotypically less variable than D×T hybrids involving heterozygous talls.

6.2.2 Mass Production of Hybrids

Production of seed nuts of intervarietal crosses is laborious, expensive and time consuming. Despite all efforts, only a few seed nuts are produced per palm. The concept of seed gardens is used to overcome some of these problems. In seed gardens, the two parental varieties are planted in a given proportion and the female parent is emasculated to allow them to be crossed naturally from the pollen shedding from surrounding male palms. This phenomenon is called directed natural pollination. However, at present, techniques have been developed for the spraying of processed pollen onto the female parents, thereby getting rid of the need for having a large population of male parents within the same locality. This technique further allows the production of different crosses with the same mother stock at different time periods. However, in both types of situations the seed gardens need be sufficiently isolated from alien pollen to ensure legitimacy of the intended hybrid. This isolation is achieved by a natural barrier such as a water mass (sea, reservoir), surrounding forest plantation, planting a strip of a different crop or by having a sufficient number of guard rows of coconut with the pollen parent.

6.2.3 Complex Hybrids

Normally in coconut breeding, promising hybrids are identified at the F_1 stage rather than proceeding to advanced breeding lines mainly because several rounds of crossing involve a long period of time. However, this termination at F_1 stage limits the complete exploration of possible phylogenic recombination of a cross. Nevertheless, a few countries are reported to be testing multiple crosses to develop varieties with desirable multiple traits.

For example, Thailand is reported to be testing a three-way cross hybrid (T×T)×T and (D×T)×T. Comparing the levels of heterogeneity of these two kinds of combinations will reveal important information although a greater variability is expected for the cross (D×T)×T due to segregation for dwarfism than (T×T)×T. In Ivory Coast, MYD was crossed with the hybrid WAT×RIT in 1976. This three-way hybrid yielded only 77% more than the WAT control. However, single cross hybrids of

MYD×WAT and MYD×RIT have recorded yields 97% and 129%, respectively, over the control variety WAT (Anonymous 1988).

6.3 Synthetic Varieties

Exploiting heterosis through hybridization is an efficient method in increasing productivity. However, it is expensive and rather time consuming to establish and maintain seed gardens for the mass production of recommended hybrids of coconut. Moreover, the offspring of the resultant original cross cannot be used as seed material because the genetic vigour is not maintained due to segregation of alleles. Apart from that, hybrids narrow down the diversity which is an important advantage for further extraction of genetic gains out of cultivated material.

Synthetic varieties have been suggested as a way of overcoming some of these problems. The assessment of morphometric traits of different phenotypes formed the theoretical basis of synthetic varieties that are being produced in the Philippines (Santos and Rivera 2002). The parental base of a synthetic variety is a composite of selected parental lines which combine well in all combinations through natural crossing. Consequently, prospective parental genotypes are first tested for their combining ability or additive gene effects for them to qualify to enter the mating pool. Therefore, the most critical stage in developing a synthetic variety is the selection of parents for the composite. However, the main drawback for synthetic varieties in coconut would be the need for several cycles of intermating for the combined genes favouring the desired trait to attain equilibrium because, in general, purification of parental lines is prevented in coconut. Similarly, interspadix self-pollination which may occur seasonally in the tall coconuts need to be prevented to avoid inbreeding depression. Removal of "unwanted" inflorescence in seed gardens, at critical seasons when the inflorescence emission is faster, provides a way of overcoming this problem.

7 Biotechnological Applications

The advent of molecular markers paved way for a rapid enhancement in breeding research in many crop plants, as they deal directly at genetic level unmasked by the environmental effects. The applications of marker systems are wide, ranging from the assessments of genetic diversity to development of genetic linkage maps.

7.1 Molecular Markers for Genetic Diversity Studies in Coconut

Conventional coconut breeding programmes using standard conventional breeding techniques have been relatively successful. However, the biotechnological techniques

offer prospects to overcome some of the inherent constraints in coconut breeding. Application of molecular genetics in coconut breeding, particularly the molecular markers, has so far been mainly concerned with assessing genetic diversity and creating genetic linkage maps. Use of molecular biological tools in coconut breeding initially aimed at assessment of coconut genetic diversity and genetic relatedness at the DNA level, using universal marker techniques such as RAPD (Ashburner et al. 1997; Duran et al. 1997; Everard 1996; Dassanayake 2003; Dassanayake et al. 2003), RFLP (Lebrun et al. 1998, 1999), AFLP (Perera et al. 1998; Teulat et al. 2000) and ISTR (Duran et al. 1997; Rohde et al. 2000). With the need for coconut specific markers, two sets of microsatellite markers were isolated by two groups of scientists independently using the cultivar Sri Lanka Tall (Perera et al. 1999) and Tagnanan Tall (Rivera et al. 1999). Microsatellites as co-dominant markers have been particularly useful in analysing highly heterozygous coconut for genetic diversity and genetic relatedness estimates. Germplasm characterization and development of co-collections (Perera et al. 2000, 2001, 2003; Teulat et al. 2000; Dassanayake et al. 2003; Meerow et al. 2003), hybridity testing (Perera et al. 2004) and detecting somoclonal variation in tissue cultured coconut plants and construction of genetic linkage maps (Herran et al. 2000; Lebrun et al. 2001; Baudouin et al. 2006) are other areas where microsatellites have been used. A microsatellite kit comprising 14 primers and an associated software for data analysis has also been developed (Baudouin and Lebrun 2002). More recently, DArT markers for coconut have been developed and used for diversity studies (Perera and Killian 2008). Among these markers, SSRs have been the most widely and extensively used in analysing coconut genome.

Genetic diversity studies using microsatallite marker have revealed a high level of genetic diversity in a worldwide collection of 130 tall coconut individuals and 49 dwarf coconut individuals (Perera et al. 2000, 2003). Perera (1999) observed a reduction in the amount of genetic diversity in dwarfs in comparison to tall coconuts with a comparable reduction in the number of alleles in dwarf coconuts. Rivera et al. (1999) analysed 20 coconut varieties from South East Asia and the Pacific, and Teulat et al. (2000) studied 14 coconut varieties and reported similar results to Perera (1999). These studies revealed a non-uniformity of genetic diversity between the tall and the dwarf groups of coconut; the tall group reporting comparably higher genetic variation and higher heterozygocity (30%). Although, the dwarfs are naturally self-pollinating a low frequency of 2.5% heterozygosity was reported.

The findings of molecular studies on the genetic diversity of coconut formed the basis for the changes in germplasm collection strategies and selection of parents for hybrid breeding programmes. Several countries attempted and succeeded in coconut germplasm exchange programmes. For example, Sri Lanka exchanged germplasm with India, Papua New Guinea and Ivory Coast and even initiated hybridization programmes with exotic pollen imported from Ivory Coast in order to exploit maximum heterosis resulting from the higher genetic diversity of genetically distant parental material (Meegahakumbura et al. 2008).

In the context of germplasm collection, molecular genetic analysis helped identify redundant accessions in the germplasm collection thereby increasing the

efficiency of conservation strategies. The results of RAPD analysis (Ashburner et al. 1997) revealed a low but variable rate of gene migration between South Pacific populations with possible finder effects and subsequent human collection. Based on the results, Ashburner et al. (1997) proposed that germplasm collection in the South Pacific region should focus on populations rather than individuals due to the high variation observed among populations. However, Perera et al. (2001) reported contrary findings with respect to Sri Lankan tall coconut germplasm representing different geographical regions showing very low (5%) population differentiation and the need for adjusting conservation strategies accordingly.

7.2 Genome Mapping of Coconut

Selection of a mapping population is one of the most critical decisions in constructing a linkage map with DNA markers. A mapping population should comprise of the parents and their segregating progeny. Segregating populations such as F_2s and back crosses or advanced segregating populations such as Recombinant Inbred Lines (RILs) or Doubled Haploid Lines (DHLs) are the commonly used mapping populations at present for linkage mapping in self-pollinated crops (Kuittinen et al. 1997; Marquez-Cedillo et al. 2001). The choice of a mapping population depends upon the biology of the organism and the power of the different methods and the heritability value of the trait of interest. However, the development of the above-mentioned typical mapping populations for coconut is hindered by the predominantly cross pollinating nature of coconut.

Genetic linkage mapping in outbred species such as coconut, thus poses additional challenges in QTL mapping. As a result, in QTL mapping ventures in outbred species (e.g. most of the trees, humans, etc.), the information is restricted to existing pedigree populations. The problems involved in obtaining information from pedigree populations are the substantially smaller family sizes compared with the commonly used segregating populations and the direct unavailability of information about the linkage phase of genes at the marker loci and QTL. To solve these problems, respectively, a large number of families and complicated statistical tools are needed for modelling the inheritance of genes within a multiple generation pedigree (Mackay 2001; Kearsey and Luo 2003). In considering the breeding behaviour of coconut, it has been established that tall coconuts are cross pollinating while dwarf coconuts are self-breeding. Therefore, a segregating population in coconut can be obtained by crossing self-pollinating homozygous dwarf coconuts with cross pollinating heterozygous tall coconuts (Bandaranayake and Kearsey 2005). However, one critical consideration in such an occasion is the selection of tall parents possessing sufficient levels of heterozygosity resulting in a considerable level of polymorphism in the subsequent progeny.

The major constraint in producing a fairly large mapping population in coconut is the limited number of seeds produced from a particular mother palm and also the

low rate of success in artificial pollination. As a result, obtaining a sufficient number of progeny for mapping will take a long time which is not desirable because there will be a considerable age gap among the individuals of the resulting mapping population. Combining several separate half sib families to produce a single mapping population could be taken as an alternative for increasing progeny size within a shorter time span. In view of this understanding, it is appropriate to choose several dwarf palms from a population as female parents and a single highly heterozygous male tall coconut as pollen donor to construct the mapping population and therefore selecting a dwarf parent is essential to obtain half sib families to determine linkage phases accurately (Perera 2010).

Several coconut genome maps have already been derived despite the difficulty of developing proper segregating families. The first genetic linkage map thus produced for coconut was using an F_1 population obtained by crossing East African Tall × Laguna Tall and genotyped using ISTR markers (Rohde et al. 2000). A second mapping population was developed in the Philippines by crossing MYD with Laguna tall and the linkage map was developed by genotyping with AFLP, ISTR, RAPD and ISSR markers. The framework map of this population included 382 makers in 16 linkage groups. Proceeding with QTL analysis with this population, six QTLs responsible for early germination were placed on this map (Herran et al. 2000). With the earlier established positive correlations among early germination and flowering and high yield, this QTL map provides the opportunity for marker-assisted selection in coconut. The same QTL map was later enriched with QTL for growth parameters, leaf production, girth and stem (Ritter et al. 2000). A third mapping population was developed in Ivory Coast by crossing Cameroon Red Dwarf and Rennel Island tall. Two hundred and eighty markers were placed on the 16 linkage groups of the resultant framework map in addition to locating QTL for yield parameters nut number, number of bunches and fruit components (Lebrun et al. 2001; Baudouin et al. 2006).

7.3 Physical Maps and Synteny Studies

Physical maps provide the likely order of defined genomic DNA segments along the chromosomes. If contiguous regions of overlapping cloned DNAs exist, the physical map provides an estimate of the true distance in base pairs between distinct location on and specific chromosome. Physical maps serve many purposes such as genome-wide gene discovery, EST mapping (functional genomics) or comparative genomics (synteny studies). Efforts are underway to develop physical maps for coconut and oil palm by deriving cosmid clones of genomic DNA and mapping them onto the molecular linkage maps of coconut and oil palm by association to mapped polymorphic AFLP markers, which will result in high density molecular linkage reference maps for both coconut and oil palm.

7.4 *In Vitro Culture Methods for Coconut Breeding*

Lack of a viable vegetative propagation method hinders clonal propagation of improved planting material. Tissue culture protocols have been improved by a few laboratories and clonal plantlets have been derived from zygotic tissues (Chan et al. 1998; Verdeil et al. 1999; Fernando et al. 2003). However, clonal propagation through tissue culture is still economically not viable due to varying and poor responses.

Embryo culture techniques have been successfully used for regenerating certain phenotypes, such as Dikiri coconut from Sri Lanka. The embryo culture protocols are being refined for different coconut varieties for safe and efficient trans-boundary exchange programmes (Dr. K Weerakoon, Coconut Research Institute of Sri Lanka, personal communications, 2010).

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Chapter 9
Cotton

Lori Hinze and Russell Kohel

Abstract Cotton is a significant agricultural commodity throughout the world that is used primarily for its fibers to manufacture textiles, but with notable secondary value for its seeds. As cotton oil mills began to operate and products other than whole cottonseed became available, the value of cottonseed increased. This increase in the value of cottonseed spurred research efforts to improve the protein and oil quantity and quality of cottonseed. This chapter concentrates on several aspects of cotton as an oilseed crop, including seed quality, seed processing, uses of cottonseed, and prospects for future improvement in cottonseed quality. Cottonseed oil and meal are the two most valuable products of cottonseed. Cottonseed oil is considered heart healthy and has a long shelf life. Cottonseed meal is used principally as feed for livestock and its major value is as a concentrated protein supplement. Cottonseed flour has a high quality amino acid profile. A limiting nutritional factor of cottonseed is the presence of gossypol. Gossypol binds with protein causing a lysine deficiency and has toxic effects when ingested by nonruminant animals. Despite this limitation, the seed component of cotton production cannot be ignored, and the production of gossypol-free seed would enhance the overall value of cotton. The industry is beginning to see cottonseed as a viable source of revenue, thereby adding value to each and every acre of cotton.

Keywords Cotton (*Gossypium* spp.) • Cottonseed • Protein and oil quantity • Gossypol • Lysine deficiency • Molecular breeding

L. Hinze (✉)
USDA-ARS, Southern Plains Agricultural Research Center, 2881 F&B Road, College Station, TX, USA
e-mail: Lori.Hinze@ars.usda.gov

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1 Introduction

Cotton (*Gossypium* spp.) is a significant agricultural commodity, primarily commercially grown for its fibers, but with secondary value as an oilseed crop. The focus of this chapter is on the value of cotton as an oilseed crop that produces oil and meal for animal and human consumption.

Several thorough reviews have been published telling the history of cotton, including its origin, domestication, and taxonomy. For the most recent review, please consult Hague et al. (2009). There are 49 recognized species of cotton, and four of these species are domesticated: *G. hirsutum* and *G. barbadense* are tetraploids and *G. arboreum* and *G. herbaceum* are diploids. These four domesticated species are cultivated throughout the world between 37°N and 32°S latitude (Hague et al. 2009).

Cotton production, processing, and uses of its raw material (primarily cotton fiber) are detailed in a review by Campbell and Hinze (2010). When cotton leaves a producer's field, it goes to a gin where the seeds are separated from the long cotton fibers. The ginned seeds are then marketed to the cottonseed crushing industry while the fibers are sent to textile manufacturers. Cotton production, seed crushing, and fiber processing are highly mechanized in many countries of the world, and research primarily focuses on improving cotton fiber quality to meet the needs of textile manufacturers and the end-users of textile products. Research on improving cottonseed quality is gaining interest due to the increasing economic value of cottonseed.

The cottonseed is comprised of a kernel (embryo) which contains the oil and protein, and the kernel is surrounded by a hard outer shell (hull). Following ginning, which removes the long fibers, the fuzzy seed is sent to a mill for crushing to extract the oil. One ton of cottonseed will yield approximately 145 kg oil, 245 kg hulls, 413 kg meal, 76 kg short fibers that remain after ginning, and 29 kg waste products lost during processing.

In this chapter, we will discuss several measures of cottonseed that are used to assess its quality. We will discuss how cottonseed is processed to obtain oil and protein. We will also consider the effect of gossypol and the limitations it presents when using cottonseed as a source of protein and oil for humans and animals.

2 Cottonseed Quality

Relative to lint yield and quality improvement programs, few research efforts have been directed towards improving cottonseed quality. Cottonseed quality was the focus of research efforts in the 1970s and 1980s. Following this period, interest in cottonseed quality waned until recent times. An increase in the value of cottonseed has led to a resurgence of interest in improving the protein and oil quantity and quality of cottonseed. When improving seed quality, a breeder will always consider that lint quality and quantity cannot be sacrificed. Until a substantial market for improved

cottonseed develops, lint yield and quality will continue to have the greatest economic impact. The value of cottonseed can be improved by increasing oil and protein content and by modifying fatty acid and amino acid profiles to satisfy animal and human nutrition needs. In addition, the potential of using cottonseed oil as a biofuel, the possibility of tailoring cottonseed oil to other end uses, and the expanded industrial uses of the oils are influencing current research directions at all levels.

2.1 Physiology

Several research programs have studied the impact of agronomic inputs on oil and protein components of seed quality. Egyptian researchers have found that the addition of fertilizers, including potassium, zinc, and phosphorus increased oil and protein yields (Sawan et al. 2007). In addition, a high nitrogen rate was found to decrease seed oil content while increasing seed protein content (Sawan et al. 2001).

The environment has a large influence on seed quality in general. The bulk of storage reserves of the cottonseed are produced during later stages of seed development (Benedict et al. 1976). In areas of temperate climate, low temperatures at the later stages of seed development have a marked influence on seed oil. As temperatures decrease, the rate of boll development decreases, as does the seed oil content. Relative amounts of individual fatty acids change in response to temperature, but the pattern of response in cottonseed is not clear (Kohel and Cherry 1983). This relationship is made even less clear by the differences in developmental age of bolls on a plant at any given time.

Specifically, there is a large environmental source of variation for seed oil content (Kohel 1978; Shaver and Dilday 1982). Early researchers noted the environment influenced oil content greater than protein content (Turner et al. 1976a). In particular, soils with increasing salinity in Uzbekistan were found to produce cotton with decreased oil content (Yuldasheva et al. 2004). The microenvironment of the cotton plant and the method of harvest also influence seed quality. Bolls located next to the main stem on branches in the center of the plant have the highest yield and quality, but seed quality declined the longer the bolls remained in the field (Kohel and Cherry 1983; Conkerton et al. 1993). As the harvest gets later, oil and protein contents of cottonseed tend to decrease.

The response of seed development and oil content to moisture varies. Chronic moisture stress has not been shown to produce any negative changes on seed oil content (Cherry et al. 1981b). An acute moisture stress at a late stage of boll development may adversely influence seed oil content because seed may be arrested in their development. Cotton plants stressed at early stages of boll development or under chronic stress generally compensate by boll shedding so that seed oil content is affected to a limited extent (Kohel and Benedict 1984).

Several classes (types) of cottons have been evaluated for oil and protein quality. Kohel (1978) surveyed over 1,300 accessions in the U.S. *G. hirsutum* L. germplasm

collection for seed oil content. In a second publication, Kohel et al. (1985) surveyed the same accessions for protein content. The accessions came from two large groups of germplasm designated as "TX" and "SA." These surveys identified the "TX" germplasm having consistently more oil and less protein than the "SA" group. These tetraploid members of the U.S. collection were evaluated for their compositional quality (Kohel 1978; Kohel et al. 1985), and as one would expect, there was wide variation in the germplasm for seed composition. The greater variability was found in the unimproved cottons. However, these cottons varied for other seed properties such as seed size and hull thickness, which had a large impact on compositional content.

Shaver and Dilday (1982) evaluated seed quality in Mexico for a select group of "TX" germplasm. They identified some accessions with higher oil and protein than commercial checks, thus having the potential to increase these seed quality factors in a breeding program. Variability within *G. arboreum*, several wild species, and cotton hybrids were measured by Indian research programs. The *G. arboreum* germplasm had the largest seed oil percent, with a range from 18 to 25% (Agarwal et al. 2003). Among the wild species, *G. lobatum* had 22.9% seed oil followed by *G. harknessii* with 22.2% (Gotmare et al. 2004). *Gossypium stocksii* had the lowest seed oil percent (10.3%). Cotton varieties are commonly grown commercially as essentially pure lines that have been selfed to achieve a certain degree of uniformity. India pioneered the cultivation of hybrid cotton for commercial use. These hybrid cottons range in oil content from 17.9 to 23.1% and range in protein content from 36.0 to 44.3% (Rajput et al. 2007).

2.2 Genetics/Breeding

Cotton breeders have made efforts to understand the relationships of seed quality parameters with lint yield and lint quality in various genetic materials. When comparing to lint yield parameters, these relationships often varied from one study to another. In one study, increased oil and protein were negatively correlated with increased lint yield (Mert et al. 2005). In a separate study, Wu et al. (2009) found protein to be positively correlated with lint yield. Yet another study established that oil and protein had no significant correlations with yield (Turner et al. 1976b). Seed index was highly correlated with both the seed protein and seed oil indices (Wu et al. 2009). For fiber quality, conflicting relationships were also found. Oil was positively correlated with strength and negatively correlated with fiber length (Mert et al. 2005). Protein percent was significantly negatively correlated with oil percent (Turner et al. 1976b; Song and Zhang 2007).

Both Azhar and Ahmad (2000) and Khan et al. (2007) estimated narrow sense heritability for seed oil percent as moderate in the F_1 and high in the F_2. Heritability estimates for seed oil ranged from 35 to 53% in the F_2 primarily due to additive effects (Kohel 1980; Ramos and Kohel 1987; Wu et al. 2010). Therefore, selection for improved oil content could be both quick and effective using a recurrent selection program.

In contrast, separate genetic analyses have shown that protein and oil content were primarily under nonadditive (dominance) gene effects (Singh et al. 1985; Dani and Kohel 1989; Ashokkumar and Ravikesavan 2008). Though studies disagree over whether additive or dominance effects primarily control seed and protein content; overall, favorable genetic effects provide evidence that these seed traits can be genetically improved.

The oil-bearing tissue of the cottonseed is the embryo; therefore, the embryo derives its genotype from both parents. However, the maternal parent provides the nourishment for the growing seeds. The findings of research projects disagree whether cytoplasmic or maternal effects are more significant in determining seed oil content. Wu et al. (2010) have found that cytoplasmic effects are important in the inheritance of seed oil content. In a comparison of glanded and glandless cottons, Ramos and Kohel (1987) found that glandless genotypes, on average, have a higher seed oil percent, and maternal effects were not significant among these genotypes. Other studies identify the maternal plant rather than cytoplasmic effects as a greater influence when improving oil and protein indices (Dani and Kohel 1989; Ye et al. 2003).

3 Molecular Biology/Biotechnology

With the increased identification and use of molecular markers in cotton, studies have been designed to identify regions of the cotton genome (quantitative trait loci, QTL) that determine the oil content of cottonseeds. In the first report of seed quality QTL, Song and Zhang (2007) identified genomic regions on chromosome D8 as responsible for oil percent and on chromosome D9 as responsible for protein percent. Subsequent research using different genetic material associated chromosome 4 and the short arm of chromosome 9 with oil percent (An et al. 2010; Wu et al. 2009) and chromosomes 2, 9, and 12 with protein percent (An et al. 2010).

Biotechnology approaches may be applied to improve food security by making more food available. In addition, this technology may be used to enhance nutritional composition or health value of foods in both the developed and developing world. Cotton is well positioned for the application of biotechnology to nutrition. Whole, fuzzy cottonseeds are composed of 20% crude fat and 23% crude protein. Cottonseed is primarily used as an animal feed, but cottonseed oil is desirable as a vegetable oil for human consumption because it is trans-fat free oil. Worldwide, most cottonseed oil is produced (Table 9.1) and consumed (Table 9.2) in China. The oil contains a 2:1 ratio of polyunsaturated to saturated fatty acids. The fatty acid profile of cottonseed oil is compared with other common vegetable oils in Table 9.3.

To further enhance the health value of cottonseed oil, researchers aim to alter the fatty acid profile by increasing stearic and oleic acids while reducing the palmitic acid content. There are scattered reports of breeding attempts to improve the compositional quality of cottonseed. These reports are characteristically positive, but do not appear to represent any continuous effort to breed for improved seed quality (Cherry et al. 1981b). Lukonge et al. (2007) have evaluated the fatty acid profile

Table 9.1 World production of cottonseed oil

	Production (thousand metric tons)		
	2008/2009	2009/2010	2010/2011
China	1,600	1,466	1,493
India	1,030	1,045	1,089
Turkey	116	100	109
United States	301	277	306
EU-27[a]	47	50	52
Other	1,751	1,737	1,832
World total	4,845	4,675	4,881

Source: USDA-FAS (2010)
[a]EU-27: Economic and political group of 27 states that comprise the European Union

Table 9.2 World domestic consumption of cottonseed oil

	Consumption (thousand metric tons)		
Country	2008/2009	2009/2010	2010/2011
China	1,595	1,463	1,490
India	1,038	1,049	1,085
United States	225	234	250
Turkey	140	103	113
EU-27[a]	47	54	54
Other	1,768	1,746	1,838
World total	4,813	4,649	4,830

Source: USDA-FAS (2010)
[a]EU-27: Economic and political group of 27 states that comprise the European Union

Table 9.3 Range of typical fatty acid composition (%) of various vegetable oils

Fatty acid	Cottonseed	Olive	Palm	Peanut	Rapeseed (canola)	Soybean	Sunflower
Myristic (C14:0)	0.5–2.0	0.1–1.2	0.5–5.9	<0.2		<0.5	<1.0
Palmitic (C16:0)	17–29	7–16	32–47	6–15.5	3–6	7–12	2–10
Stearic (C18:0)	1–4	1–3	2–8	1.3–6.5	1–4	2.0–5.5	1–10
Palmitoleic (C16:1)	<1.5			<1.0		<0.5	<1.0
Oleic (C18:1)	13–44	65–85	34–44	36–72	55–75	19–30	14–65
Linoleic (C18:2)	40–63	4–15	7–12	13–45	15–25	48–58	20–75
Linolenic (C18:3)	0.1–2.1	<1.5		<2.0	8–22	5–9	<1.5

Source: http://www.connectworld.net/whc/images/chart.pdf

in seed of cotton accessions and have shown that stearic and palmitic acids were positively correlated. These data suggest that it would be difficult to increase stearic acid while decreasing palmitic acid using selection. Where a plant breeding approach may be difficult, molecular approaches have been taken to modify the biosynthetic pathways of these fatty acids.

Several groups have successfully engineered high-oleic acid transgenic cottonseed lines through suppression of key enzymes in the fatty acid biosynthetic pathway (Chapman et al. 2001; Liu et al. 2002a, b; Sunilkumar et al. 2005). Liu et al. (2002a, b) were able to increase oleic acid from 15 to 77% and, in a separate transgenic line, increase stearic acid from 2 to 40%. However, the lines with increased stearic acid had poor germination and reduced survival.

4 Processing Cottonseed

For every pound of fiber, the cotton plant produces approximately 1.6 lb of cottonseed. This seed is fed to cattle, used as raw material in the cottonseed processing industry, and a small amount is exported. Throughout its history, cotton has been grown primarily for its fiber. With the development of the crushing industry and the more recent interest in cottonseed for biodiesel and for human food, however, the use of cottonseed on a commercial scale is gaining interest. Until the crushing industry developed, cottonseed generally had no cash value. Small quantities of seed were used for planting the next year's crop, for fertilizer, and for livestock feed. As cotton oil mills began to operate, the value of cottonseed increased.

The components of cottonseed are separated at an oil mill in a process called crushing. In the first step of crushing, ginned seeds are cleaned using screens to remove any leaves, twigs, or other trash. After cleaning, the short fibers still attached to the seed (linters) are removed with delinting machines. The delinting machines are similar to cotton gins and use circular saws to cut off the short fibers. After the linters are removed, the protective hull, which surrounds the cottonseed kernel, is cut and loosened. The hulls are separated from the kernels. After separation, the hulls are ready for marketing as animal feed. The kernels, or meats, are further processed and oil is extracted.

According to the National Cottonseed Products Association, in the last 50 years, major changes have been made in methods of removing oil from cottonseed. Extracting the oil was initially performed using a labor-intensive hydraulic press. Today, oil is removed from the seed primarily by mechanical screw presses, by solvent extraction, or both. For both processes, meats (kernels) pass through a series of heavy rollers that form the meats into thin flakes. In screw pressing, the flakes are first "cooked" to reduce moisture. They then move into the screw press. The screw press operates similar to a meat grinder. Oil is forced from the meats and flows through small openings in the barrel of the press to a chamber below. From there it is filtered and put in storage tanks. The extracted flakes come out of the other end of the press. After cooling, the flakes are ground into meal. The newest technology uses an expander which helps release the oil and prepares the kernels for oil extraction. The expanded kernels are exposed to an organic solvent that dissolves out the oil. The solvent is recovered and can be reused. Extracted kernels are also ground into meal.

Crude cottonseed oil from the mill requires further processing before it is used in food. The oil is refined, bleached, winterized, and deodorized before it can be used as food oil. During refining, sodium hydroxide is added and combines with the soapstock or "foots" portion of the crude oil. A centrifuge separates the soapstock and heavier impurities from the oil. During bleaching, a special type of clay is added that combines with the compounds that give the oil its yellow color. This clay is then filtered from the oil. Winterizing separates the components of oil that tend to turn cloudy and become solid at lower temperatures. Finally, deodorizing removes unwanted flavors and is the final purifying step in processing before its use as food oil.

5 Utilization of Cottonseed

Whole cottonseed is a source of protein (20%), energy (87%), and fiber (22%) for livestock (Ely and Guthrie 2008). Animal nutritionists recognize ginned whole cottonseed as a premium supplement for cattle and other ruminant animals (Blasi and Drouillard 2002). Cottonseed oil and meal are the two most valuable products of cottonseed. Oil makes up 16% of the products resulting from crushing cottonseed in an oil mill. Cottonseeds contain a significant amount of tocopherols, forms of Vitamin E, which contribute to the long shelf life of cottonseed oil (Smith and Creelman 2001). In addition to stability, cottonseed oil has no cholesterol, is high in polyunsaturated fatty acids, moderate in monounsaturated fatty acids, and low in saturated fatty acids (Table 9.3). This profile is considered heart healthy by many medical professionals. Most of the cottonseed oil used in the USA is consumed as salad or cooking oil. The remaining oil is used in shortening and in margarine.

Cottonseed meal is the second most valuable product of cottonseed. It may be sold in the form of meal, cake, flakes, or pellets. Cottonseed meal is used principally as feed for livestock and its major value is as a concentrated protein supplement. Fish farms are an emerging market for cottonseed meal. Fish farmers use cottonseed meal as an economical, highly nutritious alternative to fish meal. Fish meal is composed primarily of wild-caught fish, and the price of fish meal continues to climb as natural fish stocks decline.

5.1 Gossypol

Cotton is characterized by the presence of glands in the aerial vegetation and in the seeds. The glands form in the space left following lysis of cells. These lysigenous glands contain gossypol, which is an antinutritional compound, and dark pigments. Chemically gossypol is a sesquiterpene, a class of hydrocarbons which acts as a natural defense mechanism. In the aerial vegetation, gossypol and its precursors are present in the glands. In the seeds, gossypol and its isomeric forms are present in

highly compartmentalized glands. Gossypol is sequestered in the glands and does not accumulate in other tissues.

Glanded cottonseeds can be fed to ruminant animals where microbial action in the rumen breaks down gossypol. There is some limitation in the amount of cottonseed, as a source of protein, which can be fed to lactating cows because high concentrations of glanded cottonseed in the feed can lead to the presence of gossypol in the milk. In monogastric animals, the gossypol in glanded cottonseed binds with protein causing protein deficiency and toxic effects. Because of this action of gossypol, it has been used as a male contraceptive (Wen 1980; Tsui et al. 1983). It is apparent that the removal of gossypol glands/gossypol from cottonseed would be beneficial (Hess 1976; Anonymous 1977; Rathbone 1977; Kohel and Yu 2007). In cottonseed crushing, oil is extracted and the meal is heat treated to bind gossypol. In the crushing process, some gland components are extracted with the oil that requires further refinements. These contaminants also limit oil storage so that periodic bleaching is required under long periods of storage. The heat treatment to bind gossypol binds it primarily with lysine. This lowers the quality of the amino acids in the meal. Supplemental lysine must be added in feed rations to compensate for the lysine lost during crushing.

If glands/gossypol were not present in the seeds, simpler and less energy-intensive processing could be utilized for oil extraction (Rathbone 1977). Such simpler processing would produce better quality oils and meal, and the meal would have a wider use potential. It could be a feed for both ruminant and monogastric animals as a higher quality protein source with greater economic value. A gland-free/gossypol-free cottonseed could be a human food source. Cotton is a global crop, and many areas of production are areas where human diets are deficient in proteins. The uses of glandless cottonseed as a food protein have been summarized by Lusas and Jividen (1987). The cottonseed kernel is the most common cottonseed product used commercially in food products. Flours, concentrates, and isolates from glandless cottonseed also have potential food uses due to diverse functionality and a protein content comparable with soy.

During the 1970s and 1980s there was a renewed interest in cottonseed, with emphasis on gossypol-free products for human food (Hess 1976; Anonymous 1977; Rathbone 1977). Several methods were developed to remove gossypol from cottonseed. They included chemical, mechanical, and genetic. Chemical methods were developed and used experimentally for gossypol removal (Cherry and Gray 1981). The most advanced mechanical separation method was the liquid cyclone process (Vix et al. 1971; Gardner et al. 1976). A pilot plant was built and successfully operated by the Plains Cottonseed Cooperative. However, the plant was not built for food-grade production, and unrelated financial problems halted further development and the project was terminated.

McMichael (1960) discovered two recessive genes ($gl_2gl_2gl_3gl_3$) that produced gland-free cotton plants. The genes originated in semiwild, nonadapted cottons. There was considerable linkage drag when trying to develop adapted cultivars. However, several commercial companies and public breeders developed improved agronomic cultivars. The most notable of these was Rogers Delinted Cottonseed Company.

This company not only bred glandless cottons, but they obtained FDA approval for a human food product. The approval was for a dehulled, roasted, whole kernel product sold under the name of "Cot-N-Nuts®." They marketed to the confectionary trade, and the main user was an energy bar product. Unfortunately, this product was labeled as hypoallergenic. Cottonseed, as other oilseeds, contains the same fraction of proteins that can cause allergenic reactions (Coulson et al. 1941, 1943). When consumers of the energy bar developed allergenic reactions, and lawsuits followed, Rogers Delinted Cottonseed Company went out of business.

Furthermore, the absence of gossypol glands in aerial vegetation of the cotton plant made the plants more attractive to other pests. Chewing insects fed vigorously on glandless plants, rabbits fed on young plants, and rodents would eat the seeds (Hinze et al. 2011). The degree to which these were major problems to prevent the successful production of glandless cultivars was never fully evaluated. These problems were risks that were a disincentive to the adoption of glandless cultivars. Breeding programs, other than Rogers Delinted Cottonseed Company, had not made a major commitment to glandless cultivars, and the two recessive glandless genes, with linkage drag, were a further hurdle to overcome. The later report of a single semidominant gene ($Gl_2^eGl_2^e$), which produced glandless cotton plants and seeds, failed to invigorate additional interest (Kohel and Lee 1984). The perceived production problems, and no clearly identified product potential, left glandless cotton breeding in a state of limbo.

The obvious advantages of a glandless/gossypol-free cottonseed continue to spur research efforts. Researchers have tried without success to introduce the glanded plant/glandless seed trait from *G. sturtianum* (Dilday 1986; Altman et al. 1987; Mergeai et al. 1997; Vroh et al. 1999). The use of biotechnology tools offers hope of producing a glanded plant with glandless seeds. Such a plant would avoid the understandable reservations of growing a plant that is glandless.

Different biotechnological approaches to produce gossypol-free cottonseed have been proposed: from the stopping of gossypol synthesis, to the degradation of gossypol, and to the prevention of gland formation (Koshinsky et al. 1994; Chen et al. 1995, 1996; Yu et al. 2000a, b; Decanini et al. 2001; Kohel et al. 2001; Martin et al. 2003). The theory of the approaches is rather straightforward; however, the implementation has proven to be difficult and complex. To date there has been successful interruption of gossypol synthesis to the extent that gossypol has been reduced, but not eliminated (Sunilkumar et al. 2006).

Cotton is the second most important oilseed. However, since the fiber is cotton's primary and most valuable product, the major thrust of breeding programs is devoted to fiber. The seed cannot be ignored, however, and the production of gossypol-free seed would enhance the overall value of cotton production. Cottonseed oil is an established industry that would not have to change, but it could be enhanced with better quality and lower costs with gossypol-free seeds. However, the main change with the production of gossypol-free seed would be the protein component of the seed. Not only would the quality be enhanced by no longer binding lysine, but also wider uses could be found as livestock feed, pet foods, and human food.

5.2 Chemical Composition

Numerous studies have reported on the variability of cottonseed constituents and chemical composition (Stansbury et al. 1956; Pandey and Thejappa 1975; Sood et al. 1976; Cherry et al. 1978, 1981a; Kohel 1980, 1998; Cherry and Leffler 1984; Kohel and Cherry 1983; Kohel et al. 1985). Currently, the National Cotton Variety Tests report on the percentage of oil, protein, and gossypol in the cottonseed samples. Values are usually reported as a percentage of the whole seed, flour, protein fraction, or oil fraction. The use of percentages is useful in merchandizing ginned whole cottonseed, but they are more difficult to interpret when trying to determine specific sources of variation and specific effects because percentage values are interdependent. In general, the experimental results reported variability associated with years, locations, and cultivars. Cultivars generally show the largest source of variability. The average cottonseed constituents for the national standards in the National Cotton Variety Testing Program (2007) were seed index = 9.4 g/100 seed, oil = 20.04%, protein = 23.38% (nitrogen% × 6.25), and gossypol = 1.27%. These values are based on fuzzy cottonseed. Acid-delinted seeds have higher percentage of the constituents because the variable amount of fuzz is removed, but the oil- and protein-bearing tissue, the kernel/embryo, is the tissue of interest. The seed coat represents about 38% of the acid-delinted seed so that there is about 40% oil in the kernel/embryo (Kohel 1978). The oil is made up of primarily three fatty acids, palmitic (C16:0) = 24.18%, oleic (C18:1) = 17.51%, and linoleic (C18:2) = 54.23%. The key remaining fatty acids are myristic (C14:0) = 0.89%, palmitoleic (C16:1) = 0.71%, and stearic (C18:0) = 2.60%.

Cottonseed flour has a high quality amino acid profile (Table 9.4). Glandless/gossypol-free flour would not be reduced in quality by the heat binding of gossypol to amino acids (in particular, lysine). Gland-free/gossypol-free flour could be used as a protein source in combination with other ingredients to produce various products. The original "Incaparina" formulation (protein-rich dietary supplements based on cottonseed flour, or soya and vegetables, Bender 2005) included 38% glanded cottonseed flour (Call and Levinson 1973; Popkin and Latham 1973; Orr 1977). Such nutritional products would be enhanced with the availability of gossypol-free flour. Experimentally, various products were produced to evaluate the degree to which cottonseed flour could be added to produce an acceptable product (Cherry et al. 1981a; Cherry 1983, 1985). A project was reported to use glandless cottons to improve the nutrition of the Ivory Coast diets (Bourely 1988). Another extensive project was in Egypt in which glandless cottonseed enhanced cookies were produced to provide a protein supplement for school children. Despite the success of this activity, widespread glandless cottons were not adapted to sustain this effort (Anonymous 1980).

Although gossypol-free flour has a wide range of uses, protein extracts should provide higher value uses in human food products. Protein extracts can be isolated from storage proteins, nonstorage proteins, or the total protein fraction (Lawhon et al. 1977; Lusas et al. 1977; Cherry and Berardi 1982; Rhee 1988). Proteins of

Table 9.4 Composite amino acid profile of fat-free cottonseed flour

Amino acid	(g/100 g sample)
Alanine	2.13
Valine	2.17
Glycine	2.21
Isoleucine	1.56
Leucine	3.24
Proline	1.97
Threonine	1.73
Serine	2.38
Methionine	0.76
Phenylalanine	2.82
Aspartic acid	4.92
Glutamic acid	10.42
Tyrosine	1.50
Lysine	2.46
Histidine	1.39
Arginine	5.42
Half cystine	0.67
Total	47.74

Source: Cherry et al. (1978, 1981b) and Salunkhe et al. (1992)

cottonseed are highly digestible in humans. The relative human digestibility of proteins from several sources is cottonseed flour (90%), soy flour (86%), milk and cheese (95%), peanuts (94%), and rice flour (88%) (Anonymous 2007). The amino acids in cottonseed flour make up about 50% of the defatted flour. The amino acid profile is variously expressed as a percentage of the flour or percentage of the protein fraction (Table 9.4).

6 Future of Cottonseed

Cottonseed is currently consumed as oil, meal, and a whole seed animal feed. From this review of the research on cottonseed quality, it is apparent that the presence of the antinutritional constituent, gossypol, is a limiting factor in its utilization. The technology and genetic resources are available to remove glands/gossypol from cottonseed and its products. The limitations to do this are the funding for their implementation and the economic drivers for markets for this new cottonseed and its products. A gossypol-free cottonseed would compete as new products in already existing markets.

Products from gossypol-free cottonseed could be used as feed for monogastric livestock and in the higher valued pet and human food product industries. However, as a human food, only dehulled, roasted, whole-kernel, glandless cottonseeds have FDA approval. Therefore, regulatory approval would have to be obtained for additional human food items.

From the production side, the growing of glanded and gossypol-free cotton cultivars would have to be segregated to prevent contamination. This segregation would have to continue through the ginning and processing of the cottonseed. The human food maximum allowable limit of gossypol is 450 ppm, which would require vigorous control of the gossypol-free cottonseed production. Mechanical or chemical removal of glands/gossypol would not require such controls of the growing or ginning of cottonseed. However, they require changes and large initial investments in these technologies. The long-term advantage is in genetic development of glandless/gossypol-free cottonseed cultivars. Such was the prediction in 1954 (Eckey 1954).

Cottonseed oil is considered to be premium cooking oil in that it is trans-fat free. According to the National Cottonseed Products Association, since New York City announced it would ban trans-fats from restaurants, the demand for cottonseed oil has doubled. The demand is expected to continue with bans in Philadelphia, statewide in California, and more bans likely to come. In addition, cottonseed oil can be certified Kosher. Many legumes are not considered Kosher, so cotton's primary competitors, including peanut, soybean, and even corn oil due to formulations blending corn with legumes, cannot compete in this marketing opportunity.

New opportunities for food use are now also possible because of recent biotechnological advances in producing seed with reduced gossypol (Sunilkumar et al. 2006; Townsend and Llewellyn 2007). The process currently used to remove gossypol from cottonseed damages protein value (Freidman 1996). If seed can be produced completely gossypol free, then the protein value of cottonseed should also inevitably improve.

Historically, the largest market for cottonseed has been the dairy industry. That has recently changed as the best market for cottonseed is now the food processing industry due to their demand for cottonseed oil. Those seeds that were once sold only to offset ginning costs are now being viewed as an increasingly important source of income. It's not just about the fiber value per acre anymore. The industry is viewing cottonseed as a viable source of revenue, thereby adding value to each and every acre of cotton.

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Chapter 10
Castor

José M. Fernández-Martínez and Leonardo Velasco

Abstract Castor (*Ricinus communis* L.) is a very ancient oilseed crop cultivated because of the high oil content of the seeds, which ranges between 42 and 58%. The oil contains a high proportion (84–90%) of ricinoleic acid, a monounsaturated hydroxy fatty acid with multiple industrial applications such as paints and varnishes, cosmetics, polymers, biolubricants and biofuels. This chapter summarizes breeding objectives and crop improvement methods and techniques used to breed cultivars in castor. The most important breeding objectives are related to plant architecture and adaptation to mechanized harvest, development of male sterility systems for exploitation of heterosis, agronomic traits associated with high yield and yield stability, adaptation to specific environments, resistance to biotic and abiotic stresses, high seed oil content, diversification of seed oil quality and elimination of toxic compounds of the seeds. Despite being a highly cross-pollinated species, castor shows little inbreeding depression, which determines that breeding methods for self-pollinated crops together with common methods for cross-pollinated species such as recurrent selection are suitable for castor breeding. Additionally, hybrid breeding as a means of exploitation of heterosis has been an important aspect of cultivar development. Major landmarks in castor breeding have been the identification of dwarf-internode mutants, male sterility systems that facilitated the development of commercial hybrids, the identification of strains with high oleic acid content and low content of toxic compounds, and the development of efficient regeneration and transformation protocols. In the near future, the increasing demand for the use of vegetable oils in non-food applications such as biofuels and biolubricants is expected to stimulate the development of castor as an industrial crop that do not compete in the food markets.

J.M. Fernández-Martínez (✉)
Institute for Sustainable Agriculture (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain
e-mail: cs9femaj@uco.es

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1 Introduction

Castor (*Ricinus communis* L.) is a very ancient oilseed crop. It is cultivated because of the high oil content of the seeds, which ranges between 42 and 58%. Castor oil is not suitable for edible purposes because it contains a high proportion (84–90%) of ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid), a monounsaturated hydroxy fatty acid. Instead, the oil has been extensively used in local medicines mainly as a laxative, as a lamp oil, and more recently in the manufacturing sector (Brigham 1993; Weiss 2000).

Currently castor is a minor crop at a world scale, with castor oil accounting for less than 0.15% of the international vegetable oil market (Scholz and Nogueira da Silva 2008). Castor bean production is concentrated in India, China, Brazil and Mozambique, which together account for more than 90% of castor world acreage and production (Table 10.1). Average seed yield is 944 kg/ha, but there are large differences between major producing countries, from 357 kg/ha in Mozambique to 1,181 kg/ha in India. This is probably due to the fact that much of the harvested crop in some countries is obtained from seeds collected from semi-wild plants instead of commercially cultivated fields, and accordingly statistics are largely based on estimates (Atsmon 1989). Nevertheless, in some countries castor is cultivated as an annual crop making use of improved production techniques and high yielding cultivars well adapted to mechanized cropping.

Castor can be regarded as an underutilized oilseed crop. It has a great potential because of multiple industrial applications of its high-ricinoleic seed oil such as paints and varnishes, cosmetics, polymers, biolubricants and biofuels (Brigham 1993; Weiss 2000). Future prospects for an increasing demand of castor oil are largely based on its use for biodiesel production. However, the high viscosity of the methyl ester of ricinoleic acid, which exceeds the maximum values for kinematic viscosity in biodiesel standards, hampers the use of castor oil for biodiesel production (Knothe 2008). The discovery of a castor mutant in which ricinoleic acid is partly replaced by oleic acid, which accounts for 78% of the total fatty acids (Rojas-Barros et al. 2004) opens up new perspectives for large-scale use of castor oil as a biodiesel feedstock.

Castor scientific breeding traces back to the 1920s, when S.C. Harland and J.E. Peat in the UK, O.E. White in the US and G.M. Popova and V.E. Borkovskii in the USSR initiated genetic and breeding studies on this crop (Moshkin and Dvoryadkina 1986). Major landmarks in castor breeding have been the identification of dwarf-internode mutants (Krug et al. 1943), male sterility systems that facilitated the development of commercial hybrids (Claassen and Hoffman 1950; Zimmerman and Smith 1966), the identification strains with high oleic acid content (Rojas-Barros et al. 2004) and low content of toxic compounds (Auld et al. 2003), and the

Table 10.1 World castor bean production area, seed yield and total seed production of major producing countries*

Country and continent	Production area (Ha)	Seed yield (kg/ha)	Total production (tonnes)
World	1,456,924	962	1,404,916
Asia	1,070,681	1,109	1,190,123
China	235,000	936	220,000
Cambodia	1,400	1,000	1,400
India	795,240	1,181	944,420
Indonesia	6,473	175	1,151
Pakistan	5,750	557	2,659
Thailand	13,310	805	10,715
Vietnam	7,000	714	5,000
Africa	206,490	402	83,014
Angola	13,500	259	3,500
Ethiopia	5,460	1,029	5,600
Kenya	13,000	231	3,000
Madagascar	7,240	345	2,500
Mozambique	140,000	357	50,028
South Africa	8,000	612	4,900
America	189,190	748	141,954
Brazil	174,924	725	127,237
Ecuador	1,363	1,602	2,185
Haiti	2,600	546	1,420
Paraguay	9,800	1,100	10,760
Europe	998	517	532
Russian Federation	950	517	522

*Average data from 2004–2008 (FAOSTAT, 2009)

development of efficient regeneration and transformation protocols (Sujatha and Sailaja 2005; Ganesh Kumari et al. 2008). The castor genome has been already sequenced though the results have not been published yet (Foster et al. 2010), which will lead to the development of novel molecular tools for castor breeding in the short term.

2 Origin and Domestication

Castor is one of the oldest cultivated plants. Castor seeds have been found in some 4,000-year-old tombs of famous personages of the ancient Egypt, in particular priests (Scarpa and Guerci 1982). Castor oil was extensively used in medicine in ancient Egypt, as a component of drugs, healing unguents, and as laxative (Aboelsoud 2010). Medicinal uses of castor oil were exported from Egypt to Greece, Roma, India and China (Scarpa and Guerci 1982). Classical authors such as Herodotus, Diodorus, Strabo and Pliny mention the use of castor oil in Egypt as a lamp oil. Strabo adds that the poorer people used the oil for anointing the body (Manniche 1989).

The castor plant (*R. communis* L.) belongs to the monotypic genus *Ricinus* of the *Euphorbiaceae* (spurge family), which contains some 280 genera many of them

tropical (Weiss 2000). It is a diploid species with basic chromosome number $n = 10$. Castor is indigenous to East Africa and most probably originated in Ethiopia as suggested by the large diversity of plants (Vavilov 1951). Moshkin (1986a) also considered East Africa and Ethiopia as the centre of diversity of castor and proposed four additional centres of origin of castor cultivation in Northwest Asia, Mediterranean and Southwest Asia, India and China. Nowadays, castor has a worldwide distribution in the warmer regions (Weiss 2000).

Several authors have classified *R. communis* into different species or subspecies on the basis of morphological traits and geographical distribution (Schultze-Motel et al. 1982; Popova and Moshkin 1986). However, none of them are nowadays accepted as true species or subspecies and they are merely local types adapted to environmental pressure or human selection (Weiss 2000).

3 Genetic Resources

Castor bean genetic resources can be categorized as ex situ resources, i.e. germplasm accessions preserved in seed banks and in situ resources. The latter include landraces of castor cultivated as an oil plant as well as ornamental plants in gardens. Also, castor plants commonly escape from cultivation and are found in disturbed sites such as roadsides, stream banks, abandoned lots and edges of cultivated fields. Additionally, it is considered an invasive weed throughout much of its range of distribution (Weber 2003). These feral semi-wild castor populations constitute very valuable sources of germplasm of potential use in breeding programs, especially for characters related to adaptation to localized diseases and pests and to specific environmental conditions (Auld et al. 2009).

Extensive ex situ collections of castor germplasm are maintained in several countries. Auld et al. (2009) estimated 6,588 accessions at 39 gene banks. The major germplasm collections are maintained at the National Crop Gene Bank of China, with 2,073 accessions (http://icgr.caas.net.cn/cgrisintroduction.html, accessed 1 July 2010), the United States Department of Agriculture National Plant Germplasm System, with 1,043 accessions (http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?31896, accessed 1 July 2010), EMBRAPA Genetic Resources and Biotechnology of Brazil, with 778 accessions (http://www.cenargen.embrapa.br/recgen/sibrargen/bradenom-port.html, accessed 1 July 2010), the N.I. Vavilov Institute of Plant Industry of Russia, with 731 accessions (http://www.vir.nw.ru/data/dbf.htm, accessed 1 July 2010), and the Institute of Biodiversity Conservation of Ethiopia, with 436 accessions (http://www.ibc-et.org/conservation/database-ms, accessed 1 July 2010).

Castor genetic resources are an invaluable source of traits of interest for castor breeding, such as traits related to insect and disease resistance, agronomic performance, oil quality and male sterility. Specific examples on the role of genetic resources in castor breeding are given in the next section.

4 Breeding Objectives

The most important breeding objectives are related to plant architecture and adaptation to mechanized harvest, development of male sterility systems for exploitation of heterosis, agronomic traits associated with high yield and yield stability, adaptation to specific environments, resistance to biotic and abiotic stresses, high seed oil content, diversification of seed oil quality and elimination of toxic compounds of the seeds.

4.1 Adaptation to Mechanized Harvesting

Plant ideotype for mechanized crop harvesting includes short stature, determinate growth habit with reduced number of racemes per plant, dense racemes and indehiscent capsules that are easy to hull (Vanozzi et al. 1983; Baldanzi and Pugliesi 1998; Savy Filho 2007a).

4.1.1 Plant Height

Bhardwag et al. (1996) evaluated plant height in the USDA castor germplasm collection and found a broad variation from 64 to 242 cm. Plant height is determined by the number of nodes and the length of internodes (Laureti and Brighan 1987). The shortest plants are derived from dwarf-internode germplasm identified in Brazil before 1936 (Krug et al. 1943) and introduced in U.S. breeding programs in 1938 (Brigham 1970a). The trait is controlled by a single recessive gene, which determines reduced internode length but does not affect the number of nodes (Zimmerman 1957), which allows selection for plant height within germplasm carrying the dwarf-internode gene (Laureti and Brighan 1987). Examples of dwarf-internode germplasm and varieties showing significant differences for plant height are the composite germplasm T55001 (0.5–0.9 m; Brigham 1973), and the cultivars Lynn (0.9–1.2 m; Brigham 1970c) and Dawn (1.1–1.5 m; Brigham 1970a). Genotypes other than those derived from the dwarf-internode mutant have been developed by recurrent selection for reduced number of nodes and reduced internode length (Laureti and Brighan 1987; Oliveira and Zanotto 2008). Heritability of plant height in castor has been estimated in 0.60 (Carvalho do Amaral 2003).

4.1.2 Number of Racemes and Determinate Growth Habit

Mechanical harvesting requires simultaneous maturation of all racemes in the plant. Savy Filho (2007a) recommended cultivars producing preferably one raceme per plant, or a maximum of two racemes, for mechanical harvesting. Castor is naturally

multi-branched, with one primary raceme and a varying number of secondary and tertiary racemes (Weiss 2000). Branching is largely influenced by environmental and genetic factors, the latter being mainly additive (Baldanzi and Pugliesi 1998). Modern cultivars are characterized by a low number of secondary racemes. Laureti et al. (1998) evaluated five cultivars in Italy and observed an average number of racemes per plant of 1.3. Koutroubas et al. (2000) reported average number of secondary racemes between 1.9 and 4.0 in the evaluation of 19 cultivars in six environments in Greece. At the cultivar level, the average number of secondary racemes per plant ranged between 0.3 and 1.4 in cultivar B9 and between 4.4 and 6.3 in cultivar 929.

4.1.3 Capsule Indehiscence

Capsule indehiscence is the result of thinner capsule walls and is controlled by one to three recessive genes (Zimmerman 1958; Moshkin and Dvoryadkina 1986). Despite the trait is recessive, its introgression into dehiscent cultivars is achieved in a few generations (Banzatto et al. 1963).

4.2 Male Sterility

The castor inflorescence is a monoecious raceme of cymes. The more general situation is a separate distribution of female or pistillate flowers and male or staminate flowers, with pistillate flowers in apical position and staminate flowers in basal position (George and Shifriss 1967). The percentage of pistillate and staminate flowers usually ranges from 30 to 50% and 50 to 70%, respectively, even though these percentages vary with genotypes and growing environment (Claassen and Hoffman 1950). However, other patterns of sex differentiation can be found, such as strictly pistillate, interspersed staminate flowers in the apical pistillate region, and entirely interspersed inflorescences (Shifriss 1956).

Two different types of male sterility, with strictly pistillate inflorescences, have been identified in castor. The N-pistillateness type is controlled by recessive alleles at a single locus and is also affected by modifying genes and environmental factors (Claassen and Hoffman 1950; Shifriss 1956; Zimmerman and Smith 1966). The N-pistillateness is maintained by sib-mating, as the progeny from seed produced on female plants segregates in a 1:1 ratio for female and monoecious plants. For F_1 seed production, monoecious plants are rogued before anthesis. The S-pistillate type was obtained by selection within sex-reversal variants in Israel (Shifriss 1960). Sex-reversals are plant variants which start out as female and revert to normal monoecism at any time after the first raceme. However, it is possible to develop true-breeding female lines through continuous inbreeding of late reverters (Shifriss 1960). Even though the inheritance of S-pistillateness has not been completely resolved, this male sterility system allows efficient F_1 hybrid seed production and requires to rogue a lower proportion of plants than the N-pistillate system.

Zimmerman and Smith (1966) developed a temperature-sensitive pistillate strain that combines the temperature-insensitive N-pistillate gene and temperature-sensitive genes for interspersed staminate flowers. The strain is widely used in the production of commercial F_1 hybrids, which is normally pistillate under moderate temperatures, and interspersed staminate under high temperatures (Zimmerman and Smith 1966; Brigham 1980). Differentiation of environmentally sensitive staminate flowers is determined by a system of polygenes (Zimmerman and Smith 1966), whereas two major genes underlie temperature-insensitive occurrence of interspersed staminate flowers (George and Shifriss 1967).

4.3 Seed Yield

4.3.1 Yield Components

For a given plant density, castor seed yield ultimately depends on the number of racemes per plant, the number of capsules per raceme and the thousand seed weight (Hooks et al. 1971; Giriraj et al. 1974; Koutroubas et al. 2000; Solanki and Joshi 2000; Sarwar and Chaudhry 2008). The number of capsules per raceme is in turn determined by raceme length and density, defined as number of capsules per unit of raceme length (Laureti and Brighan 1987). The relative weight of yield components on seed yield determination largely depends on the genetic structure of the germplasm and the local environmental conditions. Once determined the yield components with greater effect on seed yield, selection for yield components is advantageous as they show significant additive effects and a higher level of heritability than seed yield (Sarwar and Chaudhry 2008).

4.3.2 Combining Ability

Despite inbreeding in castor results in little loss of plant vigour, F_1 hybrids express a significant heterosis (Zimmerman 1958), which is partially maintained in the F_2 (Voskoboinik 1986a). Maximum heterosis values for seed yield around 30% have been reported (Zimmerman 1958; Hooks et al. 1971; Voskoboinik 1986a; Golakia et al. 2008).

Breeding for seed yield of F_1 hybrids involves the selection of inbred lines with high combining ability, i.e. their aptitude to produce hybrids with high seed and oil yields. Selection of useful inbred lines is most commonly based on measurements of general combining ability (GCA) with one or several tester lines or hybrids (Laureti 1987). In a further step, the best female lines are crossed with the best male lines and further yield trials are necessary to determine the best specific combining abilities. All these trials are an expensive part of breeding programmes (Bonjean 1991).

4.4 Adaptation to Specific Environments

Adaptation to particular environments may require selection for specific traits, e.g. short stature in areas with great lodging risk, germination at suboptimal temperatures and early maturity in areas prone to summer drought, absence of spines to facilitate manual harvesting, etc. (Atsmon 1989; Brigham 1993).

4.4.1 Day-Length Reaction and Earliness

Castor is basically a long-day plant, but adapts with some loss of yield to a wide range of day length (Weiss 2000). However, mutants with loss of day-length sensitivity have been developed (Donini et al. 1984).

The development of early genotypes, able to mature earlier than standard types, is often required in areas where high temperatures, severe droughts, or even frosts in Northern latitudes may occur during the latter part of the growing season, in order to allow the crop to escape these unfavourable conditions (Laureti 1987). In India, where 80% of castor production is located in rainfed areas prone to drought, several extra-early accessions that matures in less than 100 days after planting, compared to more than 120 days in conventional early cultivars, have been identified (Anjani 2010). In Brazil, cultivars are considered as early maturing when they mature in less than 140 days (Savy Filho 2007a).

4.4.2 Germination at Low Temperatures

The minimum soil temperature for castor germination is 16°C (Thomas 1960), with an optimum between 18 and 23ºC (Bonjean 1991). For castor to be grown as a spring crop in Mediterranean-type climates, the development of genotypes able to germinate at lower temperatures to escape summer droughts would be required. Genetic differences in susceptibility to low seedbed temperatures were reported in Russia, where seedbed temperatures of 8–12ºC did not affect emergence of local cultivars (Weiss 2000). Large-scale studies on the variability available in germplasm for germination at suboptimal temperatures have not been carried out yet.

4.4.3 Spineless Capsules

Extremely spiny capsules are a deterrent for manual harvesting. Germplasm with complete absence of spines in the capsules is available and the trait is controlled by recessive alleles at a single locus (Smith 1963).

4.5 Resistance to Biotic Stresses

4.5.1 Diseases

There are a number of diseases occurring on castor, but only a reduced number of them are important at a world scale. The most important ones are *Alternaria* blight caused by *Alternaria ricini* (Yoshii) Hansford, bacterial leaf blight caused by *Xanthomnas campestris* pv. *ricini*, *Botrytis* gray rot caused by *Botrytis ricini* Godfrey (anamorph)/*Botrytonia ricini* (Godfrey) Whatzel (telomorph), sclerotial wilt or root rot caused by *Macrophomina phaseolina* (Tassi) Goidanich, seedling blight caused by *Phytophthora nicotianae* Breda de Haan var. *parasitica* (Dastur) Waterhouse, and wilt caused by *Fusarium oxysporum* f.sp. *ricini* Nanda and Prasad (Dange et al. 2005). *B. ricini* may infect the racemes but also participates together with other unidentified mould fungi in a devastating disease complex known as capsule drop (Culp 1966). Wilt caused by *F. oxysporum* may occur in combination with the reniform nematode *Rotylenchulus reniformis* Linford and Oliveira, which increases the severity of the symptoms (Chattopadhyay and Reddy 1995).

Several sources of resistance to bacterial leaf blight have been identified. The first source was the cultivar Cimarron (Calvert et al. 1953), from which other resistant cultivars such as Hale (Brigham 1970b) and Lynn (Brigham 1970c) were developed. Singh et al. (1976) also identified several sources of resistance in India.

Fusarium wilt is one of the most devastating castor diseases. Incidence of this disease has been reported to reach 28% in Russia (Sviridov 1986) and 85% in North Gujarat, India (Dange 2003). Important efforts have been devoted to evaluate germplasm for resistance to *Fusarium* wilt by cultivation in wilt sick plots as well as using root dip inoculation in conidial suspensions of the fungus, which has led to the development of several sources of genetic resistance (Prasad and Bhatnagar 1981; Sviridov 1984, 1986; Anjani et al. 2002; Lavanya et al. 2003; Anjani 2005; Dange et al. 2005). *Fusarium* wilt resistance in the female line VP-1 was developed by mutagenesis (Lavanya et al. 2003). Dange et al. (2005) emphasized the role of the reniform nematode in breaking down resistance to *Fusarium* wilt in India, which encouraged further evaluations to identify germplasm resistant to the *Fusarium*-nematode wild complex. Several studies reported different modes of inheritance of resistance to *Fusarium* wilt, which may depend upon the castor genotype, the virulence of the pathogen, and interactions with other pathogens such as the reniform nematode. Moshkin and Dvoryadkina (1986) reported that resistance is recessive and complex, as resistant plants isolated in the F_2 continued to segregate. However, the authors also reported dominant inheritance in crosses with cultivar VNIIMK 165. Desai et al. (2001) found resistance to be polygenic with both additive and non-additive gene action.

M. phaseolina is a soilborne fungus that attacks the roots and the basal portion of the stem from the seedling stage to harvest. Genetic resistance to this pathogen was identified in a small-seeded accession from Madagascar by Grezes-Besset et al. (1996), who demonstrated that resistance was expressed during the entire crop cycle.

No resistance to *Botrytis* gray rot has been identified. However, Thomas and Orellana (1963) reported that plants with compact inflorescence and dwarf internodes as well as racemes with interspersed staminate flowers are more susceptible to the disease.

The disease complex capsule drop is one of the most devastating diseases of castor in humid areas, with yield losses of up to 85% (Culp 1966). Its control is essential for efficient mechanical harvesting. Two resistant accessions were identified in the United States in 1957–1958 (Stone and Culp 1959). Resistance was controlled by two dominant genes, one of them being closely linked to the gene(s) underlying the short-pedicel trait (Culp 1966).

Resistance to other minor diseases such as *Verticillium* wilt caused by *Verticillium albo-atrum* Reinke and Berth (Brigham and Minton 1969; Brigham 1970a, b, c) and *Alternaria* leaf spot caused by *Alternaria tenuissima* (Nees and T. Nees) Wiltshire (Brigham 1970a, b, c) have been reported.

It has been claimed that the anthocyanin of purple genotypes increases the resistance to some fungal diseases (Atsmon 1989) which is in agreement with a multiple resistance to *Fusarium* wilt and serpentine leafminer (*Liriomyza trifolii* [Burgess]) identified in purple-coloured morphotypes (Anjani 2005). However, Moses and Reddy (1989) found no differences for *Botrytis* gray rot infection between varieties with purple and green stem.

4.5.2 Insects

The castor plant is a host to a number of insect pests. More than 100 species of insects and mites have been found to feed on different organs of castor plants causing plant injury and a varying degree of yield loss depending upon the infestation severity (Barteneva 1986). The most damaging insect pests are different in different growing regions (Barteneva 1986). The most common pests include cutworms (larvae of *Agrotis* spp., Lepidoptera; Noctuidae), which eat off seedlings, *Achaea* spp. caterpillars (Lepidoptera; Noctuidae), which can cause serious defoliation, stink bugs (Heteroptera; Pentatomidae) and mirid bugs (Heteroptera; Miridae), which attack the inflorescences, leafhoppers or jassids (Hemiptera; Cicadellidae), which cause phytotoxemia known as hopperburn in the plant (Jayaraj 1967). Castor capsule borer (*Dichocrocis punctiferalis* [Guenee]; Lepidoptera; Crambidae) and castor seemilooper (*Achaea janata* [Linnaeus]) are the most destructive pests in India (Bilapate 1978; Malathi et al. 2006).

Jayaraj (1967) identified several castor genotypes with genetic resistance to leafhopper (*Empoasca flavescens*). Germplasm resistant to capsule borer (*D. punctiferalis*) (Weiss 2000) and leafminer (*L. trifolii* [Burgess]; Diptera; Agromyzidae) (Anjani et al. 2010) has been identified as well. Purple stems and leaves (Jayaraj 1967; Anjani et al. 2010) and waxy coat or bloom (Atsmon 1989) are traits closely associated with insect resistance. The colour of castor stems is controlled by at least three independent genes (Peat 1928). First studies on the inheritance of waxy coat

on the stem and petioles of leaves found that the trait was controlled by a dominant gene (White 1918). Further studies distinguished different types of waxy coat and identified three dominant genes whose combination determines strong waxy coat on the stem, petiole and dorsal side of leaves (Peat 1928; Zimmerman 1958; Moshkin and Dvoryadkina 1986).

In recent years, breeding for insect resistance is being approached by transgenic breeding, particularly in India. Efforts are being directed to the development of transgenic castor plants resistant to castor semilooper (Malathi et al. 2006; Sujatha et al. 2009) and other foliage feeders such as tobacco caterpillar (*S. litura* [Fabricius]; Lepidoptera; Noctuidae) (Sujatha et al. 2009).

4.6 Resistance to Abiotic Stresses

4.6.1 Drought and High Temperatures

Castor is cultivated in semi-arid regions where it is considered as drought tolerant (Babita et al. 2010), but water shortage considerably reduces seed and oil yield (Laureti et al. 1998; Koutroubas et al. 2000). Such a reduction depends on the drought resistance of the cultivar (Moshkin 1986b). Wilting of plants, falling of leaves, wrinkled seeds and low oil content are the main symptoms of low drought resistance (Moshkin 1986b). Accordingly, selection against these traits is an adequate strategy in breeding for drought resistance in a given environment. Spines of capsules (Moshkin 1986b) and high osmotic adjustment (Babita et al. 2010) are important traits for resistance to drought. The latter trait is associated with accumulation of greater levels of proline, total soluble sugars, total free amino acids and potassium (Babita et al. 2010).

Castor suffers from extremely high temperatures (Zimmerman 1958). Symptoms include severe wilting of the leaves, blasting of flowers, failures in seed set and reduction of oil and protein content (Zimmerman 1958; Weiss 2000). In cultivation areas where water shortage and high temperatures occur at the end of the crop cycle, the development of early maturing cultivars is an efficient means of escape from these adverse conditions (Anjani 2010).

4.6.2 Salt Tolerance

Castor is ranked at the bottom of plants considered to have medium salt tolerance (Zimmerman 1958). The salinity threshold level for emergence and stand establishment has been identified at 7.1 dS m^{-1} (Zhou et al. 2010). Breeding salt-tolerant cultivars is important, as the area affected by salinity is increasing in the major areas of castor cultivation (Weiss 2000). Cultivar differences for salt tolerance have been reported (Weiss 2000; Raghavaiah et al. 2006) but breeding research for this trait has been scarce thus far.

4.7 Seed Oil Content

Seed oil content in castor germplasm ranges from 42 to 58% (Zimmerman 1958). The trait is under polygenic control (Zimmerman 1958; Moshkin and Dvoryadkina 1986). Some studies revealed predominance of additive effects (Moshkin and Dvoryadkina 1986; Rojas-Barros 2001), but significant dominant effects have been reported as well (Zimmerman 1958; Hooks et al. 1971; Chakrabarty 1997; Okoh et al. 2007). Oil content is under sporophytic control, with the pollen having little or no effect (Rojas-Barros 2001). There is a negative correlation between oil and hull content in the seed (Zimmerman 1958; Gomes de Albuquerque et al. 2008). The latter trait shows large variation and is under polygenic control, with low hull content being partially recessive over high hull content (Moshkin and Dvoryadkina 1986).

4.8 Seed Oil Quality

Castor seed oil is unique amongst oilseeds, as it contains a high proportion of ricinoleic acid, a monounsaturated hydroxy fatty acid of great value for industrial application but no use as a food (Scholz and Nogueira da Silva 2008). The high ricinoleic acid content confers a high viscosity to castor biodiesel, which must be corrected through the use of blends (Conceiçaõ et al. 2007). Most castor cultivars have ricinoleic acid content between 84 and 90% of the total fatty acids. A greater variation between 58.5 and 92.3% has been reported in the literature (Rojas-Barros et al. 2004). A mutant in which hydroxylation of oleic acid to ricinoleic acid was partially blocked resulting in an unusual accumulation of oleic acid (78% compared to 4% in conventional castor oil) was discovered in the USDA accession PI 179729 (Rojas-Barros et al. 2004). The trait is recessive and controlled by alleles at two loci showing dominant-recessive epistatic interaction. Additionally, it is controlled by the genotype of the developing embryo, with no maternal influence, which allows selection at the single-seed level (Rojas-Barros et al. 2005). Castor seeds contain a high tocopherol content mainly made up of gamma- and delta-tocopherol (Velasco et al. 2005) though no studies on variability for this trait in castor germplasm have been conducted.

4.9 Toxic Compounds and Allergens

The presence of toxic compounds and highly allergenic storage proteins are serious obstacles to castor processing (McKeon et al. 2000). The most toxic compound in castor seeds is ricin, a toxic glycoprotein present in the endorsperm that inactivates ribosomes and prevent protein synthesis in eukaryotes. The lethal dose for rabbits is

40 μg per kg of body weight, which is twice as toxic as cobra venom (Balint 1974). Initial germplasm evaluation for reduced ricin content in seed endosperm was reported by Khvostova (1986), who identified two cultivars (Kruglik 5 and Early hybrid) with reduced ricin content. The same author reported that high temperature during flowering reduces ricin accumulation, whereas its synthesis occurs more intensively under irrigation conditions.

Agglutinin is also a toxic glycoprotein of the seed endosperm, but it is much less toxic to mammals than ricin (Harley and Beevers 1982). Immunological methods used to measure ricin concentration do not discriminate between ricin and agglutinin, due to their immunologic relatedness (Harley and Beevers 1982). Pinkerton et al. (1999) evaluated a castor germplasm collection for the sum of ricin and agglutinin, which ranged from 1.9 to 16 mg g^{-1} seed. The germplasm with the lowest levels of toxic proteins was crossed by a semi-dwarf cultivar, which led to the development of the cultivar Brigham with a tenfold reduction in the level of ricin (Auld et al. 2009). Transgenic approaches to develop ricin-free castor are under way (Chen et al. 2007).

Ricinine is an alkaloid mainly found in vegetative tissues, capsule shells and seed hulls, whereas endosperm content is very low (Bukhatchenko 1986). The author reported a strong negative correlation between seed oil and ricinine contents, as well as environmental effects on ricinine accumulation in different plant tissues. Ricinine may act as a feeding deterrent for herbivores and especially aphids (Holfelder et al. 1998).

Castor plant and castor meal produce severe immune reactions mainly caused by a glycoprotein named 2S albumin, composed of two dimeric proteins (McKeon et al. 2000). Both proteins are encoded by a single gene (Chen et al. 2004). The gene has been cloned and sequenced, thus opening up the possibility of molecular breeding solutions to suppress the synthesis of allergens in castor (Chen et al. 2007).

5 Breeding Methods and Techniques

Breeding strategies are strongly determined by the mode of reproduction of the crop. Despite being a highly cross-pollinated species, with average rates of cross-pollination in different studies ranging from 36 to 76% (Domingo 1944; Stein 1965; Brigham 1967; Myczkowski et al. 2006), castor shows little inbreeding depression (Voskoboinik 1986a). Molecular studies on naturalized castor populations revealed a high coefficient of inbreeding (Foster et al. 2010). Therefore breeding methods for self-pollinated crops have been used to develop cultivars in this crop, together with common methods for cross-pollinated species such as recurrent selection. Additionally, hybrid breeding as a means of exploitation of heterosis has been also an important aspect of cultivar development in castor. Crop improvement methods and techniques extensively used to breed cultivars in castor as well as methods for increasing and preserving variability are described below.

5.1 Development of Initial Material

5.1.1 Exploitation of Natural Variation: Introductions and World Collections

Plant introduction, i.e. the acquisition of superior varieties by importing them from other areas, is the simplest method of crop improvement. One objective of plant introductions is to identify genotypes that surpass the best local materials. This allows the release of superior varieties in a short time, either by direct increase from the introduced stock or after slight selection. A second objective of plant introductions is to identify genotypes containing desirable traits (e.g. disease resistance or capsule indehiscence) that will be hybridized with local varieties. Castor introductions have been effective for achievement of both objectives. For example, the first small-seeded Russian cultivars such as Chervonnaya were developed from an introduction of Iran (Moshkin 1986c), US dwarf varieties such as Dawn originated from the introduction of an internode-dwarf mutant from Brazil (Brigham 1970a), whereas the first indehiscent Brazilian varieties such as Campinas originated from the cultivar Cimarron, an introduction from the United States (Savy Filho 2007a). Introductions also played an important role in the initial development of varieties in other countries such as Italy (Laureti and Brighan 1987) and France (Bonjean 1991).

Because of the importance of plant introductions in plant breeding, large efforts have been devoted to form large world collections of this crop, the most important of which have been summarized above. The first systematic collection and evaluation of castor germplasm was initiated in 1922 by G.M. Popova at the All-Union Research Institute of Plant Industry (VIR) of the former USSR (Moshkin 1986c). Castor germplasm collections are invaluable sources for identification of novel traits such as insect and disease resistance (Moshkin 1986d; Anjani 2005), agronomic traits (Moshkin 1986c) and seed quality traits (Khvostova 1986; Auld et al. 2003; Rojas-Barros et al. 2004).

5.1.2 Creation of Novel Variation

Even though mutagenesis is a widely used approach for creating novel variation in oil crops, its use in castor breeding has been scarce as compared to other oilseed crops (Ashri 1994). The efficacy of chemical mutagenesis is limited due to the hard seed coat of the seeds, which represent a barrier to the absorption of the mutagenic solution, even after scarification and blanching. Tepora (1994) proposed an alternative consisting in puncturing the seed coat at the point of attachment of the caruncle, presoaking the seeds in water for 24 h, and then applying the mutagenic treatment for 4 h. However, no valuable mutants were identified in that study. On the contrary physical mutagenesis, mainly through gamma-ray irradiation, has allowed the induction of castor mutants with loss of day-length sensitivity (Donini et al. 1984), early maturity (Ankineedu et al. 1968), pistillateness (Kulkarni and Ankineedu 1966; Chauhan et al. 1992), dwarfness and determinate plant growth

(Tepora 1994), resistance to *Fusarium* wilt (Lavanya et al. 2003) and improved agronomic performance (Sarwar and Chaudhry 2008; D'Souza et al. 2009). It is particularly noteworthy the case of the early-maturing cultivar Aruna, developed by fast-neutron mutagenesis (Ankineedu et al. 1968), which has become an important cultivar in India (D'Souza et al. 2009). Aruna cultivar has particular relevance in areas prone to drought such as the Telangana region of Andhra Pradesh, where rains frequently fail from September onwards. The short cycle of Aruna is a mechanism to escape from drought, which not only helped to stabilize the yield of castor in the region, but also facilitated double cropping in years with favourable winter rainfall (Swaminathan 1983).

5.2 Breeding Methods

5.2.1 Mass Selection

In mass selection, individual plants with desirable traits are chosen, harvested, and the seed composited without progeny test to produce the following generation. The method is well suited for developing improved castor cultivars from local landraces and introduced materials which are often very heterogeneous and for increasing gene frequencies for traits which are easily seen or measured (e.g. plant height or earliness). Mass selection is more effective for traits with high heritabilities. One essential condition for successful application of this method is a sufficient number of plants of the genetically variable original population. Moshkin (1986d) suggested a minimum of 100–150 plants for the improvement of a variety through mass selection. There are many examples on the use of mass selection for developing improved varieties in castor. Between 1923 and 1927, V.S. Pustovoit and V.E. Borkovskii developed the variety Karkazskaya from a North Caucasus landrace (Moshkin 1986d). In Brazil, the dwarf castor cultivar IAC-38 was developed in 1943 (Krug et al. 1943; Teixeira Mendes and Ferreira de Sousa 1945), remaining as the most important cultivar in that country during many years. Several other cultivars such as IAC-80 (Savy Filho et al. 1984), Guarani-2002 (Savy Filho 2005) and BRS 188 Paraguaçu (EMBRAPA 2004) were developed by mass selection. In the former USSR, mass selection has been frequently used in combination with self-pollination, for example in breeding programs for resistance to *Fusarium* (Moshkin 1986d).

5.2.2 Progeny Selection

This is a modification of mass selection in which the progeny of each individual plant selected in the previous generation is tested. This method was effectively used in Brazil for the improvement of castor populations with high levels of genetic variability (Carvalho do Amaral 2003). The Brazilian cultivar BRS 149 Nordestina was developed by progeny selection from the cultivar Balanita (EMBRAPA 2004).

5.2.3 Pedigree Method

This is a classical method of self-pollinated crops that has become the main breeding method in castor as this cross-pollinated crop shows low inbreeding depression (Voskoboinik 1986a). The method involves hybridization of parents with desirable traits followed by several generations of self-pollination and selection within families, usually till F_5 or F_6 generation where most families are expected to be substantially homozygous. Then emphasis shifts to selection among families and the best homogeneous F_7 or F_8 plots are used to obtain breeder's seed of new candidate cultivars. Many castor cultivars have been developed using this method, e.g. Hale (Brigham 1970b) and Lynn (Brigham 1970c) in the USA and Guarani (Banzatto et al. 1977), IAC-226 (Savy Filho et al. 1990) and IAC-2028 (Savy Filho et al. 2007b) in Brazil or VNIIMK 165 (Moshkin 1986d) in the former USSR.

5.2.4 Backcross Breeding

The backcross method of breeding provides a way to introgress new genes into the cultivar to be improved to produce a new cultivar with exactly the adaptation, agronomic performance and quality characteristics of the original cultivar, but superior to it in the traits for which breeding was undertaken. The method involves an initial cross between the cultivar to be improved (recurrent parent) and the germplasm containing the desired genes (donor parent), followed by a sufficient number of backcrosses to the recurrent parent in order to reconstitute it to a high degree. Backcrossing has been used if castor for introgressing traits such as capsule indehiscence into dehiscent cultivars (Banzatto et al. 1963).

5.2.5 Recurrent Selection

Recurrent selection involves selection and intercrossing of the best genotypes in a variable population, which facilitates a progressive accumulation of favourable alleles for the desired traits when conducted during several cycles. Recurrent selection has been used in castor to improve population morphological traits such as plant height (Laureti and Brighan 1987; Zanotto et al. 2004; Oliveira and Zanotto 2008) and pistillateness (Bonjean 1991). The latter authors reported a reduction in plant height of 64 cm after four cycles of recurrent selection within the cultivar Guarani. Recurrent selection has been widely used in the castor breeding program of the V.S. Pustovoit All-Union Scientific Research Institute of Oil Crops (VNIIMK) in Krasnodar, Russia for absence of dehiscence of capsules, height of stem, sparse branching and other traits related to productivity and suitability for mechanical harvesting (Moshkin 1986d).

5.2.6 Hybrid Breeding

Several studies have shown the existence of heterosis for different traits in castor. Initial studies conducted in 1947 on hybrids between Russian varieties Kruglik 5 and Sanguineus resulted in a yield increase of 140 kg/ha over the best variety (Voskoboinik 1986a). Zimmerman and Parkey (1954) and Hooks et al. (1971) reported significant levels of heterosis for days to flowering, number of racemes per plant, volume weight of seeds, oil content and seed yield. Other studies have also reported significant levels of heterosis for several traits including seed yield and seed oil content (Solanki and Joshi 2000; Lavanya et al. 2006; Okoh et al. 2007; Golakia et al. 2008). Hybrids have also been reported to show faster seedling emergence than varieties (Brigham 1965). The main yield advantage of F_1 hybrids has been associated with their strong female tendency, which increases the number of capsules per inflorescence (Atsmon 1989). Maximum heterosis for seed yield has been reported to occur in crosses involving a parent with a large number of racemes and another one with a large number of capsules per raceme (Bonjean 1991). Studies on hybrids between VNIIMK 165 and Sanguineus 401 reported that heterosis for seed yield is maximum at the F_1 generation (8–22%), then decreases at the F_2 (3–7%) and F_3 (1.5%) generations (Voskoboinik 1986a). Commercial production of F_1 hybrid seeds in castor is feasible thanks to the availability of several systems of femaleness, which have been reviewed above.

The most important task in castor hybrid breeding is the optimization of all steps of the method used to identify the best hybrid combinations. Voskoboinik (1986a) suggested the following scheme: (1) *Nursery for selection of self-pollinated lines*. Initial selections (varieties, introductions, individual selections) are self-pollinated during 5 or 6 years until uniformity is achieved. The lines are classified into female and monoecious; (2) *Nursery for evaluation of GCA*. The lines are evaluated for GCA by crossing with a set of two or three testers of diverse origin, either female for male lines or monoecious for female lines. Selection of good testers for GCA is crucial for successful hybrid breeding. Examples of good GCA testers in the VNIIMK breeding program are the cultivars VNIIMK 165 improved, Fioletovaya 2753, and Chervonnaya (Voskoboinik 1986a). Laureti (1987) used a commercial hybrid as tester for GCA. F_1 plants are evaluated for GCA in replicated field trials with the testers as checks; (3) *Nursery for resistance to* Fusarium. All the lines and hybrids are evaluated for *Fusarium* resistance, either in the field or in the greenhouse; (4) *Nursery for evaluation of lines*. Selected lines are evaluated for agronomic performance; (5) *Nursery for crosses of lines*. Male and female selected lines are produced on spatially isolated plots and F_1 seed is produced; (6) *Initial trial of hybrids*. Evaluation is conducted on two-row plots of 12 m^2 in three replications; (7) *Advance trial of hybrids*. The best hybrids are sown in four-row plots of 24 m^2 in three to four replications; and (8) *Competitive trial of hybrids*. Evaluation is conducted over 3 years to identify the best hybrid combination to undergo commercial production.

5.2.7 Synthetic and Composite Cultivars

Synthetic cultivars involve hybridization in all combinations among a number of selected genotypes. Hybrid seed is bulked to form the cultivar, which is maintained from open-pollinated seed. Composite cultivars are developed by blending equal quantities of seed from several lines. The composite cultivar is maintained by open-pollination after it is initially constituted. The main advantage of both types of cultivars is that they facilitate exploitation of the heterosis derived from line intercrossing with low seed production costs as compared to F_1 hybrid seed (Allard 1960). Lowery et al. (2007) developed a castor synthetic population by intercrossing 12 F_8 inbred lines with low concentration of ricin and agglutinin in seeds and dwarf-internode growth habit. Several composite cultivars have been released such as CMR-1, resistant to capsule mould (Stafford 1973) and T55001 with extremely short stature (50–90 cm) (Brigham 1973).

5.3 Breeding Techniques

5.3.1 Self-Pollination and Artificial Hybridization

For self-pollination or artificial hybridization, castor inflorescences must be bagged before flower opening. Most commonly used bags are made of kraft or parchment paper (Brigham 1980; Moshkin 1986c). Primary racemes are preferred because they are larger and produce more seed than secondary racemes. One or two adjacent leaves and the vegetative buds at the base of the inflorescence should be removed to facilitate the attachment of the bag and to avoid new shoots growing inside the bag. Moshkin (1986c) recommended covering with cotton the base of the inflorescence. Relevant data such as the date of bagging, emasculation and pollination, the source of pollen or any relevant plant characteristic are written on the bag. The bag is secured by folding them against stem and placing a staple or a paper clip, but the latter should be avoided in windy areas. The bags should be checked periodically and changed to larger sizes if necessary (Brigham 1980).

To ensure a high seed set rate in racemes bagged for self-pollination, the bags are carefully agitated 5–7 days after bagging to force the pollen to move upward (Brigham 1980). Alternatively, the bags are removed and the stigmas are pollinated by hand using staminate flower from the same plant. Bag removal should be accomplished when wind movement is at a minimum. For self-pollination of female racemes, all opening flowers and 25% of the buds are eliminated at the beginning of flowering, which should stimulate the formation of buds of staminate flowers. If they are not formed, the procedure is repeated after 5 or 6 days (Moshkin 1986c).

For artificial hybridization, male flowers are removed with forceps until flowering. Particular care must be taken to remove any staminate or hermaphroditic flowers which may be interspersed among the pistillate flowers (Brigham 1980).

Staminate flowers of the male parent are collected in the morning shortly before or after anthesis and stored in small paper bags or Petri dishes. Pollen will remain viable several days at room temperature or for a longer period if the flowers are dried slightly and stored in a freezer (Moshkin 1986c). Pollination is accomplished by dusting pollen on the stigmas, either using a single staminate flower or with a small brush when pollen has been collected in bulk. Fingers, forceps and brushes should be cleaned with alcohol when changing from one male parent to another (Brigham 1980). Additional pollinations can be made in successive days at 2–3 days intervals as new pistillate buds open.

5.3.2 Techniques Used for Agronomic Evaluation

Initial selections as well as advanced breeding materials must be evaluated under field conditions. In most programs, the most important traits under evaluation are seed yield and seed oil content. In some cases, selection is conducted for traits such as plant height, raceme length, days to maturation, etc. In these cases, the plants in the selection plot are grown very sparsely (70×70 or 70×35 cm) under strict uniformity of all other variables that influence the development of the plants (Moshkin 1986d). The optimum plant density for seed yield evaluation should be determined at each location, depending on the local conditions. The size of the plot, the number of replications and the frequency of the check varieties depend upon the characteristics of the material under evaluation. For evaluation of traits with high heritability, the experiment may be conducted without replications. For advanced breeding material, the plot size, the number of replications and the frequency of check varieties should be greater than in the nurseries of initial material. Moshkin (1986d) recommended plot sizes between 24 and 50 m^2, as rectangular as possible, and between two and five replications. More attention should be paid to the number of replications than to the plot size. Border effects must be considered and border plants eliminated, as they are more branched, taller and give more yield.

Disease resistance is one of the most important traits in areas prone to disease. In the case of soil borne pathogens, evaluation can be conducted in infested soils with the use of both resistant and susceptible checks. A comparative study conducted over 5 years on evaluation for *Fusarium* resistance under field conditions and in the greenhouse using artificially infested soil revealed correlation coefficients between 0.75 and 0.89 (Sviridov 1986).

5.3.3 Laboratory Techniques for Seed Quality Evaluation

Breeding programs to improve seed oil quality traits require the availability of adequate screening techniques to measure them. Breeding for reduced ricin and agglutinin content in the seeds only started after methods to evaluate the levels of these compounds were developed. In the former USSR, Khvostova (1986) reported the

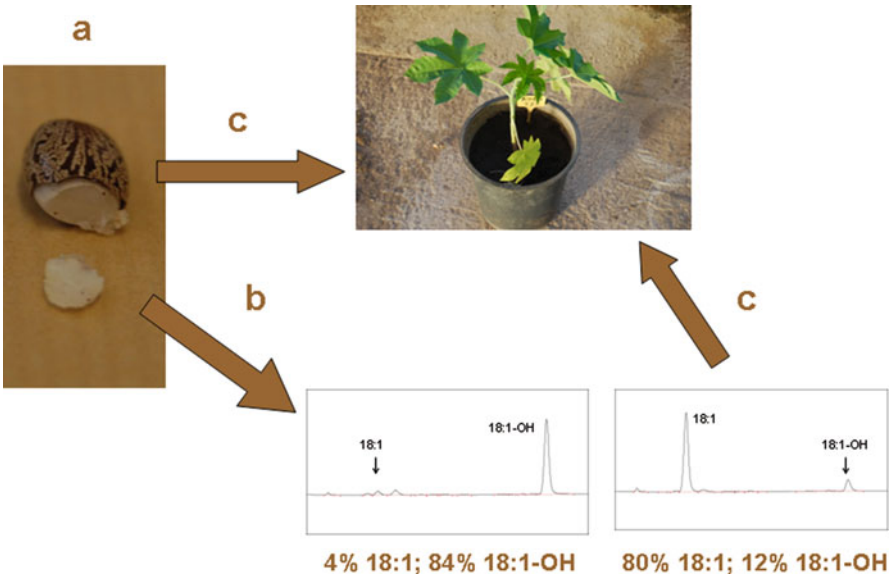

Fig. 10.1 Schematic representation of the use of half-seed analysis for selection for fatty acid profile in single seeds of castor: (**a**) a small seed portion distal to the embryo is excised; (**b**) the seed portion is analysed for fatty acid profile; (**c**) only the seeds with the desired fatty acid profile, in this example high oleic acid (18:1) and low ricinoleic acid (18:1-OH) content, are germinated

evaluation of castor germplasm for these toxic compounds based on the ability of the protein to agglutinate blood erythrocytes. The ongoing breeding program in Texas to develop castor cultivars with low levels of ricin and agglutinin is based on the measurement of these toxic compounds using a radial immunodiffusion assay (Auld et al. 2009).

Studies in other oilseeds have demonstrated that genetic changes underlying modified fatty acid profiles are mainly under gametophytic control, i.e. they are governed by the genotype of the developing embryo (Velasco and Fernández-Martínez 2002). Accordingly, selection for these oil quality traits can be conducted at the single-seed level using the half-seed technique, initially developed for nondestructive analysis of the fatty acid composition of single seeds of rapeseed, *Brassica napus* L. (Downey and Harvey 1963). The technique has been adapted to the analysis of castor seeds (Rojas-Barros et al. 2004). It consists in the removal of a small seed portion in the seed extreme distal to the embryo in such a way that the germination capacity of the seed is not affected. The excised half seed is used for chemical analysis whereas the other half seed containing the embryo can be sown to produce a viable plant (Fig. 10.1).

The use of this technique allowed the identification of a natural castor mutant with high oleic acid content (Rojas-Barros et al. 2004) and it is being routinely used to introgress the trait into castor cultivars.

6 Molecular Research and Biotechnology

6.1 Molecular Breeding

Research on DNA-based markers for castor molecular breeding started very recently, more than a decade later than the major oilseed crops. Some research was conducted in the 1980s on the use of several isozyme systems such as peroxidase, esterase and glutamate dehydrogenase as biochemical markers (Sujatha et al. 2008). Isozymes were revealed as successful biochemical markers to discriminate tall and medium tall genotypes from dwarf genotypes (Sathaiah and Reddy 1985) as well as to estimate positive heterobeltiosis for seed yield in F_1 seedlings (Sathaiah and Reddy 1986).

Only a few studies on the use of DNA-based markers have been conducted in castor, in all cases focused on assessing genetic diversity in castor germplasm. Allan et al. (2008) studied 41 accessions from 35 countries of five continents using three primer sets of amplified fragment length polymorphism (AFLP) markers and nine simple sequence repeats (SSR) or microsatellite markers. The authors identified low genetic diversity in castor germplasm, with the majority of genetic variation being found within accessions rather than among accessions, which pointed to a low genetic differentiation of castor germplasm at a worldwide scale. These conclusions were basically confirmed in a further study on 152 germplasm accessions using 232 high-quality single nucleotide polymorphisms (Foster et al. 2010). The authors also investigated the genetic structure of 13 naturalized populations from Florida, observing larger molecular variance within populations than among populations. They concluded that most accessions are a mixture of genotypes resulting from multiple introductions rather than gene flow among established populations. Additionally, the observation of a high coefficient of inbreeding suggested high rates of self-pollination. Gajera et al. (2010) used 35 random amplification of polymorphic DNA (RAPD) and five inter-simple sequence repeats (ISSR) markers to evaluate genetic diversity among 22 castor genotypes. Bajay et al. (2009) reported the development of 12 polymorphic microsatellite markers useful for genetic diversity studies in castor.

Even though molecular breeding research in castor has been scarce thus far, the foundations for the development of high-throughput molecular tools are being laid. A whole genome shotgun sequencing of the ~400 Mbp castor genome with a 4X sequence coverage was announced in 2006 (Chan et al. 2006). According to the information provided by the Castor Bean Genome Database (http://castorbean.jcvi.org/index.php), the 4X draft of the castor genome has already been sequenced and assembled and ~50,000 expressed sequence tags (ESTs) from different tissues have been produced to help gene discovery and annotation. Such a large number of ESTs complement those developed in previous studies (Van de Loo et al. 1995; Doering-Saad et al. 2006; Lu et al. 2007). More than 62,500 ESTs from castor are currently available in Genebank (http://www.ncbi.nlm.nih.gov/genbank/; accessed 1 July 2010).

6.2 Transgenic Breeding

The lack of an efficient system for in vitro plant regeneration has been a bottleneck for castor transgenic breeding. In castor, callus initiation and plantlet regeneration from vegetative explants are restricted to young seedling tissues. Callus can be initiated from the seedling explants but plant regeneration via organogenesis proved to be difficult (Sujatha et al. 2008). Athma and Reddy (1983) studied the differences in callusing ability of different seedling explants from root, shoot and cotyledonary leaf tissue. The authors identified maximum callusing ability of the shoot tissue (90–98%) followed by root (88–91%) and leaf tissue (73–83%). After solving some problems related to low reproducibility or low efficiency, several reliable protocols for high frequency plant regeneration by in vitro shoot proliferation from meristematic explants of castor were developed (Sujatha and Reddy 1998; Ahn et al. 2007; Ganesh Kumari et al. 2008, Alam et al. 2010).

Based on the plant regeneration protocol reported by Sujatha and Reddy (1998), Sujatha and Sailaja (2005) developed a protocol for genetic transformation of castor plants via *Agrobacterium*-mediated gene transfer. Using this protocol, Malathi et al. (2006) produced transgenic castor plants expressing *cry1Ab*, encoding a *Bacillus thuringiensis* delta-endotoxin that conferred resistance against the castor semilooper (*Achoea janata*). Sujatha et al. (2009) used both *Agrobacterium*-mediated and particle gun bombardment transformation methods to produce castor transformants expressing the *cry1EC* gene that conferred resistance to both castor semilooper and tobacco caterpillar (*S. litura*). Additional *cry* genes are being used for genetic transformation of castor plants in order to produce transformants resistant to major foliage feeders (Sujatha 2008). Genetic transformation experiments to produce ricin-free strains are also underway (Sujatha 2008).

7 Seed Production

The castor breeder identifies lines, populations or hybrid cultivars that perform better than the currently used cultivars. The seed of this material, referred to as breeder seed or prebasic seed, is maintained and increased by the breeder and it is genetically the purest source of a cultivar. When applied to hybrid cultivars, it refers to the seed of parental (male and female) lines. The initial increase of breeder seed, referred to as foundation seed, is also grown, directly or indirectly, under the supervision of the plant breeder. Registered seed is the progeny of breeder and foundation seed handled under procedures acceptable to the certifying agency to maintain satisfactory genetic purity and identity. Certified seed is the progeny of breeder and foundation seed.

The increase and production of breeder, foundation and certified seed is critical and requires highly qualified seed producers. The standards of purity and identity and certification procedures vary from country to country. In Russia, three categories

for seed quality have been established, with a recommendation to maintain the standards of the first category. This includes a varietal purity not below 99.8%, a minimum germination capacity of 95%, moisture content below 9%, a minimum castor seed content of 98% with no more than 2% of hulled seeds, a maximum of six seeds of other plants per kilogram of seed with no more than four seeds of weeds and a maximum of 0.2% of seeds infected by *Fusarium* (Blagodyr 1986).

The increase of breeder/foundation seed of open-pollinated cultivars as well as hybrid parental lines requires a layout of isolated blocks. Brigham (1980) recommended an isolation distance of 800 m and not down wind from other castor plantings. All wild castor plants growing along roadsides within 800–1000 m of the isolated planting should be removed. Voskoboinik (1986a) recommended isolation distances of 1,000 m for multiplication of female and dwarf lines, 500 m for multiplication of male parents and 150–200 m for hybrid production plots.

The procedure for hybrid seed production is much more complicated. Current castor hybrid seed cultivars are single F_1 hybrids based on two parentals (female and male) inbred lines using the systems of male sterility described above. For hybrid seed production, the planting ratio (female:male) is a crucial aspect for an economical hybrid seed production. Usually two rows of male pollinator parent are planted for every 10–12 female rows (Brigham 1980) although 1:3 and 1:4 (male:female) have also been used (Atsmon 1989). Voskoboinik (1986b) suggested male:female proportions of 2:4, 2:6 or even 6:6. Plantings should be oriented so that prevailing winds blow across the rows for best pollen distribution. In order to achieve flowering synchronization, the male should be earlier flowering or sowing of the parental lines is staggered. Roguing off-types in male and female parents must be carried out just prior to blooming and the female parent must be rogued for any pollen-shedding plants at the early stage of the primary raceme (Voskoboinik 1986a).

8 Conclusion

An increasing demand for the use of vegetable oils in non-food applications such as biofuels and biolubricants will stimulate in the short term the development of industrial crops that do not compete in the food markets. Castor is probably the best suited crop for such applications. Further improvements of seed quality such as the development of cultivars with toxin-free seeds and seed oils with modified fatty acid profiles are required. Molecular research on castor started later than in other oil crops but spectacular advances are being made, with castor genome sequencing being nearly finished and efficient in vitro plant regeneration and transformation protocols already developed. The integration of such novel genomic and biotechnological technologies into conventional breeding programs will lay the foundations for the development of a new generation of castor cultivars producing high-quality seed oils and by-products for energy and industrial applications and adapted to a wide spectrum of environments.

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Chapter 11
Olive

Aurora Díaz

Abstract The scarce knowledge about the genetics of the olive tree is not comparable to the great impact of its cultivation on the economy and culture of Mediterranean countries. Actually, the polyploid nature of some *Olea europaea* subspecies has been recently confirmed by the use of new techniques and methodologies, like microsatellite markers and flow cytometry analyses.

The most extended idea among the researchers is that the origin of olive cultivation goes back to the Prehistory in the Eastern Mediterranean. The use of cytoplasmic DNA markers to trace olive migration routes has allowed identifying, at least, two possible centres of origin for the olive tree, located to the east and the west of the Mediterranean Sea, Near East and Maghreb. Nowadays, the olive tree cultivation is concentrated in Mediterranean-type climate regions with benign winters and dry and hot summers.

Modern olive oil industry requires more competitive cultivars better adapted to the new trends in olive growing. Breeding programmes undertaken have focused in obtaining new cultivars with a combination of superior characteristics, like high productivity, low vigour and compact plant architecture, earliness of flowering and fructification, resistance to pathogens and pests (i.e., leaf spot, Verticilium wilt and olive knot), among agronomic traits; and high oil content and quality, as oil traits.

The detection of a large number of mislabellings, homonyms and synonyms has raised the need of easy and accurate cultivar identification methods to manage properly the rich olive biodiversity. Up to date, morphological traits are the only markers accepted and used by the International Plant Genetic Resources Institute (IPGRI, Rome) and the International Olive Oil Council (IOOC), though their usefulness is being constantly strengthened by molecular markers to unambiguously discriminate among individuals. The use of molecular markers can speed the breeding programmes up, not only being used in identification and compatibility studies, but in

A. Díaz (✉)
Instituto de Biología Molecular y Celular de Plantas-CSIC/Universidad Politécnica de Valencia, Laboratory 0.08 Ciudad Politécnica de la Innovación, Ingeniero Fausto Elio, s/n-Escalera 8G, 46022, Valencia, Spain
e-mail: audiaber@ibmcp.upv.es

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the selection of individuals with desirable agronomic characteristics in an early stage (marker-assisted selection, MAS). Isozymes became the biochemical markers most widely used in plant breeding, though they have been superseded by genetic markers. Most of them have been used with identification purposes, some cases of homonyms and synonyms being solved, and to estimate the genetic distances among very diverse sources of material (wild, feral and cultivated forms). In this sense, microsatellite markers have revealed the exotic germplasm as a source of new variability, wild genotypes being grouped together in a different gene pool than the cultivated forms. Clusterings of olive cultivars according to economically important traits have been described, what could be very useful when it comes to design breeding crosses. And the genetic relationships among olive cultivars and genotypes selected from a breeding programme that ultimately has rendered a new variety have been elucidated. Furthermore, microsatellites have become tremendously useful for checking the paternity of olive progenies from controlled crossings and exploring the compatibility relationships among olive cultivars, which is vital to design effective crosses in breeding programmes. Linkage maps in olive are needed, so markers linked to the traits of interest can be identified. Up to date, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and microsatellite markers have been used to construct linkage maps.

Genetic transformation can significantly contribute to plant breeding by generating additional genetic diversity and introducing alleles that encode desirable traits into superior cultivars. The progress in the genetic transformation methodologies in olive must be accompanied by the design of efficient regeneration protocols, via organogenesis and somatic embryogenesis.

Real-time quantitative PCR (qPCR) and real-time quantitative reverse-transcription PCR (qRT-PCR) have contributed to monitor the sanitary status of olive plants that is essential to undertake successful breeding programmes. These techniques have also been used to infer the resistance or susceptibility level of particular cultivars to olive leaf spot, this application being very valuable as a breeding tool.

From MAS to expression studies, without forgetting genetic transformation, the olive research community has used these technological innovations to acquire a deeper knowledge of the species and to transfer it to breeding programmes, what is providing the first promising results.

Keywords Olive • Morphological markers • Biochemical markers: isozymes • Genetic markers • RFLPs • RAPDs • AFLPs • SCARs • Real-time quantitative PCR • Genetic transformation

1 Introduction

The olive tree (*Olea europaea* L.) is a subtropical, evergreen oil-producing tree belonging to the family *Oleaceae*, the subfamily Oleoideae, the tribe Oleeae, the genus *Olea* and the subgenera *Olea* (Heywood 1978). The genus *Olea* comprises

more than 40 species including the cultivated, wild and feral forms under the name *O. europaea* L. (section *Olea*). However, *O. europaea* is the only species producing an edible fruit. Enormous confusion prevails around the taxonomical classification in this family. There is lack of consensus over the nomenclature adopted to distinguish the cultivated forms from the wild forms. At least three different ways of naming the olive tree, cultivated in the Mediterranean region, can be found in the literature (*O. europaea* subspecies *sativa*, *O. europaea* subspecies *europaea* var. *sativa* and *O. europaea* subspecies *europaea* var. *europaea*). A similar situation can be found in case of the wild olive trees, popularly known as acebuches (*O. europaea* subespecies *sylvestris*, *O. europaea* subespecies *europaea* var. *sylvestris* and *O. europaea* subespecies *europaea* var. *oleaster*).

Despite the huge impact of the olive tree cultivation on the economy and culture of Mediterranean countries, the knowledge about the genetics of this species is very limited. High number of chromosomes ($2n=46$) is an indicator of its polyploid (tetraploid) origin (Taylor 1945; Brousse 1987). Stergiou et al. (2002) speculated on the possible role of tropical and subtropical species, like *Olea chrysophylla* Lam. and *Olea excelsa* Ait., in its evolution. The hypothesis that domesticated olive comes from an ancient polyploid is supported by molecular data, as some microsatellite markers have been reported to show multi-locus amplification in modern olive cultivars (Cipriani et al. 2002; Diaz et al. 2006a). An alternative explanation is argued by Minelli et al. (2000), who point out the existence of chromosome fusion and rearrangements in a primitive genome, consisting of 48 chromosomes, as the probable origin of the current chromosome set. The range of possibilities (contradictory, in many cases) about the olive genome structure and origin exemplifies the scarcity of knowledge about the species and the need for undertaking a deeper investigation on its genetic behaviour. Actually, microsatellite patterns and flow cytometry analyses have confirmed the hexaploid and tetraploid nature of *O. europaea* subspp. *maroccana* and *cerasiformis*, respectively (Besnard et al. 2008; Brito et al. 2008), and the triploid status of some individuals belonging to *O. europaea* subsp. *laperrinei* (Batt. & Trab.) Ciferri (Besnard and Baali-Cherif 2009).

2 Origin and History

Some authors date the beginning of olive cultivation around the Copper Age, 4000–3000 BC (Loukas and Krimbas 1983). This long lapse of time was more delimited by Zohary and Hopf (1994), suggesting that olive domestication took place between 5,500 and 5,700 years ago. In any case, its origin is very ancient and its cultivation goes back to the Prehistory, even if it has not been possible yet to determine certainly the course of its progressive or intermittent propagation through the time (Civantos 1999).

Different hypothesis have been proposed regarding the place in which the olive tree was used as a crop for the first time (Chevalier 1948; Moazzo 1994). One of them postulates that it comes from the coasts of Syria, Lebanon and Israel while another one considers the olive tree native to Asia Minor. The historian and man of

letters De Candolle is among those supporting Syria as the centre of origin of *O. europaea* L. (De Candolle 1884). Some other authors locate the beginnings of the olive cultivation in a region around Palestine, Crete and Egypt. There are still others who maintain that the olive tree originated in Ethiopia. However, the most extended idea among the researchers is that the olive tree originated in Eastern Mediterranean, more precisely, to the north of the Dead Sea (Zohary and Spiegel-Roy 1975; Loukas and Krimbas 1983). The first documentary evidences of the olive tree cultivation go back to 4,000 years before the present era when it appeared in the oriental regions of the current Syria and Iran. More recently (2500 BC), the Minoic small clay boards named "Ebla boards" show the olive trees and bear witness to the use of the olive oil at the court of king Minos.

In the last decade, the use of cytoplasmic DNA markers to elucidate migration routes of some plant species has become very common (Tomaru et al. 1998; Sinclair et al. 1999; Gugerli et al. 2001). The study of the mitochondrial DNA polymorphism using amplified fragments length polymorphism (AFLP) markers has helped to conclude that there are, at least, two possible origins for the Mediterranean olive tree (Besnard and Berville 2000; Besnard et al. 2001a). These are, on one hand, the extensive area known as Near East and, on the other hand, Maghreb. So, two different centres of origin are proposed, located to the east and the west of the Mediterranean Sea. This conclusion is based on the discovery of a unique mitotype (ME1) to the populations of wild olive trees from Near East, whereas other two out of the four possible mitotypes (MOM and MCK) could only be found among the wild forms from the west area.

3 Olive Tree Cultivation Spread

At first, it is assumed that olive tree spread from its centre of origin, on one hand, to Cyprus and Anatolia and, on the other hand, to Crete and Egypt. There are some discrepancies in this theory, since some authors support that the olive tree went from Crete to Egypt, Asia Minor, Palestine and Greece, spreading from there to the whole Mediterranean from the second century BC. Some of the researchers consider Palestine being a primary centre of origin of the olive tree. However, Besnard et al. (2001a), who proposed two clearly differentiated areas for its origin, postulated that dispersion of olive tree took place from the oriental region of the Mediterranean toward the western shore, in clear parallel with the migratory movements of men during colonization of the European continent. This would explain why the mitotypes MOM and MCK are only present in the western area.

During the fifteenth, sixteenth and seventeenth centuries, olive cultivation spread all over the Iberian Peninsula. At the beginning of the nineteenth century it was taken to Australia by Italian emigrants. It is grown in many other countries of Latin America and has been spread even up to California. At present the countries around the Mediterranean Basin represent more than 99% of the total hectares dedicated to cultivating olive trees all around the world, Spain being the world's largest olive oil-producing country (FAOSTAT 2008). World olive oil production is around

2.9 million tonnes, the Mediterranean countries being the major contributors (International Olive Council 2009, http://www.internationaloliveoil.org/downloads/production1_ang.PDF). Up to date, there are 103 germplasm banks disperse around five continents and 25 countries which aim to preserve these resources (Olea databases 2008, http://www.oleadb.it).

4 Breeding Objectives and Procedures

The olive tree is the sixth most important oil crop in the world and in spite of its great economic importance, the most widely planted cultivars are ancient and come from the empirical selection made by growers throughout the centuries (Rallo et al. 2005). Its long juvenile period delays development of new cultivars through breeding methods involving selection among progenies generated from crossing of parents (Rallo 1995). However, the modern olive oil industry requires new and more competitive cultivars that have high oil content, better oil quality, low alternate bearing, increased productivity, suitability for mechanical harvesting and resistance to pests. In the case of table olives, other features to be considered are the shape and size of fruit; uniformity in ripening and a high pulp:stone ratio (Lavee 1994). Some cultivars, such as 'Barnea' (Lavee 1986) in Israel and 'FS 17' (Fontanazza et al. 1998) and 'Briscola' (Roselli and Donini 1982) in Italy, were obtained as a result of breeding programmes. Nevertheless, they are still not widely grown. A new cultivar has been obtained from the crossbreeding programme started in Cordoba (Spain) in 1990 (Rallo 1995). The traits considered for selection were earliness of bearing, high productivity, oil production efficiency, increased oleic acid content, a certain degree of resistance to olive leaf spot and suitability to mechanical harvesting. For this, 'Arbequina,' 'Frantoio' and 'Picual' were chosen as progenitors. More recently, another cultivar called 'Chiquitita,' which combines the outstanding characteristics of both parentals ('Arbequina' and 'Picual') has been developed (Luis Rallo et al. 2008a; Pilar Rallo et al. 2008b). It shows early bearing, high oil content and yield efficiency on one hand and, at the same time, it has low vigour and a compact architecture.

Breeding programmes undertaken have focused on obtaining new cultivars with a combination of superior characteristics. The traits of interest in breeding and improvement of olive are shown below.

4.1 Agronomic Traits

4.1.1 Productivity

Many factors can affect the productivity of an olive tree, ranging from environmental and cultural management to genetic factors. The alternate bearing conditions greatly affect the productivity. Some cultural practices contribute to diminish this

phenomenon; and there is a genetic component also as in some cultivars the alternate bearing effects are less pronounced. Another genetic characteristic (though influenced, to some extent, by the environmental conditions) is the rate of bud differentiation to reproductive inflorescences or vegetative shoots. The fruit set is also determined by the self- and cross-compatibility of the cultivars (and the nature of the pollen available), apart from other factors having an impact on the tree reproductive ability, like those causing ovary abortion.

4.1.2 Vigour and Plant Architecture

Dwarf olive trees or shrubs are desirable in order to be cultivated in a hedgerow design that would facilitate the mechanical pruning and harvesting. Several studies have evaluated the suitability of some olive cultivars to the modern high-density orchards (De la Rosa et al. 2007; Leon et al. 2007b). As a result, low-vigour cultivars, like 'Arbosana' and 'Arbequina' showed the highest productivity and oleic acid content (De la Rosa et al. 2007), the latter rending no differences in fruit oil content and moisture when grown at different densities (Leon et al. 2007b).

4.1.3 Earliness of Flowering and Fructification

The reduction of the juvenile period or the lapse of time in which the plant is not able to produce flowers, is one of the most promising olive breeding objectives. As in many other trees, the juvenile period in olive is very long, which is a handicap for growers and breeders. In an attempt to predict the juvenile/adult shift, Moreno-Alias et al. (2009) recorded a number of leaf parameters, finding an organized layer of subepidermal cells in the abaxial mesophyll exclusive to adult trees. So, the authors proposed to use this feature as a criterion of phase change in the olive tree. From a breeding perspective, there is need of an early and easy to measure trait as an indicator of the juvenile period length. In this sense, a correlation between seedling vigour parameters (i.e., height and stem diameter) and the length of the juvenile period has been observed in different studies (De la Rosa et al. 2006; Luis Rallo et al. 2008a; Pilar Rallo et al. 2008b) and is being suggested as precocity selection criterion at early developmental stages. However, Pritsa et al. (2003) observed no such correlation between vigour parameters and earliness of first flowering. Interestingly, in a comparative field assay including 15 selections, a high number of early-bearing genotypes has been reported (Leon et al. 2007a).

4.1.4 Resistance to Pathogens and Pests

Among the most important diseases affecting the olive tree, in terms of economical losses, the olive leaf spot, the *Verticilium* wilt and the olive knot are the most remarkable ones.

Olive leaf spot (also named peacock spot, peacock eye and bird's eye spot) is caused by the fungus *Spilocaea oleaginea* and it dramatically decreases the olive productivity. An extensive classification of more than 300 olive cultivars according to their resistance or susceptibility to this disease has been carried out (Trapero and Lopez-Doncel 2005). More precisely, 28 and 20 cultivars were described as resistant and highly resistant, respectively. In Israel, a cultivar resistant to *S. oleagin*a was obtained (Lavee et al. 1999) and a molecular marker linked to the locus conferring the resistance to the disease identified (Mekuria et al. 2002) which could assist in the early selection of resistant individuals in subsequent breeding programmes.

The disease known as Verticilium wilt is caused by the fungal pathogen *Verticillium dahliae*. An evaluation of 23 olive cultivars carried out by Lopez-Escudero et al. (2004) revealed three cultivars ('Frantoio,' 'Oblonga' and 'Empeltre') being moderately susceptible and resistant to the defoliating (highly virulent) and non-defoliating (mildly virulent) pathotypes, respectively. As expected, 'Empeltre' showed a remarkable recovering ability to both fungal isolates, as it is considered a valuable cultivar for inclusion in breeding programmes aimed to develop new genotypes resistant to *Verticillium* wilt. In this context, new technologies, like the real-time PCR (qPCR), have been optimized to be used as a way to identify the resistant or susceptible nature of individuals in early developmental stages (Mercado-Blanco et al. 2003) and this will undoubtedly facilitate the selection in breeding programmes.

The bacterium *Pseudomonas savastonoi* is the causal agent of olive knot disease, which infects through wounds. In an attempt to classify olive cultivars into resistant or susceptible types to knot disease Peñalver et al. (2006) inoculated 29 cultivars with two pathogen strains at two different doses and found that none of them was immune to the disease. They categorized olive cultivars as high, medium, or low susceptible to knot disease based on their reaction upon inoculation. According to these authors, some cultivars, widely included in breeding programmes for having outstanding characteristics, like 'Frantoio' and 'Picual', were among those showing low susceptibility. Nonetheless, these results should be taken cautiously, as 'Frantoio' has been reported to be the most susceptible cultivar among those tested by Hassani et al. (2003). The use of potent diagnostic tools, as the qPCR, already employed to detect *P. savastonoi* in olive samples (Bertolini et al. 2003a, b), will hopefully be useful in such studies.

4.2 Oil Traits

4.2.1 Oil Content

In all selection schemes, oil quantity and quality are the criteria invariably taken into consideration. In this context, the work carried out in the Olive Germplasm Banks of Cordoba and Catalonia (both in Spain), to measure several olive fruit characteristics is very valuable (Del Rio et al. 2005; Tous et al. 2005). The oil content (among other fruit characteristics) was measured in 112 (Del Rio et al. 2005) and 30

(Tous and Romero 2005) olive cultivars. In both cases, the cultivars were classified into five different categories according to the oil content, weight and pulp:stone ratio of their fruits, ranging from "very high" to "very low."

4.2.2 Oil Quality and Composition

Similarly to the work carried out in the Olive Germplasm Banks of Cordoba and Catalonia (both in Spain) on oil content, the composition of virgin oils coming from 74 (Uceda et al. 2005) and 28 (Tous et al. 2005) olive cultivars has been analysed. Though there were differences among the cultivars with respect to oil quality and composition obtained in both locations (as expected, due to the influence of environment and cultural practices), some important cultivars, like 'Picual', show excellent values (i.e., monounsaturated:polyunsaturated fatty acids ratio) in both cases.

Furthermore, in order to identify the relevant genes regulating the fruit metabolism and phenolic content (quality trait) during ripening, Alagna et al. (2009) undertook a transcriptomic study of 'Coratina' (cultivar with a very high phenolic content) and 'Tendellone' (an oleuropein-lacking cultivar), in both cases using material at the beginning and at the end of fruit development. Some of the genes expressed differentially at both stages coded for enzymes involved in the metabolic pathway for terpenoid biosynthesis.

5 Genetic Engineering and Molecular Biology

5.1 Types of Markers and Their Use in Olive

The genetic variability existing in the cultivated olive is enormous. To date, 2,600 different olive cultivars have been described (Rugini and Lavee 1992) and large numbers of mislabelling, homonyms and synonyms have been reported (Barranco and Rallo 2000). The preservation of this valuable genetic patrimony is of paramount importance to avoid its erosion, which would lead to an irreversible narrowing of the genetic background, as it is occurring in many other crops. During the last few decades, considerable exploration, harvesting, characterization and evaluation works of the most outstanding olive cultivars have been performed. Therefore, easy and accurate cultivar identification is an urgent necessity to manage properly the rich olive biodiversity. All the markers listed below have played an important role in the identification and evaluation of the variability present in the species, as well as in the breeding programmes and incompatibility studies.

Besides, even if the juvenile period in olive tree has been considerably shortened by forced growth techniques (Santos Antunes et al. 1999), it is still too long to make a breeding programme viable. The use of molecular markers can speed up this process. They are being used not only in identification and compatibility tasks, but also in the selection of individuals with desirable agronomic characteristics in an

early stage (marker assisted selection, MAS). For this, obtaining linkage maps in olive is needed, so that markers linked to the traits of interest can be identified.

5.1.1 Morphological Markers

Before the availability and routine use of molecular markers, a great number of morphological traits were studied in an attempt to identify and characterize the enormous variety of forms existing in most of the species of agricultural importance. In olive, the organs most commonly used for this purpose are leaves, inflorescences, whole fruits and seeds (Barranco and Rallo 1984), though pollen grains are also being used as the exine pattern seems to be a valuable discriminating character (Lanza et al. 1996). Important morphological traits used for different studies include the size, the area, the perimeter, or the longitudinal diameter (in leaves, fruits, seeds and pollen), weight (in fruits and seeds), and other organ-specific measurements, like the number of flowers or their density, in the case of the inflorescences. From these data, new parameters can be developed, like the pulp:seed weight ratio or the weight ratio in the whole fruit (pulp and stone) and the seed. Categories based on qualitative characters can also be established, like the leaf or fruit colour and shape. At present, the International Olive Oil Council (IOOC, Madrid) employs the cultivar classification system described by Barranco and Rallo (1984).

The difficulty in evaluation and the influence of general (cultural management and environmental conditions) and plant-specific (age, phenological state, etc.) factors are among the most important handicaps in the use of phenotypic characters as markers. Massei and Hartley (2000) reported a clear example of these effects. Furthermore, these authors observed that the selection aimed to increase the yield and the growth rate in the species *O. europaea* has led to a decrease in the defence mechanisms deployed by the plant. Another additional problem, considering the high cost of the orchard maintenance, is that the olive tree shows a prolonged unproductive period and most of the phenotypic characters are evaluated when the plant has reached the adult state.

At first, morphological markers were employed to discriminate olive cultivars (Barranco and Rallo 1984, 1985; Leitão 1988; Cimato et al. 1993; Prevost et al. 1993; Tous and Romero 1993; Cantini et al. 1999; Barranco et al. 2000a). Up to date, they are the only markers accepted by the International Plant Genetic Resources Institute (IPGRI, Rome) and the IOOC, though its usefulness is being constantly strengthened by molecular markers (Claros et al. 2000; Sanz-Cotes et al. 2001; Roselli et al. 2002; Rotondi et al. 2003; Corrado et al. 2009; Rao et al. 2009), as sometimes they reveal themselves insufficient to discriminate among olive forms, cultivated and oleaster trees (Hannachi et al. 2008).

5.1.2 Biochemical Markers: Isozymes

Undoubtedly, the biochemical markers most widely used in plant breeding have been the isozymes. They represent the different biochemical forms of an enzyme,

easily distinguishable by electrophoresis and coded by different alleles of the same gene (Soltis and Soltis 1989).

The methodology needed to develop and use these codominant markers in the olive tree is relatively easy, quick and affordable (Trujillo et al. 1995). They have been extensively used in olive cultivar identification due to their high level of polymorphism (Pontikis et al. 1980; Loukas and Krimbas 1983; Trujillo et al. 1990; Ouazzani et al. 1993, 1995, 1996; Hilali and El Antari 1994; Trujillo et al. 1995). However, in recent times, breeders are making use of DNA-based markers to acquire genetic knowledge about the olive tree because isozymes have many disadvantages. They are gene products, so their expression is affected by the environment, sometimes is tissue-specific and can be subjected to selective processes. Besides, less than one percent of the genetic variations involve changes in the electrophoretic mobility of the proteins, the number of isozyme systems is limited and many species are monomorphic for these markers, particularly those with a high degree of endogamy or with a narrow genetic base.

5.1.3 Genetic Markers

In last two decades, plant breeding has experimented major advances in the field of genetics with the development of new molecular markers. Genetic markers analyse directly the genotype (DNA), quite the opposite than the previous ones, which were based on the phenotypes and the gene products. For this reason, they are the most extensively used presently. Among them, PCR-based markers, amenable to automation, seem to be the most suitable ones for breeding purposes.

Restriction Fragment Length Polymorphisms

This type of markers detects the polymorphism present in the DNA strand when the target site of a restriction enzyme is altered by a mutation or by changes in a segment of the molecule, like deletions and insertions (Botstein et al. 1980). They are codominant and robust markers but their development is time consuming and expensive, and show little polymorphism in species with a small gene pool.

In the last decade, DNA coming from cytoplasmic organelles has been used to unravel origin of the olive tree and the history of its domestication. Mitochondrial (Besnard and Berville 2000; Khadari et al. 2001b; Besnard et al. 2002a; Bronzini de Caraffa et al. 2002) and chloroplastic (Amane et al. 1999; Lumaret et al. 2000; Besnard and Berville 2002; Besnard et al. 2002a, b) RFLPs have been the markers most widely used for this purpose. The discoveries reported by Amane et al. (1999) are especially interesting as they have found a correlation between a concrete chloroplastic genotype (clorotype V) and the olive male sterility. Similar results were reported by Besnard et al. (2000), who associated the male sterility with the CCK chlorotype and the MCK mitotype. The value of these markers linked to male sterility in olive breeding is enormous as they allow establishing the fertility status in

early stages of the trees. There are also works in which the polymorphism revealed by nuclear RFLPs is used to elucidate the moment of the genetic divergence of the different taxons included in the species *O. europaea* (Besnard et al. 2001a). Finally, they have been employed to construct the first olive linkage map, along with other molecular markers (De la Rosa et al. 2003).

Random Amplified Polymorphic DNAs

Random amplified polymorphic DNAs (RAPDs) were the first PCR-based DNA markers developed (Welsh and McClelland 1990; Williams et al. 1990). Arbitrary, short primers (around ten nucleotides) are used to amplify genomic DNA, what renders a band profile considered as a dominant marker.

In olive, a massive use of RAPDs has been made to achieve cultivar identification (Bogani et al. 1994; Vergari et al. 1996; Claros et al. 2000; Bandelj et al. 2001; Belaj et al. 2001; Besnard et al. 2001b; Khadari et al. 2001a, b; Sanz-Cotes et al. 2001; and a lot more besides), some cases of homonym and synonym being solved (Wiesman et al. 1998; Mekuria et al. 1999; Barranco et al. 2000a, b). It has been even possible to detect intra-cultivar polymorphism in some of the most important Portuguese cultivars intended for oil production (Gemas et al. 2000, 2004). RAPDs have been also used to estimate the genetic distance among the wild, feral and cultivated forms and within those groups (Besnard and Berville 2000; Belaj et al. 2001, 2002; Besnard et al. 2001a, b, c; Bronzini de Caraffa et al. 2002; Sesli and Yegenoglu 2009), as well as to study the olive propagation in the Macaronesian region (Hess et al. 2000). In this last study, the authors employed ISSR (inter-simple sequence repeat) markers too. The results obtained by Mekuria et al. (2002), in which a clear segregation of the RAPD band patterns in the progeny from crosses among resistant, semi-resistant and sensitive cultivars to olive leaf spot is observed. Thus, markers linked to these disease-resistance gene(s) have been identified are becoming of great interest to the olive breeding programmes. As mentioned above, the data coming from the use of different sorts of molecular markers, RAPDs among them, have been used to construct the first linkage maps of the *O. europaea* L. genome (De la Rosa et al. 2003; Wu et al. 2004).

Amplified Fragment Length Polymorphisms

The methodology necessary to develop amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995) is more complicated than the one used in the case of the RAPD markers. Firstly, the total genomic DNA of the species must be digested and the resulting fragments ligated to adaptors used as priming sites in the following rounds of PCR.

In the olive tree, AFLP markers have been used to study the intra-cultivar (Belaj et al. 2004) and inter-cultivar variation (Sanz-Cortes et al. 2003) showed by different Spanish varieties and to explore the genetic diversity and relationships of Slovene

(Bandelj et al. 2004) and southern Italian cultivars (Rao et al. 2009). Owen et al. (2005) extended the study including cultivars from Eastern, Central and Western Mediterranean Basin and found a grouping fashion that supports the East–West divergence of olive. Interestingly, Grati-Kamoun et al. (2006) reported the clustering of olive cultivars grown in Tunisia but coming from different areas of the Mediterranean Basin according to their fruit size but not to their geographical origin when they studied their genetic relationships using AFLPs. Montemurro et al. (2005) observed cultivars from different Mediterranean countries grouped according to their end-use (oil, table or dual purpose) when they analysed data generated from AFLP and microsatellite makers. The associations based on traits of such economic importance could be very useful when it comes to decide crosses in breeding programmes, as sometimes the genitors are chosen not only because of their outstanding characteristics but also because they are not genetically close, minimizing in this way inter-incompatibility issues. Together with RAPDs, AFLPs have been employed to investigate the relationship between the feral and the cultivated olives (Angiolillo et al. 1999; Baldoni et al. 2000). Gallitelli et al. (2001) carried out the same kind of study about the genetic distance among cultivated varieties, including an evaluation of the usefulness of AFLPs compared to RAPDs. Finally, AFLPs are among the markers used for obtaining the first linkage map in olive (De la Rosa et al. 2003).

Sequence Characterized Amplified Regions

Any of the DNA markers mentioned above can be transformed into an easier to use and more specific and robust sort of marker named sequence characterized amplified region (SCAR) (Paran and Michelmore 1993). For this purpose, it is necessary to clone and sequence one of the fragments obtained previously and design specific primers to amplify the region by PCR. In the olive tree, the development of several SCAR markers from RAPD (Hernandez et al. 2001a, b; Bautista et al. 2003), AFLP (Busconi et al. 2006; Pafundo et al. 2007) and selective amplification of microsatellite polymorphic loci (SAMPL, Busconi et al. 2006) bands have been reported. Though these markers are less polymorphic than others due to their dominant nature, they can be successfully used for identification purposes. In this way, the ten markers obtained by Bautista et al. (2003) were sufficient to unambiguously discriminate the 22 geographically related cultivars in study.

Microsatellites

Microsatellites or “simple sequence repeats” (SSRs) are short (1–6 bp) tandemly repeated DNA motifs (Hamada et al. 1982). They are multi-allelic, hypervariable, codominant and amenable to automation by PCR markers.

Up to date, 120 microsatellites are available in the olive tree (Rallo et al. 2000; Sefc et al. 2000; Carriero et al. 2002; Cipriani et al. 2002; De la Rosa et al. 2002; Wu et al. 2004; Diaz et al. 2006a; Sabino-Gil et al. 2006). Even if a great effort has

been made to develop good, reliable molecular tools such as microsatellite markers that could assist breeding programmes, compared to other fruit trees, like the apple tree (*Malus domestica* B.) or the peach tree (different species of *Prunus*), it is inevitable to reach to the conclusion that they are still insufficient. All of them have been used for cultivar identification, detecting even intra-cultivar variation in some cases (Cipriani et al. 2002). Their high discrimination power have made possible to solve many cases of homonyms, synonyms and misnamings (La Mantia et al. 2005; Cantini et al. 2008). Additionally, microsatellites have been widely employed for elucidating genetic relationships among olive cultivars (Carriero et al. 2002; Belaj et al. 2003; Bandelj et al. 2004; Diaz et al. 2006a) and among the first 17 selections of an olive breeding programme (Diaz et al. 2007a) that ultimately has rendered a new variety (Luis Rallo et al. 2008a; Pilar Rallo et al. 2008b). Microsatellites have revealed a certain tendency of the cultivars to group together according to their geographical origin and routes of propagation (Rallo et al. 2003; Bandelj et al. 2004; Diaz et al. 2006a; Sarri et al. 2006), though some clustering based on their end-use have been reported too (Montemurro et al. 2005; Rekik et al. 2008). Furthermore, it has been verified that microsatellites can be transferred to related species belonging to the genus *Olea* (Rallo et al. 2003) or even to other genera in the family *Oleaceae* (De la Rosa et al. 2002), since microsatellites flanking sequences are highly conserved. And, more interestingly, the microsatellites developed in other species belonging to the same family have been employed, together with other markers, for elaborating a linkage map of the olive genome (De la Rosa et al. 2003). Different types of DNA markers, including microsatellites, have been used to construct a new olive linkage map (Wu et al. 2004).

Microsatellites have revealed themselves to be very useful for checking parentage of olive progenies from controlled crossings (De la Rosa et al. 2004; Diaz et al. 2006b, 2007a, b) since their great polymorphism makes it possible to obtain high parentage exclusion probabilities and, in some cases, to assign the paternity to concrete genotypes. De la Rosa et al. (2004) proved the enormous contamination present among the offspring coming from selfings and out-crosses within an olive breeding programme (64.4% of the seedlings had a different pollen donor from the nominal one) using this methodology. Similarly, Diaz et al. (2006b) found that none of the seeds coming from the self-pollination of 'Picual' and 'Arbequina' olives were really products of self-fecundations. Interestingly, when the offspring from controlled crosses was analysed, the pollen contamination rate was either almost total or almost null depending on the cultivars chosen as genitors (Diaz et al. 2007b). All this supports the idea of an incompatibility system acting in some olive cultivars. Microsatellites have also been used to assign the paternity to olive seeds coming from free-pollination in Australia (Mookerjee et al. 2005). However, these results themselves are not sufficient to affirm that the cultivars chosen as mother trees are self-incompatible, since their flowers were not subjected to self-pollinations; they only corroborate that foreign pollen competes favourably with its own pollen, as it has been extensively reported in the literature (Fernandez-Escobar and Rallo 1981). The same can be argued for the intercompatibility relationships established. The knowledge of the cross-compatibility

relationships in olive (Diaz et al. 2006c, 2007b) is vital to design effective crosses in breeding programmes and the microsatellites seem to be the suitable tools to verify the paternity of the seedlings. Genotyping the individuals at an early developmental stage means time and effort savings since it makes possible to discard the unwanted ones (i.e. those coming from pollen contamination) before reaching the adult phase, when it is feasible to carry out a morphological characterization.

The wild olive germplasm represents a valuable source of variability with a huge potential in breeding programmes. The transfer of both qualitative and quantitative traits from wild into domesticated forms could become an attractive objective in olive breeding programmes. In this sense, an attempt to elucidate the genetic relationships within and between wild and cultivated olives using microsatellites has been made (Erre et al. 2010). This study shows the wild genotypes clustering together in a different gene pool than the cultivated forms, revealing the exotic germplasm as a source of new variability. Regarding the structure of wild populations from north-western Mediterranean, Belaj et al. (2007) observed high and low levels of diversity within and among populations, respectively, using microsatellite markers. They hypothesized that the hybridization with cultivars and the exchange of cultivated genetic resources among different Mediterranean regions could be behind the limited genetic differentiation among populations and the lack of grouping according to their geographical origin.

Actually, microsatellites have shown a higher level of polymorphism when they were compared to other markers, like AFLPs (Belaj et al. 2003; Bandelj et al. 2004; Montemurro et al. 2005) and RAPDs (Belaj et al. 2003). In this context, enterprises like the molecular database, included in the olea databases (http://www.oleadb.it), where the allelic profile of a wide set of olive cultivars for 12 microsatellite markers can be consulted, facilitate access to information continuously being expanded. At the same time, a standardization of some of the microsatellites available is starting to be carried out (Doveri et al. 2008; Baldoni et al. 2009), with the same cultivars being genotyped with a set of markers in different laboratories. This kind of work is aimed to compare the results obtained using diverse methodologies in different laboratories and to rank the markers according to their usefulness in cultivar discrimination.

Single Nucleotide Polymorphisms

In recent years, a new generation of molecular markers has entered into the molecular biology field, particularly in the human diseases diagnostic area. These are termed as single nucleotide polymorphisms (SNPs) and consist of single DNA base differences (single base pair changes or deletions) between homologous genomes in which the minor allele is present in 1% of the cases or more (Cooper et al. 1985). The considerable increase in sequences available in databases has revealed the high frequency of these DNA variations in genomes. This abundance turns SNPs into good genome coverage supplier markers, their frequency in several crop species being an order of magnitude higher than that of microsatellites (Kwok et al. 1996). Other desirable characteristic also present in SNPs is their codominant inheritance.

The first SNP-based markers were recently developed in olive (Reale et al. 2006). They were used for cultivar identification purposes, allowing the authors to verify the authenticity of samples coming from the same cultivar but collected in different geographical locations. They made possible to unambiguously discriminate 77% of the cultivars studied. Additionally, an assumed case of synonym between 'Ottobratica' and 'Mirtolia' was clarified, as both rendered different genotypes for the 11 markers tested (nine SNPs among them). This confirms the usefulness of molecular markers in clearing confusion in olive nomenclature. Methodologies to process a high number of samples with a large number of markers (due to their low polymorphism) are complicated. In this context, Consolandi et al. (2007) have used a microarray-based approach to identify 49 olive cultivars using 17 new SNPs. Similarly, Muleo et al. (2009) successfully used a high-resolution melting (HRM) analysis for identification purposes. Nonetheless, the most important drawback in scoring SNPs is the high cost and the necessity of sophisticated equipments for employing the majority of the methodologies developed. For this, the transformation of SNPs into codominant PCR and gel-based markers, like cleaved amplified polymorphic sequences (CAPS), as proposed by Reale et al. (2006), seems to be a good way of getting an easy to use and low-cost method.

Although the development and use of SNPs in olive are still in an early phase, these are markers with an enormous potential in a broad range of applications such as genetic diversity studies, evolutionary and population genetics, mapping, quantifying linkage disequilibrium and marker-assisted plant breeding. In this sense, SNPs located in coding or functional regions of the genomes are especially useful in MAS, since associations between the markers and particular traits allow a more efficient and cost-effective phenotype selection. In short, olive SNPs can be appropriate for the study of genetic diversity and cultivar identification at first, and in future for studies of associations with economically valuable traits.

5.2 Genetic Transformation

Genetic transformation can significantly contribute to plant breeding by generating additional genetic diversity, which can be subsequently subjected to selection through classical and molecular approaches, but also introducing alleles that encode desirable traits into superior cultivars. Successful genetic transformation has been undertaken in some economically important crop species, such as maize, rice, cotton and soybean. Concisely, two methodologies have been developed to transfer an engineered gene into a plant chromosome, the *Agrobacterium*-mediated transformation and the microprojectile bombardment. The first strategy employed in the case of the olive tree was the agro-transformation (Rugini 1986). This technique has been used with the aim of reducing the olive tree size and enhancing its rooting ability (Rugini and Fedeli 1990). The authors employed *Agrobaterium rhizogenes* to transform immature zygotic embryos of 'Morailo' cultivar. Though the transgenic nature of the calli selected was confirmed molecularly, the regeneration

was a limitation. Mencuccini et al. (1999) succeeded in obtaining transgenic calli using adult material (leaf petioles) from 'Dolce Agogia' cultivar as starting material. However, they failed to regenerate the whole plant. Regarding the biolistic technique, Lambardi et al. (1999) detected GUS transient expression in somatic embryos derived from 'Canino' cultivar. Though encouraging results were obtained with cotyledon explants, nowadays, the protocols for olive genetic transformation through biolistic methods are still under optimization (Perez-Barranco et al. 2009).

What becomes clear from above is that the progress in the genetic transformation methodologies in olive must be accompanied by the design of efficient regeneration protocols, via organogenesis and somatic embryogenesis.

5.3 Real-Time Quantitative PCR and Real-Time Quantitative Reverse-Transcription PCR

It is well known that plant diseases impact negatively on yield and fruit quality. For this reason, to develop efficient methods aimed to monitor the sanitary status of the plants is essential to undertake successful breeding programmes. The development of real-time quantitative PCR (qPCR) and real-time quantitative reverse-transcription PCR (qRT-PCR) has allowed the routine and reliable quantification of PCR products with a great specificity and sensitivity, becoming a valuable diagnosis tool (Schaad and Frederick 2002). The qRT-PCR has become a powerful diagnosis tool as in many cases, the viruses can be latent or the symptoms are cultivar-specific, the visual inspections being unreliable. Faggioli et al. (2002) employed one-step qRT-PCR protocol to correlate the infection of olive trees by the strawberry latent ring spot virus (SLRSV) with leaf symptoms. Furthermore, multiplex qRT-PCR has been optimized in olive, allowing to detect a number of different viruses infecting the tree in a single step (Bertolini et al. 2001; Luigi et al. 2009; Varanda et al. 2010). A variant of this technique, the nested qRT-PCR has been successfully used to detect four RNA viruses and the bacterium *Pseudomonas savastanoi* simultaneously in more than 240 olive samples belonging to 15 different cultivars (Bertolini et al. 2003a; Bautista et al. 2003). The high sensitivity of qRT-PCR made possible to diagnosis infection even when the amount of the viral RNA was below the minimum threshold required by other techniques (Grieco et al. 2002; Alabdullah et al. 2009; Varanda et al. 2010). Interestingly, the quantification of DNA coming from highly virulent (defoliating) and mildly virulent (nondefoliating) *Verticillium dahliae* by qPCR has allowed the establishment of a correlation between those values and the susceptibility of olive cultivars to Verticillium wilt (Mercado-Blanco et al. 2003). This methodology has rendered satisfactory results when combined with others, such as doubled-stranded RNA (dsRNA) analysis and dot blot hybridization, complementing and/or improving the data shed by the latter (Montemurro et al. 2008; Alabdullah et al. 2009).

Due to its sensitivity, among many other applications, qRT-PCR can be used to compare the gene expression of samples subject to different treatments. Benitez et al. (2005), coupling this technique to differential display, identified olive genes involved in signalling, transcriptional control and stress response, whose transcript levels were significantly raised after the infection with the fungus *Schinia oleagina*. Interestingly, the induction of those genes was higher and earlier in the resistant cultivar 'Lechin de Sevilla' compared to the susceptible cultivar 'Picual'. Additionally, the basal expression of some of those genes was increased in the resistant cultivar compared to the susceptible one even when uninfected, suggesting that a constitutive activation of the response pathways could be under its invulnerability. The authors propose the measure of the basal expression of those genes as a way of inferring the resistance or susceptibility level of a particular cultivar, as they observed a correlation between both parameters when different cultivars were analysed. The potential use of this type of assays as a breeding tool to identify and select resistant individuals is obvious.

6 Conclusions

Genetic engineering techniques cannot be considered as substitutes for classical methods in plant breeding. Quite the opposite, the new advances in Genetics and Molecular Biology should be used in combination with conventional breeding, which will facilitate the work of breeders, as it is actually happening in the case of the olive tree. From MAS to expression studies, without forgetting genetic transformation, groups working in olive have known how to incorporate them to their fields of study to generate basic knowledge and to apply it to the breeding of the species.

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Chapter 12
Soybean

Aditya Pratap, S.K. Gupta, Jitendra Kumar, and R.K. Solanki

Abstract Soybean (*Glycine max* (L.) Merrill.) is one of the most important oil crops of the world which also has tremendous importance as a food legume. Soy oil finds a variety of uses for domestic and industrial purposes besides its use in several food preparations and animal feed. Having 53% global production share of all oilseed crops, soybean finds an important place in most of the agricultural production systems of major countries including USA, China, Brazil, Argentina and India. It has found an important place in major crop improvement programs and consequently, there has been a considerable increase in its production and productivity over the last two decades. Soybean is a diploidized ancient tetraploid. Though it has a relatively large and complex genome, significant progress has been made towards using methods of genome analysis and molecular cytogenetic tools to elucidate its special function as well as to develop improved cultivars. A number of stable, high-yielding and biotic and abiotic stress resistant varieties have been developed using various traditional and modern crop improvement tools. Definite strides have been made in alien gene introgressions, molecular marker technology, micropropagation, genetic transformation, and marker-assisted breeding. Herbicide-tolerant transgenic soybean has witnessed a huge commercial success and made it a leading biotech crop. At the same time, modification of fatty acid profile of soy oil and improvement in protein content and nutritional quality have established soybean as one of the most viable commercial crop. This chapter discusses soybean as a crop in detail covering all major aspects related to its history and domestication, cytogenetics, breeding behavior, genetic improvement as well as its oil and nutritional quality.

Keywords Soybean • *Glycine max* (L.) Merrill. • Genetic improvement • Transgenics • Molecular breeding • Oil content

A. Pratap (✉)
Crop Improvement Division, Indian Institute of Pulses Research, Kanpur 208024, India
e-mail: adityapratapgarg@gmail.com

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1 Introduction

Soybean (*Glycine max* (L.) Merrill.) occupies a premier position among agricultural crops, being the most important source of good quality concentrated proteins as well as vegetable oil. Seeds of soybean have been used in Asia and other parts of the world for many centuries to prepare a variety of fresh, fermented and dried foods (Probst and Judd 1973). Soy-based nutritious food products such as tofu, soy milk, soy sauce, miso, etc. have been developed for human consumption while oil extracted soy meal is used as a nutritious animal feed. Besides its use for domestic purposes, soy oil finds multifarious uses in industries related to production of pharmaceuticals, plastics, papers, inks, paints, varnishes, pesticides and cosmetics. Recently, use of soy oil as biodiesel has opened up another possibility of renewable sources of energy for industrial uses. As a legume crop, soybean is capable of utilizing atmospheric nitrogen through biological nitrogen fixation and is therefore less dependent on synthetic nitrogen fertilizers. Keeping in view its vast utilities, there is ample justification for its significant involvement in major crop improvement programs throughout the world.

Soybean belongs to the family Leguminosae and subfamily Papilionaceae. The cultivated soybean has been proposed to be named correctly as *G. max* (L.) Merrill by Ricker and Morse in 1948 (Gazzoni 1994). The genus *Glycine* consists of two subgenera: *Glycine* (perennials) and *Soja* (annuals). The perennials consist of 22 recognized species and the annual two species, *G. max* L. Merrill. (cultigen) and *G. soja* Sieb. & Zucc. (wild species and progenitor of *G. max*) (Hymowitz 2004). Natural cross-pollination is usually less than 1% in the highly self-pollinated annual *G. max* though it may sometimes reach up to 2–3%. The perennial species have been reported to have up to 60% out-crossing for *Glycine argyrea* and *Glycine clandestina* (Brown et al. 1986). Both the cultivated and wild soybeans are paleopolyploids with $2n = 2x = 40$ and these are perfectly cross-compatible (Hymowitz 2004). Soybean has a relatively large genome (1.12×109 bp) (Arumuganathan and Earle 1991) and about 55% of its genome consists of highly repetitive sequences (Danesh et al. 1998).

Owing to the concerted efforts of the crop scientists and soybean growers, the world production of soybean has increased steadily during the last decade, rising from 155.1 million metric tons in 1999 to 210.9 million metric tons in 2009 (www.soystats.com, Fig. 12.1). Among all oilseed crops, soybean alone has maximum global production share (53%), followed by rapeseed (15%), cottonseed (10%) and peanut (9%). Among the soybean producing nations, United States of America has the biggest production share with about 38% world soybean production, followed by Brazil (27%), Argentina (15%), China (7%) and India (4%) (www.soystats.com, Fig. 12.2). Soybean development is highly sensitive to environmental fluctuations and water is the major factor having great impact on its productivity. Further, the effects of temperature and photoperiod are also important which determine the cultivation of soybean over space and time. Despite several biotic and abiotic stresses and production constraints, there have been remarkable gains in this wonder crop over the years. This chapter discusses the soybean crop with a special focus on its genetic and crop improvement aspects.

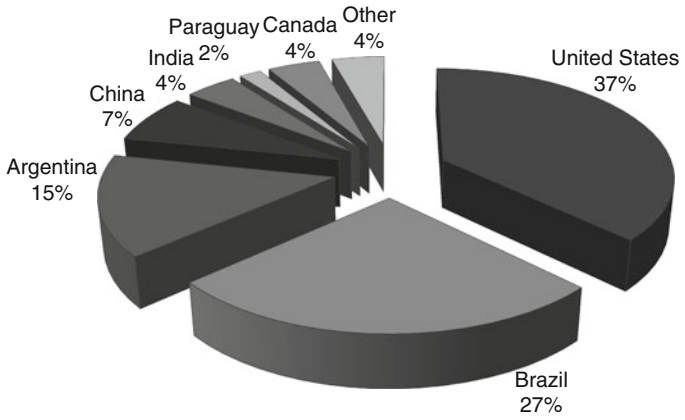

Fig. 12.1 Major soybean producing countries (2009) of the world

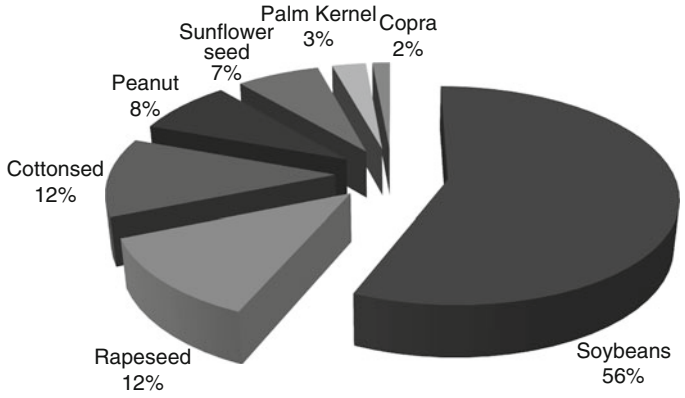

Fig. 12.2 Production of different oilseeds (2009) of the world

2 History, Origin and Evolution

Soybean as a crop is cultivated over a vast area throughout the globe and its history has been discussed well by various researchers (Hymowitz 1970; Guo 1993; Singh and Hymowitz 1999; Guo et al. 2010). Evidences suggest that soybean emerged as domesticate during the Shang dynasty in the eastern half of northern China during ca. 1700–100 BC (Singh and Hymowitz 1999). As one of the oldest cultivated food legumes, it has been known to man for over 5,000 years and therefore proposes it as a candidate place of its domestication (Hymowitz 1970). Molecular diversity studies conducted on soybean populations collected from north and south regions suggested that this crop was also domesticated in South China (Ding et al. 2008). Thus evidences suggest that both North and South China regions were involved in domestication of soybean since ancient times. The oldest records of soybean cultivation

appear in bronze inscriptions and in early writings that date not much earlier than 1100 BC. With the expansion of the Shang dynasty, trade of soybean migrated to South China, Korea, Japan and South East Asia. By the first century AD, soybeans were probably distributed throughout China by trade missions and with time to other Asian countries. The earliest Japanese reference to this crop was found in the *Kojiki* (records of ancient matters) that was completed in 712 AD. In the sixteenth and seventeenth centuries, there are several references to native soy foods in writings of European visitors to China and Japan. The first soybeans were brought to the United States by Samuel Bowen in 1765, a seaman employed by the East India Company, who planted them in "Greenwich," a few miles east of Savannah, Georgia. Mr. Bowen used soybean to produce soy sauce and a soybean noodle for export to England (http://www.soymeal.org/pdf/HistorySoybeanUse.pdf).

The late type soybean from South China was found closer to the wild type and it is expected that wild soybean is a common ancestor for cultivated type of South China, from which early cultivated types originated during the process of dissemination to North China (Gai et al. 2000). The higher genetic diversity among the South China population compared to North China population also supported the origin of soybean in South China (Ding et al. 2008).

3 Polyploid Nature of Soybean

The genus *Glycine* is of an ancient polyploid origin and its genome has been reported to have gone through two major rounds of duplication events during speciation (Schlueter et al. 2004; Van et al. 2008). The haploid genome studies also suggested that soybean is a diploidized ancient tetraploid (Safari and Schlueter 2011). Studies showed that these duplication events occurred at ~14.5 and 45 MYA (Schlueter et al. 2004; Blanc and Wolfe 2004). The genetic map of soybean revealed multiple nested duplications that appeared to reflect an even more ancient round of polyploidy at some point in the ancestry of the genus (Shoemaker et al. 2006). It has been suggested that the ancestral "diploid" genome donors of modern "allopolyploid" soybean were themselves stabilized paleopolyploids from an earlier round of genome duplication.

The soybean genome has been described as having both allo- and autopolyploid origin. An allopolyploid soybean genome was first hypothesized based on cytogenetic (Singh and Hymowitz 1985) and molecular studies (Shoemaker et al. 1996), while the phylogenetic analysis of nuclear genes has hypothesized its origin to be of autopolyploid nature (Doyle et al. 2003; Straub et al. 2006). A novel molecular cytogenetic tool, the fluorescence in situ hybridization (FISH) has clearly distinguished ten chromosome pairs in soybean suggesting that there are two distinct and co-resident genomes in its nucleus having two types of centromeres, which reflect divergence in its two diploid progenitors (Udall and Wendel 2006).

Most of the molecular studies suggest that cultivated species, *G. max*, has close phylogenetic relationship with wild species, *G. soja*, which is known as a progenitor

of this species. The North Asian subgenus *soja* has been suggested to be the probable wild progenitor of cultigen *G. max* (L.) Merrill. (Doyle et al. 2003). A number of perennial diploid relatives of *Glycine* have been found throughout Australia and Papua New Guinea. Among these, there are reports of intercrossing among the diploid species which resulted in some allopolyploid taxa (Doyle et al. 2004). Doyle et al. (2004) has defined the *tomentella* and *tabacina* complexes, which have been described as allopolyploids found in the wild. These resulted from various combinations of diploid progenitors, which support that these polyploids have clearly arisen through multiple origins.

4 Biology and Breeding Behavior

Soybean is a hairy annual with an extensive tap root system, most of it in the top 15 cm of the soil. The tap root may grow as deep as 2 m and adventitious roots grow from the hypocotyls (Chaturvedi et al. 2011). Cultivated soybeans have an erect growth habit though procumbency is not uncommon in germplasm resources (Burton 1997). The modern cultivars of soybean are mostly erect, bushy, 20–180 cm tall, usually with a few primary branches and no secondary branches. Exceptionally prostrate and freely branching forms are also found, particularly in those varieties which are meant for forage purposes. The leaves are trifoliate and alternate with long petioles and small stipules and stiples; the leaflets are ovate to lanceolate with mucronate tip.

In soybean, flowering and maturity are greatly influenced by photoperiod. The flowers are typical papilionaceous, white or pale purple, with a tubular calyx of five unequal sepal lobes and a five-member corolla that consists of a posterior standard petal, two lateral wing petals and two anterior keel petals (Guard 1931). The androecium is diadelphous with 9 + 1 arrangement. The single pistil is unicarpellate and has 1–4 campylotropous ovules (Palmer et al. 2001). The style curves back toward the posterior stamen and is surrounded by a knob-like stigma (Carlson and Lersten 1987). Each flower is subtended by two bracteoles and has a hairy calyx of five pointed sepals united for about half of their length. The pods are short stalked and occur in groups of 3–15, 3–7 cm long and hairy, light brown at maturity and slightly constricted between the seeds. The seeds vary greatly in shape, size and color though these are mostly often round and yellowish, brown or black with epigeal germination.

Soybeans are mostly self-pollinated, though rates of natural cross-pollination have been observed to be between 0.03 and 1.14% in natural conditions (Culter 1934; Caviness 1966). The wild annual soybean, *G. soja* is predominantly self-pollinated, while the perennial wild relative, *G. argyrea* (Ting.) and its closely related species, *G. clandestine* (Wendl.), have both self-fertilized cleistogamous as well as chasmogamous flowers on the same plant (Brown et al. 1986; Schoen and Brown 1991; Palmer et al. 2001). The chasmogamous flowers are frequently visited by insect pollinators leading to cross-pollination. Small insects such as thrips and

honeybees are mainly responsible for natural outcrossing in soybean, but other insects are also observed to be working on soybean flowers. Insect-mediated cross pollination in soybean has been discussed in detail by (Palmer et al. 2001). Self-pollinating soybean flowers have 3–4 ovules, which reach maturity prior to anthesis (Stelly and Palmer 1985). Flowers open and normally self-pollinate at anthesis. For planned controlled pollination, first the sepals and petals are carefully removed from the young unopened flowers. This is followed by removal of anthers by forceps or tweezers though removal of anther is not always necessary (Stelly and Palmer 1985). The remaining flowers on the inflorescence are also removed. Pollination is done the next morning. For this, the flowers which are about to open should be taken. In these, with the help of forceps, first sepals are removed, followed by removal of the standard petal, wing and keel petals to expose the anthers. These anthers are then gently brushed on the stigma till the pollen is clearly visible on it. A small pod is usually visible in 6–7 days.

5 Molecular Cytogenetics

The molecular cytogenetic tools and the methods of in situ hybridization, viz., genomic in situ hybridization (GISH), FISH, multicolor FISH and extended DNA fiber mapping have revolutionized our understanding of the structure, function, organization and evolution of genes and the genome (Chaturvedi et al. 2011). These methods have made it feasible to link the molecular data about DNA sequence with chromosomal expression and information at the tissue, cellular and sub-cellular levels and hence changed the way we apply cytogenetics to agriculture (Schwarzacher and Heslop-Harrison 2000). In soybean, these tools have also been extensively used to resolve various issues related to the origin of the species, assessment of variability and physical mapping of chromosomes besides physically mapping the whole genomes and the targeted alien introgressions.

In soybean, the cytological study of metaphase chromosomes is a challenging task due to its small size (1–2 μm), large number (2n = 40) and very little morphological diversity (Sen and Vidyabhusan 1960; Clarindo et al. 2007). With the exception of a single acrocentric pair, soybean chromosomes are all metacentric or sub-metacentric making them difficult to distinguish in routine mitotic preparations. The first cytological description of domesticated soybean (*G. max*) was developed using pachytene chromosomes, which were numbered from 1 to 20 on the basis of total chromosomes length, arm length ratios and relative proportions of euchromatin and heterochromatin (Singh and Hymowitz 1988).

In situ hybridization of DNA probes to soybean chromosomes was first reported by Skorupska et al. (1989) and later by Griffor et al. (1991). Soybean repetitive DNA has been used to develop fluorescent in situ hybridization probes that could differentially label mitotic chromosomes in root tip preparations. These karyotyping tools were applied to wild soybean (*G. soja*), which represents a large gene pool of potentially agronomically valuable traits. Reciprocal chromosome translocations

between chromosomes 11 and 13 in two accessions of wild soybean were identified and characterized. The translocation is wide spread in *G. soja* accessions and likely accounts for the semi-sterility found in *G. soja* × *G. max* crosses.

Apart from identification of chromosomes, molecular cytogenetics has also been used to suggest polyploidy in *G. max*. Two soybean centromere-specific satellite repeat classes in its genome suggest the existence of two sub-genomes (Gill et al. 2009). The ancestor of soybean and the remainder of the genus *Glycine* have been hypothesized as being formed via a polyploidy event within the last 15 million years (Shoemaker et al. 2006). However, it remains unclear whether the event was allo- or autopolyploidy (Kumar and Hymowitz 1989; Straub et al. 2006). Lackey (1980) suggested that there have been several rounds of polyploidization and segmental duplication in soybean on the basis of chromosome number. Similarly, it was also repeated on the basis of multiple hybridizing RFLP fragments (Shoemaker et al. 2006) and on the basis of implicated ESTs (Blanc and Wolfe 2004; Schlueter et al. 2004).

6 Genetic Improvement

Improving seed characteristics for oil and protein content, plant characteristics and resistance to biotic (diseases and insect-pests) and abiotic (mainly environmental) stresses are the major breeding objectives in soybean. History of soybean breeding started with domestication, which is believed to have first occurred in the eastern half of China during ca. 1700–1100 BC (Singh and Hymowitz 1999). In the sixteenth and seventeenth centuries, it was brought into Europe by missionaries and was likely introduced into North America in 1765 and after that reintroduced several times (Sleper and Shannon 2003). During 1860–1900, the introduced soybeans were grown in all agricultural research stations across the United States and research was conducted on their improvement (Probst and Judd 1973). In USA, plant introduction of various lines has contributed significantly in genetic improvement for yield potential of soybean (Pathan and Sleper 2008). Introduced lines or cultivars from the United States were the important early sources of adapted materials for planting soybean at latitudes of 22°N to 30°S in Brazil (Ferraz de Toledo et al. 1994).

Keeping in view the self-pollinating behavior of soybean, the steps used for its cultivar development are: (a) hybridization of the selected parents (in single, three-way or multiple combinations) or (b) increase in homozygosity by selection (by pedigree, mass selection or single seed descent methods), and (c) yield testing. Conventional breeding is generally based on phenotypic selection of superior individuals from segregating populations, which takes about 8–10 years to complete the cycle starting with making the crosses to release of variety/germplasm (Pathan and Sleper 2008). Over the past years, pedigree, bulk, mass selection, single seed descent and early generation testing methods have been commonly followed for the development of improved soybean cultivars, with little efforts towards population

improvement and hybrid breeding. Pedigree selection is effective in incorporating desirable qualitative traits into breeding material. However, it is generally used in small breeding programs because it is highly labor intensive. With pedigree selection, desirable families are selected in each generation and one or more plants from each family are advanced to next generation through inbreeding/selfing. Bulk or population method is now widely used due to its simplicity and also because natural selection also gets a chance to operate. Single-seed descent (SSD) method is the most widely used method to increase homozygosity in soybean. This method has been further modified as single pod descent (SPD) by breeders to hasten the harvesting process. The major emphasis has been laid on high seed yield, oil content and quality, resistance to biotic and abiotic stresses and maturity duration. Efforts have also been directed toward development of plant types.

Early generation testing developed in Canada as a modification of bulk method has also been shown to be very feasible for improving the characters showing additive and additive × additive genetic components of variance and has been found to be efficient and successful in soybean (Cooper 1990). It has an advantage over late generation testing due to reduction of population load as inferior lines are discarded in early generations. However, F_2, F_3 and even F_4 families are subjected for early generation selection depending upon the target trait and environmental conditions (Burton 1997). In soybean, various recurrent selection methods have also been used or proposed. These include S1 family selection for yield (Kenworthy and Brim 1979; Rose et al. 1992) and protein (Brim and Burton 1979), mass selection for oil (Burton and Brim 1981) and seed weight (Tinius et al. 1991), and half-sib family selection for seed yield (Burton and Carver 1993) and oil quality (Carver et al. 1986). Successful application of recurrent selection in soybean could be due to the availability of male sterile lines and it has been employed for improvement of yield (Tinius et al. 1991), oil and protein content (Burton and Brim 1981) and fatty acid (Carver et al. 1986). Soybean breeding in USA has been viewed as a process of cyclic recurrent selection in which superior cultivars are selected and released, then combined and tested and four such cycles in the selection of MGI-IV cultivars between 1933 and 1971 were identified by Luedders (1977), from which yield increase were reported to the tune of 1 and 0.6% per year in the simultaneous tests of such cultivars.

Breeding populations are often developed by 2-way, 3-way or 4-way crosses of cultivars and/or breeding lines. If unadapted germplasm is used, at least one backcross to the adapted parent is often used (Burton 1997). This is usually done for random mating purposes in recurrent selection population improvement programs (Brim and Stuber 1973; Burton et al. 1990; Tinius et al. 1991). However, controlled biparental pollinations are also possible (Nelson and Bermard 1984; Lewers et al. 1996).

Increasing both protein and oil concentration in seeds is an important breeding goal in soybean, but these are negatively correlated (Brim and Burton 1979). It has been reported that oil content in soybean is governed by additive gene effects, additive × additive epistatic interaction and complementary epistasis (Rahangdale and Raut 2002) and therefore, use of recurrent selection schemes can be the most effective for increasing oil content (Burton and Brim 1981).

High phytic acid (PA) in soybean seeds causes mineral malnutrition in human beings. Therefore, systematic studies have been conducted on this aspect. Recently, it has been observed that total phosphorus (P) and phytate P (PhyP) are controlled by dominant recessive epistasis, which can help us to develop low phytate containing varieties (Sompong et al. 2010). The quality of soybean oil is also determined on the basis of polysaturated fatty acid/saturated fatty acid ratio, mono-unsaturated fatty acid and ratio of essential fatty acids such as linoleic/linolenic. High linolenic acid in soybean oil has poor oxidative stability (Patil et al. 2004). Isoflavon in soybean oil is another important target to improve the oil quality. For this trait, epistatic interactions have been observed, except for malonyldiadzin (MDZ). For obtaining the largest selection gains for this trait, importance has to be given for exploiting the additive genetic variances in superior lines or the cytoplasmic effect and the epistatic interactions between cytoplasmic and nuclear genes (Chiari et al. 2006). Lutein is a major carotenoid in soybean seed beneficial for eye health. This component is positively correlated with oleic acid and negatively correlated with lenoleic and lenolenic acid contents (Lee et al. 2009).

Lodging resistance is another important target for improvement in soybean cultivars. Erect growth habit reduces mechanical harvest loss and has maximum light penetration through plant canopy. Besides this, soybean breeders have used several other traits with mixed results such as narrow leaflets, brachytic stem (short internode), stem termination change to alter height, and more fibrous rooting (Wells et al. 1993). Change in the length of reproductive period has also been focused for adaptation to specific environments. However, in practice, lengthening the pod-filling period and/or changing the rate of dry matter accumulation in pods have given minor improvement in yield improvements, despite positive correlation between these two traits (Smith and Nelson 1986).

6.1 Distant Hybridization

Since soybean is a highly inbreeding plant and during the last many decades, soybean breeding has been limited to mainly gene-pool 1 (GP1), most of the present day cultivars have a narrow genetic base. Plant breeders have mostly used existing germplasm and land races to develop new varieties for desirable agronomic traits. However, there has not been remarkable achievement in yield and other traits partly because enough genetic diversity is missing for some of the traits to make progress. This could be due to genetic bottlenecks that occurred during the domestication process (Tanksley and McCouch 1997). Wild species/relatives are useful reservoirs of genes for various quality traits including resistance genes to many biotic and abiotic stresses. However, their transfer from wild species to the elite cultivars through conventional breeding have been limited, due to pre- and post-fertilization barriers and the associated transfer of undesired alleles (linkage drag). Efforts have been made to recover/transfer the favorable alleles in elite germplasm that were left behind by the domestication process more efficiently using embryo rescue techniques, hormonal manipulations and innovative genomics-assisted breeding strategies such as molecular maps and integrative QTL analysis.

Based upon the success rate of hybridization among the species, Harlan and de Wet (1971) proposed the concept of primary (GP-1), secondary (GP-2) and tertiary (GP-3) gene pools. The soybean cultivars, land races and their wild annual progenitor, *G. soja*, have been placed in GP-1 (Singh and Hymowitz 1999). However, going by the definition of GP-2 by Harlan and de Wet (1971), which states that "all species that can be crossed with GP-1 with at least some fertility in F_1," none of the species qualifies to fall in GP-2 of soybean. The GP-3 includes 16 wild perennial species of the subgenus *Glycine*. All these species are indigenous to Australia and are geographically isolated from *G. max* and *G. soja* (Singh and Hymowitz 1999). These species are extremely genetically diverse, grow in very diverse conditions and have a very wide geographical distribution (Kollipara et al. 1997; Singh and Hymowitz 1999). From reports, it is indicated that gene transfer from wild perennial species to soybean is possible. Consequently, intersubgeneric hybrids have been produced and fertile modified diploid lines have been obtained. The wild progenitor of soybean, *G. soja*, despite having a number of undesirable traits, may be an excellent source of genetic variability, continuous backcross breeding and selection may be practiced to remove the undesirable traits. Despite a number of attempts to hybridize wild perennial *Glycine* spp. with the soybean, only a few sterile intersubgeneric F_1 hybrids have been reported. Initially, attempts to broaden the genetic base of *G. max* by utilizing *G. soja* were reported by Hartwig (1973), Ertl and Fehr (1985), and Carpenter and Fehr (1986). Later, limited numbers of interspecific crosses were attempted between *G. max* and *G. soja* (Palmer and Kilen 1987; Singh and Hymowitz 1988). Singh et al. (1990, 1993a, b) successfully produced backcross-derived fertile progenies using soybean and a wild perennial *G. tomentella*. AB-QTL approach has also been used in soybean. For instance, Chaky et al. (2004) generated 296 $BC_2F_{4:6}$ backcross introgression lines (BILs) from the cross *G. max* (Dunbar) × *G. soja* (PI 326582A). This study provided several QTL for seed yield, seed protein and oil as well as some BILs that were late-maturing and taller.

6.2 Male Sterility and Hybrid Development

Hybrid breeding program has not been much successful in soybean owing to its highly self-pollinating nature, absence of stable male sterility–female fertility systems, lack of efficient pollen transfer mechanisms, low number of seed set per pod and poor natural crossing (Singh and Hymowitz 1999; Palmer et al. 2001). Due to the small size of the soybean flowers, manual cross-pollination to produce large quantities of hybrid seed is also difficult and time consuming. Still, possibilities of development of hybrid varieties have been explored and efforts have been made on identification of male sterility systems. Several genic male sterile lines (*ms1*, *ms2*, *ms3*, *ms4*, *ms5* and *ms6*) have been identified (Graybosch and Palmer 1988; Skorupska and Palmer 1989; Palmer and Skorupska 1990; Palmer et al. 2001) and the different types of male sterility systems have been discussed (Palmer et al. 2001).

Table 12.1 Male-sterile, female-fertile mutants of soybean reported in United States

Mutant	Phenotype	References
ms1 (North Carolina)	Male sterile	Brim and Young (1971)
ms1 (Urbana)	Male sterile	Boerma and Cooper (1978)
ms1 (Tonica)	Male sterile	Palmer et al. (1978)
ms1 (Ames 1)	Male sterile	Palmer et al. (1978)
ms1 (Ames 2)	Male sterile	Skorupska and Palmer (1990)
ms1 (Danbury)	Male sterile	Skorupska and Palmer (1990)
ms2 (Eldorado)	Male sterile	Bernard and Cremeens (1975)
ms2 (Ames 1)	Male sterile	Palmer (2000)
ms2 (Ames 2)	Male sterile	Cervantes-Martinez et al. (2005)
ms3 (Washington)	Male sterile	Palmer et al. (1980) and Graybosch and Palmer (1987)
ms3 (Flanagan)	Male sterile	Chaudhari and Davis (1977) and Graybosch and Palmer (1987)
ms3 (Plainview)	Male sterile	Skorupska and Palmer (1990)
ms4 (Ames)	Male sterile	Delannay and Palmer (1982)
ms4 (Fisher)	Male sterile	Skorupska and Palmer (1990)
ms5	Male sterile	Buss (1983)
ms6 (Ames 1)	Male sterile	Palmer and Skorupska (1990) and Skorupska and Palmer (1989)
ms6 (Ames 2)	Male sterile	Ilarslan et al. (1999)
ms7	Male sterile	Palmer (2000)
ms8	Partial male sterile	Palmer (2000) and Frasch et al. (2010)
ms9	Male sterile	Palmer (2000)
Msp	Partial male sterile	Stelly and Palmer (1980)

Source: Palmer et al. (2004)

The interest in hybrid soybean developed after the identification of the first male-sterile, female-fertile mutant (Brim and Young 1971). Its use in recurrent selection breeding programs (Brim and Stuber 1973; Lewers and Palmer 1997) increased the awareness of the potential to produce commercial hybrids in soybean. Several nuclear recessive genes are reported to confer male sterility in soybean, which are used by soybean breeders for insect-mediated pollination. Genetic mutations affecting microsporogenesis and microgametogenesis in soybean have generated male-sterile, female-fertile lines. A detailed list of genes controlling sterility and their corresponding phenotype is shown in Table 12.1. Palmer and Lewers (1998) reported that male sterility in soybean is controlled by single recessive gene, but the local conditions need to be addressed to support chances of pollination and pollination vectors for hybrid seed production (Perez et al. 2008).

A workable CMS system with appropriate maintainers and restorers is a prerequisite for commercialization of a hybrid (Wang et al. 2009). Several CMS systems have been identified in soybean (Zhang et al. 1999a, b; Sun et al. 1994, 1997; Bai and Gai 2003; Zhao and Gai 2006; Li et al. 1995; Xu et al. 1999). Stability of CMS lines, restores and maintainers has been well documented in China (Wang et al. 2009). Using these systems, world's first commercial soybean hybrid was

released in 2003 in China. The identification of cytoplasmic-nuclear male-sterile lines along with their maintainers and restorers has also been achieved by intraspecific (*G. max*×*G. max*) and interspecific (*G. max*×*G. soja*) hybridizations (Davis 1987; Sun et al. 1994, 1997; Zhang and Dai 1997; Ding et al. 1998; Bai and Gai 2003; Zhao and Gai 2006).

The degree of heterosis is an important consideration in commercial hybrid seed production program. Heterosis studies have shown that levels, above the better parent, are possible (Brim and Cockerham 1961; Manjarrez-Sandoval et al. 1997; Ortiz-Perez et al. 2007; Perez et al. 2009; Sun et al. 1999). Manjarrez-Sandoval et al. (1997) recorded heterosis as high as 11% across locations. In some cases, the better hybrids yielded between 10 and 20% more than the better parent (Palmer et al. 2001). However, many of the studies in hybrid soybean have been conducted in single rows with spaced plants, conditions that are different from commercial fields and therefore may not give reliable indications on superiority of hybrids. In other studies, where more hybrid seed was available, yield tests were done in replicated plots in several environments.

Upon obtaining a stable male sterility system, it is necessary to transfer the pollen from the male parent to the female parent. Entomophilus cross-pollination of male-sterile soybean plants may facilitate the production of hybrid seed (Nelson and Bermard 1984; Ortiz-Perez et al. 2007). Pollinator insects such as honeybees (*Apis melliphera*) and alfalfa leaf cutter bee (*Megachile rotundata* F.) are attracted to soybean flowers and can be used in hybrid production. Some wild native bees, primarily from families Megachilidae, Halictidae, Anthophoridae and Andrenidae, could be efficient pollinators (Ortiz-Perez et al. 2007).

7 Biotechnology

Despite the systematic and continuous breeding efforts through conventional methods, significant genetic gain in soybean production is yet to be achieved. Among the major yield constraints, a large amount of genotype×environment (G×E) interactions on the expression of important quantitative traits and susceptibility of soybean genotypes to biotic and abiotic stresses have led to slow progress in its genetic improvement and yield stability. In recent years, biotechnological tools have opened up new avenues to complement traditional plant breeding and both of these can work together for accelerated improvement of not only soybean but all agricultural crops also. Various biotechnological tools such as plant tissue culture, genetic transformation, molecular breeding and marker-assisted selection can play a major role in developing superior cultivars.

7.1 Micropropagation and Somaclonal Variation

Micropropagation is the process of in vitro multiplication of donor plant to produce a large number of true to type progenies and its goal is to obtain a large number of healthy plants in a short period with minimized expenses (Skrzypek et al. 2011).

Micropropagation is often used to speed up the breeding process. Through micropropagation, a cell or group of cells from somatic tissues such as roots, cotyledons, stems, leaves or reproductive organs form an embryo. It mostly occurs indirectly via an intervening callus phase, or sometimes embryos may arise directly from the explant surface, likely from epidermal or sub-epidermal layers. Successful micropropagation may greatly help in the generation of additional variability through isolation of somaclonal/gametoclonal variants as well as genetic transformants. There are reports on morphological variants in soybean through cell and tissue cultures (Graybosch et al. 1987; Bailey et al. 1993). Somatic embryos and young callus tissue can be an object of genetic transformation, or they can be used to start cell or protoplast suspension culture, suitable to alternative methods of transformation or in vitro mutagenesis. The success of micropropagation protocols relies on many factors, viz., genotype of the plant from which explant is taken, culture and environmental conditions, explant used and its disinfection, pretreatment, composition of nutrient media, length of treatment during subsequent culture phases, subcultures of calli, ex vitro acclimatization of the plantlets and conditions of further growth.

Cotyledonary nodes from mature seeds have been the most responsive for the induction of multiple shoots via organogenesis in soybean (Barwale et al. 1986). Initially, Barwale et al. (1986) succeeded in obtaining fertile plants in 54 soybean genotypes using callus cultures derived from immature embryos on basal MS medium (Murashige and Skoog 1962) with B5 vitamins (Gamborg et al. 1968), supplemented either by 8 mg/L naphthaleneacetic acid (NAA) or 3 mg/L benzylaminopurine (BAP) and 0.037 mg/L NAA. These embryos were successfully regenerated into plants on the medium supplemented with 0.38 mg/L BAP and 0.04 mg/L indol-3-butyric acid (IBA). Later, Finer and Nagasawa (1988) elaborated the suspension culture system based on high level of synthetic auxin analogue 2,4-D in the induction medium. This protocol was also applied for soybean transformation (Trick and Finer 1998; Santarem and Finer 1999) and in vitro mutagenesis (Van et al. 2008). Bailey et al. (1993) made further improvements of the protocol, testing additional growth regulators, source of carbohydrates and other medium additives. Several other modifications were done to the media and culture conditions for improving the plantlet recovery frequency from the cultured explants (Walker and Parrott 2001; Schmidt et al. 2005). Genotypes have been reported to influence the protocol's efficiency (Barwale et al. 1986; Parrott et al. 1989; Tomlin et al. 2002; Van et al. 2008).

7.2 Doubled Haploid Breeding

Haploids induced by in vitro culture of gametophytic cells, particularly male gametophytes, are of tremendous importance in crop improvement programs. Doubled haploid (DH) breeding enables the breeders to develop completely homozygous genotypes from heterozygous parents in a single generation and allows fixing the recombinant gametes directly as fertile homozygous lines (Pratap et al. 2006). DH lines may be used as mapping populations for molecular linkage maps, besides their use in mutation breeding and genetic engineering. Above all, in vitro screening for

complex traits such as drought, cold and salinity tolerance can be done during the culture process (Pratap et al. 2005). Over the past three decades, several attempts have been made to develop anther and microspore culture systems for soybean (Cardoso et al. 2004; Ye et al. 1994). Several initial studies reported induction of callus from anthers (Ivers et al. 1974; Liu and Zhao 1986), shoot organogenesis (Yin et al. 1982; Jian et al. 1986) and embryo-like structures (ELS) from anther-derived callus (Hu et al. 1996; Kaltchuk-Santos et al. 1997). In a few cases, a low number of plants were regenerated, though the haploid origin of the plants was uncertain (Yin et al. 1982; Jian et al. 1986; Hu et al. 1996; de Moraes et al. 2004; Tiwari et al. 2004).

Genotypic and donor plant growth conditions can have a profound effect on embryogenic response. Similarly, growth media also plays an important role in any micropropagation program. In soybean, most of the protocols have used anthers collected from the field (de Moraes et al. 2004; Cardoso et al. 2004) in contrast to most other species where donor plants are grown under controlled conditions. Several basal media and their modifications have been tried to induce haploids in soybean with varying results. In general, B5 medium with 16 organic compounds (Zhuang et al. 1991) and with Yeung's amino acids (Yeung and Sussex 1979) is appropriate for anther culture. De Moraes et al. (2004) obtained one confirmed haploid plant (n = 20), following induction of embryogenic calli from anthers on this basal medium supplemented with 2.0 mg/L 2,4-D, 0.5 mg/L BAP, 9% sucrose and 0.25% phytagel. This result further confirms the finding of Hu et al. (1996) that 2,4-D is essential for soybean microspore callus induction. There is no general consensus regarding the most appropriate microspore developmental stage and the effect of pretreatment stress on androgenesis from soybean. Yin et al. (1982) and Ye et al. (1994) found that the early- to mid-uninucleate stage was best for induction while several other authors reported mid- to late-uninucleate and early binucleate stage of pollen development to be more appropriate (Kaltchuk-Santos et al. 1997; Cardoso et al. 2004). Among pretreatments, temperature stress has been most frequently studied (Liu and Zhao 1986; Rodrigues et al. 2005). However, despite these efforts any major breakthrough is yet to be achieved in haploidy breeding in soybean.

7.3 Molecular Breeding

Plant breeding is moving fast since the molecular breeding tools have become available. Use of molecular markers for improving breeding efficiency was initially suggested in 1989 (Tanksley et al. 1989). Since then, identification of molecular markers associated with traits of interest to breeders has witnessed tremendous progress in many crops including soybean. Two main approaches, viz., linkage mapping and association mapping, are now being routinely used for identifying marker-trait associations in soybean. Linkage mapping-based approaches have been extensively used for mapping genes for various biotic stresses such as sclerotinia stem rot (Guo et al. 2008),

Brown stem rot (Patzoldt et al. 2005), Phytophthora stem rot (Han et al. 2008; Wang et al. 2010), Asian soybean rust (Hyten et al. 2009; Chakraborty et al. 2009), Soybean Mosaic Virus (Shi et al. 2008), sudden death syndrome (Kazi et al. 2008) and cyst nematode (Wu et al. 2009; Vuong et al. 2010). Similarly for biotic stresses, QTL mapping has been successful for water-logging (Githiri et al. 2006), salt stress (Lee et al. 2009; Tuyen et al. 2010), manganese toxicity (Kassem et al. 2004), iron deficiency chlososis (Li et al. 2000), aluminum tolerance (Qi et al. 2008) and phosphorus deficiency (Li et al. 2005). In fact, soybean is the most successful legume crop where use of markers in breeding programs is routine and several improved lines/varieties for resistance to different SCN races (Concibido et al. 1996; Arelli and Young 2009), Phytophthora root rot and brown stem rot, insect resistance (Walker et al. 2002; Warrington et al. 2008), low linolenic acid content (Sauer et al. 2008), yield (Concibido et al. 2003), mosaic virus resistance (Saghai Maroof et al. 2008; Shi et al. 2009) have been developed. Further, a number of varieties (JTN-5503, JTN5303, DS-880, JTN-5109) have been released in soybean for resistance to diseases and Soybean cyst nematode resistance, most of them in USA (Arelli et al. 2006, 2007; Arelli and Young 2009; Smith 2010).

7.4 Genetic Transformation

There has been huge commercial success of transgenic soybean since the first reports on its genetic transformation (Hinchee et al. 1988; McCabe et al. 1988) appeared. There has been continuous dominance of transgenic soybean among the different global biotech crops, with the entire soybean crop being herbicide tolerant. In 2007, the global area of transgenic herbicide tolerant soybean was 58.6 million hectares which was 64% of the total global area under soybean cultivation (James 2007). *Agrobacterium*-mediated transformation is the most widely used transformation technology (Eapen 2008), partly because it often gives rise to simple transgene integration patterns, which is desirable for correct and stable transgene expression. On the other hand, among the direct gene transfer techniques, particle bombardment is by far the most popular because it is expected to be less genotype dependent in contrast to *Agrobacterium*-mediated transformation. This technology has been applied with different legumes including soybeans (Rech et al. 2008). However, a disadvantage of this technique is that it sometimes results in complex transgene integration patterns, thus enhancing the likelihood of transgene silencing (Travella et al. 2005; Yang et al. 2005). An example of this phenomenon is a study concerning transformation with isoflavone biosynthetic genes in soybean (Zernova et al. 2009). The transgenic lines carried multiple transgene inserts and although the lines were transformed with sense constructs aiming at overexpression of isoflavone biosynthetic enzymes, the transgenic lines actually contained lower levels of isoflavones, suggesting co-suppression of the homologous soybean genes (Zernova et al. 2009). Over the last one decade, a number of transgenic lines have been produced in soybean for different traits (Table 12.2).

Table 12.2 Some recent examples of soybean lines improved through genetic engineering

Introduced gene(s)	Trait	References
Ribozyme terminated fatty acid desaturase and thioesterase	Modified seed oil composition	Buhr et al. (2002)
Gly m Bd 30 K	Allergen elimination	Herman et al. (2003)
Borago officinalis fatty acid Δ^6 desaturase	Modified seed oil composition	Sato et al. (2004)
cry1Ab	Insect resistance	Dufourmantel et al. (2005)
Coat protein of soybean mosaic virus	Virus resistance	Furutani et al. (2006)
Inverted repeat of coat protein of soybean dwarf virus	Virus resistance	Tougou et al. (2006)
Fatty acid Δ^6 desaturase and Δ^{15} desaturase	Modified seed oil composition	Eckert et al. (2006)
RNAi construct targeting cyst nematode MSP gene	Nematode resistance	Steeves et al. (2006)
Fatty acid Δ^6 desaturase, fatty acid elongase and fatty acid Δ^5 desaturase	Modified seed oil composition	Chen et al. (2006)
4-Hydroxyphenylpyruvate dioxygenase	Weed control	Dufourmantel et al. (2007)
Dicamba monooxygenase	Weed control	Behrens et al. (2007)
Heat labile toxin (LT) B subunit	Oral vaccine	Moravec et al. (2007)
SLC1	Increased oil content	Rao and Hildebrand (2009)
Oxalate decarboxylase	Fungal resistance	Cunha et al. (2010)
Mutated anthranilate synthase	Nutritional quality improvement	Ishimoto et al. (2010)

Source: Angenon and Thu (2011)

Using genetic transformation, fatty acid metabolism has been manipulated in soybeans with a reduction in saturated and polyunsaturated fatty acids and a concomitant strong increase in oleic acid (Buhr et al. 2002) and high amounts of long chain polyunsaturated fatty acids (Chen et al. 2006; Eckert et al. 2006). Padgette et al. (1995) reported that a stable glyphosate-tolerant soybean line had been developed using the *Agrobacterium*-mediated gene transfer. Kinney (1996) produced a high oleic acid content (84%) soybean line by particle bombardment method. Transgenic plants from which the major allergens have been eliminated have also been obtained, resulting in a strong decrease of binding of IgEs from allergic patients to extract these transgenic seeds (Herman et al. 2003). Soybean has also been used as a platform to produce biopharmaceuticals such as vaccines and antibodies during the last decade (Ma et al. 2005; Kaiser 2008) resulting in the production of edible vaccines (Moravec et al. 2007). Plastid transformation in soybean has first been reported by Dufourmantel et al. (2004) and has subsequently been used to obtain high level expression of Cry1Ab protein and 4-hydroxyphenylpyruvate dioxygenase, conferring strong insecticidal activity and herbicide tolerance, respectively

(Dufourmantel et al. 2005, 2007). One major concern in transgenic soybean is the removal of marker genes. Two main strategies for marker gene removal are available: the co-transformation strategy and the use of site-specific recombinase systems such as Cre-*lox*, R-*RS* or FLT-*FRT* (Darbani et al. 2007). If the marker gene and the gene of interest are integrated at different loci, they can segregate independently and marker-free progeny can be obtained. This strategy has been adopted for the production of marker-free transgenic soybeans (Sato et al. 2004; Behrens et al. 2007).

8 Oil Extraction

For extraction of oil from soybean seeds, hydraulic presses, screw presses and solvent extraction are the commonly used methods. In the 1930s, hydraulic or screw presses were more commonly used. However, the modern oil extraction industries prefer to use solvent extraction process which removes more oil from soybean, hexane being the most common solvent used (Carrao Panizzi and Gontijo Mandarino 1994). The extraction process is completed in several steps. The first step involves cleaning the soybean seeds to remove the foreign material and dirt and drying them to a moisture level of 9.5%. This follows cracking the seeds by passing them through corrugated rolls of roller mills and dehulling of the cracks and heating the cracked soybean meats to about 165°F to soften them before flaking. The heated and cracked meats are then passed through a roller mill equipped with smooth surface rolls to produce flakes. The flakes are then placed into a vapor sealed percolation extractor and the solvent is percolated through a bed of soy flakes, dissolving the oil. The mixture of oil and solvent (micella) leaves the bottom of the bed through perforated plate. After this, hexane is removed leading to the production of crude soybean oil, the step completing in two-stage steam-heated evaporator. The crude soybean oil is then subjected to a refining process which includes degumming, neutralization, bleaching, deodorization and hydrogenation. The refining process does not change the composition of glycerides in the oil though it removes impurities such as waxes, free fatty acids, sterols, pigments and minerals such as P, Fe, Na and Cu from the crude oil. The byproduct soy flakes with the oil removed are conveyed to a desolventizer toaster for removing undrained hexane. This process removes the hexane and destroys anti-nutritional factors such as trypsin inhibitors, ureases and hemagglutinins. Then the meal is dried to about 13–14% moisture in a dry-cooler and then screened and ground to produce uniform size prior to shipment to the end user.

9 Oil Content and Protein Quality

Soybean has approximately 40% protein, 20% lipids, 17% celluloses and hemicelluloses, 7% sugars, 5% crude fibers and about 6% of ash. On oil extraction, the crude oil requires further treatment called refining to convert it into a bland, stable

Table 12.3 Fatty acid composition of soybean oil

	Fatty acid composition (wt.%)	
Component fatty acid	Range	Average
Saturated		
Lauric	–	0.1
Myristic	<0.5	0.2
Palmitic	6–12	10.7
Stearic	2.0–5	3.9
Arachidic	<1.0	0.2
Bohenic	<0.5	–
Total	10–19	15.0
Unsaturated		
Palmitoleic	<0.5	0.3
Oleic	20–50	22.8
Linoleic	38–60	50.8
Linolenic	2–13	6.8
Eicosenioc	<0.1	–
Total	–	80.7

and nutritious product, which is used for edible purposes. Soybean oil is important edible oil which provides us with calories, essential fatty acids and fat soluble vitamins. It is widely used in various food products, including salad and cooking oil, shortenings, margaine, mayonnaise and also in salad dressings. Soybean oil has a high content of linoleic acid, an essential polyunsaturated fatty acid as well as linolenic acid (Table 12.3). Linoleic and linolenic acids are more important because mammals including human beings cannot synthesize them though their essentiality in humans has been debated for years. Availability of these two fatty acids depends only on the dietary supplies. Soybean oil is an excellent source of these essential fatty acids because unhydrogenated soybean oil contains about 53% linoleic acid and 8% linolenic acid while partially hydrogenated oil contains about 23% linoleic acid and 3% linolenic acid.

The linolenic acid is responsible for poor keeping quality of oil. The presence of 7–8% of linolenic acid contributes to less oxidative stability than that of more saturated fats, but the linolenic acid content is lowered to a considerable extent by selective hydrogenation during processing of the oil into food products. Attempts are being made to breed the linolenic acid directly by genetic transformations and indirectly by breeding for high-oleic lines. Hymowitz et al. (1972) and Hammond and Fehr (1983a, b) have reported the variability of linolenic acid content up to 3%. Reduced palmitic acid was reported by Fehr et al. (1991) and Wilcox and Cavins (1990). In addition to the desired high concentration of polyunsaturated fatty acids, soybean oil has several minor constituents that are valuable commercial products. These minor constituents, which include lecithin, phytosterols and tocopherols, are made available as a result of the high volume of soybean oil processed.

Protein content in soybean varies from 35 to 50%. The essential amino acids are also important factors in soybean proteins and these are considered a measure of quality. Soy protein is rich in lysine, which is markedly deficient in cereals and also low in

sulfur containing amino acids such as methionine and cystine. Howel et al. (1972) have attempted to increase methionine content in soybean protein. Mutation breeding is found useful and also applied to improve soybean oil and protein content.

Oil and protein content in soybean are negatively correlated traits; therefore increasing one usually results in decrease in the other (Brim and Burton 1979). Bhatnagar et al. (1992) were able to break this negative association through mutagenesis and obtained stable high protein and high oil containing genotypes. Index selection has also been used to simultaneously increase the both (Burton 1997). The genetic factor is a major contributor for protein and oil content with some influence of environment in which the variety is grown (Fehr et al. 1992). Genotypes having high oil content invariably contain relatively higher oil content under different growing environments. In the low temperature environments, the soybean contains relatively high oil percentage in the seed.

The oil content in soybean has been reported to be controlled by maternal influence (Brim et al. 1968; Singh and Hadley 1968) while additive gene action has been reported to be important for this trait (Singh and Hadley 1968; McKendry et al. 1985; Raut et al. 2000). The percentage of oil also greatly depends upon extraction method used. The solvent extraction method is reported to extract more oil. Owing to breeding efforts, the oil content has been increased up to 21% in modern soybean varieties. In India, elite lines with higher oil percentage (approximately 24%) have been developed at ARI, Pune through conventional breeding method using early generation testing (Raut et al. 2002).

10 Soybean Oil for Industrial Uses

There is considerable potential for an increased use of soybean oil as a renewable source of industrial oil, provided its physical and chemical properties are modified. Soybean oil is a complex mixture of five fatty acids (palmitic, stearic, oleic, linoleic and linolenic acids), which all have different melting points, oxidative stabilities and chemical functionalities (Cahoon 2003). The traditional solution to oil instability has been to partially hydrogenate the oil by addition of hydrogen atoms across the double bonds in the unsaturated fatty acids. However, keeping in view the huge demand of soybean oil and cost involved in its hydrogenation, changing of oil composition through breeding efforts offers a long-term solution. Breeders have worked successfully to increase the proportion of oleic acid vs. linoleic and linolenic acids in soybean (Kinney 1997). The high oleic acid soybean has about 80% oleic acid in comparison to 25% levels in conventional soybean oils, which is the highest among all oilseed crops. For this, the expression of *FAD2* genes that code the enzyme that converts the monounsaturated oleic acid to the polyunsaturated linoleic acid was down-regulated. This prevents the addition of second double bond to oleic acid to form linoleic acid, resulting in greatly increased levels of oleic acid. High oleic soybean also has lower saturated fatty acid content than the conventional soybean oil, making it more useful for the consumers. More recently, soybean with even higher oleic acid contents (85%) of the total oil has also been generated by down-regulating the *FAD2* gene along with

the FATB genes that control the production of palmitic acid (Buhr et al. 2002). High oleic soy oils and their blends are very good alternatives to partially hydrogenated oils and have found very good applications in the snack food preparation industry, feedstocks, pharmaceuticals, cosmetics and machine lubricants.

Genetic engineering can also be used to produce high linolenic acid soybeans, a polyunsaturated fatty acid with low oxidative stability. By increasing the expression of FAD3 gene, soybean seeds with linolenic acid content of more than 50% of the total oil have been generated and these soybean lines have linolenic acid content comparable to linseed oil (Cahoon 2003) and are suitable for coating applications such as paints, varnishes, polishes and inks.

11 Conclusion

Keeping in view the importance of soybean as a protein and oil rich crop, its ability to improve soil quality and its multifarious uses in domestic and industrial sectors, there are enough reasons to dedicate more research efforts for its genetic improvement. Over the past years, significant progress has been made for improvement of soybean varieties and a number of promising cultivars with good yield potential have been developed. Significant amount of research has been dedicated to improving plant type, oil content and quality, protein content and nutritional profile of this crop. However, still there is a huge gap between the production realized and the production demand of this crop. This is more important keeping in view the increasing demand of its oil owing to increasing population, diversification of uses and improved paying capacity of the people, particularly in developing nations. Therefore, the challenges include increasing seed yield as well as oil and protein content. For this, a comprehensive soybean improvement program is essential for which adequate genetic variation is required for exploitation. Prebreeding and distant hybridization aided by hormonal manipulations and embryo rescue are required to be exploited for developing additional variability. Despite soybean genome being large in size, several genomic tools have been developed in this crop. There has been significant improvement in molecular marker technology which could assist in marker-assisted selection and target-oriented marker-assisted back cross breeding for specific traits. Genetic transformation has also been most rewarding in this crop. Therefore, all these strategies will have a definite role to play for improvement of soybean and move along with traditional plant breeding to develop superior and stable cultivars.

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Chapter 13
Groundnut

A. Mothilal

Abstract Groundnut (*Arachis hypogaea* L.) primarily considered as an oilseed crop in developing countries is an important source of protein and also serves as fodder for livestock industry. The genus *Arachis* consists of 80 annual and perennial wild species which include diploids and tetraploids distributed in nine sections. All the species are occurring in South America east of the Andes, south of the Amazon, north of La Plata and from northwest Argentina to northeast Brazil. Cultivated groundnut is a segmental allopolyploid having the genomic constitution of AABB, which is believed to be originated through single hybridization between two diploid species. Cultivated groundnut is broadly classified into two subspecies (*hypogaea* and *fastigiata*) and six botanical types (*hypogaea*, *hirsuta*, *fastigiata*, *peruviana*, *aequatoriana* and *vulgaris*). Two botanical types viz. *vulgaris* and *hypogaea* occupied major groundnut growing area. A total of 14,310 genotypes including local land races, breeding lines, and genetic stocks collected across the world are being maintained at International Crop Research Institute for Semi-Arid Tropics (ICRISAT), India. The breeders hesitate to utilize the germplasm directly because of lack of knowledge about the germplasm, unavailability of descriptive characters, and evaluation methods. Hence, core and minicore subset were developed for multiple environmental condition, earliness, nutritional quality, and Sclerotinia blight resistance. Various biotic (foliar and fungal diseases, bacterial and viral diseases, nematodes, *Aspergillus* and insects) and abiotic stresses (drought and salinity) limits groundnut production. Concerted efforts have been made to tackle the stresses by developing improved cultivars of groundnut with inbuilt resistance/tolerance along with enhanced nutritional quality to meet the demand of farmers, traders, and consumers. Earlier objectives were fulfilled through conventional breeding approaches

A. Mothilal (✉)
Regional Research Station, Tamil Nadu Agricultural University,
Vridhachalam, Tamil Nadu, India
e-mail: mothiezhil@gmail.com

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such as mass selection and pure line selection. Later both intra- and interspecific hybridization has been made extensively to fulfill the goals by following pedigree and backcross method of breeding. Mutation breeding also played a significant role in developing several promising high-yielding cultivars in India and China. Although cultivars with inbuilt resistance were developed through conventional approaches, resistance is always linked with poor pod and kernel features which are very difficult to break. Biotechnological approaches such as marker assisted selection (MAS) and genetic transformation helps to develop ideal groundnut cultivar with inbuilt resistance and improved pod and kernel features and pave the way to introgress genes from incompatible species. SSR markers are widely used for genotyping, construction of linkage map, and for MAS due to its codominant and easy to detect nature from a small amount of DNA. QTLs for pod and seed traits were identified for yield improvement. Markers were also identified for resistance to foliar diseases, Sclerotinia blight, aflatoxin, nematode, and drought. Both conventional breeding and MAS makes the selection more stringent and helps achieve the goal. Transgenic groundnut was successfully developed by integrating a nonheme chloroperoxidase (*cpo-p*) gene which inhibits *Aspergillus flavus* hyphal growth which in turn reduces aflatoxin contamination. Introduction of *cry*1A(c) gene in groundnut protects the crop from lesser cornstalk borer damage. The transgenic groundnut can also be utilized as a donor parents in conventional breeding for developing cultivars for resistance to bacterial and fungal diseases.

Keywords Groundnut (*Arachis hypogaea* L.) • Allopolyploidy • Interspecific hybridization • Mutation breeding • Biotechnological approaches • Marker assisted selection

1 Introduction

Groundnut (*Arachis hypogaea* L.) is a highly self-pollinate crop with peculiar geocarpic fruiting habit. It is a rich source of oil (44–56%), protein (22–30%), and carbohydrate (10–25%). Also, it is a very good source of minerals (calcium, phosphorus, magnesium, zinc, iron) and vitamins (E, B and K) (Savage and Keenan 1994). It is the fourth important source of edible oil and third most important source of vegetable protein. It is widely distributed in the tropical, subtropical, and temperate regions of the world. However, commercial cultivation is restricted to a latitude of 40°N and 40°S. The major groundnut growing countries are China, India, Indonesia, Myanmar, and Vietnam in Asia; Nigeria, Sudan, Democratic Republic of Congo, Chad, Mozambique, Zimbabwe, Burkina Faso, Uganda, and Mali in Africa; USA in North America; Argentina, Brazil and Mexico in Latin America and the Caribbean. Groundnut assumed commercial importance only during 1800 in South Carolina where it was used for oil and food. Portuguese and Spaniards invaded the New World and found the groundnut growing areas in Mexico and introduced it to Europe, to both the coasts of Africa and Asia, Pacific islands,

and finally to south eastern United States (Reddy 1988). Domesticated groundnut varieties are divided into two subspecies (subspecies *hypogaea* and *fastigiata*) and six varieties such as var. *hypogaea*, var. *hirsuta,* var. *fastigiata*, var. *vulgaris*, var. *aequatoriana*, and var. *peruviana*. Later two varieties are sporadically grown in Central and South America; while in the United States, 70% of groundnut area is cultivated with *hypogaea* runner group having smaller seeds, large seeded *hypogaea* occupied 20% area; Spanish and Valencia contributed 10 and 1%, respectively.

2 Origin, Evolution, and Taxonomy of Groundnut

2.1 Origin and Evolution of Cultivated Groundnut

Cultivated groundnut (*A. hypogaea* L.) is believed to have originated in the southern Bolivia to northern Argentina region of South America. Archeological survey indicates the occurrence of *A. hypogaea* L. in the Huarmey Valley near the Peruvian coast around 5000 year BP (Bonavia 1982). Recently, pod samples collected in the Casma Valley on the Pacific coast of Peru which dates between 3500 and 3800 year BP resembles three wild *Arachis* species (Simpson and Faries 2001). Extensive explorations were made to collect pod samples from various regions where the cultivated groundnut was believed to be originated, to study the variations found in the cultivated groundnut. Based on the variability observed in the cultivated groundnut, six regions were proposed as the center of diversity for *A. hypogaea* L. (1) Bolivian region (southwest Amazon) was considered as the primary center of diversity; (2) Guarani region (Paraguay – Parana), (3) Goias and Minas Gerais region (Tocantins, San Francisco), (4) Rondonia and northwest Mato Grosso (south Amazon), (5) Peruvian region (upper Amazon and west coast) were classified as secondary centers of diversity; (6) North-eastern Brazil was recognized as the tertiary center of diversity (Gregory and Gregory 1976; Krapovickas 1968, 1973).

Section *Arachis* is characterized by having tap roots, no rhizomes, vertical pegs, and standards with no red veins in the ventral side. The species *A. hypogaea* L. is a segmental allotetraploid (AABB) believed to be evolved from a single hybridization between two diploids followed by chromosome doubling (Husted 1936). The "A" genome species have a distinctive pair of chromosomes that is conspicuously smaller than any others has been identified and designated as "chromosome A," while some other diploids have a chromosome pair with secondary constriction and a satellite, which has been designated as "chromosome B" (Smartt et al. 1978a). Based on morphological, karyomorphological, and cross compatibility studies, several diploid species have been proposed as the donor of A genome species which includes *Arachis cardenasii* Krapov. and W.C. Gregory (Smartt et al. 1978b; Singh and Moss 1982); *Arachis duranensis* Krapov. and W.C. Gregory (Seetharam et al. 1973; Gregory and Gregory 1976; Singh 1988); *Arachis villosa* Benth. (Kirti et al. 1983); *Arachis correntina* Krapov. and W.C. Gregory (Murty and Jahnavi 1986); and *Arachis trinitensis* Krapov. and W.C. Gregory (Fernandez and Krapovickas 1994).

Palynological studies revealed the presence of triangular oblate pollen grains in *A. correntina* and *A. cardenasii* indicating that one of the species might be the probable donors of A genome (Chandran and Pandya 2000). The probable B genome donors were *Arachis batizocoi* Krapov. and W.C. Gregory and *Arachis ipaensis* Krapov. and W.C. Gregory (Smartt et al. 1978b; Singh and Moss 1984; Singh 1988; Klosova et al. 1983; Fernandez and Krapovickas 1994).

Most of the molecular markers suggested a single origin for cultivated groundnut *A. hypogaea* L. Restriction fragment length polymorphism (RFLP) analysis revealed the involvement of *Arachis duranensis* Krapov. and W.C. Gregory (A genome species) and *A. ipaensis* Krapov. and W.C. Gregory (B genome species) in the evolution of cultivated groundnut (Kochert et al. 1991, 1996). Randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis suggested *A. villosa* Benth. (A genome species) and *Arachis ipaensis* Krapov. and W.C. Gregory as the progenitors of domesticated groundnut (Raina et al. 2001). Microsatellite markers revealed *Arachis duranensis* Krapov. and W.C. Gregory (A genome species) and *Arachis ipaensis* Krapov. and W.C. Gregory as the progenitors (Moretzsohn et al. 2004). However, polymerase chain reaction (PCR) amplified fragment length polymorphism (AFLP) revealed either *Arachis helodes* Martius ex. Krapov. and Rigoni or *Arachis simpsonii* (both are A genome species) and *Arachis ipaensis* Krapov. and W.C. Gregory are the progenitors (Milla et al. 2005). Mallikarjuna et al. (2005) reported the involvement of *Arachis hoehnei* Krapov. and W.C. Gregory (B genome donor) in the evolution of cultivated groundnut. Simple Sequence Repeats (SSRs) markers recognized *Arachis duranensis* Krapov. and W.C. Gregory (A genome species) and *Arachis ipaensis* Krapov. and W.C. Gregory as the progenitors of the groundnut (Koppolu et al. 2010). Fluorescent in situ hybridization (FISH) showed that *A. villosa* Benth. and *Arachis ipaensis* Krapov. and W.C. Gregory are the most probable donors of the A and B genome (Raina and Mukai 1999). Double genomic in situ hybridization (GISH), involving *Arachis duranensis* Krapov. and W.C. Gregory (A genome) and *A. ipaensis* Krapov. W.C. Gregory (B genome) appeared to be the best candidate donors because they yielded the most intense and uniform hybridization pattern when tested against the corresponding chromosome subsets of *A. hypogaea* L. (Seijo et al. 2007). In spite of several cytogenetical and molecular studies conducted during the past still there is no conclusive evidence and the debate is continuing of which diploid species contributes the A and B genome to the cultivated groundnut.

2.2 Taxonomy

The genus *Arachis* belongs to the family Fabaceae, tribe Aeschynomeneae, and subtribe Stylosanthinae (Rudd 1981). The main features associated with the subtribe Stylosanthinae are presence of tubular hypanthium, pinnate leaves, and straight embryo. The genus *Arachis* is characterized by the subterranean fruiting habit and

geocarphic peg. The botanical name of the domesticated groundnut *A. hypogaea* L. was described by Linnaeus in 1753. Until 1839, *A. hypogaea* L. is the only described species. Later, Bentham (1841) described five species: *A. glabrata*, *A. prostrata*, *A. pusilla*, *A. tuberosa*, and *A. villosa*. Chevalier's (1933) system of classification is a mile stone of taxonomy of *Arachis*. He attempted to classify the genus *Arachis* based on aerial and root characteristics. Hoehne (1940) described a key to distinguish the species based on leaflet characters. Gregory et al. (1951) paved the way of modern taxonomic classification based on the branching pattern. Krapovickas (1969) first proposed the subgeneric taxonomic classification of *Arachis* into sections. Gregory et al. (1973) and Krapovickas (1973) described the species within the sections and series. Based on morphological, geographic distribution, chromosomal features, cross compatibility, and pollen stainability of interspecific hybrids, Krapovickas and Gregory (1994) proposed nine sections in the genus *Arachis* viz., *Arachis*, *Erectoides*, *Heteranthae*, *Caulorrhizae*, *Rhizomatosae*, *Extranervosae*, *Triseminatae*, *Procumbentes*, and *Trierectoides*. The species in sections *Trierectoides*, *Erectoides*, *Triseminatae*, *Extranervosae*, and *Heteranthae* are the most ancient than species in other sections.

2.3 Classification of Cultivated Groundnut (Arachis hypogaea L.)

Based on growth habit, branching pattern, location of fruiting branches, and also presence or absence of dormancy and maturity (Krapovickas and Rigoni 1960), the cultivated groundnut can be classified as follows:

1. Subspecies *hypogaea*: Main stem without flowering; alternate pairs of vegetative and reproductive nodes on lateral branches (alternate flowering); inflorescence simple; dark green foliage; and seed dormancy usually present.
   (a) Var. *hypogaea*: Leaflets glabrous on ventral side; two seeded; pods very large or small; less hairy; and medium late maturity.
   (b) Var. *hirsuta*: Leaflets with hairs on ventral side; 2–4 seeded; more hairy; and very late maturity.
2. Subspecies *fastigiata*: Main stem with flowering; vegetative and reproductive nodes present themselves in no specific order (sequential flowering); inflorescence simple or compound; light green foliage; and seed dormancy usually absent.
   (a) Var. *fastigiata*: Leaflets glabrous on ventral side; 2–4 seeded (rarely 5); reproductive branches usually short and slender; and little branched, curved branches.
   (b) Var. *peruviana*: Leaflets glabrous on ventral side; reproductive branches usually long and robust both on the main stem and branches; less hairy; and deep pod reticulation.

(c) Var. *aequatoriana*: Leaflets with hairs on ventral side; reproductive branches usually long on the branches; very hairy; deep pod reticulation; purple stems; more branched; and erect.
(d) Var. *vulgaris*: Leaflets with hairs on ventral side; two seeded; pods clustered around the base of the plant; more branched; and upright branches.

3 Floral Biology

Gregory et al. (1973) reported the floral morphology, anatomy, and reproductive development of the genus *Arachis*. Groundnut is indeterminate in growth and first flowering appears between 14 and 55 days after emergence (Simpson et al. 1993b). The inflorescence is a reduced monopodium and either simple or compound. Although one to five flowers are borne in the axils of cataphylls or foliage leaves, only one flower reaches anthesis on a day. The flower is sessile. However, a stalk like structure i.e., calyx tube (hypanthium) bears the papillonaceous flower. One simple and bifid bract surrounds the flower bud. The calyx consists of five lobes. One of them occurs opposite the keel petal while the other four lobes almost fused except at their tips occur on back of the standard petal. The corolla consists of standard, wing, and keel petals. The standard is usually yellow but pastel, orange, reddish orange, cream-white, and white color also found in the genus. Inside standard crescent area is usually marked with lines of dark color mostly deep purple. Wing petals are usually yellow. Keel petals are hyaline, colorless to faintly yellow which surrounds the stamens and pistil. Each flower consists of ten stamens usually monadelphous. The staminal column surrounds the ovary. Four oblong anthers are alternating with four globose anthers; two anthers are sterile staminodes. Xi (1991) studied microsporogenesis in groundnut. Microspore matures in 6–8 h prior to anthesis (Pattee et al. 1991). The stigma is supported by a long filiform style with two sharp bends. The stigma is club-shaped or clavate. Most of the annuals have larger stigmas and not surrounded by hairs and easy to pollinate (Lu et al. 1990). The pistil consists of a single ovary with 2–4 ovules occasionally 5–6 ovules. The flower is usually cleistogamous. Cross-pollination is also reported from 1.0 to 3.9% (Gibbons and Tattersfield 1969). Bees act as a pollen carrier and cross-pollinate *Arachis* even up to 8.0% (Knauft et al. 1992). Anthesis takes place at the time of flower opening or just before flower opening. Fertilization takes place within 12 h after pollination (Gregory et al. 1973; Pattee et al. 1991) and the flowers wither within 5–6 h. The proembryo undergoes four to five mitotic divisions and becomes dormant. Meanwhile the intercalary meristem located below the ovary becomes active and produces stalk like structure called peg (or ovary stalk or carpophore or gynophore). The peg bears the ovules at its tip. The peg is supposed to be positively geotropic. The peg after penetrating the soil begins to grow horizontally (diageotropic) and the fertilized ovary starts developing into a fruit. Both genetic and environmental factors influence the peg length. Pods reach maturity at about 60 days after the fertilization. The seed contains two cotyledons, a radical, a hypocotyl, and an epicotyl. Cotyledons are considered as storage tissues for the germinating seedlings.

4 Genetics of Qualitative and Quantitative Traits

4.1 Inheritance of Quantitative Traits

Genetic studies on qualitative characters help to focus the breeding approach in a desired direction. Qualitative traits show discontinuous variation. Also, these traits serve as genetic markers and helps in the identification of true F_1 hybrids in hybridization program. Certain pod (reticulation, constriction, beak and size) and kernel traits (shape, color of testa) helps to distinguish different cultivars. Although most of the characters are governed by nuclear genes, cytoplasmic factors may also modify or alter the expression of the phenotype. Van der Stok (1910) initiated the studies on genetics of various qualitative traits. Seshadri (1962), Hammons (1973), Wynne and Coffelt (1982) and Reddy (1988) reviewed extensively the genetics of qualitative traits.

4.2 Inheritance of Quantitative Traits

Most of the economically important traits are polygenic which are highly influenced by the environment. These characters will not follow the Mendelian inheritance. Unlike the qualitative traits, quantitative traits show continuous genetic variation. These include yield and yield components. A thorough knowledge on the inheritance of quantitative traits, extent of genetic variability, nature of gene action, and correlation analysis helps to formulate a suitable breeding strategy to isolate a genotype with desirable attributes.

(a) Assessment of genetic variability
Groundnut improvement depends on the magnitude and extent of genetic variability present in the germplasm/segregating population. The nature and quantum of variability present in the gene pool decides the extent of improvement for a character under consideration. Partitioning the variability into heritable and nonheritable components will enhance the efficiency of selection. Simple biometrical tools such as phenotypic coefficient of variation, genotypic coefficient of variation, heritability, and genetic advance will help to quantify the genetic variability. Genetic variability has been extensively studied in germplasm lines and segregating populations. In all the experiments, phenotypic coefficient of variation is higher than genotypic coefficient of variation suggesting that the trait of importance is not only influenced by the genotype but also by the environment (Vasanthi and Raja Reddy 2002; Kavani et al. 2004; Wani et al. 2004; Mothilal et al. 2004, 2005; Shoba et al. 2009; Meta and Monpara 2010). Heritability in broad sense for various yield and component characters is moderate to high. The heritability estimates includes both additive and nonadditive (dominance and epistasis) variances. Additive component is fixable while the nonadditive component is nonfixable. Genetic advance measures the genetic

gain under selection. High heritability along with high genetic advance renders the selection effective (Johnson et al. 1955). The estimate of genetic advance is invariably low in all the traits. High heritability coupled with low genetic advance indicates that nonadditive mode of inheritance and selection for such traits would not be effective.

(b) Nature of gene action
The genotypic variance is divided into additive genetic variance, variance due to dominance deviation (intra allelic interaction) and variance due to epistasis (inter allelic interaction). Epistatic component is further subdivided into additive × additive, additive × dominance, and dominance × dominance components. Both additive and additive × additive type of epistasis alone is fixable. The nonfixable component such as additive × dominance and dominance × dominance type of epistasis may not serve the purpose of breeding. Knowledge on the nature of gene action for a particular trait is essential to adopt a suitable breeding strategy to improve the trait of interest.

– Combining ability
The gene action for a particular trait can be estimated through combining ability analysis. General combining ability is attributed to additive gene effects while specific combining ability is due to dominance and epistasis. General combining ability helps to identify desirable parents which could be used in hybridization program to evolve superior hybrids. Specific combining ability helps to isolate superior cross combinations. Combining ability can be estimated through several mating designs such as line × tester, complete diallel, half diallel, and triallel analysis. In certain experiments, both additive and nonadditive gene actions were equally important in the inheritance of yield and yield components (Venkateswarlu et al. 2007). However, pod and kernel yield is predominantly under the control of nonadditive gene action as reported by Mathur et al. (2003), Dasaradha Rami Reddy and Suneetha (2004), Mothilal et al. (2007), Manivannan et al. (2008), Rekha et al. (2009), and Mothilal and Ezhil (2010).

– Epistasis
Combining ability does not provide the estimate of epistatic variance. Generation mean analysis is a useful kit to draw more information on various genetic components (additive, dominance and epistatic variance viz., additive × additive, additive × dominance and dominance × dominance) and the type of epistasis viz., complementary epistasis and duplicate epistasis. The presence of duplicate epistasis would be detrimental for rapid progress, making it difficult to fix genotypes with increased level of character manifestation, because the positive effect of one parameter would be canceled by the negative effect of another. Hence, early generation intermating besides accumulating the favorable genes and maintaining heterozygosity in the population is likely to throw out desirable recombinants. Complementary epistasis helps in effective execution of pedigree method of breeding. Such detailed

information helps to decide a suitable breeding technique for the improvement of various yield components. Hammons (1973) and Wynne (1976) reported preponderance of epistatic gene action for most of the quantitative traits in groundnut. In spite of the limited scope of exploitation of nonallelic interactions in groundnut, the information on nonallelic interactions would be of value to groundnut breeders. Dobaria et al. (2004) reported dominance gene action for kernel yield, while Parameshwarappa and Girish Kumar (2007) observed dominance and additive × additive type of gene action for yield and yield components. However, Rathnakumar et al. (2007) noticed additive, additive × additive, and additive × dominance gene action for pod yield and kernel weight.

– Heterosis
Heterosis is a phenomenon in which the F_1 hybrid shows superiority over its respective parents. Exploitation of hybrid vigor is more common in cross-pollinated crops. Commercial utilization of heterosis in autogamous crops like groundnut is very much limited due to difficulties in hybridization and production of F_1. Heterosis is due to dominance and epistatic gene interactions. If the character is under the control of additive type of epistasis, it may be possible to fix alleles to preserve heterotic effects (Isleib and Wynne 1983). Pungle (1983) indicated the possibilities of getting productive superior segregants in later generations (F_6) when the observed heterosis is more in F_1. Magnitude of heterosis depends on the extent of genetic diversity. Heterosis for various attributes has been extensively studied for yield and yield components. Manivel et al. (2003) observed heterosis for early flowering and pod weight per plant. Significant heterosis for dry pod yield per plant and kernel yield per plant has been reported by Vyas et al. (2001), John and Vasanthi (2006), and Mothilal and Muralidharan (2006). Groundnut breeders in general hesitate to make intragroup crosses because of lack of visible phenotypic variability. However, Arunachalam et al. (1982) observed heterosis in both intra- (between SB × SB, VL × VL, VB × VB and VR × VR) and intergroup crosses (between SB × VL and VB × VR).

(c) Association of traits
The ultimate objective of groundnut breeding program is to evolve varieties with high yield. Selection based on per se performance of yield alone will mislead the success of a breeding program. Yield is a complex trait which includes several component characters. Association of these characters with yield is essential to formulate a selection criterion. Correlation analysis gives overall information about the co-inheritance of two characters. Also, it is possible to know the degree and direction of relationship between characters. Since, groundnut possesses subterranean fruiting habit the association between aerial characters with underground traits is essential to determine the relationship between them. In many instances, number of primaries, number of mature pods, 100 kernel weight, and harvest index (HI) are considered as important yield contributing traits. Venkataravana et al. (2000), Singh et al. (2000), Mathews et al. (2001), Golakia et al. (2004), Suneetha et al. (2004), Kavani et al. (2004), Nagda

and Joshi (2004), Apte et al. (2008), and Nigam et al. (1984) studied correlation in various germplasm lines and found significant association of various yield components with pod and kernel yield. Similarly, Pimratch et al. (2004), John et al. (2008a, b), Songsri et al. (2008), Venkataravana et al. (2004), and Parameshwarappa et al. (2008) analyzed correlation in segregating populations and noticed significant association of yield components with pod and kernel yield. Path coefficient analysis measures the direct influence of one character on other and separates the correlation coefficient into direct and indirect effects. Venkataravana et al. (2000), Mathews et al. (2001), Suneetha et al. (2004), Nagda and Joshi (2004), Nikam et al. (2008), and Parameshwarappa et al. (2008) examined the influence of direct and indirect effects of various yield components (HI and 100 kernel weight) with pod and kernel yield.

5 Germplasm Resources

5.1 Collection of Wild Species

Explorations were extensively made in areas where wild species of *Arachis* are found abundantly. Wherever possible, seeds of wild species were collected and deposited in the gene bank. However, Rhizomatous or stolaniferous plants are collected as a whole as they are devoid of seeds. Similarly, cuttings were made from the perennial species for conservation as they do not have rhizomes or stolens for perpetuation. The mission of collecting the wild species made a remarkable success with the support of Krapovickas and Simpson. Till date the genus *Arachis* consists of 80 annual and perennial species, 69 species described by Krapovickas and Gregory (1994) and 11 species described by Valls and Simpson (2005).

5.2 Germplasm Collections

The Chaco region which lies between southern Bolivia and northwestern Argentina is considered as primary center of diversity (Krapovickas 1969; Gregory and Gregory 1976). The Spaniards took three-seeded Peruvian types (including hirsuta) to Indonesia and China upto Madagascar from the west coast of South America via the western Pacific in the early sixteenth century. The groundnut also moved to North America from Africa as well as from the Caribbean Islands, Central America, and Mexico. Local markets are flooded with different land races or local types of domesticated groundnut in the primary and secondary centers of diversity and these are mixtures of several purelines. Samples were drawn from the market and separated based on pod and seed features. Simpson (1985) suggested that the optimum size of the collected sample may be 1 kilo. Stalker and Simpson (1995) developed 86 pure lines from a sample collected in one market at Brazil. To strengthen the

germplasm collections, 40 collection trips were made from 1959 in South America. Large numbers of accessions were introduced in United States from Africa and Israel during 1960s and 1970s, respectively. The ICRISAT in India is maintaining 14,310 groundnut collections and 413 wild species in the gene bank collected from 92 countries. These collections include accessions, landraces, named cultivars, breeding lines, and genetic stocks. These collections were evaluated and characterized following the groundnut descriptor published by International Board for Plant Genetic Resources (IBPGR), ICRISAT (1992) and the USDA (Pittman 1995). The USDA germplasm collections consist of 8,000 accessions of *A. hypogaea* and 800 related wild species (Stalker and Simpson 1995). Groundnut germplasm collections are also being maintained at Texas A&M, N.C. State University. Similarly, at National Center for Genetic Resources (CENARGEN), Brazil several collections are being maintained.

5.3 Core and Minicore Collections

In groundnut, enormous genetic variability is found in the germplasm maintained in the gene banks. However, the breeders hesitate to utilize the germplasm directly because of lack of knowledge about the germplasm, unavailability of descriptive characters, and evaluation methods. Jiang and Duan (1998) reviewed the utilization of groundnut genetic resources in China. He concluded that only exotic germplasm and wild relatives are utilized for the cultivar development. In USA, the cultivar Dixie Giant is most frequently used as parent for most of the runner market type groundnut while, Small White Spanish 1 was the major source of parent in more than 90% Spanish cultivars (Knauft and Gorbet 1989). Although numerous donors are available for developing disease resistant lines, only three genotypes (J 11, NcAc 17090 and PI 259747) have been most frequently used in India (Nigam 2000). Available genetic resources could be effectively utilized by drawing samples of accessions having desirable agronomic traits with resistance/tolerance to biotic or abiotic stresses. The concept of core collection is becoming important to enhance utilization of genetic resources in crop improvement program. A core collection is a subset of accessions from the whole accessions that represents most of the available genetic diversity of the species (Brown 1989a, b). Brown (1989a) using a sampling theory accommodated 10% of the total accession in a core subset. This is considered as the optimum size and retains 70% of alleles of the entire collection. Based on six morphological traits viz., plant type, pod type, seed size, testa color, seeds per pod, and seed weight, Holbrook et al. (1993) developed a groundnut core collections consisting of 831 accessions from 7,432 U.S. groundnut accessions. A core subset of 1,704 accessions was developed on the basis of taxonomic, geographic, and morphological descriptors of 14,310 groundnut accessions maintained in the gene bank of ICRISAT (Upadhyaya et al. 2002a). The core subset consists of 584 accessions belonging to variety *vulgaris*, 299 to *fastigiata*, 27 to *peruviana*, 6 *aequatoriana*, 784 *hypogaea*, and 4 *hirsuta* types. Still the number of accessions

in the core collections was too large. Hence, these genotypes were further evaluated for various morphological, agronomic, and quality traits. Rajagopal et al. (2003) also constituted a working collection comprised of 213 accessions involving 41 Virginia bunch, 18 Virginia runner, 85 Spanish bunch, and 69 valencia bunch genotypes. Ward's method of clustering was employed to separate the core collections into groups. From the core collection, a minicore subset of 184 accessions was developed (Upadhyaya et al. 2002b). The core collection represented 100% range of entire collection for primary seed coat color, growth habit, rust, early leaf spot, and rosette virus resistance (Upadhyaya et al. 2003). Since the core subset represented the entire collection, the sources of resistance can be identified rapidly. Minicore collection has been established for Asia (Mallikarjuna Swamy et al. 2003), multiple environmental conditions (Upadhyaya et al. 2005), earliness (Upadhyaya et al. 2006), Valencia (Dwivedi et al. 2008), nutritional quality (Dean et al. 2009) and Sclerotinia blight resistance (Damicone et al. 2010).

5.3.1 Goals of Groundnut Breeding

Breeding objectives are designed to rectify the specific defects or to improve the trait of interest. Though numerous high-yielding cultivars are developed throughout the world, only few varieties are in the limelight and occupy the major groundnut areas. Duration of the crop makes them to fit in a particular cropping sequence. Mostly, late maturing Virginia bunch and Virginia runner varieties are dominating the areas in United States and in China. However in India, farmers are widely cultivating Spanish bunch varieties due to their early maturity and suitability in a cropping sequence.

Breeding cultivars with wider adaptability is difficult owing to high G×E interaction. Hence, the breeders aimed to develop location specific genotypes which suits well for a particular zone. Similarly, to overcome the losses due to biotic and abiotic stress, strategies were made to develop inbuilt resistance/tolerance to such constraints. The most economic and eco-friendly approach to control pests and disease is to grow resistant cultivars. Cultivars resistant to diseases with acceptable level of yield have been developed and introduced in areas where the disease/insect damage is severe. Resistance has also been introgressed from the wild compatible species. Though numerous high yielding cultivars with desirable agronomic traits were released based on conventional breeding approaches, it has its own limitations. Measuring the traits such as drought resistance, aflatoxin resistance, and quality in the segregating populations developed through conventional approach is cumbersome and requires huge resources. Genetic engineering paves the way to improve the traits and to pyramid desirable genes distributed in several parents into a single common genetic background. Molecular markers are highly preferred in such cases and presence of trait can be easily detected in the early stages itself and one need not wait until the expression of the trait. Genetic transformation helps to transfer the genes across the plant kingdom. Several genotypes with resistance to viral and bacterial diseases were engineered through this approach. Very few genetically

modified cultivars are successfully cultivated. In many occasions, the transformed genotype serves as a potential donor to transfer the virus or bacterial resistance to the adapted variety through conventional breeding. This review encompasses most of the genetics and breeding aspects of groundnut and will give an overall idea of happenings in groundnut breeding around the world.

6 Breeding Groundnut

6.1 Breeding Objectives

The main objective of any crop breeding is to evolve high-yielding variety with desirable agronomic traits. However, there are instances were a particular trait needs to be improved or altered without changing the genetic architecture of the cultivar. Various specific objectives of groundnut are (1) high yield, (2) earliness, (3) incorporation of 2–3 week fresh seed dormancy in Spanish and Valencia types, (4) resistance/tolerance to foliar diseases, (5) resistance/tolerance to seed/soil borne diseases, (6) resistance/tolerance to nematodes, (7) resistance/tolerance to *Aspergillus flavus*, (8) resistance/tolerance to insect pests, (9) tolerance to drought, (10) tolerance to low fertility and acid soil conditions, (11) tolerance to iron chlorosis, (12) improved quality, (13) high oil content (14) improved forage potential, and (15) improved biological N fixation.

6.2 Breeding Methods

Since groundnut is autogamous in nature, breeding methods suggested for the improvement of self-pollinated crops may be scrupulously followed as elaborated by Allard (1960) with little modifications. Parental divergence and combining ability of parents (Arunachalam 1993), nature of gene action governing the yield components, breeding methodology adopted and intensity of selection decides the success of groundnut breeding program. The various breeding approaches employed for the evolution of groundnut cultivars are discussed herein.

(a) Mass selection
Phenotypically similar plants with uniform pod and kernel characters are mixed together to form a new variety. Mass selection can be practiced to improve the local land races or varieties and for the purification of varieties developed by pureline selection. Mass selection is effective only for the characters with high heritability such as pod size and quality characters.

(b) Pedigree breeding
Though this method is laborious, resource and time consuming, it is the most commonly employed breeding method to develop high-yielding groundnut

varieties with desirable agronomic traits. A clear cut objective is defined before initiating the breeding program. The first and foremost important criterion is the selection of superior parents for inclusion in the crossing program. The characteristic features of parents are: (a) it should possess the character to be improved in a high order of expression (concentration of favorable genes), (b) it should have large additive gene effects and complementation effects for the character, (c) it should produce greater recombinants with transgressive segregation in the segregating populations, (d) it should be a good combiner for the character of interest, and (e) it should result in high *sca* in the resulting hybrid. The parents may be selected by following combining ability estimates and through diversity analysis. Selected parents are crossed to get sufficient crossed seeds. Many numbers of flowers may be crossed since the percentage of success is low when compared to other crops. Pool all the seeds of the same parentage. Group the crossed pods according to the objective of the breeding program. The F_1 plants are space planted to allow the plants to produce maximum number of pods. The F_2 generation is raised along with the parent. The F_2 generation is the ideal generation for imposing selection as desirable recombinants may be observed in this generation. Crosses showing low mean and low variability for the character, low heritability and low genetic advance, and low proportion of segregants combining the desirable characters in combination breeding, are ruthlessly rejected. Each individual F_2 plants are observed for various morphological characters such as plant stature, canopy characteristics, growth habit, branching pattern, and resistance to foliar diseases. After harvest, the pod size, pod shape, kernel size, kernel color, pod placement on the plant, number of mature pods, uniformity in maturity, and pod filling are examined and single plants with expected characters are selected. Selected F_2 plants are raised as progeny rows as F_3 families. Again selection of individual plants is practiced within each F_3 families keeping the observations made in mind for the F_2 generation. Selected F_3 single plants are raised as progeny rows as F_4 families. Single plant selection is made within each F_4 families. The F_5 generation is raised as progenies along with the standard check variety. Most of the plants within the progenies attain homozygosity at this stage. Based on the analysis of variance test, significantly superior families than the local check are selected and advanced to yield trails for further evaluation. For developing earliness, disease resistance, insect resistance, and drought tolerance pedigree breeding approach brings a successful result.

Two more alternatives to this breeding method viz., bulk pedigree method and modified pedigree method are suggested.

– Bulk pedigree method
  The desirable parents are crossed to produce enough F_1 seeds. The F_1 plants are space planted to allow them to produce more number of seeds. The F_2 plants are also grown by adopting normal spacing. No selection is practiced in the F_2 generation. However, seed size and plant type may be observed. The seeds of the F_2 plants are bulked to raise the F_3 generation. Seed bulking is practiced till F_6 generation. Single plant selections are made in the F_6 generation keeping in mind the morphological pod and seed characteristics. Selected

plants are raised as progeny rows. The selected plants attain homozygosity by this time. Superior families are selected and forwarded to yield trials. If the selected family is found superior than the check, then the selected lines are tested in a wide range of environments.

– Modified pedigree method
This method is the modification of the bulk pedigree method in which the segregating populations are advanced by selecting single seed from each plant from F_2 generation onwards with the objective of retaining maximum genetic variability. The selection is imposed on later generations. Rapid generation advancement is possible by growing atleast three generations per year thereby the time could be reduced. Two parents with desirable attributes are crossed and the F_1 seeds were planted to get enough F_2 seeds. The F_2 plants are raised in thick population to have a maximum number of plants in the segregating population. One seed from each individual F_2 plants are selected and harvested. The harvested seeds are utilized for raising F_3 generation. Again single seed is selected from each F_3 plant and such selection continues till F_4 generation. The F_5 populations are space planted and individual plants with desirable attributes are selected. Selected single plants are raised as progeny rows along with the check variety. The progenies showing superior performance compared to the check are forwarded to yield trails. Since the Spanish bunch genotypes do not possess seed dormancy, three generations could easily be raised per year. However, the Virginia genotypes possess seed dormancy. Hence, the seeds may be treated with ethrel solution (mix 0.5 mL of Ethrel in 2 L of water and soak kernels in ethrel-water solution overnight) before sowing.

(c) Recurrent selection
The recurrent selection differs from other methods of selection in which the genes from different plants are reshuffled to produce more of recombinants due to additional intermating in the subsequent generations. A number of parents with desirable agronomic traits are selected and intercrossed in all possible combinations. The F_1's of the two-way crosses are again crossed with each other or the progenies are observed for desirable traits and the selected plants are intercrossed. Further, the generation advancement is made through pedigree, bulk, or through modified pedigree approach. Monteverde-Penso et al. (1987) suggested that recurrent selection scheme utilizing single seed descent, a broad-based population, and low selection intensity would be effective for groundnut.

(d) Backcross breeding
Backcross method is commonly employed to introgress disease resistance genes from the related wild species into the well adapted cultivar. Simply inherited traits and resistance to groundnut rosette virus disease has been successfully transferred to nonrecurrent parents (Gibbons 1969). Similarly, high oleic trait was transferred to large seeded high-yielding varieties such as SunOleic 95R and SunOleic 97R in the USA through backcross breeding (Gorbet and Knauft 1997, 2000).

(e) Early generation testing

The advantage behind the early generation selection is the early elimination of undesirable material. Still there is no conclusive remark regarding the effectiveness of early generation testing. Wynne (1976) concluded the difficulty of predicting the yield of S_1 generation as compared to the bulk in S_4 generation. However, he suggested that the component characters such as fruit length, fancy size pods, and sound mature kernel could be improved through early generation selection. In contrast, Coffelt and Hammons (1974) advocated early generation testing for yield. They selected and advanced lines outyielding the parents in the early generations. Bandyopadhyay et al. (1985) advocated effectiveness of early generation by formulating a selection index based on physiological and yield components. Halward et al. (1990) suggested the effectiveness of early generation testing for certain agronomic traits with limitations for yield.

(f) Multilines

Multilines are early generation composites. Seeds from phenotypically similar plants or lines mixed together to form multilines from the F_3 to F_5 generation. Vegetative characters such as plant height, branching pattern, number of branches, leaf size and color and reproductive characters such as pod shape and size, kernel shape, size and color are observed before making multilines. These multiline varieties are better adapted to wide range of environmental conditions and gives stable yield.

(g) Mutation breeding

Since the available germplasm has narrow genetic base, an alternate approach to broaden the genetic base of groundnut is to induce mutation. Mutation breeding is a potent genetic tool to generate new gene recombination by treating the seeds using either physical (gamma radiation) or chemical (ethyl methane sulfonate) mutagens. The only disadvantage with respect to this approach is requirement of huge resources to screen the population. Mutation breeding is an alternate approach to break the undesirable linkages associated with pod and kernel yield. Desirable mutants with reduced plant height, increased number of pods, improved pod and kernel size, increased 100 kernel weight, improved oil content and O/L ratio, and enhanced level of resistance to rust and late leaf spot were obtained.

A minimum of 500 dry seeds with optimum moisture content are selected and treated with two different doses (200 and 300 Gy) using gamma rays or using chemical mutagens. However, the LD_{50} of the genotype may be worked out with various doses/concentrations before the initiation of breeding program. Untreated genotype may be considered as control. The treated seeds were sown immediately to observe the M_1 population. All the M_1 plants were harvested individually on a single plant basis. Seeds obtained from each M_1 plant were raised as progeny rows along with the control in the next season. Control genotype may be raised in every 15 row interval. Single plants having higher kernel yield than the control are selected from each individual M_2 progeny rows. Selected single plants are raised as M_3 families in the next season along with the control. Families showing high mean kernel yield coupled with high or low

variance are selected and single plant selection is imposed on each of the selected family. Again the selected single plants are raised as progeny rows as M_4 families. Segregating families and families showing low mean and low variance are discarded. Only the top performing families are selected. The selected lines are evaluated during rainy and post-rainy seasons in a replicated trial to assess the yield performance along with the control and released varieties. Entries showing significantly higher mean kernel yield along with desirable agronomic traits are proposed for multilocation testing.

(h) Interspecific hybridization
Most of the diploid wild species of section *Arachis* harbor disease resistance genes. However, cultivars of *A. hypogaea* are highly susceptible to fungal diseases. Cultivated species is highly cross compatible with all the diploid species and paves the way for the gene transfer. Hence, attempts were made to transfer alien gene from the related wild species to the background of highly susceptible cultivars through several pathways described further without disturbing other desirable agronomic traits.

Triploid pathway: Cultivars of *A. hypogaea* are crossed with diploid wild species to get triploid hybrids. The observed triploids are mostly perennials, more vigorous with intermediate leaflet size showing high level of resistance with prostrating habit. Occasionally, some triploids produce seeds due to the formation of unreduced gametes (2n pollen). Segregating populations show different ploidy levels (2x to 6x). Erect plants could be observed in F_5 or later generations. However, stable genotypes having desirable level of resistance with normal two seeded pods could be recovered only in F_9 or F_{10} generation.

Hexaploid pathway: Sterile triploids are treated with 0.25% hydrotropic solution of colchicine to produce hexaploids. Crossing-over between the two genomes of two species produces recombinants with low frequency. Segregants resistance to foliar diseases are back crossed to cultivar *A. hypogaea* to get stable tetraploid genotypes with higher level of resistance and desirable agronomic traits.

Autotetraploid pathway: Autotetraploids are developed from diploid species having either A genome or B genome of section *Arachis*. Autotetraploids are more vigorous with thicker stems, dark green larger leathery leaflets, flowers, and pods than the diploids. Some of them produce pods and few show seed sterility. Pollen fertility is moderate in autotetraploids. Crosses are made between cultivar *A. hypogaea* with autotetraploids. The F_1 plants are more vigorous and highly resistant to foliar diseases. The hybrids had a genomic constitution of AAAB or ABBB depending on the species involved in the hybridization (AA or BB species) (Singh 1985). The F_1 plants are backcrossed with *A. hypogaea*. The segregating populations are tetraploids resembling *A. hypogaea* having high yield with disease resistance, and which are isolated in advanced generations.

Amphidiploid pathway: Synthetic amphidiploids are produced from diploid *Arachis* species having either A genome or B genome. Amphidiploids are sterile

or partially sterile. These amphidiploids are crossed with *A. hypogaea*. The F_1 hybrids show high level of resistance to foliar diseases with undesirable pod traits. A high level of resistance is noticed in eight amphidiploids viz., *A. villosa*×*A. stenosperma*, *A. duranensis*× *A. villosa*, *A. villosa*×*A. batizocoi*, *A. cardenasii*×*A. villosa*, *A. correntina*×*A. helodes*, *A. stenosperma*×*A. cardenasii*, *A. stenosperma*×*A. kempff-mercadoi*, and *A. duranensis*×*A. stenosperma* (Vindhiyavarman 2002). These amphidiploids can be used as a genetic bridge to introgress resistance genes into cultivated groundnut by attempting few backcrosses with *A. hypogaea* and to restore the normal pod and seed traits.

6.3 Breeding for Specific Traits

Although there are several breeding objectives to satisfy the need of the growers, traders, and consumers, few objectives are considered as important ones which are most common to the breeders irrespective of the region who are actively involved in groundnut improvement program.

1. Breeding for early maturity
   Early maturing groundnut cultivars with short growing season are most appropriate to escape from the end of season drought, cooler temperature, and early frosts. Such a short duration groundnut cultivar could be accommodated in different cropping systems in South and Southeast Asia (Gibbons 1980). Even under residual soil moisture conditions, groundnut could be raised as second crop after the harvest of rice in East and Southeast Asia. Compared to the rice-rice cropping sequence, rice-groundnut cropping sequence is more remunerative and improves the soil fertility significantly. As intercrop, early maturing groundnut cultivars offers less competition with the main crop. Earliness in terms of calendar days differs in different regions. For example, cultivars with 140 days duration in United States, 120 days duration in China and 100 days duration in South and Southeast Asia is categorized as early maturity. However, in Sudan-Sahelian regions of West Africa, 90 calendar days are considered as early maturity (Virmani and Singh 1986).

2. Assessment of maturity
   Prevailing indeterminate growth and subterranean fruiting habit possess difficulty in estimating the maturity of groundnut. However, environment plays a major role in hastening the maturity. First formed flowers produce pods and reaches maturity earlier than later formed flowers. Pods of varying levels of maturity make confusion in estimating maturity. The most common and easiest method of estimating maturity is examining the internal pericarp color (Miller and Burns 1971). Spanish bunch varieties ready for harvest when 75–80% pods showing darkening of internal pericarp color while, Virginia bunch genotypes can be harvested when 70–75% pods showing the aforesaid color (Nigam and Aruna 2008). Instead of following the calendar days for screening the large segregating population for earliness, cumulative thermal time (CTT) is calculated

in day-degrees (°Cd) to assess the early maturity which is more appropriate and gives a reliable result. The optimum CTT for early maturity is 1,470°Cd (equivalent to 90 days after sowing in rainy season).

3. Genetics of earliness
   The inheritance of earliness was critically studied by many researchers and varied results were obtained. Late maturity was dominant over earliness (Badami 1923, 1928) and is conditioned by single gene. Patel et al. (1936) and Hassan (1964) observed incomplete dominance of late maturity over earliness governed by single gene. However, Holbrook et al. (1989) reported four or five genes with complete dominance of late maturity. Upadhyaya and Nigam (1994) reported a single gene with additive gene action for days to first flowering. Three independent genes with dominant–recessive (13 late: 3 early) and duplicate dominant (1 late: 15 early) epistasis was observed for early accumulation of flowers. Contrary to the earlier given results, Vindhiyavarman and Raveendran (1996) reported involvement of two recessive genes acting additively for days from seedling emergence to first flowering. Basu et al. (1986) reported good general combining ability for early maturity. Preponderance of additive genetic variation as evidenced by the higher magnitude of *gca* than *sca* for days to first flowering was reported by Nigam et al. (1988). Khalfaoui (1990) studied heredity of extreme precocity in a cross between two Spanish bunch genotypes 73-30 and Chico. From emergence to first flowering he observed additive, dominance, additive × additive, and dominance × dominance gene action with duplicate digenic inheritance. Number of flowers produced during the first 4 days of flowering indicated the involvement of more than two genes. For percentage of ripe pods, both additive and dominance effects were reported. However, additive component was greater in magnitude with the involvement of two or three genes governing the trait in two parents. Besides, Ali et al. (1999) observed the importance of both additive and dominance gene action for maturity index.

4. Components of earliness
   Khalfaoui (1990) reported days from sowing to seedling emergence, days from seedling emergence to first flowering, number of flowers produced during the first 4 days of flowering, and percentage of ripe pods at 80 days after sowing as important components for earliness. Days from seedling emergence to first flowering, days from emergence to accumulation of ten flowers, and days from emergence to accumulation of 25 flowers were reported to be important component traits (N'Doye and Smith 1993). However, Islam and Rasul (1998) and Singh and Singh (1999) reported days to 50% flowering as an important component of early maturity.

   Short duration cultivar should have a rapid emergence, dwarf growth habit, reduced internodal length, narrow (possess high radiation use efficiency) and thicker leaves (possess high transpiration efficiency [TE]), and early flowering and more number of flowers per leaf axis along with high partitioning efficiency and photoperiod insensitivity.

5. Screening for earliness
   Standardized robust screening technique is essential to screen the larger segregating populations of groundnut for earliness. Previously calendar days were followed to distinguish the early- and late-maturing genotypes. At ICRISAT, Patancheru, India, earliness is assessed based on degree-days in which test entries were screened by raising 4 m long ridges in a ridge-furrow system. A spacing of 60×10 cm for Spanish bunch and 60×15 cm for Virginia bunch genotype was adopted. Control cultivars were raised in every nine test entries. At 1,470°Cd (equivalent to 90 DAS in the rainy season) half of the plot was harvested to assess maturity. Further, the selected genotypes were again evaluated in a preliminary trial during post-rainy season at two harvest dates 1,240°Cd (equivalent to 75 DAS in the rainy season) and at 1,470°Cd. Based on pod yield, shelling percentage, and 100 seed weight, the genotypes were selected for further evaluation (Upadhyaya et al. 2006).

6. Sources of earliness
   Most of the breeding program aiming to develop early maturity cultivars utilized very few genotypes such as Chico (Selection from PI 268661), Gangapuri (a land race from Khargone, Madhya Pradesh, India) and JL 24 (Selection from EC 94943). However, Chico is most widely used source for earliness. Upadhyaya et al. (2006) identified four new early maturing land races (ICG 4558, ICG 4890, ICG 9930 and ICG 11605) with predominantly three to four seeds per pod. Bera et al. (2004) screened 768 Spanish bunch groundnut and shortlisted 16 genotypes as donors for earliness. At ICRISAT, Patancheru, India, several early maturing groundnut cultivars (ICGV 86105, ICGV 88023, ICGV 86065, ICGV 86143, ICGV 86061, ICGV 86072, ICGV 86082, ICGV 92195 and ICGV 93382) were developed and are commercially cultivated in Nepal, Pakistan, Sri Lanka, Vietnam, Zambia, Congo, Philippines, Guinea Conakry, Mali, Bangladesh, Burkina Faso, Myanmar, and India.

   Some of the donors for earliness are Luhua 6 (a gamma ray induced mutant of Baisha 1016), TxAG 1 (a gamma ray induced mutant of Spantex), TxAG 2 (R 25 (mutant)×TPL 206-6-1), TG 1E (Tall mutant×TG 9), TG 2E (Dwarf mutant×TG 3), TG 3E (TG 17×Chico), 91776 (TG 3×8068), Dh 40 (Dh 3-20×TG 2E), ALG (E) 57 (CO 2×ICGV 86687), GG 3 (GAUG 1×JL 24), GG 5 (27-5-1×JL 24), GG 12 (Shulamit×GAUG 10), TG 26 (BARCG 1×TG 23), R 9251 (JL 24×TG 23), JL 220 (JL 80×VG 77), M 522 (PG 1×F 334-AB-14), RS 138 (a selection from Brazil introduction), VRI 3 (J 11×Robut 33-1), CO 4 (TMV 10×ICGS 82), 55-437 (a selection from population of South American origin; drought resistant), 73-30 (61-24×59-127), 47-10 (a selection from Madagascar population), Te 3 (a selection from a local population from Southern Burkina Faso), TS 32-1 (Spantex×Te 3; moderately drought resistance), KH 149A (GH 119-7.I.II.III×91 Saria; rosette resistant), KH 241 D (GH 1185.s II×91 Saria), A 124 B (a selection from local Loudima Red population), ICG (FDRS) 4 (Argentine×PI 259747; rust resistant), GC 8-35, 55-21 and 55-33 (55-437×Chico; drought resistant), SRV 1-3 (a selection from recurrent selection scheme; drought resistance), SR 1-96 (a selection from recurrent selection schemes cultivation 11908-13; drought resistant), and Fleur 11 (introduced from China; drought resistant).

7. Breeding strategies
   Kirby and Banks (1981) proposed some modalities to be followed for a successful breeding for early maturity. They suggested that (1) the parents should combine well to give early maturity and throw desirable segregants in the segregating populations, (2) adopting a stringent selection for earliness by limiting the growing season, (3) imposing early generation selection in F_2–F_4 generations, (4) quick generation advancement, and (5) field evaluation of the selected lines over seasons and years. Physiological and genetic basis of selection yielded superior genotypes combining all the desirable attributes for earliness. Nigam and Aruna (2008) formulated certain strategies for efficient selection of early maturing genotypes which includes identification of genotypes having low base temperature (Tb) and CTT for various phenological phases, tolerance to high temperature, photoperiod-insensitivity, high crop growth rate and partitioning, high water use efficiency, and diversified sources of earliness.

6.3.1 Breeding for Resistance to Foliar Diseases

Diseases are the major limiting factors in groundnut production. Among the foliar diseases, early leaf spot caused by *Cercospora arachidicola* S. Hori (teleomorph *Mycosphaerella arachidis* Deighton), late leaf spot caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton (teleomorph *Mycosphaerella berkeleyi* Jenk.), and rust caused by *Puccinia arachidis* Speg. are the key destructive diseases and economically most important. Disease incidence and intensity varies between locations and seasons. Severe disease incidence gradually reduces the leaf photosynthetic area and causes complete defoliation. Epidemics of these diseases can cause pod yield loss of up to 70% (Subrahmanyam et al. 1984). Besides pod yield losses, the foliar diseases drastically reduce yield and quality of haulms significantly. In such cases, development and popularization of resistant cultivar is a suitable alternate to control the disease epidemics. Almost all the cultivars belonging to subsp. *fastigiata* are highly susceptible to leaf spots than the cultivars of subsp. *hypogaea* which exhibits varying level of disease resistance. Hemingway (1957) observed that majority of the leaf spot infection originates from the adaxial surface of the leaf. Cultivars of subsp. *fastigiata* and *hypogaea* had higher and lower number of stomata, respectively. Genotypes having stomatal length of 16 μm or more, exhibited complete resistance than those having a length of 14–15 μm. Moderately susceptible cultivars showed cell wall swelling and thickening around the infection site while highly resistant cultivars showed deposition of pectic substance on cell walls and in intercellular spaces (Mazzani et al. 1972). Intensity of leaf spots was more in larger and light green leaves with lesser palisade tissues (subsp. *fastigiata*) than smaller and dark green leaves with more palisade tissues (subsp. *hypogaea*). Resistant cultivars had smaller leaflets, fewer stomata per unit leaf area, and thicker palisade layer than susceptible cultivars. Also, the total chlorophyll and phenol content was relatively higher in resistant genotypes (Brahmachari and Kolte 1983). However, stomatal size and frequency was not associated with

resistance for rust. Rust uredospores germinated on the leaf surface and the germ tubes entered through the stomata in all the immune, resistant, or susceptible genotypes. However, the germ tube died without further development in immune genotypes. Rate and degree of development of rust mycelium in the stomatal cavities distinguishes other categories of resistance (Subrahmanyam et al. 1980). In the case of leaf spot diseases, resistance was imparted in the postentry phase.

6.3.2 Components of Disease Resistance

Identification of genotypes with desirable level of disease resistance and knowledge on components of resistance are prerequisite for an effective execution of resistance breeding program. Nevill (1981) suggested that the cultivars showing resistance mechanism are having longer latent periods, reduced sporulation, and less defoliation when compared to the susceptible one. Ricker et al. (1985) recognized the importance of longer latent period in conferring resistance to late leaf spot. However, Johnson et al. (1986) reported maximum percentage of lesions that sporulated as an important component of early leaf spot resistance. Waliyar et al. (1993) concluded that longer incubation period, reduced sporulation, smaller lesion diameter, and lower infection frequencies were conferring resistance to the early leaf spot disease. Lesion number per leaflet, lesion diameter, infected area per leaflet, infection index, type of lesion, and sporulation index were also important key components for late leaf spot resistance (Moraes and Godoy 1985). Jogloy et al. (1987) analyzed different components of resistance for late leaf spot resistance and advocated increased latent period, decreased lesion number, lesion size, spore production, and defoliation as important components. Chiteka et al. (1988) opined that the resistant genotypes had longer latent period and reduced sporulation. However, host plant resistance to late leaf spot disease is due to longer incubation and latent periods, lesser lesions per leaf, smaller lesion diameter, lower sporulation index and lesser leaf area damage, and disease score (Dwivedi et al. 2002a).

Subrahmanyam et al. (1983a) made a detailed study on components of resistance to rust disease. They observed that low infection frequency, increased incubation period and slow development and release of uredospores conferred the resistance mechanism. Sokhi and Jhooty (1982) and Zhou (1987) described "slow rusting" type in resistant cultivars which had increased incubation period, decreased infection frequency, reduced uredinial pustule size, failure of uredinia to rupture, and reduced spore production and spore germinability. Further, Reddy and Khare (1988) observed longer incubation period, lower pustule diameter and smaller pustules in resistant cultivars. Number of tannin sacs in leaves had a strong association with rust resistance. Susceptible entries had fewer (24–40/mm^2) while resistant had more (36–56/mm^2) tannin sacs. The diploid wild species recorded still higher number (42–105/mm^2). Dwivedi et al. (2002a) noted that the longer incubation and latent periods, fewer pustules per leaf, smaller pustule diameter,

lower sporulation index, and lesser leaf area damage and disease score were selective components of resistance.

6.3.3 Genetic Basis of Disease Resistance

Broomfield and Bailey (1972) noticed digenic mode of inheritance with recessive genes during the F_2 population of a spontaneous cross between rust-resistant female PI 298115 and an unknown male parent. In another experiment, Kishore (1981) reported digenic (15 susceptible: 1 resistant) and trigenic mode of inheritance (63 susceptible: 1 resistant) in a study involving three susceptible and three resistant parents. Knauft and Norden (1983) observed two recessive duplicate genes governing rust inheritance. Also, Tiwari et al. (1984) registered recessive nature of resistance. The F_2 crosses between resistant and susceptible genotypes segregated as 9 susceptible: 7 resistance. Contrary to the earlier results, Kalekar et al. (1984) reported the role of single recessive gene conferring resistance. In yet another experiment, rust resistance was governed by one or two or three recessive genes as evidenced from the segregation ratios of 3:1, 15:1, and 63:1, respectively (John Joel et al. 2006). Nevill (1980) suggested recessive nature of genes for late leaf spot resistance. He observed that three or four loci were involved in the resistance reaction. Motagi et al. (2000) reported duplicate complementary recessive genes controlling late leaf spot resistance.

6.3.4 Screening Methods

1. Field screening
   Screening of the test genotypes for late leaf spot and rust diseases are carried out by following "infector row" technique suggested by Subrahmanyam et al. (1995). Depending upon the disease situation, highly susceptible and test genotypes are planted in a 1:4 ratio and the genotypes are arranged systematically throughout the experimental area. The row length may be preferably 4–9 m with a intra-row spacing of 10 and 15 cm for the Spanish and Virginia genotypes, respectively. In order to encourage disease pressure, spore suspension are prepared and sprayed over the infector rows during the evening hours. Besides, infected leaf debris collected from the previous season's harvest are scattered throughout the experimental area to serve as additional inoculums. To enhance the relative humidity which is more congenial for the spore germination, sprinkler system of irrigation may be adopted to wet the crop and soil. Maximum number of plants per row is observed for disease severity. The test genotypes are screened twice; first at pod filling stage and second just before harvest. A modified 9-point scale is followed to assess the disease intensity/severity in the genotypes. To confirm the resistance, selected genotypes are raised in pots containing soil and manure and evaluated under glass house conditions. Spore suspensions are prepared and sprayed twice over the test genotypes and scored for disease severity.

2. Greenhouse screening
   Detached leaf technique originally proposed by Mains (1917) and modified by Mayee and Munde (1979), Sokhi (1983), and Ghewande (1990) are followed for rapid screening of test genotypes. A plastic tray is filled with sterilized river sand and moistened with Hoaglands nutrient solution. Eight week old genotypes are selected for evaluation. From each test genotype, fully opened undamaged leaves at the third node from the terminal bud of the main stem are excised through the pulvinous are washed in running tap water. Petioles bearing the leaves are inserted into moist sterilized sand medium. The trays are covered with colorless plastic sheets and incubated at 25°C for 16–24 h. Collected spores are suspended in distilled water with a few drops of wetting agent Tween 80. Using hemacytometer, the spore concentration is adjusted to 35,000 spores/mL. The plastic sheets were removed and the leaves are inoculated with spore suspension using an automizer. Again the trays are covered with plastic sheet and incubated for 20–40 days. Genotypes are screened for disease incidence using the same 9-point scale.

6.3.5 Sources of Resistance

Success of disease-resistant breeding depends on availability of stable resistant donors for the disease of interest. Very few resistant genotypes are available in the primary gene pool of *Arachis*. Two diploid species viz., *A. chacoense* and *A. stenosperma* are highly resistant to early and late leaf spot diseases. *A. cardenasii* was reported to be immune to late leaf spot but susceptible to early leaf spot (Subrahmanyam et al. 1980). Detached leaf technique to identify the sources of resistance to leaf spot diseases confirmed the resistance in SO 909 and SO 911 (Moraes and Salgado 1983). Most of the diploid species possessed a high level of resistance ranging from immunity to hypersensitivity for rust disease. Diploid species viz., *A. batizocoi*, *A. duranensis*, *A. spegazzinii*, *A. correntina*, *A. stenosperma*, *A. cardenasii*, *A. villosa*, *A. apressipila*, *A. paraguariensis*, *A. pusilla*, *A. villosulicarpa*, *A. hagenbeckii*, and *A. glabrata* are highly resistant to rust disease (Subrahmanyam and McDonald 1983). However, *A. monticola*, *A. prostrata*, and *A. marginata* are susceptible to rust disease (Subrahmanyam et al. 1983b). Also, Vindhiyavarman (2002) reported high level of resistance in *A. batizocoi*, *A. duranensis*, *A. stenosperma*, *A. helodes*, *A. villosa*, *A. correntina*, *A. cardenasii*, and *A. kempff-mercadoi* for rust diseases. Among the 3,655 entries screened, only two entries viz., B 613 and PI 341839 were observed to be resistant to both late leaf spot and ruse diseases (Ghewande et al. 1983). A gamma ray induced Spanish bunch mutant (VGM 1) for foliar disease resistance have been identified as a donor (Mothilal et al. 2010) for foliar diseases.

6.3.6 Breeding Strategies

Best genotypes with adequate level of resistance components are intercrossed to isolate progenies with desirable and durable resistance. More emphasis may be

given for percentage of defoliation while making selection in the segregating populations in the field since, it integrates most of the important components of resistance (Dwivedi et al. 2002a). However, number of lesions per leaf should also be considered as a selection criterion along with percentage defoliation while making selections in the segregating populations (Foster et al. 1981). Jogloy et al. (1987) discouraged early generation selection. He suggested making selections in the later generations for leaf spot resistance. However, selection for pod length and seed size could be achieved in the early generation as they show high correlation with disease resistance. Tallury et al. (2009) advocated the possibility of combining high level of resistance with superior yield and quality factors.

6.3.7 Breeding for Resistance to Seed/Soil Borne Diseases

Seed/soil borne diseases develop either from fungi present in the seed or when fungi from the soil directly invade the seedling and cause damage such as preemergence seed rot or postemergence seedling mortality. Among the seed/soil borne diseases, Sclerotinia blight (*Sclerotinia minor* Jagger), Cylindrocladium black rot (CBR) (*Cylindrocladium parasiticum* Crous, Wingfield & Alfenas), root rot (*Macrophomina phaseolina* (Tassi) Goid *Rhizoctonia bataticola*), and stem rot (*Sclerotium rolfsii* Sacc.) are of economic importance. These diseases cause severe damage to the plants and reduce the pod yield considerably.

Sclerotinia blight was first reported in the United States in 1971 in Virginia. The fungus survives in the soil for at least 5 years even without any alternate host. Genotypes having less canopy permits free air circulation which in turn reduces humidity which is more congenial for the fungus to develop (Dow et al. 1988). More than 50% pod loss was noticed in severely affected fields of Sclerotinia blight (Porter and Melouk 1997). Cylindrocladium black rot causes chlorosis and wilting of the main stem which makes the plants to wilt and finally death of the plant. The root system gets collapsed due to the severe incidence of the disease. The disease was first reported in the Virginia-North Carolina groundnut growing areas in 1970. Stem rot attacks groundnut crop near the soil surface and decays the stems as a result the plants may wilt and die.

6.3.8 Genetics of Resistance

Not much work has been done to identify the genetics of seed/soil borne diseases. Very few literatures were available regarding the inheritance of resistance to diseases. For stem rot two loci were found to be responsible for the resistance mechanism in the cultivar TxAG 5 (Wildman et al. 1992). Association analysis exhibited negative genotypic correlation between CBR resistance and traits associated with insect resistance (Green et al. 1983).

6.4 Screening Methods

6.4.1 Field Screening

Screening Genotypes for Sclerotinia Blight Resistance

Field screening for Sclerotinia blight resistance is conducted in groundnut fields showing a severe infestation of the blight incidence. Randomized complete block design with three or four replicates is used to estimate the disease incidence in the field. The size of experimental plots is 1.8 × 6.1 m using standard 91 cm rows. Disease severity is estimated using a scale of 1–5 where 1 = no disease, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = >75% lesion severity (Goldman et al. 1995).

Screening Genotypes for Cylindrocladium Black Rot Resistance

Experimental fields naturally infested with *C. parasiticum* are selected for screening the genotypes. Each plot consisted of four rows of 6.1 m length. Only two rows in the middle are considered for evaluation. Approximately 45 plants can be accommodated in a single row. The plots are arranged in a randomized complete block design with 20 replications. Number of dead or wilted plants per plot is counted (Pataky et al. 1983). Inoculum density in the field is categorized as low, medium, or high if the microsclerotial population per gram of soil in a plot is estimated to be less than one, from one to three, or greater than three microsclerotia per gram of soil, respectively. Root rot intensity is assessed by following 0–5 scale.

Screening Genotypes for Stem Rot Resistance

The test genotypes are grown in plots consisting of two rows of 6.1 m length and 0.9 m apart. Isolates of *S. rolfsii* is grown in a medium containing sterile grains of oats or corn. Plants are inoculated at 55–65 days after sowing with approximately 60 cm^3 of dried, colonized grain per 6.1 m row. Optimum moisture in the experimental plots before the application of inoculum is ensured. After inoculation, the plot is irrigated twice subsequently. Disease severity is assessed by following 1–10 scale, with 1 = ≤10% disease and 10 = ≥90% of plants are dead or dying (Gorbet et al. 2004).

6.4.2 Greenhouse Screening

Screening Genotypes for Sclerotinia Blight Resistance

The test genotypes are grown in 10 cm pots containing sterilized soil and MetroMix 200 (Sun Gro Horticulture, Bellevue, WA) for 6 weeks. *S. minor* culture is grown by collecting the inoculum from diseased groundnut in potato dextrose agar (PDA)

medium for 2 days. First petiole from the base of the main stem is selected and the inoculum is applied over a freshly cut petiole. Inoculated test genotypes are kept in moisture retaining mat and misted 1 min for every 2 h during the day time and continued all along the experiment. Entire potted plants are covered with polythene sheets to increase relative humidity. Using a digital caliper, lesion length (mm) is measured on 4, 5, 6, and 7 days after inoculation (Hollowell et al. 2008) and area under the disease progress curve (AUDPC) is calculated.

Screening Genotypes for Cylindrocladium Black Rot Resistance

Two seeds of each test genotype are planted in plastic containers having a diameter of 3.81 and 20.96 cm length. A cotton ball is placed in the bottom to serve as a wick for water. The containers is filled with planting medium containing two parts of steamed soil and one part MetroMix 200 is artificially infested with 25 microsclerotia of *C. parasiticum* per g of medium. Racks of containers are placed in plastic tray containing water. Water level is maintained up to 3 cm above the bottom of the containers and the plants are allowed to grow for a period of 8 weeks. Surviving plants from the containers are removed and the roots are washed. The degree of decay is assessed on a 0–5 scale (0 = no lesions decay, 1 = few lesions on secondary and main roots, 3 = more lesions on secondary and main root, and 5 = completely decayed roots with most of the secondary roots and part of main root missing) (Hollowell et al. 2008).

Screening Genotypes for Stem Rot Resistance

Test entries are planted in 15 cm diameter pots in water bath temperature tanks adjusted to 24 or 28°C. Pots are filled with 1:2 mixture (v/v) of potting mix and pasteurized sandy loam soil (v/v) along with rhizobium inoculum. Eight weeks after sowing, eight sclerotia are added in each pot and wrapped the pot with two layers of cheese cloth or is not wrapped. Humid environment is created above the soil in pots by constantly wetting the cheese cloth through providing contact with water in temperature tanks. Approximately 500 mL of water is added in both wrapped and unwrapped pots every 1–2 days. Plants are allowed to wilt. Lesions are observed and scored (Shew et al. 1987).

6.5 Sources of Resistance

The genotype TxAG-5 seems to be highly resistant to this disease. Hollowell et al. (2008) reported that the genotype N96076L showed multiple disease resistance including resistance to sclerotinia blight. Advanced breeding lines such as N0308IT, N03088T, N03089T, and N03090T showing resistance reaction have superior yield and agronomic traits. Spanish bunch genotypes exhibited a lower incidence of

Sclerotinia blight than Virginia bunch or Virginia runner habits (Akem et al. 1992). Damicone et al. (2010) evaluated core collections for reaction to Sclerotinia blight resistance and found that entries 208, 128, 804, 582, and 273 had higher level of resistance with erect growth habit. They also observed that entry 92 possessing upright growth habit had a good yield potential coupled with resistance. Spanish groundnuts are more resistant to CBR (Coffelt and Garren 1982). The genotype NC 18016 was more resistant to this disease under field conditions (Pataky et al. 1983). In general, late maturity genotypes are consistently showing resistance to stem rot than early and medium duration varieties. However, some medium maturity entries reported higher level of resistance. The genotype AP-3 recorded the lowest disease incidence and higher pod yield followed by Carver and 90x7-3-2-1 (Gorbet et al. 2004). Shew et al. (1987) observed partial resistance of NC Ac 18416 under moderate as well as very high disease pressure. The cultivar Southern Runner had very good yield potential in spite of high populations of *S. rolfsii* in the field (Brenneman et al. 1990). Ashok et al. (2005) screened the genotypes for resistance and found that three accessions belonging to *hypogaea* bunch (TCG 1525, P 1269710 [NC Ac 38] and ND 8-2) one belonging to *hypogaea* runner (Haryanawadi), four belonging to *fastigiata* (SS 34, VRR 472, Tai son (Jewel Nut) and P 1268559 [NC Ac 10121]), and two belonging to *vulgaris* (NC Ac 18019 and RR 5290) showed resistance mechanism. Makne et al. (2004) identified three donors viz., LGN 74, LGN 69, and LGN 83 for stem rot resistance. The cultivar Bailey had significantly higher level of resistances. Some sister lines of Bailey viz., N03088T, N03089T, and N03090T also had lower level of stem rot incidence. In addition to this, five lines developed by University of Florida (FLMR 7, FLMR 9, FLMR 12, FLMR 14 and FLMR 15) and a cultivar developed by University of Georgia (Georgia 08) was found to be highly resistant to stem rot (Chapin et al. 2010). The entry GP-NC WS 12 an advanced breeding line of NC 6 × (NC 3033 × GP-NC WS 1) performed consistently well in all the isolates of *S. minor* (Hollowell et al. 2003).

6.5.1 Breeding for Resistance to Nematodes

The root-knot nematode (*Meloidogyne arenaria* Neal (Chitwood)) is an important pest of groundnut growing areas of United States especially Alabama, Florida, Georgia, and Texas (Motsinger et al. 1976; Wheeler and Starr 1987). Affected plants become yellow and exhibit stunted growth. Often they produce galls on roots, pods, and pegs. Secondary infection by stem rot (*S. rolfsii*) and Sclerotinia blight (*S. minor*) is also observed in nematode infested plants. Losses due to root-knot nematode alone were estimated to be more than one billion US dollars annually (Sasser and Freckman 1987). Yield losses due to nematode incidence could be reduced to some extent by adopting crop rotation and by application of nematicides. However, application of nematicides is not so effective because of the short-term efficacy (Dickson and Hewlett 1989; Culbreath et al. 1992) and the cost involved by the farmers. Hence, the only alternative, effective, and cheaper approach is cultivation of resistant genotypes. Unfortunately, most of advanced breeding lines and land

races are highly susceptible to plant-parasitic nematodes. Researchers are examining the source of resistance in various germplasms and other wild species.

1. Mechanism of resistance
   Host-plant resistance to plant-parasitic nematode is due to a reduction or inhibition of nematode reproduction (Taylor and Sasser 1978; Fassuliotis 1979). Resistance was characterized by lower gall rates, fewer egg-laying females and larvae, and inhibition of nematode development (Castillo et al. 1973). The first criterion of assessing the resistance is galling response of host genotypes. The second criterion for assessing the host reaction is determining the nematode population in rhizosphere at 30 days after inoculation. Roots of resistant genotypes prevent nematode invasion (Christie 1949). Resistant genotype restricts root penetration, failure to establish a feeding site and absence of host necrosis (Bendezu and Starr 2003). The wild diploid species *A. batizocoi* and *A. chacoense* confer resistance by reducing invading nematodes and increase the period to complete its life cycle. However, *A. cardenasii* inhibits nematode development and the host exhibits necrosis in roots (Nelson et al. 1990). Resistant genotypes either reduce or inhibit nematode reproduction (Taylor and Sasser 1978; Fassuliotis 1979). Nematode population is drastically reduced and no egg masses or galls are observed in *A. glabrata* infested with egg masses of *Meloidogyne* (Baltensperger et al. 1986). Due to the repellent mechanism, juvenile (J2) nematodes failed to establish the feeding site and the nematode development was delayed in the resistant cultivar COAN (Bendezu and Starr 2003).

2. Genetics of resistance
   The hypersensitive mode of resistance in *A. cardenasii* is governed by relatively few genes and easy to manipulate in a breeding program (Nelson et al. 1990). Resistance to root-knot nematode is governed by two dominant genes, one gene (Mag) inhibits root galling and another gene (Mac) inhibits egg production (Garcia et al. 1996). Burow et al. (1996) identified RAPD markers linked to a single dominant gene in the BC_5 generation breeding lines. The genetics of six breeding populations derived from BC_5F_2 generation obtained from the cross *A. hypogaea* cv Florunner×TxAg 7 and *A. hypogaea* cv NC 7×TxAg 7 were studied for resistance (Choi et al. 1999). Three lines TP 259-3, TP 262-3, and TP 271-3 had single gene conferring resistance mechanism. The lines TP 259-2, TP 263-2, and TP 268-3 had two dominant genes in a heterozygous state (AaBa). Resistance to *Meloidogyne* in the groundnut cultivar COAN is inherited as a single dominant gene (Burow et al. 2001).

3. Screening methodology

   (a) Field screening
   Heavily infested field may be selected to screen the germplasm or segregating populations. Test entries are planted in plots consisting of two rows of 1.5 m length, 80 cm apart (Holbrook et al. 1996). Normal package of practices are adopted. At the time of planting, plant rhizosphere in each plot is selected and ten soil cores of 2.5 cm diameter and 20 cm deep are collected. The soil cores

plot wise was bulked and nematodes are extracted. *M. arenaria* juvenile (J2) nematodes at mid-season and at the end of season are collected from roots eluted from the soil sample. The nematode population is estimated. Host plant resistance may be assessed by calculating the total number of nematodes at harvest divided by number of nematodes at planting.

(b) Green house screening
Test genotypes are raised in pots containing steam sterilized loamy sand. Each pot is inoculated with 3,500 eggs of *M. arenaria* race 1 at 14 days after planting. Plants are uprooted 90 days after inoculation and the soil is washed. The roots are kept in 1,000 mL beaker containing 300 mL of 0.05% phyloxine B solution for 3–5 min. Roots of host genotypes are checked for root galls and egg masses. A 5 point scale (0 = no galls or no egg masses; 1 = 1–2; 2 = 3–10; 3 = 11–30; 4 = 31–100; 5 = >100 egg masses or galls) is adopted to screen the genotypes (Taylor and Sasser 1978). Plants are classified as resistant when mean of egg masses is ≤30 (Holbrook et al. 1996).

4. Identification of donors for nematode resistance
Holbrook et al. (1996) screened 1,000 plant introductions for resistance to root-knot nematode. Only two genotypes PI 311265 and PI 298848 were found to be highly resistant with good pod yield. In another experiment, Holbrook and Noe (1990) studied the sources of resistance among diploid and tetraploid wild species of groundnut. They observed high level of resistance in the third order (which includes *A. cardenasii* Krap. et Greg. *nom nud*., *A. duranensis* Krap.et Greg. *nom.nud*., *A. helodes* Martius ex Krap.et Rig. and *A. villosa* Benth.) and fourth order gene pool species (*A. burkertii* Handro, *A. glabrata* Benth. and *A. hagenbeckii* Harms.). The wild species exhibited less plant damage and nematode reproduction. Castillo et al. (1973) reported nematode resistance in wild *Arachis* spp. P 237, P 246, P 250, and P 258. The genotype TP 135, an interspecific hybrid derived from four *Arachis* spp. was found to be resistant to *Meloidogyne* race 1 (Starr et al. 1990). The pedigree of the resistant hybrid TP 129 was [*A. batizocoi* K 9484 × (*A. cardenasii* GKP 10017 × *A. chacoensis* GKP 10602)]^{4x}. From TP 129, another hybrid T 135 was developed by crossing *A. hypogaea* cv. Florunner with TP 129. The hybrid possessed higher level of resistance; hence it could be crossed with agronomically acceptable groundnut genotypes to develop a resistant variety. Texas Agricultural Experiment Station has released two cultivars viz., COAN and NemaTam. Both of them had greater yield potential and resistance to *Meloidogynae* (Simpson and Starr 2001). Three lines COAN, AT-0812, and C209-6-13 were found to be effective in suppressing the populations of *M. arenaria* and *M. javanica* (Timper et al. 2003).

6.5.2 Breeding for Resistance to *Aspergillus flavus*

"Aflatoxins" are highly harmful and carcinogenic mycotoxin produced mainly by two fungi, *A. flavus* Link ex Fries and *Aspergillus parasiticus* Speare. Based on blue and

green fluorescence under ultraviolet light and their relative mobility by thin layer chromatography on silica gel, four major aflatoxins are recognized as B_1, B_2, G_1, and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2). *A. flavus* produces AFB_1 and AFB_2 aflatoxins while *A. parasiticus* Speare produces all the four types of aflatoxins. Aflatoxin contamination reduces the groundnut quality and poses a severe threat to groundnut industry. The European Union admits 2 ppb (parts per billion) of aflatoxins B_1 and 4 ppb of total aflatoxins in the food. However, the United States Food and Drug Administration permit aflatoxins up to 20 ppb in food or feed substrate. Every year billions of dollars worth of food stuffs were destroyed worldwide since they had exceeded the threshold level of aflatoxins. Lamb and Sternitzke (2001) estimated a loss of more than $20 million due to preharvest aflatoxins contamination (PAC) in southeast United States groundnut industry. Pre harvest seed infection was promoted by moisture and heat stress during pod development, physical damage by insect pests and nematodes, and other cultural operations (Mehan et al. 1991). Traits related with drought tolerance are also associated with preharvest aflatoxin contamination. Breeding for drought tolerance may simultaneously improve the resistance to PAC (Girdthai et al. 2010a). Improper storage of groundnut pods leads to postharvest aflatoxin contamination. Chemical control of *Aspergillus* has not yielded any successful result. Development of a resistant variety which suppresses the preharvest aflatoxin contamination, in vitro seed colonization, and aflatoxin production will be a cost-effective solution for the control of aflatoxin contamination. Jiang et al. (2006b) reported seed coat resistance during storage. Under conventional storage condition, resistance to *A. flavus* was observed up to 7 months. However, the resistance declined after 9 months which could be due to change of environmental temperature. Negative correlation was observed between oil content and resistance. Groundnut genotypes with high oil content are generally susceptible to *A. flavus* invasion.

Breeding for resistance to *Aspergillus* has been slow due to lack of reliable, efficient, and simple screening procedure to identify sources of resistance in the germplasm/segregating populations. Hence, the foremost important approach is to standardize a rapid reproducible screening technique to screen the available germplasm. Once, the procedure is developed, resistant donor can be identified very easily. The candidate genotype should be evaluated in multiple environments against variable *Aspergillus* species and strains. Genes conferring resistance to preharvest seed infection, in vitro seed colonization, and aflatoxin production are accumulated in a common genetic back ground to prevent the pathogenesis both in the field and during seed storage.

1. Genetic enhancement for *Aspergillus* resistance
   Resistance to aflatoxin contamination can be achieved through (a) resistance to fungal invasion, (b) inhibition of aflatoxin formation, and (c) resistance to insects and abiotic stress such as drought.

   (a) Physical barriers
   Liang et al. (2003b) studied the role of wax and cutin layers in imparting resistance to colonization. Resistant genotypes had thicker and coarser waxy deposit on seed coat than the susceptible one. They act as a physical barrier and prevent the seed infection and colonization. Asis et al. (2005) reported

that resistance to fungal colonization and aflatoxin contamination was found to be associated with seed coat integrity.

(b) Morphological and biochemical traits conferring resistance
Girdthai et al. (2010b) reported low to moderate heritability estimates for *A. flavus* infection and aflatoxin contamination. Positive association between specific leaf area (SLA) and aflatoxin traits was found to be significant. Similarly, SPAD chlorophyll meter reading (SCMR) was negatively correlated with aflatoxin traits. Hence, physiological-based selection approaches using SLA and SCMR might be effective for improving aflatoxin resistance in groundnut. Arunyanark et al. (2009b) also observed low to moderate heritability for seed infection and aflatoxin contamination. Selection for drought tolerance could simultaneously improve aflatoxin resistance. Physiological traits such as HI, SLA, or SCMR traits could also be considered for indirect selection of aflatoxin resistance. Higher phenol content in leaves and kernels of resistant genotypes (J 11, IC 48, ICGV 89104 and ICGS 76) also confers resistance against aflatoxin contamination (Latha et al. 2007). Drought tolerance traits viz., SLA and root length density (RLD) might be effective factors contributing to resistance to aflatoxin contamination and selection for these traits may upgrade the resistance mechanism (Arunyanark et al. 2009a).

(c) Inheritance of aflatoxin resistance
Very few reports are available regarding the inheritance of preharvest seed infection, in vitro seed colonization, and aflatoxin production. Utomo et al. (1990) and Upadhyaya et al. (2002b) reported the involvement of different genes in the inheritance of three resistance mechanism. Zhou et al. (1999, 2002) studied the inheritance of seed infection by *A. flavus* and observed that the resistance was controlled by additive gene action with a pair of major and minor genes. Xue (2004) discouraged early generation selection since the resistance was governed by nonadditive gene action.

2. Screening germplasm for resistance to aflatoxin

(a) Field screening for preharvest aflatoxin contamination (PAC)
Aflatoxin contamination occurs primarily due to heat and drought stress. Holbrook et al. (1994) suggested a large scale screening of groundnut germplasm. Experimental plot in which the test genotypes raised are covered with movable rainout shelters (9.1 m wide × 22.5 m long) 40 days prior to harvest to encourage heat and drought stress conducive for aflatoxin contamination. Artificial inoculation of *A. flavus* over the test genotypes should be made frequently by adding spore suspension solution during flowering phase. To supplement the soil population of *A. niger*, infected cracked corn may be applied to the experimental field which resulted in more fungal growth on the developing pods.

(b) Screening for in vitro seed colonization (IVSC)
Five gram of seeds is selected in each test genotype along with the resistant check J 11. Seed testa is removed manually and split the two cotyledons.

The seed halves are surface sterilized by sodium hypochlorite solution for 3 min followed by washing with 20 mL of sterile water. The seed halves are placed in a moistened sterile filter paper in 9 cm diameter sterile petri dishes. The seed halves are inoculated with 50 μL of a suspension containing approximately 2.5×10^5 conidia per mL of *A. flavus* strain NRRL 3357. The petri dishes are incubated at 28°C for 8 days. After 8 days, mycelia growth, color, and development of fluffy colonies are observed and assessed using 0 (no growth, green color or fluffy colonies) to 10 (dense mycelium, dark green color or full of fluffy colonies) scales. Seed halves are dried at 60°C for 1 day and 50°C for another 3 days. The seed halves are ground to estimate aflatoxin content in the sample (Xue et al. 2004).

3. Sources of resistance
   Xiao et al. (1999) observed that two stable genotypes N 1211 and N 1322 confers high level of resistance under artificial inoculation condition. Lei et al. (2004) and Liao et al. (2003) identified two resistant donors Taishan Zhengzhu and Zhonghua 6. Zhou and Liang (2002) reported aflatoxin resistance in PI 337494F, J 11, Zhanqiu 48, UF 71513 and Meixianhonhyi. Xue et al. (2004) screened the wild species for aflatoxin resistance and suggested that two diploid species viz., *A. duranensis* and *A. cardenasii* had higher level of resistance. The genotypes ICG 7633, ICG 4749, ICG 1326, ICG 3263, ICG 9407, ICG 10094, ICG 1859, and ICG 9610 showed consistent resistance across locations, which serve as good candidate donors for resistance breeding program (Nigam et al. 2009). The genotypes GFA1, GFA2, AR 1 to AR 4 were highly resistant to aflatoxin contamination (Mixon 1986).

4. Breeding strategies
   A strong significant correlation of SLA and SCMR with aflatoxin resistance has been observed when 140 groundnut lines in the F4:6 and F4:7 generations from four crosses were evaluated. Hence, both SLA and SCMR could be a reliable indicator for enhancing the aflatoxin resistance in groundnut (Girdthai et al. 2010a).

6.5.3 Breeding for Resistance to Insect Pests

The groundnut crop suffers from a variety of more than 350 species of insect pests. Of which leaf miner (*Aproaerema modicella* Deventer), armyworm (*Spodoptera litura* F in India, *Spodoptera littoralis* Boisduval in Africa and *Spodoptera frugiperda* (J.E. Smith) in North and South America), cotton bollworm/corn earworm (*Helicoverpa zea* Boddie in North and South America and *Helicoverpa armigera* (Hubner) in Asia, Africa and Australia), leaf hoppers (*Empoasca kerri* Pruthi), white grubs (*Holotrichia consanguinea* Blanch and *Holotrichia serrata* F in India and *Eulepida mashona* Arrow in Africa), aphids (*Aphis craccivora* Koch), thrips (*Scirtothrips dorsalis* Hood. *Caliothrips indicus* Bagnall, *Frankliniella schultzei* (Trybom) and *Thrips palmi*), Jassids (*E. kerri* Pruthi), and termites (*Microtermes* spp and *Odontotermes* spp.) are most important. An estimated yield loss due to

insect pests alone was $720 million annually (ICRISAT 1992). Besides, the aphids and thrips act as vectors for the transmission of viral diseases of groundnut. Defoliators cause severe damage to the crop plants and reduce the pod yield considerably. Army worm is the major defoliator causing significant yield loss ranging from 22 to 71% (Kulkarni 1989; Patil et al. 1994).

Frequent utilization of insecticides to control the insect pests is not only a threat to environment but the pesticide residues in food and food products may cause severe health hazards to the human being. The only way to minimize the pesticide usage is host plant resistance which is considered as most economical and eco-friendly approach to check the insect population below economic injury level (EILs).

Association of Morphological and Biochemical Traits Conferring Insect Resistance

Cotton bollworm (*H. armigera* (Hubner)): Apical leaflet shape in the main stem, basal leaflet shape in primary branches, days to maturity, hypanthium length, leaflet hairiness on main stem, peg length, and standard petal width exhibited negative correlation with *H. armigera* damage. However, basal leaflet width on main stem and main stem hairiness showed positive association with insect damage (Sharma et al. 2003).

Leaf hopper (*E. kerri* Pruthi): Resistant genotypes were evaluated to study the morphological basis of resistance (Rajamanickam 2004). Higher and varying length of trichomes was observed in adaxial leaf surface of the resistant and wild species promoting resistance. Negative correlation between leaf hopper infestation and density of trichomes revealed that the resistance was associated with trichomes. Leafhopper damage was negatively associated with apical leaflet length on primary branches, basal leaflet shape on primary branches, days to maturity, flowers on main stem, growth habit, main stem hairiness, stipule adnation length, and main stem thickness (Sharma et al. 2003).

Armyworm (*S. litura* (Fab.)): Rajendra Prasad and Gowda (2004) observed high leaf toughness conferring resistance in ICGV 86031. Also, antibiosis was observed in Mutant (28-2), NC Ac 343, R 92227, and TAG 24. The genotype Dh 53 had lower relative water content, higher laminar and epidermal thickness indicating the role of antibiosis conferring resistance mechanism (Patil et al. 2006). Rajendra Prasad and Gowda (2004) showed that antibiosis and non preference are the main mechanisms operating in resistant genotypes.

Leafminer: Leafminer resistance was negatively correlated with basal leaflet width, stem pigmentation, and bristles on leaflet margin (Sharma et al. 2003). Resistant cultivars may have biochemical or biophysical resistance. Certain biochemicals like alkaloids, isoprenoids, aromatics, glycosides, and acetogenins have been found to be responsible for resistance. Biophysical characteristics viz., toughness or thickness of leaves, stems or roots, trichome type and trichome number are associated with insect resistance. Hence, due weightage may be given to the morphological traits while making selections in the breeding program.

Thrips: Antixenosis was found to be the mechanism of resistance in the cultivar Robut 33-1 (Amin 1985). Both the spreading growth habit and dark green foliage of Robut 33-1 conferred resistance to thrips. Dwivedi et al. (1993b) reported that dark green leaf color, leaf wax, and hairy traits of ICGV 86031 were responsible for the resistance. Diploid wild *Arachis* species and its interspecific derivatives exhibited antibiosis (Lynch and Stalker 1997) mechanism of resistance.

Screening Methods

1. Field screening
   Sharma et al. (2003) conducted an experiment to screen the genotypes. The experiment was laid out in both rainy and post-rainy seasons. Wild species, test genotypes, and controls were raised in four row plots of 2 m length in a randomized complete block design with three replications. Test genotypes and control cultivars were harvested at the end of the rainy season and the wild species were allowed to grow continuously during the post-rainy season. Again the test genotypes and control genotypes were raised in the same plots adjacent to the wild species as sown in the rainy season. Observations were recorded on leaf miner damage (% leaflets with leaf miner damage), leaf feeding by *H. armigera* and *S. litura*, leaf rasping by *S. dorsalis*. Damage rating was assessed using 0–9 scales. For screening the genotypes for jassid resistance, the test entries and cow pea (infestor crop) are planted in 4:1 ratio in the experimental field. A row to row spacing of 0.75 m and plant to plant spacing of 0.15 m is adopted. Two weeks after sowing, laboratory bred jassids are released in the infector rows when the infector rows showed yellowing symptoms, the cow pea plants are cut and evenly distributed throughout the field to allow the jassids to infest the test genotypes. Number of jassid nymphs on five plants and percentage of yellowing is recorded and scored.

2. Greenhouse screening
   Screening for *S. litura*: Five seeds in each test genotype are planted in a plastic pot having 30 cm diameter and 30 cm height filled with 2:1:1 ratio of soil, sand and farmyard manure. Only two seedlings per pot are retained and the pots in a greenhouse are kept with a temperature at $28 \pm 5°C$ and RH > 65%. Only one plant in each pot is covered using a plastic jar cage (11 cm diameter, 26 cm height) having two wire-mesh screened windows and the top of the plastic cage is covered with a lid fitted with wire-mesh screen. First instar larvae are released inside the cage and allowed to infest the test genotype while other plant in the same pot is left uninfested. One week after infestation, number of surviving larvae in each caged plant is counted and damage is assessed using 0–9 scale (Sharma et al. 2002). Both infested and uninfested plants are excised at the base separately and the fresh weight was assessed individually. Plants are dried in an oven at 65°C for 5 days and the dry weight of infested and uninfested plants is measured. Loss in biomass weight due to feeding of larvae is calculated in relation to the biomass of the uninfested control.

Sources of Insect Resistance

1. Resistance to defoliators
   Accessions belonging to *A. cardenasii*, *A. duranensis*, *A. kempff-mercadoi*, *A. monticola*, *A. sternosperma*, *A. paraguariensis*, *A. pusilla*, and *A. triseminata* showed multiple resistances to leaf miner, *H. armigera*. Few species viz., *A. cardenasii* (ICG 8216), *A. duranensis* (ICG 13242), *A. ipaensis* (ICG 8206), *A. paraguariensis* (ICG 8130), and *A. appressipila* (ICG 8946) showed resistance to leaf feeding and antibiosis to *S. litura* under green house conditions (Sharma et al. 2003). Species belonging to section Rhizomatosae (*A. glabrata*), Extranervosae (*A. macedoi*), and *Arachis* (*A. villosa*, *A. stenosperma*, *A. batizocoi*, *A. monticola*) caused high larval and pupal mortality (Campbell and Wynne 1980). Diploid wild species such as *A. villosa*, *A. cardenasii*, and *A. correntina* have been found to be potential donors for the transfer of resistance to *S. litura*. All the wild species exhibited antibiosis for the defoliator (Lynch et al. 1981).

   Dharne and Patel (2000) screened 32 genotypes for resistance to *S. litura*. The entries viz., ICGVs 86156, 86400, 86528, 87128, 87141, 87290, 87411, and 91214 recorded lowest leaf damage of 5%. Rajagopal et al. (1988) screened groundnut entries for reaction to tobacco caterpillar. Two Virginia bunch genotypes (V 40 and Ah 6429) and 3 Virginia runner (NCA 17840, NFG 79 and EC 21989) genotypes exhibited highest level of resistance.

2. Resistance to sucking pests
   Diploid wild species *A. batizocoi* was highly resistant to thrips, whereas *A. duranensis* had immunity for leaf hopper damage (Vindhiyavarman 2002). Amin (1985) screened the germplasm for jassids and classified the genotypes into four groups as: highly resistant (NcAc 2214, NcAc 2240 (DP), NcAc 2230), moderately resistant with low percentage of yellowing (NcAc 2242, NcAc 2243 (DP), NcAc 2232, NcAc 2243 (T), NcAc 489, NcAc 1705, NcAc 2462, 13 and Gujarat Narrow leaf), tolerant, and susceptible. Also, they observed lesser thrips damage in NcAc 2242, NcAc 2214, NcAc 2243 (T), NcAc (DP), NcAc 2232 and NcAc 2230.

   The genotype Georgia-01R is a multiple pest resistant runner cultivar possessing higher level of resistance to both leaf hopper and thrips (Branch and Todd 2004).

6.5.4 Breeding for Resistance/Tolerance to Drought

Drought stress is defined as a reduction in grain yield attributable to plant water deficit. Drought resistance is based on the plant ability to obtain water or to use water efficiently when water is limited by drought. Nearly two-thirds of groundnut production globally is from rainfed areas of Semi-Arid Tropics which receives insufficient and scanty rainfall which leads to severe drought which in turn limits groundnut production (Wright and Nageswara Rao 1994; Reddy et al. 2003). Imposing stress in first 40 days after sowing was not detrimental and resulted in yield almost equal to the crop without stress (Patil and Gangavane 1990). However,

water deficit during late flowering and pod development stages (71–105 days after sowing) is detrimental to groundnut yield (Stansell and Pallas 1985; Roy et al. 1988). End of season drought predisposes groundnut to aflatoxin contamination (Cole et al. 1982, 1985; Blankenship et al. 1983, 1984; Hill et al. 1983; Wilson and Stansell 1983). Leafminer incidence was found to be higher in the most stressed plants (Wheatley et al. 1989). Loss due to drought was estimated to be over 6.7 million metric tons (Subbarao et al. 1995). The estimated yield loss of 56–85% was experienced depending on crop growth stage (Awal and Ikeda 2002; Reddy et al. 2003), drought intensity, and drought duration (Nautiyal et al. 2002; Nigam et al. 2005). Therefore, development of resistant varieties offers a great scope to alleviate the problem and a long-term solution to mitigate the drought stress.

Drought Patterns

Drought patterns can be classified in to three groups as (1) Early season drought, (2) Mid-season drought, and (3) End of season drought (Nigam et al. 2002a). Early season drought does not have significant impact on pod yield. A brief spell of 20–25 day moisture stress in the early stage followed by irrigation to release the stress may induce uniform flowering which might lead to higher productivity. Mid-season drought influences the pod yield considerably. End of season drought inhibits the pod development and thereby reduces the yield significantly. Early maturing varieties are the only option to escape from the end of season drought.

In the past, empirical approach was followed in drought resistance breeding program to develop a variety with high yield and drought tolerance. Though, direct selection for yield is effective (White et al. 1994), huge resource investment and higher genotype × environment interaction (G × E) for pod yield slow down the progress of breeding (Branch and Hildebrand 1989; Cooper and Hammer 1996). Passioura (1977) developed a simple physiological model under moisture-limited condition, in which pod yield is determined by a simple equation.

$$Y = T \times \mathrm{TE} \times \mathrm{HI},$$

where

Y = yield (kg/ha),
T = amount of water transpired (mm/ha),
TE = transpiration efficiency (kg ha/mm) or WUE (total biomass production per unit of water transpired),
HI = harvest index.

Hubick et al. (1986), Wright et al. (1988, 1994), and Nageswara Rao et al. (1993) demonstrated significant genotypic variation for T, TE, and HI. Application of this physiological model for developing drought resistance variety is cumbersome and difficulties are experienced in measuring T and TE under field conditions. However, Farquhar and Richards (1984) and Farquhar et al. (1989) reported a close association

between carbon isotope discrimination (Δ) and TE. Hence, (Δ) has been widely used as an indirect selection criterion to assess the variability for TE (WUE) in groundnut germplasm (Hubick et al. 1986; Wright et al. 1988, 1994; Roy 1995; Udayakumar et al. 1998). Higher TE (WUE) was associated with increased root growth (Wright et al. 1994). However, TE is negatively correlated with HI (Hubick et al. 1988; Wright et al. 1988).

SLA (ratio of leaf area to leaf dry weight) exhibited negative correlation Δ with and hence WUE (Nageswara Rao et al. 2001; Upadhyaya 2005). SLA could be used as a surrogate measure of WUE. SLA and specific leaf nitrogen (SLN) could be assessed rapidly by using SCMR. SCMR is an indicator of photosynthetically active light transmittance characteristics of the leaf and chlorophyll content (Akkasaeng et al. 2003) and chlorophyll density (Arunyanark et al. 2008) and WUE (Sheshshayee et al. 2006). In a large segregating population, it has now become easier to measure SLA or SLN. SCMR could be used as a screening tool to evaluate the TE in groundnut (Bindu Madhava et al. 2002). Drought resistant genotypes could be developed rapidly through trait-based selection (Nigam et al. 2005; Arunyanark et al. 2008; Jongrungkland et al. 2008; Pimratch et al. 2008) such as HI, WUE or TE, SLA, and SCMR. Both SLA and SCMR have been used as surrogate traits for WUE (Wright et al. 1994; Nageswara Rao and Wright 1994; Sheshshayee et al. 2006; Nigam et al. 2005). A significant correlation in positive direction was observed between SLA and ribulose 1–5 biphosphate carboxylase (Rubisco) indicating that photosynthetic capacity per unit leaf area is the key factor responsible for variation in WUE (Nageswara Rao et al. 1995). Progressive developments in identifying mechanisms of resistance and rapid screening techniques have paved a way for faster development in drought resistance breeding. Both empirical and trait-based selection can be simultaneously compared and effective strategy may be devised for identifying genotypes with resistance if not tolerance to drought.

Genetics of Drought Resistance

Songsri et al. (2008) studied heritability estimate in four crosses both under stressed and nonstressed conditions. Higher heritability estimates were observed for HI, SLA, and SCMR. SCMR reading is potentially useful as a selection trait for drought resistance because of its high heritability. Nigam et al. (2001) and Surihan et al. (2005) reported predominant role of additive gene action in SLA and HI. Painawadee et al. (2009) reported low to intermediate heritability estimates for drought resistance traits and pod yield and yield components. HI and days to maturity had very high heritability estimates (Wallace et al. 1993). Makne (1992) reported predominant role of nonadditive genetic variance for HI. However, Dwivedi et al. (1998) observed additive genetic variance for HI. Lal et al. (2005) observed additive gene action for SLA, SCMR, and HI. Early generation selection will be effective in improving these traits. Two crosses, TMV 2 NLM × ICGV 86031 and ICGV 86031 × SMG 84-1 were identified as good specific combiners for SCMR and SLA as they involve parents with high gca effects suggesting additive × additive type of epistasis operating in these crosses. Additive × additive epistasis is a fixable variance

in the early generation in the absence of repulsion phase linkages. However, preponderance of nonadditive gene action for total dry matter and fodder yield indicated that the selection may be postponed to later generations. Nigam et al. (2001) studied gene effects for SLA and HI in three crosses and reported additive and additive × additive type of gene action, which indicated the possibility of early generation selection. Moderate heritability estimates was observed for number of pods per plant in both stress and nonstress conditions (Chavan and Dhoble 1994). Jayalakshmi et al. (1998) reported high heritability estimates for SLA and HI in three crosses (ICGV 86031 × JL 24, ICGV 86031 × TG 26 and TAG 24 × JL 24). Six F_2 populations of groundnut studied for the inheritance of leaf chlorophyll content in groundnut revealed that the leaf chlorophyll content was governed by nonadditive gene action (Babitha et al. 2006). They inferred the effectiveness of SCMR in later segregating generations.

Association of Drought Resistance and Agronomic Traits

Songsri et al. (2008) reported strong and negative genotypic correlation between SLA and SCMR under both stressed and nonstressed conditions. Also, they observed a strong association of drought tolerance index for pod yield and drought tolerance index for biomass. They noticed a significant association between HI with pod yield and number of mature pods per plant under stressed and nonstressed conditions. Nigam and Aruna (2008) also observed correlation between SLA and SCMR at end of season drought condition. Upadhyaya (2005) showed negative correlation between SCMR and SLA and SCMR values were more strongly correlated with pod yield and other economic traits such as 100 seed weight. Significant inverse relationship between SLA and RWC was observed under water-limited conditions (Nautiyal et al. 2002).

1. Screening for drought resistance
   Nigam et al. (2002a) suggested field screening for drought tolerance in groundnut. Sprinkler irrigation system was used as source of irrigation in the experimental area. The germplasm and segregating populations were evaluated for mid-season drought and end of season drought. Segregating populations were evaluated for mid-season drought by withholding irrigation from 40 to 80 days after sowing. Similarly, screening for end of season drought may be carried out by withholding irrigation from 80 days after sowing until harvest. Pod and kernel yield alone was considered for selection in drought screening trial.

Identification of Drought Tolerant Types

Rucker et al. (1995) suggested that genotypes possessing larger root systems have the ability to avoid drought. They recommended two entries, PI 318740 and PI 315628 as donors as they had higher yield levels coupled with drought resistance. At ICRISAT, several lines with superior performance under different kinds of drought (ICG# 3086, 3141, 2738, and 1163 and ICGV# 91151, 94127, 92209 and

911109 for mid-season drought; ICG 2213, ICGS 76, ICGV# 90226, 91074, 91185, 91192, 92004, 92022, 92023, 92028, 92029 and 92033 for end of season drought) were identified as donors in breeding program (Nigam et al. 2002a). Nautiyal et al. (2008) identified *A. glabrata* and *A. paraguariensis* as heat-tolerant genotypes as they observed low relative leaf injury (RI).

6.6 Breeding for Improved Quality

Utilization of groundnut differs between countries. Developed countries focus the breeding approach to enhance the quality characteristics as it utilizes groundnut as a food crop rather than oilseed crop. Groundnut seed contains 44–56% oil and 22–30% protein and 10–20% carbohydrate on a dry seed basis and is a valuable source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (E, K and B) (Savage and Keenan 1994). Groundnut proteins are classified into arachin, conarachin, albumin, and glutelin. However, arachin and conarachin together constitutes nearly 87% of the total protein. Though groundnut is a rich source of protein, it is deficient in certain essential amino acids such as lysine, methionine, and threonine. Starch, sucrose, and reducing sugars constitute the major portion of carbohydrate in groundnut.

Groundnut quality depends on end uses. For oil extraction, traders prefer cultivar with high oil content. Consumer prefers quality oil which refers to oil with high O/L ratio. Higher ratio is an indicator of higher shelf life and stability. The quality characteristics of confectionery groundnut depend on color, size, shape, flavor, and texture of kernels. However, as a snack food, it should have desirable level of protein and mineral composition.

In general, the quality characteristics are broadly classified as physical and chemical attributes. Physical quality includes uniformity in kernel size, shape, and color with good market acceptability. On the other hand, chemical attributes includes nutritional composition, anti-nutritional factors, culinary, and organoleptic properties.

Higher free fatty acid content indicates immaturity, mold growth, and other ester hydrolysis activity which encourages rancidity in groundnut oil. Tocopherols are potent antioxidants and determine shelf life of oil. The most abundant forms of tocopherols are α and γ chemical forms. Processors require groundnut with desirable sensory quality characters which includes flavor and texture of the groundnut. Free amino acids and reducing sugars are important precursors of roasted groundnut. Sucrose content decides the taste and flavor of roasted groundnut. Vitamins and minerals increase the nutritive value of the groundnut. Allergen causes IgE-mediated hypersensitivity. Glycoproteins such as *Ara hI*, *Ara h II*, and *Ara h III* are the major contributors of allergens. Allergen sensitivity is highest in infants but the sensitivity decreases with increasing age. Saponin promotes bitterness and flatus sugars causes flatulence. Presence of flatus sugars such as raffinose and stachyose sugars reduce the quality of groundnut. Presence of enzyme inhibitors reduces digestion of protein. However, the enzyme inhibitors become inactivated while heating or cooking. Lectins interfere with absorption of essential nutrients. Goiterogens inhibits the

uptake of iodine and causes goiter in humans while, phytic acid reduces availability of dietary minerals. Aflatoxins are highly toxic secondary metabolites and causes liver cancer. Breeding program aims to improve the desirable quality traits by simultaneously reducing the anti-nutritional factors especially allergens, saponins, flatus sugars, lectins, goiterogens, phytic acid, and aflatoxin contamination.

6.6.1 Oil Quality

Unlike other edible oil, groundnut oil is subject to oxidation which results in rancidity. Shelf life of the oil gets reduced due to rancidity. The oxidative stability index (OSI – length of time until rapid oxidation of the oil begins) is a measure of stability of oil. Oil stability depends on O/L ratio, antioxidant concentration, concentration of specific fatty acids, and concentrations of copper and iron. Saturated fatty acids are less prone to oxidation than the unsaturated fatty acids. Among the 12 fatty acids in groundnut oil, oleic (18:1), linoleic (18:2), and palmitic fatty acids together account for more than 80% of the total fatty acids (Norden et al. 1987; Dwivedi et al. 1993a, b). Reduced level or elimination of long chain fatty acids in groundnut oil would be an ideal breeding objective for improving oil quality. Groundnut oil with lower levels of iodine value could be stored for longer period of time without rancidity. However, considering the human health, increased level of iodine value is highly preferred by the consumers as they had higher level of unsaturation.

Composition of two most important fatty acids viz., oleic (monounsaturated fatty acid) and linoleic acids (polyunsaturated fatty acid) determines the oil quality and oil stability in groundnut oil. Linoleic acid is less saturated and less stable than oleic acid. Andersen et al. (1998) observed negative correlation between oleic acid to iodine value and positive correlation to the ratio of unsaturated to saturated fatty acids. Oleic acid (O) and linoleic acid (L) ratio (O/L) and iodine value (IV) are important factors deciding oil stability and shelf life in groundnut products (James and Young 1983; Branch et al. 1990; Worthington et al. 1972; Young et al. 1972; Brown et al. 1975). The O/L ratio ranges from 1.0 to 4.0. However, this ratio may reach 35–40 in some mutant lines (Norden et al. 1987). Andersen et al. (1998) also recorded ratios of oleic to linoleic acids from 23.1 to 32.1 for high oleic lines and 2:1 to 3:1 for normal lines. Bera and Das (1998) observed a wide variation for O/L ratio of 10.5–45.0. High oleic content lowers iodine value which in turn increases oil stability. The O/L ratio increases with groundnut maturity (Young et al. 1972; Sanders et al. 1982) and it depends on the genotype and environmental factors. Introduction of high oleic groundnuts lowered LDL cholesterol and triglycerides, while improved the HDL cholesterol which is considered as healthier one.

Inheritance of Fatty Acid Content

Inheritance of fatty acid content is quantitative (Khan et al. 1974; Mercer et al. 1990; Mason and Matlock. 1967; Tai 1972; Tai and Young 1975). Role of additive

gene action was reported by Khan et al. (1974), Moore and Knauft (1989), Mercer et al. (1990), Knauft et al. (1993), and Singkham et al. (2009). However, Tai and Young (1975) and Bansal et al. (1992, 1993) reported additive and nonadditive gene action. Moore and Knauft (1989) reported that high oleic content is controlled by two recessive genes ol_1 and ol_2. Aruna and Nigam (2009) reported additive gene action for oleic acid content, O/L ratio and iodine value; hence early generation selection was recommended to improve these traits.

Lopez et al. (2001) indicated that both the Ol_1 and Ol_2 loci control the high oleate trait. Some genetic modifiers may be involved in the expression of the O/L ratio in some crosses.

6.6.2 Nutritional Quality

Cultivars belonging to subsp. *fastigiata* var. *vulgaris* showed a higher protein level than those found in varieties of subsp. *hypogaea* var. *hypogaea* and var. *hirsuta* (Grosso et al. 1999). Ajay et al. (2008) reported that protein content ranges from 22.22 to 30.33%. Genotypes having high protein content are GPBD 4 (30.3%), Dh 43 (29.69%) and Dh 3-30 (29.05%). Chavan et al. (1991) observed a wide variation for protein content (39.5–48.6%), total sugars (2.03–5.5%), starch (3.1–11.6%), methionine (0.72–1.45 g/16 g N), and tryptophan (0.58–1.16 g/16 g N) content among 44 genotypes. Similarly, Shinde et al. (1993) evaluated six table purpose groundnut and nine large seeded types for the nutritional composition. They observed a wide variation for protein (20.6–29.4%), starch (6.8–12.6%), and sugar (4.8–12.7%) content. Bangar et al. (1997) studied ten genotypes along with one control to determine the nutritional composition. They recorded higher amount of methionine (1.98 g/16 g N) in RHRG 95 and TG 26.

6.6.3 Flavor Quality

Flavor quality decides the preference of roasted groundnut as snack food or groundnut butter. Genetic variability for flavor quality has been observed in breeding lines (Isleib et al. 2001). Commercial producers emphasize the need to improve flavor quality in the newly released varieties. Flavor quality is not only cultivar dependent, but environment and interaction effects also play a vital role in the expression of this trait (Isleib et al. 2008). Though several hundred chemical compounds are involved in imparting flavor and aroma of the groundnut, few compounds are considered as important as they significantly contribute to the quality attributes. Methanethiol produces abusive drying while acetone, pentane, and dimethylsulfide are responsible for musty after-taste. Fruity flavor is imparted by 2-methylbutanal, 3-methylbutanal and tongue/throat burn is due to the presence of pentanal. Two compounds viz., *n*-methylpyrrole and hexanal produce musty and beany flavors, respectively in groundnut. High oleate level had a positive correlation with enhanced sensory attributes of roasted groundnut. Breeding efforts to enhance the flavor quality resulted in increased level of flavor in the advanced

breeding lines. The increase was higher in Tamrun 96 with 0.6 flavor intensity unit (fiu) followed by Tamspan 90 (0.4 fiu) and F 435 (0.4 fiu) (Pattee et al. 2002).

Tailoring of New Quality Groundnut

Increased awareness among the consumers of groundnut directed the breeders to focus their attention to develop genotypes having higher level of O/L ratio, α-linolenic (omega-3) fatty acid levels, and vitamin C content, reduced level of aflatoxins, allergens, and flatus sugar content.

Approaches for Quality Improvement

Physical quality traits such as kernel size, shape, and color can easily be improved through pedigree breeding. Similarly O/L ratio could also be improved to some extent. However, elimination of allergens, flatus sugars, improved vitamin C content, protein quality and omega-3 essential fatty acids can be improved through various biotechnological approaches such as marker assisted selection (MAS) or genetic transformation techniques.

7 Molecular Breeding

Molecular markers are known to be the most effective tool to assess the genetic diversity among the cultivated and wild species of groundnut. Morphological markers are limited in number, inexpensive, and easy to score. However, they are highly influenced by the environment. Biochemical markers are cost-effective tools. Expression of biochemical markers is not neutral. DNA markers are versatile, stable, and express uniformly in a wide range of environmental conditions. Large number of loci could be detected using few markers. Though they are costly, the DNA markers express in all tissues and can be scored at any stage of the crop growth. Hence, it is highly preferred and universally accepted for the estimation of genetic diversity analysis which in turn helps to identify desirable parents in the germplasm for constructing a linkage map and for the improvement of trait of interest. The characteristics of a good marker are: (1) exhibits high polymorphism, (2) inherited codominantly, (3) random and frequent distribution of markers throughout the genome, (4) cost effective, and (5) highly reproducible. In groundnut, (1) Hybridization based markers (RFLP) (2) PCR-based markers (RAPD, SSRs, AFLP), and (3) Sequence based markers (expressed sequence tags [ESTs], single nucleotide polymorphisms [SNPs]) has been employed for genetic diversity analysis.

Genetic diversity analysis using molecular markers.

Morphological variations observed in the cultivated groundnut and wild species have been extensively studied through two methods viz., (1) metroglyph analysis

proposed by Anderson (1957) and (2) D^2 statistics proposed by Mahalanobis (1928). Morphological traits are highly influenced by the environment and show continuous variation. The DNA markers are useful to select diverse parents for developing the mapping population and to identify the DNA marker linked with the trait of interest. Genetic diversity studies also help to identify the phylogeny of the cultivated groundnut. The cultivated species may have arisen from a single polyploidization event. Low levels of variation observed among the cultivars at DNA level is due to the polyploidization combined with self-pollination (Halward et al. 1991). Also, they concluded that the tetraploid A. glabrata may have arisen due to two independent polyploidization events. Section Caulorrhizae might be evolved from section Heteranthae (Mallikarjuna et al. 2005). Phylogenic studies revealed that sections Extranervosae, Heteranthae, and Triseminatae were considered as most primitive forms while the section Arachis was most advanced. Other sections viz., Caulorrhizae, Erectoides, Procumbentes, Rhizomatosae, and Trierectoides are intermediate in evolution (Wang et al. 2010). RAPD analysis exhibited a high degree of genetic similarity between A. pietrarellii and A. villosulicarpa suggesting that these two species are closely related (Galgaro et al. 1998).

7.1 Randomly Amplified Polymorphic DNA (RAPD)

The RAPD markers are dominant markers and utilize primers of ten nucleotides long. The markers are extensively used for the diversity analysis. Bhagwat et al. (1997) studied genetic diversity among 13 mutants using 12 random primers. The primers showed polymorphism of 5.5% with an average of 1.51 polymorphic bands per primer. Only one primer OPJ06 exhibited a higher level of polymorphism. Subramanian et al. (2000) subsequently examined 48 oligonucleotide primers to estimate the diversity among 70 genotypes. Among the primers studied, 7 primers showed 14.6% polymorphism. A total of 408 bands were observed from the 7 primer pairs. In another study, genetic similarity has been reported to be varied from 59.0 to 98.8% with an average of 86.2% among the 26 accessions examined using 8 primers of 10-mer (Dwivedi et al. 2001). Raina et al. (2001) also observed a polymorphism of 42.7 and 54.4%, respectively for the RAPD and ISSR primers utilized to detect polymorphism among 13 species of section *Arachis* and one species each of section *Heteranthae*, *Rhizomatosae*, and *Procumbentes*. In general, RAPD recorded higher primer index (0.35–4.65) than SSR primers (0.35–1.73). Nobile et al. (2004) analyzed genetic variability within and among species belongs to *Rhizomatosae*. A total of 113 polymorphic bands were observed using ten random primers. Both diploids and tetraploid species that formed two separate clusters suggested that the tetraploids did not originate from the diploid species analyzed. Mallikarjuna et al. (2005) examined 32 accessions of wild species belonging to 25 species of 6 sections using 29 primers. All of them showed polymorphic bands ranging from 5 to 33 with similarity value varied from 0 to 49% with an average of 15% similarity.

7.2 Restriction Fragment Length Polymorphism (RFLP)

RFLP markers are codominant markers with high reproducibility. The markers are used to construct linkage maps and for indirect selection of genotypes. Both the recessive genes and multiple alleles could be identified using the RFLP technique (Stalker and Mozingo 2001).

7.3 Amplified Fragment Length Polymorphism (AFLP)

The AFLP markers are PCR based with the reliability of RFLP markers. He and Prakash (1997) first utilized this marker in groundnut. Out of 28 primers that have been used to generate 111 AFLP markers in cultivated groundnut, only 3% of them exhibited polymorphism.

7.4 Simple Sequence Repeats (SSRs)

SSR markers are widely used for genotyping, construction of linkage map, and for MAS due to its codominant and easy to detect from a small amount of DNA. To discriminate the 60 cultivated genotypes and 36 wild accessions from 8 sections of *Arachis*, Moretzsohn et al. (2004) utilized 12 SSR markers. Species of section *Arachis* showed microsatellite marker transferability of up to 76%. However, other sections of *Arachis* showed only 45%. The marker Ah-041 distinguished species belongs to AA genome and non-AA genome. Gimenes et al. (2007) detected 18 putative loci in cultivated groundnut by employing 14 microsatellite primer pairs. The loci Ah 51 amplified 7 alleles with a PIC value of 0.79 whereas, the loci Ah 282 amplified only two alleles with a PIC value of 0.11. A mean polymorphic percentage of 33% was observed. The microsatellite primer pairs showed high transferability rate ranging from 60% for Ah 20–100% for Ah 30. In another experiment, Tang et al. (2007) observed a maximum genetic distance of 0.992 when 34 SSR markers were utilized for the genetic diversity studies involving four botanical varieties of cultivated groundnut. The average intra-variety genetic distance was 0.59 in var. *fastigiata*; 0.46 in var. *hypogaea*; 0.38 in var. *vulgaris*, and 0.17 in var. *hirsuta*. A study was undertaken to assess the diversity and genetic relationships of 201 accessions of *A. hypogaea* and 13 accessions of *Arachis* wild species using 13 SSR markers. The primer pair amplified 108 polymorphic alleles in *A. hypogaea*. The markers detected 3–15 alleles at each locus with an average of 8.3 per marker (Naito et al. 2008). Angelici et al. (2008) assayed genetic variability among 77 accessions of four species from section *Rhizomatosae* involving tetraploid and diploid species. A total of 249 alleles were found in the 15 loci analyzed and a high degree of intra- and interspecific polymorphism was detected. Number of alleles observed ranged from 10 for the locus Ap176 to 26 for the locus Ah21 with a mean number of 20.5 alleles per locus.

7.4.1 Construction of Molecular Genetic Linkage Map

Genome size differs among the different species of section *Arachis*. The variation in genome size is mainly attributed to the amount of repetitive DNA and differences in ploidy level (Flavell et al. 1974). Halward et al. (1993) first studied RFLP-based linkage map in the F_2 population involving two diploid species (*A. stenosperma*×*A. cardenasii*). A total map distance of 1,063 cM has been recorded with 11 linkage groups. In another study, partial genetic linkage map was constructed employing bulked segregants analysis (BSA) and AFLP analysis for aphid resistance in $F_{2:3}$ population. Twelve markers were found to be mapped in five linkage groups covering a map distance of 139.4 cM (Herselman et al. 2004). Garcia et al. (2005) studied the backcross populations of *A. stenosperma*×(*A. stenosperma*×*A. cardenassii*) to construct linkage map using RAPD and RFLP markers. They observed 11 linkage groups covering a total map length of 800 cM. Moretzsohn et al. (2005) constructed a linkage map based on microsatellite markers using F_2 population involving two diploid A genome species (*A. duranensis* and *A. stenosperma*). The linkage map consists of 11 linkage groups covering 1230.89 cM of total map distance. The first SSR-based genetic linkage map of groundnut was constructed using the RIL population derived from a cross between ICGV 86021×TAG 24 (Varshney et al. 2009). A total of 135 loci covering 22 linkage groups were constructed with a total map distance of 1,270.5 cM. Subsequently, Moretzsohn et al. (2009) constructed B genome linkage map from the F_2 population of a cross between two diploid B genome species (*A. ipaensis*×*A. magna*). The map has 149 loci spread over to ten linkage groups with a total map distance of 1,294 cM. The first SSR-based genetic linkage map for cultivated groundnut was developed by Varshney et al. (2009). They screened two genotypes TAG 24 and ICGV 86031 utilizing 1,145 microsatellite markers and several unpublished markers. Only 144 markers identified 150 loci. A total of 135 SSR loci were mapped into 22 linkage groups. Construction of genetic linkage maps help to: (a) detect the markers associated with quantitative trait loci (QTL) with economically important traits, (b) indirect selection in MAS, and (c) clone desirable genes by chromosome walking (Wicking and Williamson 1991).

7.4.2 Identification of Markers Associated with Qualitative and Quantitative Traits

Foliar Disease Resistance

Dwivedi et al. (2002a) concluded that accessions ICG 405, ICG 1705, ICG 6284, ICGV 99001, and ICGV 99005 with TMV 2 would be the better cross combinations to develop recombinant inbred lines (RILs) for mapping QTL associated with resistance to ELS, LLS, and rust in groundnut. In another experiment, Dwivedi and Gurtu (2002) effected crosses between ICG 405 with ICG 9987 and ICG 10000 with ICG 405, ICG 9989, ICG 9987 and ICG 9991 to select progenies with enhanced resistance to ELS. Also they suggested developing RILs by crossing

highly susceptible genotype ICG 10914 with ICG 9987, ICG 9991, ICG 405, ICG 10000, and ICG 9989 for identification of DNA markers linked with resistance to ELS. Once the markers linked with resistance to ELS are located, they may be used in marker assisted breeding for developing genotypes with resistance to ELS. Reddy et al. (2004) studied two tolerant (GPBD 4 and Dh 22) and one susceptible parent (Dh 40) and ten recombinant progenies from a three-way cross of Dh 40 × (GPBD 4 × Dh 22). Among the ten recombinant lines studied, six were found to be resistant (RL1, RL2, RL3, RL4, RL5 and RL7) while, four were found to be susceptible (RL8, TL9, RL10 and RL 11). Among the primers, OPA-07 and OPA-15 revealed 100% polymorphism followed by OPA-09 and OPA-18 which revealed 71.4% polymorphism. In another study, Mondal et al. (2005) screened 19 genotypes with RAPD primers. Of the 50 primers screened, 11 exhibited polymorphism among the genotypes studied. The extent of polymorphism ranged from 12.55 to 76.9% with an average of 37.5%. Among the primers studied, Kit A19 revealed highest polymorphism.

Mace et al. (2006) first reported high level of genetic polymorphism in 22 cultivated groundnut with varying level of resistance to foliar diseases using 23 SSR primers. Overall, 135 alleles across 23 loci were observed among the genotypes screened. Twelve markers showed higher polymorphism (52%). In another study, AFLP and BSA were employed to identify DNA markers linked to LLS resistance based on the segregating F_2 population of the cross between susceptible (Zhonghua No. 5) and a resistant interspecific derivative (ICGV 86699). Three AFLP markers E35/M51, E37/M48, and E41/M47 linked to LLS resistant gene were identified. The map distance between the markers and the gene were 7.40, 7.40, and 8.67 cM, respectively (You-Lin et al. 2007). Leal-Bertioli et al. (2009) screened the mapping population consists of 93 F_2 plants derived from a cross between *A. duranensis* × *A. stenosperma*. They mapped 35 candidate genes and five QTLs for LLS resistance. Still several regions within the A genome species may have genes conferring resistance. Clustering the candidate genes and QTLs suggests that the upper region of LG 4 and the lower region of LG 2 are likely to control disease resistance and to harbor clusters of disease resistance genes in *Arachis*. A QTL study for late leaf spot and rust resistance involving 268 RILs of a mapping population TAG 24 × GPBD 4 identified 11 QTLs for LLS (exhibiting 1.70–6.50% phenotypic variation) and 12 QTLs for rust (explaining 1.70–55.20% variation). One major $QTL_{rust}01$ associated with rust resistance contributed 6.90–55.20% variation was identified by composite interval mapping and single marker analysis. In another cross TG 26 × GPBD 4, one SSR marker (IPAHM 103) was linked with the QTL for rust resistance was identified (Khedikar et al. 2010).

Sclerotinia Blight Resistance

Chenault and Maas (2006) used SSR primers to screen 66 cultivated groundnut genotypes. One primer pair consistently produced banding pattern which distinguishes the resistant from susceptible genotypes. Chenault et al. (2009) first reported

molecular marker associated with resistance to Sclerotinia blight in groundnut. They utilized 16 SSR primers to examine groundnut genomic DNA from 39 cultivated groundnut genotypes. Only one primer pair distinctly produced bands at approximately 145 and 100 bp consistent with either resistant and susceptibility, respectively. Cloning and sequencing of these bands revealed the highly conserved region. Identification of this marker helps in screening germplasm collections and segregating populations and pyramiding of resistant genes with other desirable traits into superior genotypes.

Aflatoxin Resistance

Seed protein markers could not identify markers for aflatoxin resistance in groundnut (Bianchi-Hall et al. 1994). Luo et al. (2005) attempted to study the identification of resistance genes in response to *A. parasiticus* infection under drought stress using microarray and real-time PCR in a groundnut genotype A 13 which is resistant to preharvest aflatoxin contamination with acceptable level of tolerance to drought. They detected 42 upregulated genes in response to drought and *A. parasiticus* infection. Twenty five genes commonly expressed in both aflatoxin contamination and drought stress conditions. The F_2 population of a cross Zhonghua 5 × J 11 (J11 is resistant to seed infection) was studied for the association of AFLP marker with resistance to *A. flavus* infection. Two markers viz., E45/M53-440 (440 bp) and E44/M53-520 (520 bp) were observed to be linked with seed infection resistance. The distance between these two markers and the resistant gene was 6.6 and 8.8 cM, respectively (Lei et al. 2005a). These markers could be used for a marker assisted breeding program.

Nematode Resistance

Nematode resistance was associated with three RAPD markers (Burow et al. 1996). Recombination between marker RKN410 and resistance and between marker RKN440 and resistance was estimated to be 5.4 ± 1.9% and 5.8 ± 2.11 per generation, respectively. Another marker RKN229 and resistance exhibited a recombination of 9.0 ± 3.2% per generation. Presence of additional genes was confirmed by different distribution of resistance in segregating populations. Two RFLP loci viz., R2545 and R2430 linked to a single gene for resistance to root-knot nematode was screened among 548 individuals from three segregating $BC_7F_{2:4}$ populations. The former locus identified 27.6, 65.1 and 29.5% of populations TP293-3-3, TP296-4-4, and TP301-1-8, respectively, as being homozygous for resistance; while the later locus identified 24.5, 50.0, and 23.5% of populations as homozygous for resistance (Church et al. 2000). A dominant marker 197/909 was developed from another marker RKN440 which was previously used to screen for the nematode resistance. The new marker is PCR based hence, the screening will be fast and effective. The marker establishes a good association with phenotype data. Since the new marker amplifies fragments of different sizes from

susceptible and resistant plants, the chance of getting erratic results can be minimized (Chu et al. 2007). In another experiment, the F_2 population of the cross between Huayu 22 and D099 which are susceptible and resistant to nematode were studied using SSR markers based on BSA. Two SSR markers viz., S32-380 and S89-140 were linked to resistance with a genetic distance of 4.421 and 7.404 cM, respectively.

Drought

Dwivedi and Varma (2002) studied the genetic diversity among 37 genotypes differing in drought tolerant traits using SSR markers. They suggested making crosses between CSMG 84-1 and ICGV 97068 to select progenies with high T, TE, and HI. The same cross could also be used for developing RILs for mapping the QTL associated with T, TE, and HI. The crosses TMV2NLM with ICGV 94106, ICGV 97068, ICGV 99247, and Chico; CSMG 84-1 with ICGV 94100, ICGV 94113, ICGV 97068, and ICGV 99235 also be used for mapping QTL for HI. Subsequently Varshney et al. (2009) studied mapping populations of TAG 24×ICGV 86031. Although 2–5 QTLs were identified for T, TE, and SCMR, the phenotypic variation ranged from 3.5 to 14.1% only. Using differential display reverse transcriptase technique (DDRT), Jain et al. (2001) studied peanut transcriptase affected due to water stress. The transcriptases are termed as peanut transcript responsive to drought (PTRD). They identified three transcriptase viz., PTRD 1, PTRD 2, and PTRD 16 to distinguish drought tolerant and susceptible cultivars.

Pod and Seed Traits

Selvaraj et al. (2009) first reported the identification of molecular markers associated with pod and kernel traits of cultivated groundnut through BSA using SSR markers. One QTL was identified for each of the quantitative traits such as seed length, seed weight, number of pods per plant, plant weight, pod maturity, and oil content. However, two QTLs were identified for pod length. They also observed that one marker may be linked to more than one character. The SSR marker PM 375 were linked to seed length, pod length, and 100 seed weight; while another primer Ah-041 linked to number of pods and plant weight. It indicted that chromosomal regions identified by these markers may contain linked genes with pleiotropic effect on multiple traits.

7.5 Marker Assisted Selection

Marker assisted selection was first proposed by Sax in 1923 (Arus and Moreno-Gonzalez 1993). The concept behind the MAS is to select traits which are not having discrete morphological differences or characters with low heritability. The characteristics of a good marker are: (1) it should distinguish the homozygotes from

heterozygotes, (2) it should have early expression in the plant, and (3) there should not be interaction with other markers. Most of the quantitative characters are highly influenced by the genetic and environmental factors. Markers facilitate the identification of genetic factors governing the trait and increase the efficiency of selection. Also it reduces time and space which are high in traditional approaches. The success of MAS depends on identification of markers associated with the trait of interest. However, the construction of linkage map helps to locate the genes. For MAS irrespective of the stage of the crop, genomic DNA are extracted from any tissues of the crop plant. Recently, a nondestructive method for extracting genomic DNA from mature dry groundnut seed was first reported by Chenault et al. (2007). Since this method is first of its kind, the seed can be subsequently germinated to produce healthy mature plants, making this technique useful tool for the application of MAS and in screening large segregating populations.

The success of marker assisted breeding depends on: (1) availability of donors for the trait of interest, (2) appropriate mapping population for the trait, (3) high-throughput screening protocol for phenotyping the mapping population, (4) identification of appropriate markers linked with QTL of interest, and (5) PCR-based marker technology to facilitate screening the large segregating populations. Mapping populations such as near-isogenic lines (NILs), bulked segregant analysis (BSA), and RILs are widely used for mapping of major genes or polygenes. MAS could be effectively used for the characters with low heritability when additive and nonadditive genetic variance are associated with the marker loci (Dwivedi et al. 2003). When the marker is tightly linked with the QTL, the expected efficiency of MAS is high. However, when the marker and the QTLs are loosely linked, the efficiency will be low.

Available germplasm shows enormous variability for traits such as plant height, number of mature pods, pod size and shape, pod yield, kernel yield, shelling outturn, 100 seed weight, sound mature kernel percentage, seed color, seed dormancy, and maturity. These traits could be easily improved through conventional breeding techniques. Several genetically improved high-yielding varieties are occupied the major groundnut growing areas. Location specific varieties are also bred to suit to the particular zone. Though enough varieties are developed for resistance to various pests and diseases, the resistant genes are tightly linked with undesirable pod and kernel characteristics which in turn reduce the acceptability. In order to break the undesirable linkage, MAS could be effectively employed. Only moderate level of resistance to the diseases and insect pests are observed in the germplasm. Crossability barriers and sterility due to ploidy level differences associated with the wild diploid species hampers introgression of genes. However several mechanisms have been employed to harness the incompatibility reactions in wild species. MAS helps to introgress the resistant gene(s) into the cultivated groundnut without altering the other desirable agronomic traits. Similarly, the traits associated with oil quality (O/L ratio) and drought is difficult to breed as these traits are uneconomic to measure in large breeding populations. Breeding lines were developed by pyramiding the disease resistant traits with high O/L using MAS (Chu and Ozias-Akins 2009). Attempt was made to combine high oleic trait with nematode resistance by crossing resistant cultivar COAN with susceptible cultivars HULL, Norden and F89/OL 14-1-4-1-1-1-2 using the SCAR marker (Z3/265) and a RFLP marker (R2430E).

Lines showing high, moderate, and low oleic trait with nematode resistance were isolated (Varma et al. 2006). Milla-Lewis et al. (2006) employed AFLP markers associated with reduced aflatoxin accumulation in the *A. cardenasii*-derived germplasm lines. They found that 36, 46, and 36 markers were associated with reduced accumulation of aflatoxin B_1, aflatoxin B_2, and total aflatoxin ($B_1 + B_2$). Also high G×E interaction influences the expression of drought tolerant traits such as SLA, total transpiration, water use efficiency, and partitioning. In such cases, MAS holds good to develop genotypes with desired level of resistance.

7.6 Genetic Transformation

The advent of recombinant DNA technology helps to isolate and clone the gene of interest into the cultivated groundnut for combating biotic and abiotic stresses (Bhatnagar-Mathur et al. 2008). The success of genetic transformation depends on the effective regeneration system. Well-established transformation protocols helps to establish transgenic groundnut with desirable trait. The transgenic plants retained the desirable market trait along with the resistance. However, the yield is comparable with that of the parental line (Chenault et al. 2006). Hu et al. (2009) observed similarity between the Sclerotinia-blight resistant transgenic peanut and their non-transgenic parents in respect to various pod and kernel characters.

7.6.1 Tissue Culture

An ideal tissue culture technique with maximum regeneration capacity holds good for various biotechnological applications. In groundnut, in vitro regeneration occurs via embryogenesis (Chengalrayan et al. 2001; Akasaka et al. 2000; Lakshmanan and Taji 2000; Little et al. 2000) and organogenesis (Chengalrayan et al. 2001). Cotyledon explants proved to be an excellent source to get large number of transformed groundnut plants (Sharma and Anjaiah 2000). Plants were successfully obtained through in vitro techniques using the cotyledon and immature leaflet explants. The percentage of embryogenic mass induction, embryo development and conversion was genotype-dependent. However, the number of embryos produced per explant and germination to plantlets was genotype-independent. In general, the runner market types were more responsive than Virginia market types (Chengalrayan and Gallo-Meagher 2004). However, Rohini and Sankara Rao (2000) suggested a non-tissue culture-based approach for generating transgenic plants.

7.6.2 Genetic Transformation

The most common method of DNA transfer is *Agrobacterium*-mediated gene transfer in groundnut. Other methods such as electroporation and microprojectile bombardment were also employed for direct and physical DNA delivery against

fungal and viral disease, insect resistance, and drought resistance and also for quality improvement. For *Agrobacterium*-mediated gene transfer, the transgene must be integrated into the T-DNA of Ti-plasmid before introduction into the actively growing groundnut cells.

Fungal Disease Resistance

The fungus *A. flavus* produces a secondary metabolite called aflatoxin. Aflatoxin reduces the quality of groundnut and it is the major threat to groundnut industry. Though varieties resistant to *Aspergillus* were evolved through conventional breeding approaches, the level of resistance was not up to the mark. Genetic engineering approach addresses the problem. Stilbene is a phytoalexin synthesized in groundnut in response to the invasion of the fungus *Aspergillus*. The phytoalexin checks the fungal growth and inhibits spore germination. The enzyme stilbene synthase is responsible for the production of stilbene. The gene responsible for the synthesis of the enzyme has been characterized and successfully introduced in tobacco. Transgenic groundnut with the gene of interest not only improves the quality but also improves the livelihood of small-scale farmers. Genes responsible for the synthesis of hydrolytic enzymes such as chitinase and glucanase degrades the fungal cell wall and will be good candidate gene for the development of resistant cultivar (Eapen 2003). Chenault et al. (2002) successfully introduced chitinase gene from rice and a β 1-3-glucanase from alfalfa in the somatic embryos of the groundnut cultivar Okrun via microprojectile bombardment. Transgenic groundnut expressed hydrolase activities up to 37.0% than the nontransformed plants. Niu et al. (2009) developed a transgenic groundnut by integrating a nonheme chloroperoxidase gene (*cpo-p*) in embryogenic tissues of groundnut cv. Georgia Green through particle bombardment. The *cpo-p* gene inhibits *A. flavus* hyphal growth which in turn reduces aflatoxin contamination. In an attempt to develop a transgenic groundnut plant, Anuradha et al. (2008) cloned the complete cDNA containing an ORF of 243 bp of a defensin gene. The transgenic groundnut plant showed enhanced resistance against the ELS and LLS diseases. Rohini and Sankara Rao (2001) obtained fertile transgenic groundnut cv. TMV 2 expressing tobacco chitinase gene using an *Agrobacterium*-mediated transformation system. The transgenic plants were highly resistant to the fungal pathogen. Oxalic acid plays a vital role in imparting pathogenicity of many Sclerotinia species. Three Virginia cultivars (Perry, Wilson and NC 7) were engineered with oxalate oxidase gene which degrades the oxalic acid synthesis of Sclerotinia blight (Livingstone et al. 2005). The transformed plants showed uniform resistance without altering the crop characteristics (Grabau 2009).

Virus Disease Resistance

Some of the important viruses causing irreparable loss to the cultivated groundnut are tomato spotted wilt virus (TSWV), peanut stripe virus (PStV), peanut bud

necrosis virus (IPCV), peanut mottle virus (PMV), groundnut rosette assistor virus (GRAV), and tomato spotted wilt virus (TSWV). High level of resistance could be achieved through recombinant DNA technology. Transgenics with high level of resistance to PStV were developed through microprojectile bombardment of embryogenic callus. Coat protein genes such as CP2 and CP4 with N-terminal truncation have been successfully introduced to impart high level of resistance (Higgins et al. 2004). The transgenic plant serves as an important donor for groundnut breeding. Yang et al. (1998) isolated nucleocapsid protein (N) gene of the lettuce isolate and introduced through microprojectile bombardment to impart resistance against tomato spotted wilt virus (TSWV). The transgenic plant expressed N protein. Transgenic plants having TSWV resistance were evaluated under both field and green house conditions (Yang et al. 2004). The transgenic plant observed significantly lower incidence of spotted wilt when compared to the nontransgenic checks.

Insect Resistance

Lesser cornstalk borer (*Elasmopalpus lignosellus*) causes severe damage to the groundnut crop and reduces the yield and quality. Through microprojectile bombardment, *cry*1A(c) gene was introduced into groundnut. Insect feeding bioassay of transformed plants indicated various levels of resistance to the lesser cornstalk borer from complete larval mortality to a 66% reduction in larval weight (Singsit et al. 1997). Also, the *cry*1A(c) gene strongly prevents the fungal entry by reducing insect damage to the groundnut tissues.

Drought Resistance

A transcription factor DREB1A from *Arabidopsis thaliana*, driven by the stress inducible promoter from the rd29A gene was introduced in a drought sensitive groundnut cultivar JL 24 through *Agrobacterium*-mediated gene transfer. All the selected transgenic plants were able to maintain a transpiration rate equivalent to that of control. Most of the transgenic plants exhibited higher TE. Under the stress situations, one of the selected transgenic plant showed 40% higher TE than the untransformed control (Mathur et al. 2007).

Quality Improvement

Three transgenic groundnut lines 188, 540, and 654 were evaluated along with the parental like Okrun for oil, protein, ash, moisture, total dietary fiber, mineral, and fatty acid compositions. There is no significant difference between the transgenics and the parental line for oil content. All the quality components are within the range observed among the groundnut genotypes. However, the transgenic line 188 showed

significantly higher level of protein than the parental line (Jonnala et al. 2005). 12-fatty acid desaturase (FAD) gene plays a vital role in regulating the synthesis of oleic acid (Jung et al. 2000; Lopez et al. 2000). The regulatory mechanism of FAD gene in fatty acid metabolism has been extensively studied for incorporation of the gene in groundnut (Dongmei Yin and Dangqun Cui 2006).

8 Summary and Conclusion

Unlike other oilseed crops, groundnut improvement through hybridization is rather slow due to low recovery of crossed pods, restricted recombination, poor reproductive efficiency, smaller F_2 population, inherent genetic problem associated with segmental allopolyploid, and lack of clear cut understanding between canopy and reproductive traits. Despite this, a spectacular improvement has been made during the past to overcome various biotic and abiotic stresses influencing the yield. Extensive explorations have been made in South American countries especially Argentina, Bolivia, Brazil, Paraguay, and Uruguay which assembled 80 annual and perennial wild species. Besides, enormous local land races collected from the six centers of diversity have been deposited in the gene bank. A vast collection of wild species, land races, accessions, breeding lines, and improved cultivars are being maintained at ICRISAT and USDA. Though enormous genetic variability is found in the germplasm, breeders seldom utilize the accessions in the breeding program because of unavailability of descriptive characters and evaluation methods. Hence, core and minicore collections have been established for earliness, disease resistance and nutritional quality, etc. Utilizing the genotypes, numerous potential high-yielding cultivars have been developed and released for commercial cultivation. However, exploitation of genotypes from the core collection still needs to be enhanced since only few genotypes are most frequently used in developing cultivars for earliness and disease resistance. Ploidy level differences between the cultivated and wild species hamper interspecific breeding. However, compatible diploid wild species such as *A. stenosperma*, *A. chacoense*, *A. cardenasii*, and *A. duranensis* are extensively used to introgress resistant genes for pests and diseases into cultivated species. Higher level of resistance is linked with poor pod and kernel features which in turn reduce the market acceptability. However, tolerant genotypes possess acceptable level of kernel characteristics. Hence, one has to compromise the yield to some extent to have good kernel traits by way of preferring tolerant cultivars. In general race, nonspecific resistance is good than race specific resistance. The second most important constraint is drought which severely affects crop growth and reduces the yield considerably. Early season drought arrests excessive vegetative growth and promotes yield while mid-season drought and end of season drought affect the yield significantly. Drought tolerant genotypes are also endowed with aflatoxin resistance. Both trait- and empirical-based approaches have been followed for the evolution of cultivars resistant/tolerant to drought. Quality varies with consumers, growers, and traders. Oil quality is important for consumers. Oil quality and oil stability depends

on proportion of two fatty acids viz., oleic and linoleic acids. High oleic content is mostly preferred as it reflects higher shelf life. Flavor quality decides the acceptance of roasted groundnut as snack food or groundnut butter. Cultivars with high O/L ratio has been identified and released. To meet the demand of the growers, improved cultivars with fresh seed dormancy and early maturity have also been bred through conventional breeding approaches with certain shortcomings. Breeding cultivars resistant to drought and improved nutritional quality requires thorough screening of segregating populations which requires huge resources and is cumbersome. Recent biotechnological approaches such as MAS pave the way to identify the gene of interest and help to pyramid the desirable genes in otherwise agronomically superior genotypes. Major genes conferring resistance to bacterial and viral diseases have been identified across the plant kingdom and genotypes with high level of resistance have also been developed through genetic transformation techniques.

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