Chapter 4
Sunflower

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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

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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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