Chapter 6
Sunflower

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6.1 Introduction

Sunflower (*Helianthus annuus* L.) oil is the fourth most important vegetable oil in world trade at present with an annual production of around 9 million tonnes and a cultivated acreage of over 22 million hectares, mainly concentrated in the Russian Federation, Ukraine, India, and Argentina, which totalize more than 50% of sunflower world acreage. Although of North American origin, where it was domesticated by the Native American Indians for its edible seeds (Heiser et al. 1969), the transformation of sunflower into a major oilseed crop only took place in the second half of the 20th century due to two major breeding achievements: the drastic increase of oil percentage in sunflower achenes achieved in the Former Soviet Union from 1920 to 1960 (Gundaev 1971), and the development of a cytoplasmic male sterility system (Leclercq 1969) combined with fertility restoration by nuclear genes (Kinman 1970) that enabled the commercial production of hybrid seed. The subsequent development of short-stemmed, high yielding hybrid cultivars with high oil content well adapted to mechanised cropping represented the transformation of sunflower into a cash crop and sunflower oil into a major commodity in world trade.

Conventional sunflower produces a healthful oil with great consumer acceptance because of its high content of monounsaturated and polyunsaturated fatty acids as well as high vitamin E content. In recent years, new sunflower oil types for specific applications, mainly in the food industry, have been developed through conventional breeding approaches. Such specialty oils are called to play an important role in a further development of the sunflower crop.

Unlike other oilseed crops such as soybean and canola, commercial sunflower has not been subject to transgenic breeding so far. However, sunflower breeders have been very successful in attaining a wide diversity of breeding objectives, from developing novel seed oil quality types to incorporating genetic

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resistance to most of the pests and diseases that threaten the crop. The history, current status, and future prospects of breeding advances in sunflower are reviewed in this chapter.

6.2 Origin and Domestication

The cultivated sunflower (*Helianthus annuus* L.) is a member of the family Compositae (Asteraceae). The basic chromosome number is n = 17. The genus includes diploid, tetraploid and hexaploid species. The closest relatives appear to be *Tithonia*, *Viguiera* and *Phoebanthus* (Heiser et al. 1969). The common sunflower (*H. annuus*) is the most important species grown commercially, although other species are also cultivated, e.g. *H. tuberosus*, which is grown for production of edible tubers, and several other species grown as ornamentals. The name *Helianthus* is derived from the Greek words "helios," meaning sun, and "anthus," meaning flower. The Spanish name for sunflower, "girasol," and the French name "tournesol" literally mean "turn with the sun," a trait exhibited by sunflower until anthesis, after which the capitula (heads) face east.

Heiser et al. (1969) proposed a species classification of the genus *Helianthus* including 14 annual and 36 perennial species from North America (in three sections and seven series) and 17 species from South America. More recent classifications (Schilling and Heiser 1981; Jan and Seiler 2007) have introduced some modifications. The new classification brings the number of species to 51, with 14 annual and 37 perennial species (Tables 6.1 and 6.2).

Prior to the arrival of the European explorers to the New World, the progenitor of cultivated sunflower, the wild *H. annuus* was restricted to the southern U.S. (Heiser 1978). Wild *H. annuus* was used for food by the Native American Indians and, due to its association with humans, it became a camp-following weed that was introduced into the central part of the U.S., where it was domesticated and carried to the east and southwest (Heiser et al. 1969). The earliest evidence of domesticated sunflower has been dated at 4,625 B.P. (Crites 1993).

The origin of cultivated sunflower has been also investigated using molecular techniques. The use of randomly amplified polymorphic DNA (RAPD) markers supported the hypothesis that wild *H. annuus* was the progenitor of cultivated sunflower (Arias and Rieseberg 1995). Further studies using allozyme variation (Cronn et al. 1997) concluded that wild *H. annuus* from the Great Plains include the most likely progenitor of domesticated sunflower. More recent results based on the genetic relation between wild and extant domesticates (Harter et al. 2004) support the hypothesis that extant domesticated sunflowers arose from wild populations in the central part of the U.S. Other investigations using QTL analysis have studied the identity of traits that were the primary targets of strong selection during domestication (Burke et al. 2002). They concluded that strong directional selection for increased achene size appears to have played a central role in sunflower domestication.

Table 6.1 Infrageneric classification of annual *Helianthus* species (n = 17)

Section[a]	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

[a] Schilling and Heiser (1981); Jan and Seiler (2007)

The domesticated sunflower was introduced from North America into Europe by the early Spanish explorers in 1510 (Putt 1997), where they initially gained popularity as a garden ornamental. The agronomic development of sunflower as an oilseed crop and for use as edible achenes (confectionery types) took place in Russia, where a number of landraces had been developed by the late 1800s. Initial selection emphasis was given to early maturity, disease and pest resistance, and high seed oil content. Sunflower was reintroduced from Russia to North America in the latter part of the 19th century (Putt 1997).

6.3 Varietal Groups

There are three major groups of varieties of cultivated sunflower (*H. annuus*): those used for the extracted seed oil (oilseed types), those for the direct consumption of the seeds (confectionery types), and those used as ornamentals.

Table 6.2 Infrageneric classification of perennial *Helianthus* species

Section[a]	Series	Species	Chromosome number (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* (Torr. & A. Gray) Heiser	17
		H. pauciflorus Nutt.	
		subsp. *pauciflorus*	51
		subsp. *subrhomboideus* (Rydb.) O. Spring & E.E. Schill.	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

[a] Schilling and Heiser (1981); Jan and Seiler (2007)

Hybrid varieties are nowadays predominant for all three groups. By far, the major portion of sunflower production is devoted to oil extraction (Miller and Fick 1997). Sunflower oil has been traditionally viewed as a healthful vegetable oil and it is considered a premium oil for salad, cooking, and margarine production. The seeds of confectionery sunflower varieties are used as snack food as well as for feeding birds and small animals. The main characteristics that differentiate oilseed and confectionery sunflowers are oil content and seed size. The oilseed varieties have small black seeds with low hull content and very high oil content (about 50%). Conversely, confectionery sunflower varieties have larger seeds, which are usually black with white stripes, with lower oil content (about 30%) and a higher hull percentage.

Oilseed sunflower varieties are divided into three groups according to their oleic acid content: linoleic, mid-oleic, and high oleic. Linoleic (traditional) varieties have linoleic acid content between 45 and 75%, depending on the environment. It is considered a healthy vegetable oil suitable for salad and margarine production. The seed oil of mid- and high-oleic varieties has an oleic acid content of 55–75% and 85–90%, respectively. These oils are characterized by a better thermooxidative stability, which makes them more appropriate for frying purposes.

The main criterion of quality of confectionery sunflower is the seed size (Lofgren 1997). The largest size (>7 mm) type goes into the in-shell market to be used as snack. Medium-size seeds are hulled for the kernel market and those with the smallest size go into the bird and pet feeding market.

The last group of sunflower varieties includes those grown for ornamental purposes. There is great diversity for floral colour (yellow, cream, orange, rose, red, burgundy and bicolour) and morphology as well as for plant height (Fick 1976). Head diameter can vary from 10 to more than 30 cm and plant height from 50 to 500 cm (Miller and Fick 1997). Ornamental sunflower cultivars are used in gardens, home landscapes or as cut flowers. Cultivars used in home gardens are usually classified in groups based on plant height, which include giant or very tall (2.5–5 m), semi-dwarf (1–2.5 m), and dwarf types (<1 m). The cultivars used as cut flowers are pollen-free types which incorporate the cytoplasmic male-sterility trait. Ornamental cultivars usually incorporate genes from wild *Helianthus* spp.

6.4 Genetic Resources

Genetic resources are the base of crop improvement. They consist of the total pool of variability that exists in the cultivated species and also in related species that are sexually compatible with the cultivated one. Cultivated sunflower can be crossed with most of the 51 *Helianthus* spp. Sunflower germplasm resources can be categorized as *ex situ* resources (accessions preserved in seed banks) and *in situ* resources (wild populations and land races).

6.4.1 Germplasm Collection and Maintenance

6.4.1.1 *Ex Situ* World Collections

Aggressive collection of wild and cultivated sunflower germplasm for preservation in seed banks is crucial to make it easily available to sunflower breeders. Given the tenuous situation of some wild species in their natural habitats and the replacement of local landraces by outstanding high-yielding improved cultivars, seed banks may provide the only way to preserve these germplasm resources for posterity.

Systematic collection, introduction and conservation of sunflower germplasm were carried out by the N.I. Vavilov All-Union Scientific Research Institute (VIR) at St. Petersburg, Russia and by the U.S. National Plant Germplasm System (NPGS) Ames, Iowa, U.S.A. These two institutions maintain the two largest world collections of sunflower. The VIR collection has about 2,811 accessions, including 493 accessions of wild species and 2,318 of cultivated origin, most of them collected in the former USSR (Omelchenco 2001). The NPGS sunflower collection maintained at the North Central Regional Plant Introduction Station (NCRPIS) in Ames contains 3,860 accessions from 59 countries. The collection includes 1,670 accessions of cultivated *H. annuus*, 1,006 accessions of wild *H. annuus* forms, 430 accessions from other 11 wild annual *Helianthus* species, and 754 accessions representing 37 perennial *Helianthus* species (Marek et al. 2004). It is currently the largest and most genetically diverse *ex situ* sunflower collection of the world. NCRPIS not only conserves this genetically diverse *Helianthus* collection, but conducts germplasm-related research, encourages the use of germplasm and associated information for crop improvement, and distributes the accessions all around the world.

Other large collections of wild and cultivated sunflower are maintained at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, at the Dobroudja Agricultural Institute (DAI) at General Toshevo, Bulgaria, at the Research Institute for Cereals and Industrial Crops in Fundulea, Romania, and at the Station d́ Amélioration des Plantes in Montpellier, France.

6.4.1.2 Preservation of *In Situ* Resources

Preservation of in situ resources (wild species and landraces) in their natural habitats is critical, especially for wild sunflower populations, because of the lack of resources necessary to preserve all the wild species in seed banks. Moreover, a significant proportion of the wild diversity is likely to be lost while regenerating banked germplasm accessions. Unfortunately, the long-term preservation of some wild sunflower populations in their natural habitats is not always promising and some species are endangered or even extinct (Seiler and Rieseberg 1997). The U.S. Department of Interior, Fish and Wild Life Service listed several endangered and threatened species (Seiler and Rieseberg 1997). These species

included the annuals *H. paradoxus* and perennials *H. eggertii* and *H. schweinitzii*. Other candidate species for federal protection were the annuals *H. anomalus*, *H. deserticola*, *H. exilis* and *H. niveus* subsp. *tephrodes* as well as the perennials *H. laevigatus*, *H. carnosus*, *H. smithii* and *H. verticillatus*.

As for other genera, the primary obstacle for long-term preservation of wild *Helianthus* populations is human activity. This is for example the case of *H. exilis*, restricted to serpentine soils in the Inner Coastal Range of California, where mining activities destroyed several populations (Seiler and Rieseberg 1997). Another example of the impact of human activities was the case of *H. nuttallii* subsp. *parishii*, whose populations were drastically reduced by urbanizations around Los Angeles in Southern California (Rogers et al. 1982). In addition to the direct destruction of wild populations by development, their disturbance by human activity favours their hybridization with more widespread species (Rieseberg 1991). The resulting hybrid plants usually have lower fitness than locally adapted populations. For example, several rare annual sunflowers such as *H. anomalus*, *H. paradoxus* and *H. deserticola* occur sympatrically and occasionally hybridize with the common sunflower, *H. annuus*, what might imply a threat for the existence of these species. Another threat for the preservation of some rare species with small population sizes (e.g. *H. paradoxus* and *H. deserticola*) is their low level of genetic diversity (Rieseberg 1991).

6.4.1.3 Core Collections

Large germplasm collections usually contain duplications and they are difficult to manage in the evaluation of the existing variability for useful traits. Accordingly, the establishment of core subsets of the sunflower collections is imperative. Of the 1,624 cultivated accessions of the U.S. NPGS sunflower collection, a core collection of 112 accessions (7%) was established based on 20 descriptors (Brothers and Miller 1999). The accessions in this core collection represented 38 of the 57 countries of origin of the whole sunflower collection and contained 2 ornamental accessions, 7 breeding lines, 12 landraces, and 91 cultivars.

6.4.1.4 Genetic Stock Collections

Genetic stocks comprise unique mutants of different traits (morphological, chemical, physiological) as well as lines with specific characteristics (male-sterile lines, isolines, aneuploid lines) that are useful for basic research. There are about 30 sunflower genetic stocks registered by the Crop Science Society of America. Examples of these genetic stocks are characterised by nuclear male sterility (Jan 1992a), tetraploidy (Jan 1992b), dwarfness (Velasco et al. 2003a), altered seed oil fatty acid profile (Miller and Vick 2002; Vick et al. 2007), or resistance to herbicides (Miller and Al-Khatib 2002).

6.4.2 Germplasm Evaluation

A first step in any breeding program is the identification of genetic variability for different target traits. Sunflower breeders have been using a wide range of germplasm of wild and cultivated sunflower to search for variation for agronomic and seed quality traits as well as resistance to insects and diseases.

6.4.2.1 Agronomic and Physiological Traits

World collections of wild and cultivated germplasm possess a wide variability for morphological and physiological traits (plant height, flowering period, leaf and achene characteristics, etc.) of interest for sunflower breeding. A wide variety of agronomic traits was examined in wild *Helianthus* species for potential use in improving the hardiness and productivity of cultivated sunflower (Laferriere 1986). Wild *Helianthus* species have also been evaluated for resistance to several environmental stresses. Blanchet and Gelfi (1980) tested 10 species for various aspects of drought resistance and recommended *H. argophyllus* as a most likely source because of its pubescent leaves that reflect sunlight, reduced water loss, low stomatal resistance, and low transpiration rates. This species has been frequently used in breeding programs for drought resistance (Blanchet and Gelfi 1980). High variability has also been found in wild species for traits related to photosynthetic efficiency such as leaf area duration (Škorić 1988).

Several species of *Helianthus* are native to salt-impacted habitats. For example, *H. paradoxus* is found in saline marshes, where it exhibits high salt tolerance attributed to great leaf succulence and leaf sodium sequestration (Lexer et al. 2003b). Variability for salt tolerance has been also identified in germplasm of cultivated sunflower (Ashraf and Tufail 1995).

6.4.2.2 Cytoplasmic Male Sterility

Cytoplasmic male sterility (CMS) is a maternally inherited trait preventing plants from producing normal pollen. CMS is used as a tool to generate F_1 hybrid seed. Based on its origin, CMS is classified as autoplasmic or alloplasmic. Autoplasmic CMS refers to the cases where CMS has arisen within the species as a result of mutational changes in the cytoplasm. Alloplasmic male sterility arises from interspecific, intergeneric and occasionally intraspecific crosses due to incompatibility between nucleus and cytoplasm. Both types of CMS have been identified in sunflower. A type of stable alloplasmic male sterility named PET1 was reported by Leclercq (1969) in the progeny of an interspecific cross between *H. petiolaris* and cultivated sunflower. The subsequent identification of dominant fertility restoration genes, especially a source

derived from wild species (Kinman 1970) allowed for efficient and economical production of commercial hybrid seed 30 years ago. Virtually all cultivated sunflower hybrids are currently based on the CMS source derived by Leclercq (1969). The identification of additional CMS sources has been an important objective to broaden genetic diversity in cultivated sunflower. As a result of these efforts, a total of 70 CMS sources were identified (Table 6.3). Most of these sources originated from progenies of crosses between 16 different wild *Helianthus* accessions (mostly annuals) and cultivated lines (Serieys 2002), but some of them arose from mutational changes within the species *H. annuus*. Fertility restoration genes, found primarily in wild *Helianthus* species and also in related genera, have been reported for 34 CMS sources. Detailed inheritance studies have been conducted for 19 of the CMS sources (Serieys 2002). Therefore several CMS systems other than the widely used PET1 are available for practical hybrid seed production.

Table 6.3 Sources of cytoplasmic male sterility in sunflower[a]

Name	Origin (species)	FAO code[b]	Name	Origin (species)	FAO code[b]
Kouban	*H. annuus lenticularis*	ANL1	HA89	*H. annuus*	MUT 10
Indiana 1	*H. annuus lenticularis*	ANL2	HA89	*H. annuus*	MUT 11
Vir 126	*H. annuus, lenticularis*	ANL3	HA89	*H. annuus*	MUT12
397	*H. annuus* wild	ANN1	Anomalus	*H. anomalus*	ANO1
517	*H. annuus* wild	ANN2	Argophyllus	*H. argophyllus*	ARG1
519	*H. annuus* wild	ANN3	Argophyllus	*H. argophyllus*	ARG2
521	*H. annuus* wild	ANN4	Argophyllus	*H. argophyllus*	ARG3
Ns-Ann-81	*H. annuus* wild	ANN5	Arg3-M1	*H. argophyllus*	ARG3-M1
Ns-Ann-2	*H. annuus* wild	ANN6	Argophyllus	*H. argophyllus*	ARG4
	H. annuus wild	ANN7	Bolanderi	*H. bolanderi*	BOL1
	H. annuus wild	ANN8	Dv-10	*H. debilis*	DEB1
	H. annuus wild	ANN9	Exilis	*H. exilis*	EXI1
Fundulea 1	*H. annuus texanus*	AMT1	Exi2	*H. exilis*	EXI2
An-67	*H. annuus*	ANN10	Cmg2	*H. giganteus*	GIG1
An-58	*H. annuus*	ANN11	Cmg3	*H. maximiliani*	MAX1
An-2-91	*H. annuus*	ANN12		*H. maximiliani*	MAX2
An-2-92	*H. annuus*	ANN13	Mollis	*H. mollis*	MOL1
	H. annuus	ANN14	Neglectus	*H. neglectus*	NEG1
Cms-G	*H. annuus*	ANN15	Canescens	*H. niveus canescens*	NIC1
Cms-Dp	*H. annuus*	ANN16	Fallax	*H. petiolaris fallax*	PEF1
Cms-Vl	*H. annuus*	ANN17	Pet/Pet	*H. petiolaris petiolaris*	PEP1

Table 6.3 (continued)

Name	Origin (species)	FAO code[b]	Name	Origin (species)	FAO code[b]
	H. annuus	ANN18	Classical Cms	*H. petiolaris* Nutt	PET1
	H. annuus	ANN19	Cmg1	*H. petiolaris* Nutt	PET2
	H. annuus	ANN20	Petiolaris Bis	*H. petiolaris* Nutt	PET3
	H. annuus	ANN21	Pet34	*H. petiolaris*	PET4
	H. annuus	ANN22		*H. petiolaris*	PET5
Hemus	*H. annuus*	MUT1	Praecox	*H. praecox*	PRA1
Peredovick	*H. annuus*	MUT2	Phir-27	*H. praecox hirtus*	PRH1
Stadion	*H. annuus*	MUT3	Praecox	*H. praecox praecox*	PRP1
Peredovick	*H. annuus*	MUT4	Ppr-28	*H. praecox praecox*	PRP2
Peredovick	*H. annuus*	MUT5	Run-29	*H. praecox*	PRR1
Voronejskii	*H. annuus*	MUT6	Resinosus 243	*H. resinosus*	RES1
HA89	*H. annuus*	MUT7	Vulpe	*H. rigidus*	RIG1
HA89	*H. annuus*	MUT8	Rig-M-28	*H. rigidus*	RIG2
HA89	*H. annuus*	MUT9	Strumosus	*H. strumosus*	STR1

[a]Serieys (2002).
[b]The coding system for CMS sources consists of three-letter abbreviations of the cytoplasm donor species or subspecies followed by a number starting with 1, depending on the time of discovery and its reaction to restoration testers.

6.4.2.3 Disease and Insect Resistance

Diseases and insects are limiting factors of production in the majority of sunflower producing countries. The most serious diseases of sunflower are caused by fungi. They include *Sclerotinia* wilt, stalk rot, and head rot (*Sclerotinia sclerotiorum*), *Verticillium* wilt (*Verticillium dahliae*), sunflower rust (*Puccinia helianthi*), *Phoma* black stem (*Phoma macdonaldii*), downy mildew (*Plasmopara halstedii*), Phomopsis stem canker (*Diaporthe helianthi*), charcoal rot (*Macrophomina phaseolina*), alternaria diseases (*Alternaria* spp.), powdery mildew (*Erysiphe cichoracearum*, *Sphaerotheca fuliginea*, *Leveillula tarucia*), and *Rhizopus* head rot (*Rhizopus* spp.). Resistance to most of these diseases is found in wild *Helianthus* species as shown in Table 6.4 for some examples.

Genetic resistance to broomrape (*Orobanche cumana*), a parasitic plant that limited early sunflower production in the former USSR, was initially introduced into susceptible sunflower mainly from the wild species *H. tuberosus* (Pustovoit and Gubin 1974). More recently, results of evaluation of sunflower germplasm for resistance to new virulent races have shown that wild *Helianthus* species constitute the major source of resistance genes, although resistance was also found in accessions of cultivated material (Fernández-Martínez et al. 2000).

Table 6.4 Reported resistance to important diseases in wild sunflower species

Disease	Species with resistance	Reference
Downy mildew (*Plasmopara helianthi*)	*H. argophyllus* *H. annuus* *H. petiolaris* *H. praecox*	Hoes et al. (1973)
Verticilium wilt (*Vericillium dahliae*)	*H. annuus* *H. petiolaris* *H. praecox*	Hoes et al. (1973)
Rust (*Puccinia helianthi*)	*H. argophyllus* *H. annuus* *H. petiolaris* *H. praecox*	Hoes et al. (1973) Quresh et al. (1993)
Alternaria leaf spot (*Alternaria helianthi*)	*H. hirsutus* *H. pauciflorus* *H. tuberosus*	Morris et al. (1983)
Powdery mildew (*Erysiphe cochoracearum*)	*H. debilis* subsp. *debilis* *H. bolanderi* *H. praecox*	Saliman et al. (1982) Jan and Chandler (1985)
Phoma black stem (*Phoma macdonaldii*)	*H. decapetalus* *H. eggertii* *H. hirsutus* *H. resinosus* *H. tuberosus*	Škorić (1985)
Phomopsis stem canker (*Diaporthe helianthi*)	*H. maximiliani* *H. pauciflorus* *H. hirsutus* *H. resinosus* *H. mollis* *H. tuberosus*	Škorić (1985) Dozet (1990)
Rizopus head rot (*Rhizopus arrhizus*)	*H. divaricatus* *H. hirsutus* *H. resinosus* *H. x laetiflorus*	Yang et al. (1980)
Sclerotinia head rot (*Sclerotinia sclerotiorum*)	*H. decapetalus* *H. grosseerratus* *H. nuttallii* *H. pauciflorus* *H. resinosus* *H. tuberosus*	Pustovoit and Gubin (1974) Mondolot-Cosson and Andary (1994) Rönicke et al. (2004)
Sclerotinia root rot (*Sclerotinia sclerotiorum*)	*H. mollis* *H. nuttallii* *H. resinosus* *H. tuberosus*	Škorić (1987)
Sclerotinia mid-stalk (*Sclerotinia sclerotiorum*)	*H. praecox* *H. giganteus* *H. maximiliani* *H. pauciflorus* *H. resinosus* *H. tuberosus*	Škorić (1987)
Broomrape (*Orobanche cumana*)	Most of the perennial species	Fernández-Martínez et al. (2000)

Although several hundreds of insect species are associated with sunflower, only a few of them are economically important pests of cultivated sunflower (Schulz 1978). An exception was the significant yield reduction caused by the European sunflower moth (*Homoeosoma nebulellum*, Lepidoptera) in the former USSR at the end of the 19th century, which encouraged the first scientific breeding research on sunflower as early as 1890. Resistant cultivars were developed by interspecific hybridization of cultivated sunflower with *H. tuberosus*, which accumulates phytomelanin in the seed, thus reducing larval feeding (Gundaev 1971). Some wild species have been found to be resistant to insect pests attacking sunflower in North America, such as the sunflower stem weevil (*Smicronyx fulvus*, Coleoptera) (Rogers and Seiler 1985) and the sunflower beetle (*Zygogramma exclamationis*, Coleoptera) (Rogers and Thompson 1980). Evaluation of germplasm of cultivated sunflower has also revealed the existence of variability for resistance to several important insect pests (Charlet et al. 2007).

6.4.2.4 Oil and Protein Content and Quality

Oil and protein content of the sunflower achene depends on both the percentage of hull and the oil and protein concentration in the kernel. Variation for hull percentage and oil and protein content has been found in extensive evaluations of cultivated sunflower germplasm (Jiménez et al. 1985; Miller et al. 1992). Variation for oil and protein content also exists in wild species. Maximum oil content reported in wild species is lower than current standards of cultivated sunflower (around 50%). Conversely, maximum values of protein content found in wild species (35–40%) are generally higher than the typical protein content of cultivated sunflower (Seiler 1984; Ruso et al. 2000).

Variation for the seed oil quality components has been also found through the evaluation of genetic resources. The evaluation of germplasm collections led to the identification of cultivated sunflower germplasm with reduced levels of saturated fatty acids (Vick et al. 2002), high palmitic acid content (Demurin 2003) or high linoleic acid content (Miller and Vick 2001), as well as wild sunflower germplasm with reduced levels of saturated fatty acids species (Seiler 2004). Variation for increased levels of beta- and gamma-tocopherol has been found in collections of cultivated germplasm (Demurin 1993; Velasco et al. 2004a).

Sunflower germplasm is also a useful source of variation for reducing antinutritive compounds of the seeds, such as chlorogenic acid, that reduces the nutritive value of the meal. Dorrell (1976) found variation for reduced levels of chlorogenic acid in germplasm of wild and cultivated *Helianthus* species.

6.5 Major Breeding Achievements

6.5.1 Development of High Oil Germplasm in the Former USSR

After its introduction to Europe in the 16th century, sunflower was mainly grown as an ornamental. The first mention of sunflower cultivation as an oil crop was in Russia in 1779 (Gundaev 1971). The crop expanded rapidly and the first local varieties were developed by the end of the 19th century. These varieties were selected in small garden plots under different environmental conditions, which led to a wide range of variation for different traits such a maturity and seed types, including well-filled, round seeds with thin hull and oil content of about 20–30%, used for oil extraction, and large, long seeds with thick hull and oil content about 15–20%, used for direct human consumption (Gundaev 1971). Moreover, there was an important local selection for resistance to the European sunflower moth (*Homoeosoma nebulella*) and sunflower broomrape (*Orobanche cumana*), which at that time jeopardized the survival of the crop.

Scientific sunflower breeding started in 1910–1912 at Krasnodar by the academician V.S. Pustovoit, based on the local varieties developed at a local scale during the previous century (Panchenco 1966). The main efforts of breeders were initially devoted to the control of broomrape and sunflower moth, but the development of varieties with high oil content by V.S. Pustovoit constituted a crucial milestone in the development of sunflower as an oil crop not only in the USSR, but also throughout the world. The local varieties cultivated in Russia in 1913 contained only 30–33% of oil in dry seeds. This percentage increased up to 43% in 1935, 46% in 1953 and 51% in 1958, when the variety "Peredovik" was released (Panchenco 1966). This progress was obtained through the use of Pustovoit's "Method of Reserves", a method of individual selection with progeny testing and controlled pollination (Pustovoit 1967), together with the use of accurate laboratory techniques for oil content analysis. Moreover, this spectacular increase of oil content of the achenes did not caused any decline in the seed yield of the varieties released. The open pollinated Russian cultivar "Peredovik", with high oil content, introduced during the 1960s in the western countries (US, Canada, Western Europe), was the base of the first sustained commercial production of oilseed sunflower in these countries (Fick and Miller 1997).

6.5.2 Utilization of the Inbred-Hybrid Method

One of the most important breeding milestones in sunflower has been the development of hybrid cultivars that made possible the utilization of heterosis. Initial studies in the former USSR (Morozov 1947) and Canada (Unrau 1947;

Putt 1962) indicated that experimental hybrids outyielded check varieties from 160 to 189%. However, the practical production of hybrid seed was hindered by the absence of a suitable type of male sterility.

The first commercial sunflower hybrids were produced in Canada during the 1950s using a female parent with a high degree of self-incompatibility and allowing cross pollination with appropriate male parents. Although this method resulted in seed lots with hybridization rates as high as 90% under favourable environments, in general the percentage of hybrid seed was usually below 50% (Putt 1962). Thus, the seed produced by the self-incompatibility system often did not meet the legal requirements for designation as hybrid seed.

The existence of nuclear male sterility was first reported in the former USSR (Kuptok 1935) and later in France (Leclercq 1966) and Canada (Putt and Heiser 1966). In most cases the trait was controlled by a single recessive gene. Nuclear male sterility was used to produce hybrid seed in France and Romania during the early 1970s. The identification of a close linkage between genes for male sterility and anthocyanin pigmentation (Leclercq 1966) facilitated the identification and removal of the male fertile plants prior to flowering, thus allowing nearly 100% hybridization. This system allowed the development of the first commercial hybrids in Romania and in France, which yielded up to 24% more than the open pollinated varieties (Vrânceanu 1974). Even though the nuclear male sterility was an important step in the development of hybrid sunflowers, it required a considerable manual labour to remove male fertile plants.

The discovery of cytoplasmic male sterility, with its inherent advantages, provided a highly efficient method for commercial production of hybrid seed. The first stable source of cytoplasmic male sterility was discovered by Leclercq in 1968 from an interspecific cross involving *H. petiolaris* and *H. annuus* (Leclercq 1969). Subsequent identification of genes for fertility restoration in wild species (Kinman 1970) and in certain obsolete sunflower cultivars (Vrânceanu and Stoenescu 1971) allowed the efficient and economical production of hybrid seed. The development of the first sunflower hybrids based on cytoplasmic male sterility in the early 1970s intensified the interest of seed companies on the crop, which led to a considerable increase of sunflower production in many countries. When comparing sunflower yields in the countries that grew open-pollinated varieties before the introduction of hybrids, seed yields increases of about 20% were estimated (Fick and Miller 1997).

6.5.3 Development of New Types of Oil

The development of new oil types has been another important achievement for the sunflower oil industry. Sunflower breeders have been tremendously successful in developing mutants with new types of oil in the last 30 years, opening the possibility of tailoring specialty oils for specific market niches

(Fernández-Martínez et al. 2004). The most relevant was the high oleic acid mutant identified in Russia in the seventies (Soldatov 1976), which has made possible the development of commercial cultivars with mid oleic acid content (55–75%) and high oleic acid content (>85%), which are currently cultivated all over the world. In the U.S., the mid oleic acid varieties, locally known as Nusun®, are estimated to represent more than 85% of the total oilseed sunflower acreage according to the data of the National Sunflower Association.

6.6 Current Goals of Breeding

6.6.1 Seed Yield

One of the basic goals of sunflower breeding is to increase grain yield. The introduction of hybrid cultivars and the consequent exploitation of heterosis represented a breakthrough that produced an increase in yield potential around 25%. No significant improvement in grain yield potential has been observed at large scale before or after this turning point (López-Pereira et al. 1999). Even though several studies have identified yield components with a direct effect on seed yield, such as number of grains per head and grain weight (Connor and Hall 1997), major achievements in improving grain yield in sunflower have been more related to improving combining ability of hybrid parents or to selection for adaptation to limiting conditions in specific areas, e.g. shorter plant stature in areas with great lodging risk (Schneiter 1992), high degree of self-fertility in areas with limited pollinator populations (Miller et al. 1982), or pronounced head inclination in areas with high temperature and intense sunlight or with high risk of bird predation (Hanzel 1992). In many sunflower production areas, improved performance of recent hybrids was related to increased disease resistance, e.g. *Verticillium* wilt in Argentina (Sadras et al. 2000), Phomopsis stem canker in former Yugoslavia (Škorić 1985), or broomrape in several European countries (Alonso et al. 1996).

6.6.2 Morpho-Physiological Traits

6.6.2.1 Plant Height

The cultivated sunflower is a tall, erect, unbranched plant with a plant height below 75 cm in dwarf types to more than 5 m in giant varieties. Most common cultivated hybrids have a stem height of 160–180 cm, although the trait is very dependent on the environment. Several genetic sources of plant dwarfness have been identified and both semi-dwarf (100–160 cm) and dwarf (50–100 cm) hybrids have been produced and compared to standard-height hybrids. In general, no clear agronomic advantages were associated with reduced plant height in standard environments (Schneiter 1992; Velasco et al. 2003a). The

major advantage of semidwarf and dwarf cultivars is their resistance to lodging in environments with risk of heavy rains and strong winds during the growing season.

Plant height in standard sunflower types is regarded as a quantitative trait (Lay and Khan 1985). Several types of reduced plant height (<100 cm) have been developed. Reduced plant height in lines with a reduced number of leaves has been reported to be controlled by a single recessive gene (Miller and Fick 1997). Reduced plant height in genotypes with reduced internode length and a standard number of leaves has been found to be quantitatively inherited (Miller and Hammond 1991), controlled by a single dominant gene (Miller and Fick 1997), or by two recessive genes (Velasco et al. 2003b).

6.6.2.2 Head Size, Shape and Inclination

Head diameter may vary from 6 to 75 cm. The head shape presents a large gradation from concave to convex, whereas the head angle may vary from 0 (horizontal facing upwards) to 180° (horizontal facing downwards). The three traits are quantitatively inherited and subject to environmental effects (Miller and Fick 1997).

Classical Russian sunflower breeders put special emphasis on the importance of optimal head size and head shape to maximize sunflower yield. Morozov (1947) considered medium-size, thin and flat head as the ideotype for sunflower. Similarly, Pustovoit (1966) considered that a medium-size head (20–25 cm), in combination with adequate plant density, was one of the main determinants of grain yield. Larger heads would increase the percentage of hull as well as the number of empty grains in the center of the head.

Specific head shape and head inclination types have been identified as advantageous under certain environments. The incidence and severity of certain diseases such as white rot caused by *Sclerotinia sclerotiorum* and gray rot caused by *Botrytis cinerea* is directly related to the angle of the head. The lowest disease incidence is observed when the head is at an angle of 45° and remains above the foliage (Škorić 1992). A pronounced head inclination around 180°, in which the head is parallel to the soil surface, is desired to prevent sun scald in areas with high temperature and intense sunlight during seed maturation (Sailsbery and Knowles 1983), as well as to reduce bird predation in combination with a concave-shaped head (Hanzel 1992).

6.6.2.3 Flowering and Maturity Dates

Cultivation of sunflower varieties with flowering and maturity dates adapted to the particular agroecological conditions of a region is essential to ensure a high productivity of the crop. Most sunflower cultivars exhibit quantitative long-day or day-neutral responses to photoperiod although there are differences in photoperiodic sensitivity of sunflower genotypes (Connor and Hall 1997). A great variation in days from planting to maturity can be found in sunflower,

from around 75 to 150 days (Fick 1978). The genetic control of flowering date is rather complex and contradictory results have been obtained depending on the germplasm used. Vrânceanu (1974) suggested that the number of days to flowering was controlled by many genes, some of them affecting photoperiodism. However, most studies have reported a high heritability of flowering date (Miller and Fick 1997).

6.6.2.4 Pollen Self-Compatibility and Flower Characteristics

Sunflower inflorescence is a capitulum, usually referred to as head, formed by an outer whorl of sterile, ligulate flowers, known as ray flowers, and a varying number of inner whorls arranged in spiral from the head centre containing fertile flowers, known as disk flowers or florets. Flower nectaries are located at the base of the style in disk flowers and they play an important role in attracting pollinators (Tepedino and Parker 1982). Wild sunflowers have a system of sporophytic self-incompatibility that promotes insect-mediated cross-pollination. The number of loci involved in self-incompatibility has been disputed. One multiallelic locus was identified by Fernández-Martínez and Knowles (1978). Self-incompatibility systems were maintained in open-pollinated populations, but hybrid breeding has been accompanied by intense selection for high levels of self-compatibility. Nowadays, most commercial hybrids are virtually self-fertile (Fick and Miller 1997). Nevertheless, pollinator (mainly bees) attractiveness is still important in fields of hybrid seed production, where the production success depends on efficient pollen transfer from male-fertile to male-sterile parents. Selection for bee attractiveness is complex, since nearly every flower trait influences attractiveness to bees. Some of the most important traits are a short corolla length, short styles, unpigmented stigmas, and total sugar content and profile in the nectar (Montilla et al. 1988).

6.6.2.5 Male Sterility

The availability of male sterility is essential for the commercial production of hybrid seed. Both nuclear male sterility (NMS) and cytoplasmic male sterility (CMS) have been identified in sunflower. Nuclear male sterility is generally controlled by a recessive gene. Eleven different genes named *Ms1* through *Ms11* have been identified in NMS lines (Jan 1992c). A total of 72 unique CMS sources have been identified (Table 3; Serieys 2002), fertility restoration genes are available for 34 of them. Most of the CMS sources require at least two genes for fertility restoration, although some of them show single-gene restoration (Jan and Vick 2007).

6.6.2.6 Oil, Protein and Fibre Contents

The fruit of sunflower is an achene, commonly known as sunflower seed or grain. The kernel to hull ratio is one of the main parameters defining the

profitability of the crop. The percentage of hull in the achenes is very variable in sunflower germplasm, from around 10 to 60% (Miller and Fick 1997). In current oilseed cultivars, the kernel represents more than 75% of the total achene weight.

More than 80% of the economic value of oilseed sunflower cultivars is obtained from the extracted oil, whereas the rest is obtained from the protein-rich meal that remains after oil extraction. One of the major breeding achievements that facilitated the expansion of sunflower as one of the most important world oilseed crops was a drastic increase of oil content of sunflower achenes, from around 30% to more than 50% (Panchenco 1966). According to Alexander (1963), two thirds of the increase in oil content was produced by a reduction of the hull percentage in the achenes, whereas one third was produced by the increase of oil content in the kernel. The increment in the kernel to hull ratio was also associated with a concomitant increase of protein content, around 17% in current cultivars, and a reduction of fibre content. Additionally, the industry uses to hull the achenes before crushing to reduce the fibre content and improve the digestibility of the meal. Accordingly, the ease of hulling is also a selection target to take into account (Denis et al. 1994).

The hull content is a quantitative trait with high heritability mainly governed by genes with additive effects (Kovacik and Skaloud 1990). The ease of hulling is also a trait with high heritability for which variation in sunflower germplasm has been identified (Denis et al. 1994). Both oil and protein contents in sunflower achenes are traits quantitatively controlled by the genotype of the plant (sporophytic control) (Pawlowski 1964), with predominance of additive gene effects and medium to high heritabilities (Fick 1975; Alza and Fernández-Martínez 1997).

6.6.2.7 Oil Quality

Sunflower oil mainly contains molecules of triacylglycerol, composed of three fatty acids attached to a glycerol skeleton, which represent more than 95% of the total oil weight. The rest are lipid and lipid-soluble compounds, some of them of great value because of the functional and nutritional properties that confer to the oil. Breeding for oil quality in sunflower has been mainly focused in the modification of the fatty acid profile of triacylglycerols, although minor compounds with important nutritional and antioxidant value such as tocopherols and phytosterols have also attracted the attention of plant breeders in recent years.

The seed oil of conventional sunflower varieties is characterised by a high proportion of the unsaturated oleic acid (18:1) and linoleic acid (18:2), which together account for about 90% of the total fatty acids (Table 6.5). The remaining 10% correspond to the saturated palmitic acid (16:0) and stearic acid (18:0). The relative proportion of oleic acid and linoleic acid is strongly influenced by the environmental conditions, especially temperature, during seed development (Harris et al. 1978).

Table 6.5 Outstanding sunflower germplasm producing seed oil types with modified fatty acid and tocopherol profiles

	Fatty acid composition[a] (%)				
Oil type	16:0[b]	18:0	18:1	18:2	Reference
Standard[c]	7	3	30	60	
Low sat[d]	4	3	40	52	Vick et al. (2002)
High 16:0	25	4	11	55	Osorio et al. (1995)
High 18:0	5	26	14	55	Osorio et al. (1995)
Mid 18:1	4	5	55	34	Vick and Miller (1996)
High 18:1	5	3	90	2	Soldatov (1976)[e]
	Tocopherol composition (%)				
Oil type	α-T	β-T	γ-T	δ-T	Reference
Standard	95	4	1	0	
Medium β-T	50	50	0	0	Demurin (1993)
High β-T	25	75	0	0	Velasco et al. (2004b)
High γ-T	5	0	95	0	Demurin (1993)
High δ-T	5	0	30	65	Velasco et al. (2004b)

[a]Percentages for the four major fatty acids are given only.
[b]16:0 = palmitic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid.
[c]Averaged from cold and warm environments.
[d]Low content of total saturated fatty acids.
[e]Data of hybrids developed by Fernández-Martínez et al. (1993) from a mutant originally developed by Soldatov (1976).

The tocopherols are a group of four lipid-soluble substances with molecular structure comprised of a chromanol ring and a saturated phytyl side chain. The four tocopherols, named alpha-, beta-, gamma-, and delta-tocopherol differ in the number of methyl substituents and the pattern of substitution in the chromanol ring (Packer and Obermüller-Jevic 2002). They exhibit differential in vivo and in vitro antioxidant activities. While alpha-tocopherol exerts a maximum in vivo activity, also known as vitamin E activity, but poor in vitro protection of the extracted oil, gamma-, delta- and to a lesser extent beta-tocopherol are powerful in vitro antioxidants with low vitamin E value (Pongracz et al. 1995). However, the tocopherol profile is only a part of the picture when designing a breeding programme aimed at improving the tocopherol fraction in sunflower seeds. The other part is the total tocopherol content. The seed oil of conventional sunflower varieties has an average total tocopherol content of 708 mg kg^{-1}, mainly in the alpha-tocopherol form, which accounts for more than 90% of the total tocopherols (Padley et al. 1994).

Phytosterols or plant sterols are essential components of the cell membranes. Their role as functional food components and nutraceuticals due to their ability to lower total and LDL serum cholesterol in humans is increasing in recent years. Chemically, they are steroid alcohols (triterpenes) synthesized from squalene in the isoprenoid pathway (Piironen et al. 2000). Vegetable oils are the richest natural sources of plant sterols. The seed oil of conventional sunflower varieties has an average sterol content of 3,387 mg kg^{-1}, mainly as

sitosterol (59.9% of the total sterols), Δ^7 stigmastenol (10.4%), campesterol (9.5%), and stigmasterol (9.5%) (Padley et al. 1994).

The optimal quality of sunflower oil depends on the intended use of the oil, either for food or non-food applications. The former include salad and cooking oils as well as oils for the food industry (margarines, shortenings, etc.). The latter comprises countless industrial sectors such as biofuels, lubricants, surfactants, surface coatings, cosmetics, plastics, etc. In general, those oil characteristics that are undesirable for a particular application are required for others. A clear example is the saturated fatty acid content in sunflower oil. Saturated fatty acids are regarded as detrimental on human health because of their contribution to raise cholesterol levels as compared with isocaloric amounts of carbohydrates (Mensink et al. 1994). Accordingly, the breeding objective to produce a healthy oil of direct consumption (e.g. salad oil) is to reduce total saturated fatty acid content. But on the other hand, a sunflower oil rich in saturated fatty acids is desirable for the industry of margarines and related products, because its semi-solid consistency reduces the need for transformations such as hydrogenation or transesterification that generate *trans* and positional isomers related to heart disease (Willett and Ascherio 1994). Accordingly, the development of sunflower germplasm producing oil rich in saturated fatty acids is also a breeding objective if the oil is intended to the production of semisolid fats.

Oleic acid (18:1, n-9) is nowadays considered as the preferred fatty acid for edible purposes, as it combines a hypocholesterolemic effect (Mensink and Katan 1989) with a much greater oxidative stability than polyunsaturated fatty acids. For this reason, selection for mid and high oleic acid contents has been a priority in sunflower (Table 6.5).

The tocopherols are another good example of contrasting breeding goals depending on the intended use of the oil. Selection for high alpha-tocopherol content would enhance the vitamin E value of the oil in human nutrition, but selection for low alpha tocopherol content would result in the accumulation of tocopherols with greater in vitro antioxidant effect and consequently in optimal oil properties for applications requiring high oxidative stability, e.g. deep frying or biolubricants.

A summary of outstanding sunflower germplasm producing seed oil types with modified fatty acid and tocopherol profiles is presented in Table 6.5. In general, genetic modifications altering either the fatty acid or the tocopherol profile have been found to be qualitative rather than quantitative, i.e. they are controlled by a reduced number of genes and they are less affected by environmental factors than quantitative traits such as oil content (Fernández-Martínez et al. 2004). Conversely, total tocopherol content in sunflower seeds is regarded as an oligogenic or polygenic trait, although no genetic studies on this trait have been conducted yet. Preliminary information suggests an important contribution of the genotype to the expression of the character (Alpaslan and Gündüz 2000; Velasco et al. 2002).

The high oleic acid content in sunflower was initially identified as a monogenic trait produced by dominant alleles *Ol* (Urie 1984), although more detailed studies identified several modifying genes affecting the *Ol* gene and producing reversal of the expected dominance (Miller et al. 1987; Fernández-Martínez et al. 1989b; Pérez-Vich et al. 2002b), which has complicated the practical management of the trait in breeding programmes. High palmitic acid content has been found to be controlled by alleles at three independent loci *P1*, *P2*, and *P3* in such a way that the high palmitic acid phenotype is expressed in genotypes that are homozygous recessive at the *P1* locus and either at *P2* or *P3* (Pérez-Vich et al. 1999a). Genetic characterization of high stearic acid content of the sunflower mutant CAS-3 concluded that the trait is controlled by partially-recessive alleles at two loci *Es1* and *Es2* (Pérez-Vich et al. 1999b). A third gene involved in high stearic acid content, *Es3* was identified in the mutant CAS-14. However, genetic recombination of *es3* alleles with *es1* and *es2* alleles from CAS-3 did not result in an increment of the maximum stearic acid content in the seeds compared to the maximum levels produced by the *es3* alleles alone (Pérez-Vich et al. 2006a). Reduced saturated fatty acid content was identified as a partially dominant trait controlled by more than one gene (Vick et al. 2002).

The genetic studies conducted so far have also revealed that the modified tocopherol profiles in sunflower seeds are also controlled by recessive alleles at a reduced number of loci. Demurin et al. (1996) found that recessive alleles at the *Tph1* locus produced mid beta-tocopherol levels, recessive alleles at the *Tph2* locus produced high gamma-tocopherol content, whereas their genetic recombination resulted in increased delta-tocopherol content. Hass et al. (2006) identified a third gene, named *Tph3*, which in combination with *Tph1* and *Tph2* produced a high delta-tocopherol content.

An important feature of the genetic control of the seed oil quality traits is, that in most cases they are determined by the genotype of the developing embryo with small or no maternal influence. The latter is crucial in breeding programs, as a low weight of maternal inheritance allows selection to be carried out at a single-seed level. It is also noteworthy that most of the genetic modifications are recessive, which determines that the modified alleles have to be introgressed in both parents in hybrid seed production (Fernández-Martínez et al. 2004).

6.6.2.8 Seed Meal Quality

Sunflower seed meal is extensively used as a protein supplement in animal feed because of its high protein content of around 40% if the achenes are hulled before oil extraction or around 28% if no hulling occurs (Dorrell and Vick 1997). The principal factors defining sunflower meal quality are a low fibre content, high protein content of good quality, and absence or low presence of minor components with detrimental properties from the nutritional or technological points of view.

Fibre is a heterogeneous chemical entity that includes those carbohydrates that are not truly digested by the animal and therefore do not contribute energy when consumed. High fibre content in the meal reduces its nutritive value and digestibility. It is predominantly associated with the seed hull. Because of the high percentage of hull in sunflower achenes compared to other oilseeds, hulling is a common practice necessary to render a meal with adequate quality for animal feed (Bell 1989).

Sunflower seed meal has a balanced amino acid composition except for lysine, for which it is deficient (Norton 1989). Accordingly, selection to increase lysine content in sunflower seeds is one of the major objectives to improve seed meal nutritive quality. Several studies have reported the existence of variability for lysine content in germplasm of cultivated (Ivanov 1975) and wild sunflower accessions (Christov et al. 1993). Even though selection efforts have been scarce, Ivanov (1975) reported good response to selection for this trait.

Sunflower seeds contain a series of minor compounds that remain in the meal after oil extraction and confer negative properties from the nutritional and/or technological point of view. One of these compounds is phytic acid (myoinositol 1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate), which is present in cereals and oilseeds. Sunflower kernels contain around 2.2% phytic acid, which determines a content around 4.5% in the seed meal (Miller et al. 1986). Phytic acid is a strong chelating agent that can bind metal ions, reducing the availability of calcium, iron, magnesium, zinc and other trace elements (Oberleas et al. 1966). Additionally, phytates form complexes with amino acids, reducing the value of sunflower meal for nonruminant livestock (Erdman 1979).

Sunflower seeds contain significant amounts of phenolic compounds, mainly chlorogenic acid and caffeic acid, which reduce the nutritive value of sunflower meal for animal feed by interacting with amino acids, denaturing proteins, and inhibiting enzymes (Sozulski 1979). Additionally, chlorogenic acid produces a yellow-green coloration following oxidation in sunflower meal, which represents a serious limitation for the use of sunflower meal for human consumption in form of sunflower flour, protein concentrates and protein isolates (Dorrell and Vick 1997). Since phenolic compounds are predominantly present in the seed kernels, hulling scarcely reduces their presence in the meal (Pedrosa et al. 2000). From the evaluation of several lines with contrasting levels of chlorogenic acid in several environments and evaluation of progenies derived from crosses involving them, Dorrell (1974) concluded a significant effect of the genotype on the expression of the trait.

6.6.2.9 Disease Resistance

Most of the pathogens affecting sunflower have only economic impact at a local scale or under specific environmental conditions, but some of them have great relevance and can produce important yield losses if no adequate control measures are adopted. Breeding for resistance is considered the most effective and sustainable means of control.

Both qualitative or vertical and quantitative or horizontal genetic resistance mechanisms have been identified in wild sunflower species and successfully transferred to cultivated strains. Single dominant genes, generally associated with race-specific resistance to important diseases such as sunflower rust (Jan et al. 2004; Jan and Gulya 2006a), downy mildew (Miller and Gulya 1988; Jan and Gulya 2006a) and *Verticillium* wilt (Radi and Gulya 2007) have been identified in wild *Helianthus* germplasm and successfully transferred to cultivated sunflower. A serious problem associated with the use of major gene resistance is the regular appearance of new races of the pathogen that overcome the existing resistance genes, determining the need for identifying additional resistance genes to be introgressed into high yielding cultivars. Since resistance genes are usually identified in germplasm of wild species, the recovery of good agronomic characteristics after the introgression of the new resistance gene is a difficult task. Therefore genetic resistance based on more durable strategies such a combination of both vertical and horizontal resistance mechanisms has been proposed (Vear 2004). Pyramiding of resistance genes has been also proposed as a strategy to develop durable resistance. Tourvieille et al. (2004) compared different methods to enhance durable resistance to downy mildew, reporting that gene pyramids were less effective in reducing the appearance of new races compared to other control methods such as the use of combinations of resistance *Pl* genes, by alternation or in "multi-hybrids".

The situation has been different for diseases produced by *Sclerotinia sclerotiorum* (wilt, stalk rot, head rot), which cause the greatest losses to sunflower on a global basis. The causal agent is a polyphagous fungus that attacks many different species, including sunflower, where infection affects the root, stem, head, and seeds (Gulya et al. 1997). In this case, resistance is expected to be complex and controlled polygenically (Jan and Seiler 2007). Genetic resistance to Phomopsis stem canker (Vrânceanu et al. 1992) and *Alternaria* leaf blight (Morris et al. 1983) have been postulated to be oligogenic.

Virus diseases are not currently a major concern in global sunflower production. More than 30 different viruses have been identified on sunflower, but only a strain of tobacco streak virus has been reported as a serious disease of sunflower in all major sunflower-growing regions of India (Ravi et al. 2001). Although resistance to this virus has not been reported yet, resistance to other viruses such as the sunflower mosaic potyvirus (Jan and Gulya 2006b) and the sunflower chlorotic mottle virus (Lenardon et al. 2005) have been identified. The latter was found to be controlled by a single dominant gene.

6.6.2.10 Broomrape Resistance

Sunflower broomrape (*Orobanche cumana* Wallr.) is a weedy parasitic angiosperm that represents nowadays a serious constrain for sunflower production in Southern Europe and in the Black Sea region. Although the use

of herbicides is being considered as a promising control measure, genetic resistance offers the most effective and feasible control against *O. cumana*. However, the introduction of new resistance has been frequently followed by the appearance of new pathogenic races overcoming the resistance and there is a continuous need for new sources of resistance. Vrânceanu et al. (1980) identified five pathogenic races, named A through E, with a set of sunflower differentials carrying the dominant resistance genes *Or1* through *Or5*, respectively that provide an accumulative resistance to broomrape races. New virulent races overcoming resistance gene *Or5* have been identified in Spain (Alonso et al. 1996; Molinero-Ruiz and Melero-Vara 2005), Romania (Păcureanu et al. 2004), Bulgaria (Shindrova 2006), and Turkey (Kaya et al. 2004).

Resistance to races A through E has been found in most studies to be under the control of dominant alleles at single loci (Vrânceanu et al. 1980; Sukno et al. 1999), although two dominant genes (Domínguez 1996) and one recessive gene (Ramaiah 1987) have also been reported. Genetic resistance to Spanish race F in germplasm derived from wild *Helianthus* spp. has been found to be controlled by a single dominant gene *Or6* (Pérez-Vich et al. 2002a), which exhibited incomplete dominance in some environments due to the effect of a second gene *Or7* (Velasco et al. 2006), whereas resistance in germplasm of cultivated sunflower was controlled by two recessive genes (Akhtouch et al. 2002). A single dominant gene is involved in the resistance to race F in germplasm developed in Romania (Păcureanu et al. 2004).

6.6.2.11 Insect Resistance

Insects do not represent a serious threat to sunflower production except in North America, where insect pests of sunflower coevolved with their native hosts (Jan and Seiler 2007). Nowadays, the principal strategy in search for resistance to sunflower infesting insects is the evaluation of wild *Helianthus* spp. and cultivated sunflower germplasm.

6.6.2.12 Resistance to Bird Depredation

Bird damage causes serious losses in sunflower production, especially during the first three weeks of seed maturation (Cummings et al. 1989). Breeding for some morphological traits contributes to confer resistance to bird predation. These traits include long involucral bracts, horizontally oriented heads facing downwards, concave heads, and long head-to-stem distances (Gross and Hanzel 1991).

6.6.2.13 Resistance to Abiotic Stresses

Sunflower is grown in areas prone to several types of environmental stresses, from drought to waterlogging, from heat to frost injury, or from poor soils to

excess salinity. The development of cultivars adapted to such conditions is critical. But adaptation not only implies the ability to survive under stress, but the plants must be able to maintain adequate yields.

Several strategies have been followed to select for drought resistance in sunflower. Avoidance of high evaporative demand periods has been advocated as a strategy to increase productivity in areas prone to drought. In southern Spain, the shift in sunflower planting dates from spring to winter resulted in a substantial yield increase (Gimeno et al. 1989). Selection for early vigour has been proposed as a means for improvement of sunflower yield under water-limited environments (Agüera et al. 1998). There is also evidence that improved osmotic adjustment capacity is a trait that contributes to yield maintenance under drought conditions in sunflower (Chimenti et al. 2002). Some wild *Helianthus* spp., especially *H. argophyllus*, have been suggested as a source of useful traits to improve water use efficiency such as higher stomatal densities and leaf pubescence, which reduce transpiration rates (Blanchet and Gelfi 1980).

6.6.2.14 Herbicide Resistance

The acreage of transgenic varieties of several major crops carrying genes for resistance to herbicides have been steadily increasing in recent years in many countries. In sunflower, resistance to imidazolinone and sulfonylurea herbicides was identified in weedy populations of *H. annuus* (Al-Khatib et al. 1998). The trait has been transferred to cultivated sunflower germplasm (Miller et al. 2006a). Herbicide resistance in sunflower is mainly controlled by one gene exhibiting partial dominance and also affected by a second gene in some genetic backgrounds (Bruniard and Miller 2001). Resistance to herbicides in sunflower is also being used to control broomrape (Domínguez et al. 2004).

6.6.2.15 Nonoilseed Sunflower

Nonoilseed sunflower seeds are used as snack food, in the confectionery industry, and for feeding birds and small pets. The seeds of nonoilseed cultivars have lower seed oil content than oilseed cultivars. Breeding goals in relation to nonoilseed sunflower largely depend on the requirements of specific markets. For example large achenes are preferred for uses as snack food, but small achenes are preferred for birdseed. In some types of soils, sunflower accumulates excessive concentrations of cadmium in the seed kernels, which has became a problem for confectionery sunflower. This has encouraged breeding for reduced cadmium uptake into kernels (Miller et al. 2006b).

6.7 Breeding Methods and Techniques

6.7.1 Breeding Methods

There are comprehensive reviews of breeding methods and techniques in sunflower (Škorić 1988; Miller 1987; Fick and Miller 1997). Four important features have to be considered in breeding sunflower: (a) obtaining or developing sources of genetic variability, (b) improving source populations, (c) improving open pollinated cultivars, and (d) developing lines or parents for producing hybrid cultivars.

6.7.1.1 Obtaining or Generating Sources of Genetic Variability

The Use of Existing Genetic Variation

Many sources of genetic variability from cultivated germplasm and wild species are available in germplasm collections to be used in breeding programs. For example, open-pollinated varieties with high oil content developed in the former USSR were the base for the initial commercial production of oilseed sunflower in western countries in the 1960s (Putt 1997). Evaluation of cultivated germplasm has been extensively used to identify useful variation for many different traits such as disease resistance (Škorić 1992) or oil quality (Demurin 2003). However, wild species represent the most diverse source of genetic variability in sunflower breeding. Genes for disease and insect resistance, oil and protein content and quality, cytoplasmic male sterility, or agronomic and physiological traits have been identified in wild *Helianthus* species and transferred into cultivated lines. The main problem using wild species as sources of variability is that many of them do not cross readily with the cultivated sunflower.

Transferring genes from wild annual species into cultivated lines can be accomplished rather easily with conventional crossing and backcrossing. For example, genes conferring resistance to several diseases such as rust and downy mildew have been transferred from *H. debilis* or wild *H. annuus* to cultivated sunflower (Quresh et al. 1993; Tan et al. 1992). Conversely, crossing perennial *Helianthus* species with the cultivated sunflower is generally difficult due to early hybrid embryo abortion as well as high levels of sterility in the F_1 or BC F_1 generations (Jan 1997). These problems can be avoided with the utilization of the embryo culture technique (Chandler and Beard 1983) and subsequent chromosome doubling of the F_1 (Jan 1988). Using these techniques, amphiploids of the wild species *H. gracilentus, H. hirsutus*, *H. strumosus*, *H. maximiliani*, *H. nuttallii* and *H. grosseserratus* have been produced and used as a bridge to transfer resistance to broomrape race F (Jan and Fernández-Martínez 2002). Positive results of transfer of rust resistance genes from *H. hirsutus* and fertility restoration genes for a CMS cytoplasm derived from *H. giganteus* have also been achieved using interspecific amphiploids (Jan and Zhang 1995; Jan 2004).

Mutagenesis

Mutagenesis has been used successfully to generate genetic variability for useful traits for the improvement of sunflower. This method is especially important if variation for a given trait is not found in germplasm collections. Useful mutants with short plant height, high oil and protein content and low hull content have been obtained in sunflower using both irradiation and chemical treatments (Voskoboinik and Soldatov 1974; Leclercq 1985; Fernández-Martínez and Domínguez 1988). Other useful variability has been obtained for rust resistance (Lofgren and Rama Raje Urs 1982) and herbicide resistance (Bervillé et al. 1992). Jan and Rutger (1988) used streptomycin and mitomycin C to produce 22 cytoplasmic and 7 nuclear male sterile mutants.

Mutagenesis results in sunflower have been particularly successful in modifying seed oil quality traits. One of the most valuable mutants produced is the high oleic acid mutant (>80%), produced at the All-Union Research Institute of Oil Crops of the former USSR, after treatment with dimethyl sulfate (Soldatov 1976). High levels of either palmitic or stearic acid (>25%) were achieved using chemical and physical mutagens (Ivanov et al. 1988; Osorio et al. 1995). Mutants with increased levels of gamma tocopherol (>95%) have been isolated following chemical mutagenesis (Velasco et al. 2004b). The mutagenic treatment is usually applied to the seeds, which after treatment are named M_1 seeds. Mutants can be detected in the M_2 generation. For fatty acid and tocopherol profile, which are mainly under embryogenic control, mutants are detected analyzing M_2 half-seeds.

6.7.1.2 Methods for Improving Source Populations

The success of isolating inbred lines with good combining ability or other desired characters by the standard procedure of inbreeding and selection depends on the frequency of superior S_0 plants in the source populations. Recurrent selection is an effective method for increasing this frequency. Sunflower breeders have used several intrapopulation and interpopulation recurrent selection methods to develop improved source populations. Most relevant has been the use of the Pustovoit's Method of Reserves described below, which is a modified method of recurrent selection extensively used for developing open-pollinated cultivars.

Two types of recurrent selection have been considered in sunflower, phenotypic recurrent selection, in which the phenotype of the S_0 plant is the base of selection, and genotypic recurrent selection, in which some type of progeny test forms the base of selection. Phenotypic recurrent selection has been used by sunflower breeders to improve populations for several traits, including yield, oil percentage and disease resistance (Fick 1978; Miller 1987). For hybrid breeding, female (B) and restorer (R) populations are generally managed separately to simplify the development of inbred lines. The initial populations are often a combination of high-performing inbred lines or a composite of lines from

one or several open-pollinated cultivars (Miller 1987). The parents selected to form the initial population are crossed to produce a random-mated population. Random-mating is accomplished by emasculating, either by hand or using gibberellic acid, plants of each line and pollinating them with pollen gathered randomly and equally from other lines. The source material or initial C_0 material is grown and individual plants are selected and selfed. In the following generation, the progenies of selected plants are sown in a separate row. The progenies (rows) are crossed in all possible combinations and the resulting hybrid seeds are bulked to form the C_1 population. This completes the first cycle of selection. The C_1 plants from the first cycle are used for the second cycle of selection. Phenotypic recurrent selection has been successfully used in sunflower for different traits, for example, self-incompatibility (Kinman 1970), resistance to *Sclerotinia* stalk and head rot (Vear and Tourvieille 1984), seed yield (Gundaev 1971), and oil content (Luduena et al. 1992).

Genotypic recurrent selection methods 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 (Fernández-Martínez et al. 1990). The S_1 progeny recurrent selection method can also be used to develop improved open-pollinated cultivars (Miller 1987). With this method, several hundreds of individual S_0 plants of the initial source C_0 population are selected 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. The recombination generation may be obtained in a greenhouse or winter nursery, which permits the completion the three generations of a cycle of selection in 2 years. By using a winter greenhouse nursery, spring evaluation, and recombination as a second crop in the fall, Fernández-Martínez et al. (1990) were able to complete one cycle per year in southern Spain.

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 selfed and simultaneously crossed with a tester the first year. These two operations can be done in single-headed maintainer plants by self-pollinating half of the head and emasculating and crossing the other half. In the case of multi-headed restorer plants, the main head is emasculated and crossed, whereas some secondary heads are selfed (Fick and Miller 1997). The type of tester used depends on the objectives of selection. For example, in selection for general combining ability (GCA) a broad base heterogeneous unrelated population is used as tester. There are not many reports on half-sib recurrent selection in sunflower breeding. Fick (1978) reported an increase of 3.5% in seed yield after one cycle of recurrent selection in an R-line population using testcross evaluation.

Interpopulation recurrent selection methods have also been used in sunflower. The reciprocal full-sib selection method, based in the production of hybrid (full-sib) progenies and selfed seed in the same plant, was adapted for

sunflower using a multi-headed restorer (R) and a single-headed maintainer (B) population (Miller and Fick 1978). The method is initiated by forming the R and B populations by random mating diverse collections of R and B lines to create the C_0 populations. Full-sib progenies are obtained by transferring pollen from a selected plant in the B line population to the emasculated main head of a plant in the R line population. Self-pollinated seed is obtained by self-pollinating secondary heads of the R line plant. The B line plant selected is also self-pollinated. Full-sib hybrids are tested the following year in replicated yield trials. On the basis of these evaluations, the self-pollinated S_1 seed from the best full-sib hybrids are bulked within each population and randomly mated in a greenhouse or winter nursery to form the C_1 populations to be used to start a new cycle of selection. A cycle of selection is usually accomplished in 2 years (Miller 1987), or even in 1 year (Fernández-Martínez et al. 1989a). Miller and Hammond (1985) reported a 6.3% increase in yield per cycle using this scheme of selection. Fernández-Martínez et al. (1989a) used the reciprocal full-sib selection method to maximize seed yield under environments prone to drought, with evaluations conducted under both irrigated and rainfed conditions.

6.7.1.3 Methods for Improving Open Pollinated Cultivars

Mass Selection

Mass selection implies the selection of individual plants from a population on the basis of their phenotype for the improvement of a cultivar or population for some specific traits. Seeds of the selected plants are mixed and planted the next generation in order to obtain new cultivars or to maintain the varietal purity of existing cultivars. Two methods of mass selection have been used in sunflower: phenotypic mass selection and family selection. Phenotypic mass selection was commonly used for improvement of sunflower during the early stages of cultivar development in the former USSR. Several important cultivars were developed using this procedure (Gundaev 1971).

The family selection method is a form of mass selection that involves selection of individual S_0 open-pollinated plants and classification of those plants for characteristics of interest. Each plant is harvested and its seed is bulked with other plants of phenotypically similar families. The various bulks are planted in isolation for cross pollination. Gundaev (1971) listed many cultivars developed by this method. Mass selection has also been used in Argentina (Luciano and Davreux 1967) and Mexico (Robles 1982) to produce improved sunflower cultivars. Mass selection is a simple and economical method of selection but its effectiveness depends on the heritability of the traits. In general, this method has not been effective in sunflower breeding for traits with low heritability such as seed yield, but it has been effective for improving other traits including earliness, oil content or insect and disease resistance (Morozov 1947; Vrânceanu 1974).

Head to Row Selection (Pustovoit's Method of Reserves)

The "method of reserves" was the most widely and successfully used method to develop open pollinated sunflower cultivars. It was developed in the former USSR by V.S. Pustovoit during the 1920s (Pustovoit 1967). The method is a form of recurrent half-sib selection that includes progeny testing and subsequent cross pollination among superior progenies (Alexander 1963). The "method of reserves", as outlined by Pustovoit (1967) is initiated by forming a heterogeneous population of germplasm including elite cultivars, intercross hybrids, and world collection entries. About 10,000–15,000 plants are selected from this population with the main criterion that plants have 500–2,000 seeds per head. The seeds are analyzed for hull and oil percentage and around 1,200–1,500 heads are selected for progeny evaluation. Half-sib seeds of these S_0 selections are evaluated for agronomic, disease resistance, and seed quality traits in single row plots with two replications. A check consisting of the best existing cultivars is included in every third plot as a control. On the basis of the observations from the first year of testing, 15–20% of the S_0 selections are planted in a second-year observation nursery. Remnant half-sib seed produced in season 1 is utilized also to plant this nursery and to test for disease resistance.

Based on performance in the first and second year nurseries, the original seed of the best 20–50 S_0 plants selected in season 1 are planted in a cross pollination nursery for random mating using an isolation distance of 200–300 m between nurseries. Undesirable phenotypes including disease infected, extremely tall or branched plants are removed. Seeds from random-mated plants are analyzed for oil content and the seeds of selected plants are bulked for a new cycle of selection or for testing at a larger scale. After three years of testing, superior populations are released as new open-pollinated cultivars. The "method of reserves" has been especially useful in increasing oil content and oil yield per hectare, developing early maturity, and resistance to diseases, *Orobanche*, and sunflower moth (Gundaev 1971). In the former USSR, cultivars developed by this method were grown in 4.6 million hectares in 1973 (Pustovoit and Gubin 1974), and it was successfully used in other countries such as Romania (Vrânceanu 1974) and former Yugoslavia (Škorić 1988). However, in spite of its success, the genetic gain per year in comparison with other recurrent selection methods is limited due to the number of years used for evaluation at each cycle of selection.

6.7.1.4 Methods for Improving Hybrid Cultivars

Inbreeding for improving sunflower was used by early Russian and Canadian breeders to isolate uniform strains with variation for different traits such as plant height, oil content, disease, and pest resistance (Voskoboinik and Soldatov 1974; Unrau 1947). However, the main value of inbreeding was to develop true breeding lines with desirable characteristics to be used in the production of

synthetic cultivars or hybrids. The first results involving hybridization of inbred lines showed significant heterosis for seed yield and other traits (Unrau 1947; Putt 1962).

The most common method for developing new inbred lines in sunflower is pedigree selection, but bulk selection and single seed descent have also been used. Backcrossing is frequently used for modifying existing lines. The germplasm sources are derived from open-pollinated source materials, populations improved by recurrent selection, or from planned crosses between inbred lines (Miller 1987).

Pedigree selection involves self-pollination of phenotypically desirable plants in the F_2 or S_0 generation, depending on the starting material. Selection is practiced for agronomic type, disease resistance, or other desired traits. The F_3 progeny of each F_2 plant is grown the next season. Plants are selected and self-pollinated within the best $F_{2:3}$ lines. The process of inbreeding and selection is continued for five generations. In the F_3 generation, pollen from selected plants within the $F_{3:4}$ lines may be used to cross to tester lines to produce testcross seed. The hybrid testcrosses are evaluated and $F_{4:5}$ lines with superior combining ability can be selected. In the case of B lines, pollen can be collected to cross to a CMS source to begin the conversion of the F_4-derived B line to CMS (A line) by the backcross method. This process generally requires five backcrosses using the F_4-derived line as recurrent parent.

The development of lines by the backcross method is probably used more frequently than any other method, except pedigree selection. Backcrossing is used in the context of transferring a trait from one genotype (donor parent) to a desirable genotype (recurrent parent). The trait being transferred is usually simply inherited. Backcrossing is usually a correctional breeding method that is used to enhance the performance of an elite inbred line, but it is also used to introgress a specific gene in an elite inbred line. If the backcrossed-derived line must be essentially identical to the recurrent parent, about six backcrosses must be made. The complexity of the genetic transference of a given trait to a recurrent parent depends on the number of genes involved, their dominant or recessive nature, and the presence or absence of maternal effects. Examples for dominant traits controlled by one gene involve the transfer of the high oleic trait to the sunflower lines HA 89 and RHA 271 (Fernández-Martínez et al. 1993) and the resistance of downy mildew to HA 89 (Miller and Gulya 1988). These traits can be easily transferred because plants or seeds with the desired trait can be identified in the F_1 backcross generation. However, in many cases the target traits are recessive and do not show up in the F_1 generation, which increases the duration of the backcross generation. Such a limitation can be overcome by using marker-assisted selection, which allows the recessive genes to be identified in the F_1 backcross generation.

The final value of an inbred line is determined by testing general or specific combining ability in hybrid combinations. For general combining ability, the choice of the tester (homogeneous inbred lines vs. heterogeneous sources) depends on the specific program objectives. The most common testers are

inbred lines that are being used in commercial hybrids. Miller et al. (1980) and Domínguez and Fernández-Martínez (1987) found that inbred lines are effective in identifying lines with the best combining ability. Evaluation for combining ability often begins at the S_3 or S_4 generation, but a system of early generation testing beginning after the first generation of selfing was reported to be effective in identifying lines with good combining ability (Shein 1978).

Inbred lines are used primarily in the production of single crosses or three-way sunflower hybrids using the cytoplasmic male sterility and the fertility restoration system. Single-cross hybrids are produced by crossing a male-sterile female (A line) with a male-fertile restorer (R line). A three-way hybrid is made by crossing an A line with an unrelated maintainer line (B line) to produce a male-sterile single-cross hybrid. This hybrid is crossed with an R line to produce a male-fertile, three-way hybrid. Generally, single crosses are higher yielding than three-way hybrids and have greater uniformity (Fick and Zimmer 1976; Miller 1987). Three-way hybrids are produced primarily to reduce seed cost, since seed yield of single-cross female parents is often 1.5–2.0 times greater than that of inbred lines, although inbred lines that yield up to 80% of their hybrids have been developed (Fick 1978; Škorić 1988). Three-way hybrids are considered more stable over environments than single crosses due to their greater heterogeneity (Fick and Zimmer 1976; Schuster and Friedt 1988). The use of double-cross hybrids in sunflower to further improve adaptation and yield stability has also been suggested (Vulpe 1974).

Inbred lines have also been used to produce synthetic varieties in Canada (Putt 1966) and in the former USSR (Voskoboinik and Soldatov 1974). Putt (1966) concluded that high-yielding synthetics could be developed from as few as three to five inbred lines. Synthetic cultivars have been evaluated with favourable results in countries where hybrid production is not practical, for example Nigeria (Ado et al. 1991) and Egypt (Shabana 1990).

6.7.1.5 Methods for Producing Hybrid Seed

Efficient and economical production of hybrid seed at a commercial scale was greatly facilitated by the discoveries of genetic and cytoplasmic male sterility (Leclercq 1966, 1969). Nuclear male sterility was used to produce commercial hybrid seed in the early 1970s taking advantage of a close linkage between genes for male sterility and anthocyanin pigmentation of the plants, which allows the identification and removal of male-fertile plants (Leclercq 1966; Vrânceanu 1974). Detailed methods of hybrid seed production using this system have been described by Vrânceanu (1974) and Škorić (1988). However, the use of nuclear male sterility to produce hybrid seed has been replaced by the cytoplasmic male sterility and fertility restoration system and, although new sources of cytoplasmic male sterility have been found, the source discovered by Leclercq (1969) is used almost exclusively in current hybrid seed production programs. This system requires a cytoplasmic male-sterile line (A line), which is maintained by crossing to a genetically identical male-fertile line with a fertile

cytoplasm (B line), and a restorer line (R line) which combines well with the A line and restores the fertility in the hybrid cultivar. The seed harvested from the A line is grown commercially as a hybrid cultivar.

6.7.2 Breeding Techniques

6.7.2.1 Procedures for Selfing and Artificial Hybridization

In order to carry out selfing and controlled crosses, sunflower heads must be isolated from insect pollination. Paper and cloth bags are most commonly used. Paper bags cost less and may be satisfactory in some environments if excessive rain does not occur during the latter part of the growing season, but cloth bags are more desirable for the standpoint of seed set and durability (Vrânceanu 1974; Roath and Pomeroy 1988). Artificial hybrids are produced by emasculation of the female parent followed by pollination with pollen of the desired male parent. Hand emasculation is commonly used, but chemical emasculation has also been extensively used. Hand emasculation involves removing the anthers of the disk flowers with forceps early in the morning before the anthers have dehisced and before the stigmas have elongated up through the anther tubes. A large head will flower over a five to six-day period with three to six rings of florets flowering each day. Stigmas will be fully elongated and receptive 1–2 h after emasculation and will remain receptive 4–5 days. Chemical emasculation with gibberellic acid (GA_3) is utilized by sunflower breeders to produce hybrid seed. A 50 to 100-ppm concentration of GA_3 is applied to sunflower buds of approximately 1–1.5 cm diameter with generally good results (Miller and Fick 1978). However, cultivars and inbred lines may show different responses to GA_3 requiring higher or lower concentrations and earlier or later application times (Piquemal 1970). Various negative effects such as incomplete male sterility, reduced female sterility, and stem elongation have been associated with the use of GA_3, depending on concentration and timing of application (Miller 1987).

Pollination can be accomplished by several methods. If the male plant is located adjacent to the female parent and is shedding pollen, the receptacles can be rubbed together to transfer the pollen from the anthers of the male to the stigmas of the female plant. However, the most common technique is to collect pollen from the male parent in paper bags or on a cloth or leaf from heads isolated with bags prior to flowering. Pollen in paper bags can be stored up to 4 weeks in a refrigerator at a temperature of 4–6°C and relative humidity of less than 4%, and several years at –76°C (Frank et al. 1982).

6.7.2.2 Techniques Used for Interspecific Hybridization

Direct crosses of annual wild species, except *H. agrestis* with cultivated sunflower are possible using conventional methods. However, crossing perennial

species, including diploids, tetraploids and hexaploids, is much more difficult and also produces sterile F_1 plants, requiring special techniques (Jan 1997). The two-step embryo culture procedure developed by Chandler and Beard (1983) avoids embryo abortion and seed dormancy and facilitates interspecific hybridization. With this technique, embryos are excised and cultured 3–7 days after pollination. The embryos are cultured in Petri dishes on an appropriate solid growth medium (Chandler and Beard 1983). For embryo germination and seedling growth, the cultured enlarged embryos are transferred after 1–2 weeks to a liquid germination medium in test tubes. An optimized method for culturing difficult hybrid embryos derived from perennial *Helianthus* species was proposed by Jan (1988), and additional modifications have been suggested (Kräuter et al. 1991).

Another technique to facilitate interspecific transfer is the use of induced polyploidy by chromosome doubling using colchicine applied to apical meristems. Jan (1988) described a modified colchicine chromosome doubling technique with a significant positive effect on backcross seed set. The apical meristems are submerged in a 1.5 g/kg colchicine solution with 2.0 g/kg DMSO (dimethyl sulfoxide) for 5 h in the dark. Then, chromosome doubling of each head is verified by pollen grain size and stainability.

6.7.2.3 Field Plot Techniques for Cultivar Evaluation

Newly developed hybrids or open pollinated populations must be evaluated before they can be recommended for releasing. Detailed procedures of field testing sunflower genotypes have been described by Miller (1987), Fick and Miller (1997), and Vrânceanu (1974). The testing process largely depends on the genetic control of the traits. For qualitative traits controlled by major genes and scarcely influenced by the environment, limited evaluations in the greenhouse or field may be adequate. For quantitative traits, such as yield or oil percentage, lines or cultivars must be tested over years and locations. A common plot size includes two to five rows from 6 to 10 m length spaced 0.75 m. Usually test plots are overplanted and thinned to a uniform stand soon after emergence. Preliminary yield evaluations are usually planted in plots with one or two unbordered rows with two to three replications at one or more locations using a simple lattice or augmented design. Check cultivars are sown every five to ten rows in the augmented design and randomly in the lattice design. Advanced yield trials are planted in plots with two to five rows with three to four replications at several locations. In the case of three to five rows per plot, only the center rows are harvested. Significant interplot competition effects may occur between cultivars showing wide differences in height and maturity (Domínguez et al. 1980). In these cases, the effect of interplot competition can be reduced by grouping cultivars by height and maturity. The most common experimental designs for advanced yield trials are randomized complete block design and simple lattice, the latter used when the number of entries exceeds 36.

6.7.2.4 Techniques Used for Greenhouses and Off-Season Nurseries

Greenhouse and off-season nurseries are frequently used by sunflower breeders to grow several generations per year and speed up the breeding program. Postharvest dormancy is a frequent problem in greenhouse and off-season plantings. Fick (1978) reported a technique to overcome dormancy consisting in soaking the seeds in a concentration of 0.6 mL ethrel/L of water for 16 h. Treatments with grow retardants to reduce the height of plants grown in the greenhouse and produce plants with short, thick stems that are easy to manage have been used for winter planting in the greenhouse (Fick and Miller 1997). Another technique that may have value in programs requiring short generation time is the application of growth regulators as desiccants (Rana et al. 1990).

6.7.2.5 Laboratory Techniques for Seed Quality Evaluation

Breeding programmes to improve seed oil quality traits require the availability of adequate screening techniques to measure them. In sunflower, both the fatty acid and the tocopherol profile of the seeds are mainly under gametophytic control, i.e. they are governed by the genotype of the developing embryo. Accordingly, selection for these oil quality traits can be conducted at the single-seed level. Nondestructive methods to measure these traits in single seeds have been developed. Downey and Harvey (1963) developed the half-seed technique for nondestructive analysis of the fatty acid composition of single seeds of rapeseed (*Brassica napus* L.). The technique has been adapted to sunflower (Conte et al. 1989). It consists of the removal of a small seed portion in the region extremely distal to the embryo in a way that the germination capacity of the seed is not affected. The excised half seed is used for chemical analysis whereas the other half seed containing the embryo can be sown to produce a viable plant. The half-seed technique has been used for the nondestructive analysis of fatty acid composition (Conte et al. 1989), tocopherol composition (Demurin et al. 1996) and total tocopherol content (Velasco et al. 2004b).

The use of near infrared reflectance spectroscopy (NIRS) has facilitated the screening for seed quality traits in sunflower. NIRS is a fast, nondestructive and cost-effective technique that permits the simultaneous analysis of multiple constituents in a single measurement. This requires the previous development of individual calibration equations for every constituent to transform NIRS spectral data into chemical information. NIRS was first applied to determine oil content in sunflower meal samples (Robertson and Windham 1981). The technique has been also used to analyse protein content, fiber content (Kaffka et al. 1982), tocopherol content, phytosterol content (Gotor et al. 2007), and free fatty acid content (Moschner and Biskupek-Korell 2006) in sunflower meal. However, the application of NIRS to sunflower breeding requires the use of small samples of intact achenes. Sato et al. (1995) used NIRS for the analysis of oil content in hulled sunflower seeds. Pérez-Vich et al. (1998) combined the simultaneous analysis of seed oil content and fatty acid profile in both intact and hulled sunflower kernels.

Selection for seed quality at a single seed level has been facilitated by the use of near-infrared spectroscopy (NIRS) for analyzing the fatty acid profile of intact or hulled individual kernels. Sato et al. (1995) demonstrated the feasibility of NIRS for measuring the concentration of linoleic acid in the oil of single hulled kernels of sunflower. Velasco et al. (1999) reported that NIRS permitted the discrimination of intact achenes for oleic and linoleic acid concentration in the seed oil. Velasco et al. (2004c) used NIRS for large-scale screening for high stearic acid concentration in hulled sunflower seeds.

6.7.2.6 Techniques for Disease Resistance and Broomrape Evaluation

Breeding for disease resistance requires the creation of a disease environment to differentiate between resistant and susceptible plants. Breeders and plant pathologists have developed effective procedures to evaluate breeding materials for most of the major sunflower diseases. These techniques have been described in detail by Škorić (1988), Gulya et al. (1997) and Fick and Miller (1997).

Downy mildew evaluations are sometimes conducted in lands naturally infected with the pathogen, but more often under controlled conditions in greenhouse or growth chamber. The procedure described by Zimmer (1974) is highly effective and is extensively used in breeding programs. It consists of the inoculation of germinated hulled seeds, with radicals 10–20 mm long, with a suspension of 10,000 or more zoosporangia per ml of distilled water for 18 h at 20°C. Then, the inoculated seeds are planted in sterile soil and maintained on greenhouse benches during 14 days at 20–25°C and 16 h day length photoperiod. Susceptibility is indicated by the occurrence of sporulation of the fungus on the cotyledons or the under-surface of the first true leaves after 18 h in a saturated humidity chamber. A PCR test has been developed to detect the presence of the pathogen in sunflower seeds (Ioos et al. 2007).

Researchers working with sunflower rust adopted much of the methodology developed by cereal rust pathologists. Techniques for spore collection and storage and inoculation under greenhouse and field conditions were reviewed by Gulya and Marisevic (1996). Greenhouse evaluations for sunflower rust are conducted on seedlings after inoculating with 10:1 mixture of talc and urediospores. Field evaluations are conducted by spraying 3 to 4-week-old susceptible border row plants with water suspension of urediospores collected from commercial fields the preceding season. Plants are sprayed in the evening when temperatures are lower and then covered overnight with metal or plastic containers to provide a high level of humidity. Infection of susceptible plants provides natural inoculum and subsequent spread of rust throughout the nursery allowing the selection of resistant plants (Fick 1978).

The most common procedure of evaluation for resistance to *Sclerotinia* stalk and head rot is field testing in naturally infected fields (Gulya et al. 1997), but the results depend on soil and environmental factors. Several field and greenhouse methods have been developed. They involve adding sclerotia or mycelia-infested cereal grains to the soil at planting time or directly to the basal stem to

increase the level of infection (Pirvu et al. 1985; Rashid 1992) or immersing the roots of 3-week-old plants in a *Sclerotinia* culture filtrate (Huang and Dorrell 1978). For head rot resistance, an effective procedure involving spraying an ascospore suspension onto the florets and covering the head with a paper bag was developed (Tourvieille et al. 1978).

Evaluations for Phomopsis stem canker caused by *Diaporthe helianthi* are best conducted in fields under intensive natural infection (Fick and Miller 1997). In the absence of high levels of natural infection, several artificial inoculation methods have been described consisting of placing mycelial explants on mature leaves, stem or petioles (Tourvieille et al. 1988), spraying ascospores on the leaves (Marisevic and Gulya 1992), or artificially infecting field plots by placing contaminated stalk segments in the field followed by sprinkler irrigation (Griveau et al. 1992).

Effective evaluation methods have also been developed for screening for resistance to other sunflower diseases such as *Verticillium* wilt, *Alternaria*, *Phoma* black stem and *Rhizopus* root rot. Field evaluation methods in naturally infested plots and artificial inoculation procedures have been reviewed in detail by Škorić (1988) and Gulya et al. (1997). Rani and Ravikumar (2007) suggested a combination of gametophytic and conventional sporophytic selection to improve selection efficiency for partial resistance to *Alternaria* leaf blight.

Evaluations for *Orobanche* resistance can be conducted in naturally infested fields, but more frequently breeders use artificial inoculation with seeds of the parasite collected in previous years (Škorić 1988). These evaluations are conducted in artificially infected fields or in pots in greenhouses and growth chambers. A soil mixture (sand: silt, 1:1) is homogeneously infested with broomrape seeds adding 250 mg of seeds per kg of soil (Panchenco 1975). Sunflower seedlings are planted in peat pots with the inoculated soil mixture and incubated in a growth chamber under controlled conditions of light and temperature during 15–20 days and then transplanted to pots in the greenhouse or to the field (Škorić 1988). Resistance or susceptibility is determined by the percentage of sunflower plants that are parasitized and the average number of broomrape plants per sunflower plant. Special procedures have been developed for non-destructive in situ monitoring the developmental stages of the parasite and its interaction with sunflower (Eizenberg et al. 2005).

6.8 Integration of New Biotechnologies into Breeding Programs

Despite the tremendous economic significance of sunflower, initial molecular research on this crop was considerably delayed in comparison to other crops of similar or even lower importance. In fact, the first reports on molecular markers development in sunflower emerged nearly a decade after the initial studies of restriction fragment length polymorphism (RFLP) mapping in plants. However, molecular research in sunflower has been considerably stimulated in recent years by significant contributions in the construction of saturated

genetic linkage maps, mapping and characterization of genes controlling important traits, and understanding its genetic make up. Conversely, transgenic approaches have been scarcely afforded. There is still a huge amount of innovative research to be conducted in sunflower, but many tools are being continuously developed and are available to sunflower breeders.

6.8.1 Genetic Markers and Genetic Linkage Maps in Sunflower

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. The latter have evolved from hybridization-based detection to polymerase chain reaction (PCR) amplification and, most recently to sequence-based systems. Both morphological and isozyme markers are limited in number. Additionally, the morphological markers are affected by the environment, and a given marker can affect other morphological traits because of pleiotropic gene action. Consequently, genome-wide analysis is not feasible using only morphological and isozyme markers. DNA markers are typically derived from a small region of DNA that shows sequence polymorphism between individuals within a species, and may be classified into random DNA markers (also known as anonymous or neutral markers), gene-targeted markers (also known as candidate gene markers) and functional markers. Random DNA markers are derived at random from polymorphic sites along the genome, whereas gene-targeted markers are derived from polymorphisms within genes. Finally, functional markers are derived from polymorphic sites within genes causally associated with phenotypic trait variation. In this section, we will give an overview of DNA marker development and mapping in sunflower. Functional genetic linkage maps created for mapping phenotypic and quantitative trait loci will be preferentially described in a later section. Linkage group nomenclature that will be used is that of the reference public map of Tang et al. (2002).

6.8.1.1 Random DNA Markers and Maps Based on them

Restriction Fragment Length Polymorphism (RFLP) Markers

In sunflower, the first DNA-based markers developed were RFLP markers. Most of the published sunflower RFLP markers were developed with anonymous cDNA clones, which yield low copy, polymorphic restriction fragments (Berry et al. 1994; Gentzbittel et al. 1994; Jan et al. 1993). RFLP markers were initially mapped in cultivated sunflower by different research groups, and genetic maps based on them were reported. The maps by Berry et al. (1995) and Jan et al. (1998) were based on individual F_2 populations, whereas those of Gentzbittel et al. (1995, 1999) and Berry et al. (1996) were composite maps based on data of different mapping populations (Table 6.6). In general, these

Table 6.6 Characteristics of sunflower linkage genetic maps ordered according to marker system and date of publication

Reference	Mapping populations	Population type	No. of individuals	Markers	No. of mapped loci	No. of Linkage groups	Map length (cM)	Average interval size (cM)
Berry et al. (1995)	HA89 x ZENB8	One F_2	289	RFLP	234	17	1380	5.9
Gentzbittel et al. (1995)	HA89 x RHA266; CX x RHA266; PAC2 x RHA266; (HA89 x CX) x HA89; (HA89 x CX) x CX	Three F_2 and two BC_1F_1	560	RFLP	237	23	1150	7
Berry et al. (1996)	ZENB8 x HA89; ZENB8 x PAC2; ZENB8 x ZENR7; BSA52 x RHA297; HA89 x RHA271 ; ZENR7 x RHA801; HA89 x ZENR9; ZENR1 x ZENR8; ZENB4 x HA300	Nine F_2	1287	RFLP	635	17	1472	2.3
Jan et al. (1998)	RHA271xHA234	One F_2	93	RFLP	271	20	1164	4.6
Gentzbittel et al. (1999)	HA89 x RHA266; CX x RHA266; PAC2 x RHA266; SD x PAC1 ; SD x CP73; CP73 x PAC1 ; GH x PAC2	Seven F_2	1115	RFLP	238	17	1534	6.7
Ungerer et al. (1998)	(*H. anomalus* ANO-1497 x *H. anomalus* ANO-1506) x CMS89		56	RAPD, AFLP, isozyme	701	17	1983	6.2

Table 6.6 (continued)

Reference	Mapping populations	Population type	No. of individuals	Markers	No. of mapped loci	No. of Linkage groups	Map length (cM)	Average interval size (cM)
Peerbolte and Peleman (1996)	CX x RHA266; PAC2 x RHA266 from Gentzbittel et al. (1995)	Two F_2	184	RFLP, AFLP	437	19[a]	1144	
Gedil et al. (1999)	HA370 x HA372	One F_2	108	RFLP, AFLP	400	17	1326	3.3
Flores-Berrios et al. (2000)	PAC-2 x RHA-266	One RIL	99	AFLP	264	18	2558	9.9
Langar et al. (2003)	HA89 x LR4	One R_9 RIL	171	DALP, AFLP	305	18	2169	6.1
Tang et al. (2002)	RHA280 x RHA801	One R_7 RIL	94	SSR	459	17	1368	3.1
Yu et al. (2003)	HA370 x HA372 from Gedil et al. (2001a)	One F_2	94	RFLP, SSR	202	17	1275	6.3
Yu et al. (2003)	RHA280 x RHA801 from Tang et al. (2002)	One R_7 RIL	94	SSR, INDEL	577	17	1423	2.5
Yu et al. (2003)	PHA x PHB	One RIL	94	SSR	264	20	1199	4.5
Rachid Al-Chaarani et al. (2002)	PAC-2 x RHA-266 from Flores-Berrios et al. (2000)	One RIL	123	AFLP, SSR	367	21	2916	7.9
Lai et al. (2005)	RHA280 x RHA801 from Tang et al. (2002)	One RIL	94	SNP, SSR	439	17	1349	
Hu et al. (2004)	83HR4 x RHA345	One RIL	129	TRAP	160	17[b]	1140	9.0
Hu (2006)	RHA280 x RHA801 from Tang et al. (2002)	One F_7 RIL	92	TRAP, SSR	760	17	1747	

[a] The authors describe 19 stable linkage groups (3 markers or more) in a total number of 23 linkage groups.
[b] The authors describe 17 linkage groups and 4 pairs of markers not assigned.

maps comprised 17 or more linkage groups that presumably correspond to the 17 haploid chromosomes of sunflower, and covered distances close to the estimated length of the sunflower genome (1,650 cM; Gentzbittel et al. 1995) (Table 6.6). Distorted segregation and duplicated RFLP loci were detected by Berry et al. (1995, 1996) and Gentzbittel et al. (1995).

Random Amplified Polymorphic DNA (RAPD) Markers

Despite the dominant and low reproducible nature of random amplified polymorphic DNA (RAPD) markers, they were used in early genetic studies in sunflower. High levels of RAPD variation were reported in sunflower species (Lawson et al. 1994; Teulat et al. 1994) with the proportion of polymorphic loci averaging more than 50% for most domesticated lines. Methods such as bulked segregant analysis (BSA) allowed the rapid identification of RAPD markers associated with agronomically important traits in sunflower, such as rust resistance (Lawson et al. 1996) or broomrape resistance (Lu et al. 2000). To overcome RAPD limitations, RAPD bands can be converted into sequence-characterized amplified region (SCAR) markers. In sunflower, Lawson et al. (1998) developed SCAR markers from RAPD bands linked to two rust resistance genes, demonstrating their robustness for the detection of these resistance genes in different genetic backgrounds, and Lu et al. (2000) reported SCAR markers linked to the Or5 gene conferring resistance to race E of broomrape.

RAPDs 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. Later on, this map was expanded and now includes 549 RAPD, 151 AFLP, and one isozyme locus (Rieseberg et al. 1995; Ungerer et al. 1998), covering 17 linkage groups and 1,983 cM (Table 6.6). RAPD maps were also developed for wild *H. annuus* and *H. petiolaris* (Rieseberg et al. 1995), based on 212 and 400 RAPD loci, respectively. These authors reported 17 linkage groups for both species covering 1,084 cM for *H. annuus* and 1,761 cM for *H. petiolaris*.

Amplified Fragment Length Polymorphism (AFLP) Markers

AFLP are powerful markers for genome mapping and genetic variability studies, since they are highly reproducible, require no prior sequence information, and have a high multiplex ratio. However, AFLP markers are typically dominant and therefore their utility is greatest for projects where dominance is not disadvantageous. AFLP 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. Peerbolte and Peleman (1996) added 291 AFLP loci to two of the F_2 populations used by Gentzbittel et al. (1995) (Table 6.6). These markers pulled two linkage groups together and permitted several previously unlinked RFLP marker loci to be mapped (Knapp et al. 2001). 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. Other AFLP maps have been developed. Flores-Berrios et al. (2000) constructed an AFLP map of 2,833.7 cM using 99 recombinant inbred lines (RILs) (Table 6.6), which was later improved with additional AFLP markers (Rachid Al-Chaarani et al. 2002). Langar et al. (2003) constructed a genetic map 2,169 cM long combining direct amplification of length polymorphism (DALP) markers and AFLP markers (Table 6.6).

Simple Sequence Repeats (SSRs) or Microsatellites

Microsatellite markers are ideal DNA markers for genetic mapping and population studies because of their abundance, high levels of polymorphism, multiallelic nature, codominant inheritance, wide dispersion in genomes, ease of assay using PCR, and ease dissemination across laboratories (Powell et al. 1996). Early studies demonstrated the presence of SSRs in the sunflower genome with (A)n, (GA)n, and (CA)n being the most abundant motifs (Dehmer and Friedt 1998a). Later on, different research groups have described the development and characterization of SSR markers (Gedil 1999; Paniego et al. 2002; Yu et al. 2002; Tang et al. 2002), summing up a total of 2,040 markers (Paniego et al. 2007).

Tang et al. (2002) constructed the public-reference SSR map of sunflower using 94 F_7 recombinant inbred lines (RILs) and 408 polymorphic SSR markers (Table 6.6). The map was 1,368 long and had a mean density of 3.1 cM. Yu et al. (2003) provided the first sunflower cross-referenced maps by mapping 701 new SSR and 89 RFLP or INDEL marker loci into three populations, two of them previously used by Gedil et al. (2001a) and Tang et al. (2002) (Table 6.6). From these maps, Tang et al. (2003a) developed a composite SSR linkage map of sunflower that integrated 657 loci in a 1,423 cM map with a mean density of 2.2 cM per locus. This map allowed the selection of 95 single-locus SSRs at an average spacing of 12.9 cM representing a near-genomewide collection for a first-pass scan of the sunflower genome, from which 13 six-locus PCR multiplex sets including 78 SSRs were developed. A different set of 78 SSR markers was selected by Zhang et al. (2005) for sunflower variety identification and diversity assessment.

The AFLP map developed by Rachid Al-Chaarani et al. (2002) was improved by increasing the number of AFLP markers and integrating 38 SSR markers (Rachid Al-Chaarani et al. 2004). In the new map, 367 AFLP and SSR marker loci were placed in 21 linkage groups covering 2,916 cM (Table 6.6). An additional improvement of this SSR-AFLP map has recently been reported by Paniego et al. (2007), who integrated 161 new SSR markers from different sources, and cross referenced this map to the public SSR map of Tang et al. (2002).

6.8.1.2 Gene-Targeted Markers and Maps Based on Them

Markers Based on Sequenced RFLP-cDNA Probes

INDEL (Insertion-Deletion) markers were developed from 81 RFLP markers by sequencing the cDNA clones, aligning sunflower cDNA and *Arabidopsis* genomic DNA sequences, predicting from such an alignment intron sites in sunflower genes, and designing flanking primers to amplify the introns and flanking coding regions spanned by the primer pairs (Yu et al. 2003). The genetic linkage map position of these markers (ZVG1 through ZVG81) integrated in the public SSR map of Tang et al. (2002) is described in Yu et al. (2003). Recently, Kolkman et al. (2007) resequenced in different inbred lines and wild sunflower populations essentially these 81 genes previously mapped as RFLP markers and identified 1078 single nucleotide polymorphisms (SNPs) and 178 INDELs.

Markers Based on ESTs

Expressed sequence tags (ESTs) are typically unedited, automatically processed, single-read sequences produced from cDNAs, and are currently the most widely sequenced nucleotide element from the plant genomes. Different EST sequencing programs have been carried out in sunflower, including the Compositae Genome Project, and other programs reported by Fernández et al. (2003), Tamborindeguy et al. (2004), and Ben et al. (2005). These programs have produced 94,111 EST entries for Helianthus annuus in GenBank (last accessed October, 2007). A comprehensive annotated sunflower EST database can be found at the database of the Compositae Genome Project (http://compgenomics.ucdavis.edu/).

EST constitutes a novel source of DNA-based markers that are physically associated with coding regions of the genome. In sunflower, EST resources have been used to develop SNP and SSR markers. Pashley et al. (2006) developed a novel suite of 48 polymorphic SSR markers surveying sunflower EST sequences to identify those containing SSRs. These authors found that SSRs based on ESTs exhibited higher transferability across species as compared to anonymous SSRs. SNP/INDEL markers from sunflower ESTs were developed by Lai et al. (2005). These authors identified 605 ESTs that displayed SNP or small insertion-deletion variation *in silico*, had apparent tissue-specific expression patterns, and/or were ESTs with candidate gene function for development, cell transport, metabolism, plant defence, and tolerance to abiotic stress. Primer pairs for 535 of these loci were designed from the ESTs. Two hundred and forty-three of these markers were mapped within a 196 SSR loci framework map from the RIL population reported by Tang et al. (2002) and Yu et al. (2003). The SNP/INDEL-SSR map was 1,349 long (Table 6.6), and constitutes the first functional map based on sunflower ESTs.

Target region amplification polymorphisms (TRAPs) are derived from a rapid and efficient PCR-based technique, which uses bioinformatics tools and EST database information to generate polymorphic markers around targeted candidate gene sequences (Hu and Vick 2003). The TRAP technique has been employed in sunflower to construct a linkage map based on 160 TRAP markers (Hu et al. 2004) (Table 6.6), and to define the sunflower linkage group ends through the use of TRAP markers based on *Arabidopsis*-type telomere repeat sequences (Hu 2006).

Sunflower ESTs have also been used as a source for developing universal markers useful for comparative mapping and phylogenetic analysis within the sunflower family, Asteraceae. Chapman et al. (2007) used alignments of a conserved orthologous set of ESTs from sunflower and lettuce and genomic sequences of *Arabidopsis* to design a suite of primer pairs that were conserved across species, but which were predicted to flank introns (variable regions) and therefore to detect polymorphism within species. One hundred and ninety-two of these primer pairs were tested for amplification across eight diverse members of the Asteraceae. From these, 85% amplified in at least one taxon, and 20% amplified in all the eight taxa tested, the majority of these loci being polymorphic within species.

6.8.1.3 Functional Markers

Functional markers are derived from polymorphic sites within the genes known to be causally involved in phenotypic trait variation. The development of functional markers requires allele-specific sequences of functionally characterized genes from which polymorphic, functional motifs affecting plant phenotype can be identified. Functional markers have been developed in sunflower for traits determining oil quality (Tang et al. 2006b), or herbicide resistance (Kolkman et al. 2004). A detailed description of these markers will be given in the section on molecular breeding.

6.8.2 Molecular Breeding

6.8.2.1 Germplasm Characterization

The characterization of genetic structures in cultivated sunflower was one of the first aims of genetic fingerprinting using molecular markers. Initial studies using RFLPs consistently separated sunflower inbred lines into sterility maintainer (B-line) and fertility restorer (R-line) groups (Gentzbittel et al. 1994; Zhang et al. 1995), reflecting breeding strategies that maximize heterosis. AFLP and SSR analyses confirmed these results (Hongtrakul et al. 1997; Paniego et al. 2002; Yu et al. 2002). Tang and Knapp (2003) performed

phylogenetic analyses on 47 domesticated and wild germplasm accessions using 122 SSR markers distributed throughout the sunflower genome. These authors found extraordinary allelic diversity in the Native American land races and wild populations, and progressively less allelic diversity in germplasm produced by successive cycles of domestication and breeding, suggesting that the contemporary oilseed sunflower pool could profit from an infusion of novel alleles from the reservoir of latent genetic diversity present in wild populations and Native American land races. Finally, Zhang et al. (1995) used RFLPs to screen inbred lines for intra-line polymorphisms. Although they found polymorphism within the four lines screened, these lines presented good uniformity of morphological characters in the field. It was concluded that the polymorphisms stemmed from residual heterozygosity or outcrossing, and proposed using RFLPs for distinctness, uniformity, and stability testing in sunflower.

Heterotic group modelling in sunflower using molecular tools has been reported (Hongtrakul et al. 1997; Cheres et al. 2000), although it failed to reveal clear heterotic groups. Cheres et al. (2000) estimated the correlation between genetic distance, heterosis, and hybrid performance using AFLPs and coancestries, and found that genetic distance alone was a weak predictor of hybrid performance in sunflower.

6.8.2.2 Molecular Mapping of Simply Inherited and Complex Traits

Oil Content

Oil content in sunflower is considered to be quantitatively inherited, and depends on both the percentage of hull weight in relation to whole seed weight and the concentration of oil in the kernel. Leon et al. (1995b) identified six QTL associated with oil content with predominant additive gene action, which accounted for 56% of the genetic variation. Two of these QTL were associated to kernel oil percentage, two of them with kernel percentage, and two of them with both components. Later studies reported the identification of three (Mestries et al. 1998), six (Mokrani et al. 2002), and eight (Leon et al. 2003) QTL associated to achene oil content, some of them consistently identified across environments. Recently, Tang et al. (2006a) identified six QTL for achene oil concentration on LG 1, 4, 9, 10, 16, and 17 in a RIL population developed from the cross RHA280 (confectionery line) x RHA801 (oilseed line). The QTL individually explained 3.1–22.5% and collectively explained 55.7% of the phenotypic variability for achene oil concentration. QTL on LG 10, 16, and 17 were centered on the phenotypic loci *B* (apical branching), *hyp* (hypodermal pigment), and *P* (phytomelanin pigment), respectively. Hajduch et al. (2007) using a proteomic approach reported 77 protein spots differentially expressed in the high oil line RHA 801 versus the low oil line RHA 280. Identification of 44 of these proteins indicated that the two main processes affecting low or high oil concentration in these lines were glycolysis and amino acid metabolism.

Oil Quality

Fatty Acids

The molecular basis of modified fatty acid profile in the seed oil of sunflower has been studied through a QTL and a candidate gene approach. A number of sunflower genes, coding for enzymes involved in the fatty acid biosynthetic pathway in seeds, have been cloned and their polymorphism studied in cultivated sunflower. Hongtrakul et al. (1998a) reported the isolation of two stearoyl-acyl carrier protein (ACP) desaturase genes (*SAD17* and *SAD6*) in sunflower that were highly expressed in seeds. The SAD enzyme desaturates stearoyl-ACP to oleoyl-ACP. Candidate gene and QTL analysis revealed the co-location of a major QTL associated to stearic acid content in the high stearic acid mutant CAS-3 (genotype *es1es1es2es2*) with a *SAD17* gene. The *SAD17A* locus was found to co-segregate with *Es1* (Pérez-Vich et al. 2002b). Using RFLP-AFLP linkage maps constructed from two different mapping populations derived from CAS-3, the *SAD17A* locus was mapped to LG 1, and it was found to underlie the major QTL affecting the concentration of stearic acid. This QTL explained about 80% of the phenotypic variance of this fatty acid (Pérez-Vich et al. 2002b) and it was named *st1-SAD17A*. Other minor QTL affecting stearic acid content, which mapped to LG 3 (*st2.1*), LG 7, and LG 13 (*st2.3*), were detected in that study, although none of them was consistent enough to be considered as a strong candidate for the *Es2* locus (Pérez-Vich et al. 2002b).

Since the highly significant effect of the macromutation *Es1* reduced the power of the QTL analyses to identify QTL with smaller effects on stearic acid levels, another mapping population in which stearic acid segregated independently of *Es1* was developed from the CAS-20 line (genotype *Es1Es1es2es2*) (Pérez-Vich et al. 2004a). An RFLP-SSR genetic linkage map from this population allowed the identification of three QTL affecting stearic acid, located on LG 3 (*st2.1*), LG 11 (*st2.2*), and LG 13 (*st2.3*). The three QTL collectively explained 43.6% of the phenotypic variation. On the basis of positional information, QTL on LG 11 was suggested to be a *SAD6* locus (Pérez-Vich et al. 2004a).

Very high stearic acid content in the sunflower mutant line CAS-14 is determined by the *Es3* gene (Pérez-Vich et al. 2006a). Using bulked segregant analysis, Pérez-Vich et al. (2006b) mapped *Es3* to LG 8 of the sunflower genetic map, and identified SSR markers closely linked to this gene (Fig. 6.1). *Ms11*, one of the genes determining nuclear male sterility in sunflower, was also mapped to LG 8 at a genetic distance of 7.4 cM from *Es3*.

Marker studies related to high oleic acid content in sunflower began with the identification of two RAPD makers linked to the *Ol1* gene (Dehmer and Friedt 1998b). 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 (high oleic) lines (Hongtrakul et al. 1998b; Lacombe and Bervillé 2001; Martínez-Rivas et al. 2001). The *Ol1-FAD2-1* locus mapped to

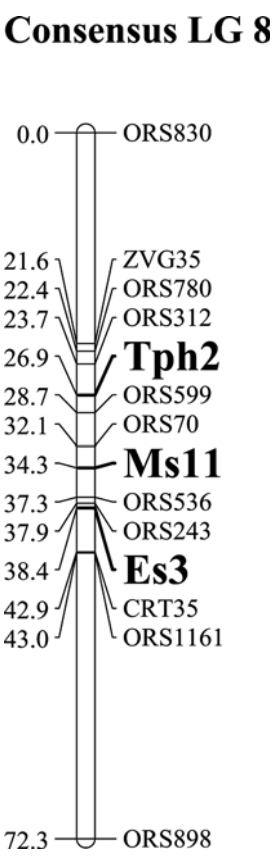

Fig. 6.1 Composite map of LG 8 containing the Tph2, Es3, and Ms11 genes determining high gamma-tocopherol content, very high stearic acid content, and nuclear male sterility, respectively. The map was constructed from the F2 mapping populations P21 × CAS-14 (Pérez-Vich et al. 2006b) and CAS-12 × IAST-540 (García-Moreno et al. 2006). The ORS and CRT prefixes denote SSR marker loci, and the ZVG prefix denotes INDEL marker loci. The cumulative distances in centimorgans are shown at the left of the map

LG 14 (Pérez-Vich et al. 2002b) 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 (Pérez-Vich et al. 2002b). Schuppert et al. (2006a) determined the physical structure of the *FAD2-1* locus and developed polymorphic sequence-tagged-site (STS) DNA markers diagnostic for the *Ol* mutation. Schuppert et al. (2005) indicated that the mechanism underlying the *Ol* mutation was a *FAD2-1* silencing by RNA interference.

Several studies have also been conducted to characterize modifying genes affecting oleic acid content. Pérez-Vich et al. (2002b) described the existence of a minor QTL on LG 8 which showed an epistatic interaction with the major QTL for oleic acid at the *FAD2-1* locus on LG 14. Lacombe et al. (2001, 2002) identified a locus that suppressed the effect of the *FAD2-1* locus, probably through a mechanism of gene silencing. Schuppert et al. (2003, 2006b) described the effect of at least seven genes from the fatty acid biosynthesis pathway, including another oleate desaturase gene (*FAD2-2*) on LG 1, acting epistatically with the *Ol1-FAD2-1* locus on LG 14.

Tocopherols

Hass et al. (2006) and García-Moreno et al. (2006) mapped the *Tph2* gene determining high gamma-tocopherol content in sunflower seeds to LG 8 of the sunflower linkage map (Fig. 6.1). In addition, Hass et al. (2006) isolated and characterized two paralogs of the gamma-tocopherol methyltransferase gene (*gamma-TMT-1* and *gamma-TMT-2*), that mapped to LG 8 and cosegregated with the *Thp2* locus. These authors also developed STS markers diagnostic for *Tph2*. However, none of these DNA polymorphisms found between wild type and mutant *gamma-TMT-1* and *gamma-TMT-2* alleles were associated with the mutant phenotype, suggesting that the mutation may disrupt regulatory sequences very tightly linked to the *gamma-TMT* locus (Hass et al. 2006).

Tang et al. (2006b) and Vera-Ruiz et al. (2006) mapped the *Tph1* gene controlling high beta-tocopherol content in sunflower seeds to LG 1. Tang et al. (2006b) determined that the *Tph1* mutation associated to the modified tocopherol phenotype was a non-lethal knockout mutation in a 2-methyl-6-phytyl-1,4-benzoquinone/2-methyl-6-solanyl-1,4-benzoquinone methyltransferase (*MPBQ/MSBQ-MT*) locus of LG 1 (*MT-1*) caused by the insertion of a 5.2 kb *Ty3/gypsy*-like retrotransposon, and developed STS markers diagnostic for wildtype and mutant *MT-1* alleles. A second *MPBQ/MSBQ-MT* locus mapping to LG 4 (*MT-2*) was also described to be associated to tocopherol composition (Hass et al. 2006; Tang et al. 2006b). This locus was epistatic to the *MT-1* locus on LG 1 and had no effect in *Tph1Tph1* or *Tph1tph1* individuals, but significantly increased beta-tocopherol content in *thp1thp1* individuals.

Disease Resistance

Resistance to downy mildew is probably one of the best examples of the use of major gene resistance. There are up to 10 resistance genes described, denoted Pl, carrying resistance to various downy mildew races and mapped to genetic maps (Vear 2004). Research on these genes has shown that there are at least three clusters of genes plus several others that segregate independently from these clusters. A cluster on LG 8 includes the Pl1, Pl2, Pl6 (from wild *H. annuus*), and Pl7 (from *H. praecox*) genes covering a large area of about 0.5 cM with two genetically distinct regions conferring resistance to different Plasmopara races (Vear et al. 1997; Bouzidi et al. 2002). A second cluster on LG 13 includes the Pl5 (from *H. tuberosus*) and Pl8 (from *H. argophyllus*) genes (Bert et al. 2001). The Pl_{arg} (from *H. argophyllus*) gene was solely mapped to a telomeric region of LG 1 (Dussle et al. 2004). Numerous resistance gene analogues (RGAs) have been found clustered and linked to the Pl clusters on LG 8 (Gentzbittel et al. 1998; Gedil et al. 2001b; Bouzidi et al. 2002; Slabaugh et al. 2003) and LG 13 (Radwan et al. 2003), and to Pl_{arg} (Radwan et al. 2007).

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 (Vrânceanu et al. 1980). The *Or5* gene has been mapped to a telomeric region of LG 3 of the sunflower genetic map (Lu et al. 2000; Tang et al. 2003b; Pérez-Vich et al. 2004b). However, recent genetic and molecular studies have revealed a more complex genetic control of broomrape resistance. Pérez-Vich et al. (2004b) 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.

Lawson et al. (1998) developed SCAR markers linked to the R_1 and R_{adv} genes conferring resistance to rust. Subsequent studies demonstrated that one of the SCAR markers linked to the R_1 gene mapped to LG 8 and was closely linked to the downy mildew cluster on this LG (Slabaugh et al. 2003). Lenardon et al. (2005) mapped the *Rcmo-1* gene for resistance to sunflower chlorotic mottle virus to LG 14.

Resistance to other diseases, such as *Sclerotinia*, Phomopsis stem canker, and black stem is more 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. 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). One particular strong QTL was found on LG 8, linked to a protein-kinase gene (Gentzbittel et al. 1998), but while it explained 50% of the variability in one cross, in other crosses it explained only 15% or was not present. 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-localise 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. 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). QTL for stem lesion detected by these authors on LG 8 and 16 were demonstrated to be consistent across generations (Micic et al. 2005a). Micic et al. (2005b) determined the effect of three to four QTL associated to *Sclerotinia* resistance by selective genotyping in a mapping population derived from crosses with a different resistant line. In addition, these authors cross-referenced previous studies of Mestries et al. (1998), Bert et al. (2002), and Rönicke et al. (2005) and found that the same six linkage groups carried QTL for *Sclerotinia* resistance in more than one population, and that LG 1, LG 9, and LG 10 had a significant effect in the majority of the populations considered. Despite the complex genetic architecture of *Sclerotinia* resistance, QTLs consistent across

environments (Bert et al. 2002), generations (Micic et al. 2005a), and mapping populations (Rönicke et al. 2005; Micic et al. 2005b) have been identified, which constitute valuable tools for the establishment of marker assisted selection programs aimed at improving *Sclerotinia* resistance.

Bert et al. (2002) found three QTLs explaining up to 20% of the variability for resistance reaction to natural attacks of Phomopsis stem canker, and other three QTL for resistance reaction to artificial infections, one of them for both types of infection. These authors also reported the co-localisation of a QTL affecting resistance to *Sclerotinia* mycelium in leaves and another QTL for resistance to Phomopsis in leaves, suggesting that these QTL could result from the same components in the mechanism of resistance to these two facultative parasites.

In two independent studies, a significant number of QTL (four to seven) with moderate effects on resistance to black stem were identified, explaining each QTL from 5 to 17.5% of the phenotypic variance (Rachid Al-Chaarani et al. 2002; Bert et al. 2004). Subsequent studies on the population from Rachid Al-Chaarani et al. (2002) testing different *Phoma macdonaldii* isolates allowed the identification of a total of 27 resistance QTL for 4 isolates (Abou Alfadil et al. 2007) and 10 resistance QTL for 2 isolates (Darvishzadeh et al. 2007), with moderate individual effects ranging from 6 to 29%. Alignan et al. (2006), using a cDNA microarray approach, identified several genes regulated in response to *Phoma macdonaldii*. These authors proposed a model in which negative regulation of a dual-specificity mitogen-activated protein kinase (MAPK) phosphatase could be implicated in the defence mechanisms against this pathogen via activation of a MAP kinase cascade that could trigger defence responses such as thaumatin biosynthesis and phenylalanine ammonia lyase (PAL) activation.

Developmental and Agronomic Traits

Male Sterility

Three out of 11 recessive genes controlling nuclear male sterility in sunflower have been mapped. The *Ms9* gene was mapped to LG 10 using TRAP and SSR markers (Chen et al. 2006), whereas the *Ms10* and *Ms11* genes were mapped to LG 11 and LG8, respectively using RFLP, SSR and INDEL markers (Pérez-Vich et al. 2005).

Molecular studies have examined the nature of different CMS sources available in sunflower. Köhler et al. (1991) suggested that a new open reading frame, orfH522, in the 3′-flanking region of the *atpA* gene was associated with the CMS phenotype. Further studies using mtDNA genes and three probes for the open reading frame clearly distinguished CMS sources by their mtDNA organization and CMS mechanism (Horn 2002). Kusterer et al. (2005) developed PCR-based markers closely linked to the fertility restoration gene *Rf1*.

Self-incompatibility and Seed Dormancy

Wild populations of *H. annuus* are self-incompatible and have deep seed dormancy, whereas modern sunflower cultivars are self-compatible and have short-lived seed dormancy. Gandhi et al. (2005) mapped QTL for self-incompatibility and seed dormancy in a backcross population from parents showing contrasting characteristics for both traits. A single locus S for self-incompatibility was identified and mapped to LG 17. The locus was tightly linked to a cluster of QTL for several domestication and postdomestication traits. Three QTL for seed dormancy with small individual effects in the predicted direction (wild alleles decreased seed germination) were identified.

Embryogenesis

Plant regeneration by in vitro organogenesis offers the possibility of obtaining a high number of regenerated shoots. Flores-Berrios et al. (2000) developed an AFLP genetic linkage map from a RIL population exhibiting variability for organogenesis traits. Six putative QTL for number of shoots per explant plated and seven putative QTL for number of shoots per regenerating explant were identified. For each trait, QTL explained 52 and 67%, respectively of the total phenotypic variance.

Days to Flowering

Days to flowering is an important trait primarily controlled by the genotype, photoperiod, and temperature. Few genetic factors for days to flowering have been reported. Mestries et al. (1998) identified two QTL that accounted for 30% of the phenotypic variation in a single environment. Leon et al. (2000) identified five QTL that accounted for 73 and 89% of the phenotypic and genotypic variations, respectively, across four locations with limited range of photoperiod. When evaluating the same population in locations with more different photoperiods, Leon et al. (2001) found that the two QTL with the strongest association with days to flowering were responsive to photoperiod.

Resistance to Abiotic Stresses

Poormohammad Kiani et al. (2007) conducted a pot experiment for mapping water status traits and osmotic adjustment associated with drought tolerance. The plants were induced to water deficit to compare QTL detection under well-watered and water-stressed environments. In general, most of the QTL detected under well-watered conditions were not detected in water-stressed conditions. Eight QTL were detected for osmotic adjustment. The largest one, located at LG 5 and accounting for 29% of the phenotypic variation, overlapped with QTL for other water status traits such as leaf water potential, relative water content, osmotic potential, and osmotic potential at full turgor.

Resistance to Herbicides

Sunflower biotypes resistant to two classes of acetohydroxyacid synthase (AHAS)-inhibiting herbicides such as imidazolinones (IMIs) or sulfonylureas (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.

6.8.2.3 Marker Assisted Selection

In contrast to the high number of reports on mapping of important traits controlled by major genes and QTL, literature on practical application of those markers in sunflower breeding programs remains very limited. There are probably several scientific and logistical issues that must be still resolved before practical marker assisted selection (MAS) strategies can flow from mapping studies. Therefore in this section we will deal with factors determining enhanced power of MAS and how they are faced in sunflower. Moreover, the few examples of practical use of molecular markers in breeding programs available so far will be highlighted.

MAS Optimization

Marker Validation and Refinement

Marker validation and refinement is one of the main factors enhancing selection power of MAS. For markers associated to simply inherited traits, marker validation and reduction of the distance between the marker and the gene of interest is fairly straightforward. Examples of marker validation in different genetic backgrounds have been reported for the Pl_2 gene determining resistance to different downy mildew races (Brahm et al. 2000), to the R_1 and the R_{adv} genes conferring rust resistance (Lawson et al. 1998), and to the *Or5* gene conferring resistance to race E of broomrape (Tang et al. 2003b; Pérez-Vich et al. 2004b). Improvement of marker accuracy for the *Rf1* gene restoring pollen fertility in PET1 based material was improved by using enlarged mapping populations (Kusterer et al. 2005).

In many cases, the marker identified in the process of fine-mapping may not be polymorphic in all the populations tested, thus requiring the identification of alternative markers for those populations. Ideal markers for selection are those based on gene mutations underlying the trait of interest. This kind of markers has been developed in sunflower for oil quality traits and other simple traits. For example, Tang et al. (2006b) determined that a non-lethal knockout mutation in a *MPBQ/MSBQ-MT* locus on LG 1 (*MT-1*) was underlying beta-tocopherol accumulation in sunflower seeds, and robust STS markers

diagnostic for wildtype and mutant *MT-1* alleles were developed. Similarly, Kolkman et al. (2004) identified a mutation in codon 205 in the acetohydroxyacid synthase gene *AHAS-1* that confers resistance to imidazolinone (IMI) herbicides, and developed a SNP genotyping assay diagnostic for it.

The situation becomes more complicated for QTL markers for complex traits. Factors such as population structure and size, parental selection and genetic background effects, epistasis, inaccurate phenotyping, or QTL x environment interactions contribute to bias the estimation of QTL effects, thus reducing the likelihood of successful use of these QTL in MAS programs. QTL validation in independent samples and in different genetic backgrounds and environments is therefore necessary before using them in MAS breeding programs. There are some good examples of QTL validation in sunflower, making the validated QTL ideal targets for MAS. For *Sclerotinia* resistance, QTL have been validated across environments (Bert et al. 2002), generations (Micic et al. 2005a), and genetic backgrounds (Rönicke et al. 2005; Micic et al. 2005b). For oil content, QTL have been also validated across generations, environments, and mapping populations (Leon et al. 2003; Tang et al. 2006a).

In addition to QTL validation, fine-mapping of QTL is very useful for identifying tightly linked markers that will not suffer from loss of linkage due to recombination between marker and QTL, and that will allow to minimize the size to the introgressed fragment. The development of a high density sunflower genetic map (one marker per 0.8 cM) through the mapping of 2,495 high-throughput DNA marker loci (Knapp et al. 2007) will contribute to map QTL with a high level of resolution. The development of specific genetic resources such as near-isogenic lines (NILs), differing in a genomic segment containing a target QTL, and RILs will also contribute to fine-mapping of QTL. In sunflower, Micic et al. (2005a) re-estimated position and effects of a number of QTL for *Sclerotinia* resistance in a RIL population developed from F_3 families where the QTL were originally identified. However, they only obtained partial recovery of QTL detected in the earlier F_3 generation in the RILs for traits such as stem lesion. Pizarro et al. (2006) have developed QTL-NILs varying in a target QTL for seed oil concentration, which allowed the authors to determine its effect with higher resolution.

Association mapping has great potential for higher-throughput QTL detection. The method relies on linkage disequilibrium (LD) to study the relationship between phenotypic variation and genetic polymorphism. The LD extent and the application of association mapping in sunflower have not been studied in depth. Recent reports by Liu and Burke (2006) and Kolkman et al. (2007), who studied patterns of nucleotide diversity in genic loci from both wild and cultivated sunflower, demonstrated that SNP frequencies and LD decay were of sufficient magnitude in wild populations (1 SNP/19.9 bp and LD decay within ~200 bp), exotic germplasm accessions (1 SNP/38.8 bp and LD decay within ~1,100 bp), and modern sunflower cultivars (1 SNP/45.7 bp and LD decay within ~5,500 bp) for high-resolution association mapping.

Assays Optimization and Cost Reduction

After the development of molecular markers and validation of their power for selection for the trait, it is often necessary to optimize the assays, with driving criteria being to reduce unit costs and turn around times while increasing throughput and minimizing errors. Technologies that speed up the implementation process, reduce laboratory requirements or errors, and lower the cost associated with scaling-up, are crucial to the success of MAS. One of the main priorities included in the "White paper: Priorities for research, education and extension in genomics, genetics and breeding of the Compositae" (The Compositae Genome project, http://compgenomics.ucdavis.edu/; 2007) for translating sunflower genomics into practical breeding programs was the reduction of total marker costs. Advances in sunflower marker technologies have been carried out in recent years. For SSR markers, PCR multiplexes for a genome-wide framework of SSR marker loci developed by Tang et al. (2003a) increased genotyping throughput and reduced reagent costs, which is essential for repetitive genotyping applications. In addition, multicolour assays, SSR primer design to facilitate "pooled amplicon multiplexing" by length in SSR development, and SSR analyses in semi-automated, high-throughput genotyping systems (Yu et al. 2002; Tang et al. 2002) resulted in time-saving and reduced costs for SSR assays. Currently, different techniques for SNP detection are being used in sunflower to type SNPs in a high-throughput, time-saving and cost-effective fashion, including denaturing high-performance liquid chromatography (DHPLC) (Lai et al. 2005), and single-base extension or allele specific primer extension (Knapp et al. 2007).

Improved QTL detection methods that reduce costs have also been proposed. Micic et al. (2005b) used selective genotyping for detecting QTL for *Sclerotinia* resistance in sunflower. This method exploits the concentration of most of the information for QTL effects in the "tails" of the quantitative trait distributions. Accordingly, population sizes can be reduced to those individuals found in these "tails". The authors concluded that selective genotyping can be efficiently used for QTL detection and analysis of congruency for resistance genes across populations, despite the limited sample size and the non-random sampling used.

MAS in Sunflower Breeding Programs

The most common application of MAS is marker assisted/accelerated backcross breeding for gene introgression. Optimally, this is based on positive foreground selection for donor trait, positive background selection for the recurrent parent genome, and negative background selection against undesirable donor parent alleles (Frisch et al. 1999). In general, marker assistance is expected to provide higher efficiency, reduced cost, and/or shorter duration of the backcross breeding scheme, compared with conventional methods.

In sunflower, marker assisted backcross breeding is currently being carried out in private breeding companies to accelerate the introgression of target genes into elite germplasm. Traits such as downy mildew resistance, high oleic acid content, and herbicide resistance are currently the main targets, although complex traits such as resistance to *Sclerotinia*, *Phoma* and Phomopsis stem canker are also taken into account. Despite there are no reports on such programs, it seems that markers routinely used in plant cultivar development in private programs are used mainly for selecting alleles with large effects on traits with relatively simple inheritance. However, dissection of complex traits such as oil content using molecular markers in sunflower is contributing to implement MAS for such traits. For example, QTL associated to different components that determine oil content (kernel percentage in the achene and kernel oil percentage) have been identified and validated. Some of these QTL are associated to the phenotypic loci *B* (apical branching), *hyp* (hypodermal pigment), and *P* (phytomelanin pigment) (Leon et al. 1996, 2003; Tang et al. 2006a). This fact was explained by Tang et al. (2006a) as a pleiotropic effect of such phenotypic loci on oil content, and allowed Leon et al. (1995a) to establish combined marker and phenotypic (based on the *hyp* locus) assisted selection for high oil content during the backcross process.

Codominant markers are most useful for marker-assisted backcrossing because selection amonguy backcross progeny involves selection for heterozygous progeny. If a dominant marker is used for selection, it will remain informative in subsequent backcross generations if the dominant allele (conferring band presence) is linked to the donor parent allele. If the recessive allele is linked to the donor parent allele, then progeny testing of each individual in each backcross generation would be required, thereby doubling the number of generations required for backcrossing. Panković et al. (2007) proposed increasing MAS efficiency in backcross programs to introgress the *Pl6* gene conferring resistance to downy mildew race 730 by using a combination of closely linked codominant cleaved amplified polymorphic sequence (CAPS) markers with dominant markers developed from resistance candidate genes.

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. To facilitate and accelerate the introgression process of genes related to disease resistance, Slabaugh et al. (2003) proposed the identification of allelic variation in wild species for specific candidate genes such as RGAs, to identify potentially useful resistance genes through disease screening, and to use markers developed from these RGAs to track the gene in the introgression process. QTL and candidate gene analyses in wild sunflower species is contributing to identify genes and QTL for adaptation to salt or drought tolerance (Lexer et al. 2003a, b; Kane and Rieseberg 2007) that could be exploited as a source of new genes to be introgressed into cultivated sunflower. Despite the use of molecular markers to assist backcross introgression of specific genes from wild species is still scarce, they have been

useful for the identification and characterization of interspecific hybrids (Natali et al. 1998; Binsfeld et al. 2001).

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. Genes controlling resistance to different races or biotypes of a pest or pathogen and genes contributing to agronomic or seed quality 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 resistance durability. For this purpose, the use of molecular markers is essential, since partial resistance conferred by these QTL can not be determined phenotypically if combined with major resistance genes. Molecular markers will be very useful to pyramid resistance genes tightly linked in clusters, a virtual impossibility in practice using phenotypic analysis alone (Slabaugh et al. 2003). For partial resistances such as *Sclerotinia* and Phomopsis stem canker, a very important step towards the improvement of the level of resistance is the use of MAS to combine different resistance QTL.

Different strategies are currently being carried out to enhance the efficiency and scope of molecular breeding. The development of BAC (bacterial artificial chromosome) and BIBAC (binary-bacterial artificial chromosome) libraries (Gentzbittel et al. 2002; Özdemir et al. 2004; Feng et al. 2006; Tang et al. 2007) and linkage group-specific clones (Jan and Seiler 2007) is providing resources and tools essential for comprehensive research of the sunflower genome. These libraries are being used for isolating and physical mapping of loci such as the *FAD2-1* locus (Tang et al. 2007), or the fertility restorer *Rf1* locus (Hamrit et al. 2006). In addition, combination of QTL mapping and gene expression analysis and function elucidation is becoming an excellent tool for dissecting QTL into Mendelian factors and improving the efficiency of molecular breeding of complex traits in sunflower, such as drought tolerance (Poormohammad Kiani et al. 2007).

6.8.3 Transgenic Breeding

Despite the increasing success of transgenic varieties of some major oilseed crops such as soybean and canola, transgenic breeding research in sunflower has been rather limited so far in comparison with the mentioned crops. A major constraint for the advance of transgenic breeding have been the limitations of the initial regeneration systems as well as problems in combining regeneration and transformation within the same cells. Nevertheless, efficient transformation protocols with high reproducibility and high transformation frequency have been developed (Mohamed et al. 2006).

Most of the development of transgenic varieties has been conducted by seed companies, which have incorporated agronomically important traits.

The most important ones for which information is available are tolerance to the herbicide glyphosate, by expression of 5-enolpyruvishikimate-3-phosphate synthase (*EPSPS*) genes from *Agrobacterium tumefaciens*, tolerance to the herbicide glufosinate ammonium by expression of phosphinothricin acetyltransferase (*PAT*) genes from *Streptomyces* spp., resistance to Lepidoptera by expression of *Cry* toxins genes from *Bacillus thuringiensis* (*Bt* genes), resistance to Coleoptera by expression of trypsin inhibitor genes from cowpea (*Vigna unguiculata*) plus lectin genes from snowdrop (*Galanthus* spp.), resistance to *Sclerotinia* by expression of oxalate oxidase genes from wheat or barley, and enhanced rubber production by expression of genes from guayule (*Parthenium argentatum*) (Cantamuto and Poverene 2007).

Transgenic lines have also been produced at the public sector. Rousselin et al. (2002) developed transgenic sunflower lines with reduced stearic acid content by expression of a delta-9 stearoyl desaturase gene from castor bean (*Ricinus communis*). Sawahel and Hagran (2006) produced transgenic plants resistant to *Sclerotinia* by expression of the human lysozyme gene.

The risks associated with the gene flow from transgenic cultivars to the wild flora are a matter of general controversy. In the case of sunflower, the risk is particular high in North America, centre of origin for the genus, but also in many other parts of the world where feral and naturalized populations of wild *Helianthus* species are present (Bervillé et al. 2005). Examples of adaptative advantages associated with the flow of transgenes to wild *Helianthus* populations have been reported by Snow et al. (2003) and Burke and Rieseberg (2003).

6.9 Seed Production

The sunflower breeder identifies inbred lines or open pollinated varieties that have better performance than the currently used ones. Once the preliminary trials suggest that an inbred line or open pollinated variety has potential, the breeder increases the seed supplies and produces larger quantities of seed for expanding testing. Seed at this stage is referred as "Breeder seed" because the breeder is responsible for maintaining purity and increasing seed supplies of the line or variety. When applied to hybrid varieties, it refers to the seed of male-sterile, maintainer, and restorer lines. The initial increase of breeder seed is known as "Foundation seed". It is handled to maintain specific genetic purity and identity. "Registered seed" is the progeny of breeder and foundation seed handled under procedures acceptable to the certifying agency to maintain satisfactory genetic purity and identity. "Certified seed" is the progeny of breeder and foundation seed handled to maintain satisfactory genetic purity and identity which is approved by the certifying agency.

Hybrid seed is the first generation of seed of a cross produced by controlling the pollination and by combining two or more lines. Single hybrid sunflower seed is produced through the controlled crossing of male (restorer or R) and

female (male-sterile or A) lines. The A-line is maintained by crossing to a genetically identical male-fertile line with a fertile cytoplasm, referred to as maintainer or B-line. The commercial seed is usually grown from hybrid seed and it is planted for any use except for seed production.

Increasing breeder seed and foundation seed of parental lines and certified seed of hybrids is a time-consuming and critical operation in breeding programs requiring full-time personnel to be in charge of operations. It requires systematic planning and management on the part of seed producers. The production, processing and marketing of the certified seed is exclusively the responsibility of the seed producers. The seed-certifying agencies set up the procedures by which each class of seed may be produced, the standards of purity and identity, and also assume the responsibility for inspecting, sampling testing, and certifying that the seed meets certification standards. Exact certification procedures vary from country to country.

6.9.1 Maintenance and Increase of Parental Lines

Increases of A, B, and R lines are initially accomplished under bags in nurseries to check for purity and stability of the cytoplasmic male sterility and to eliminate off-types. These increases are often carried out under isolation in screened cages to eliminate bagging. A hive of bees is placed inside each cage to pollinate lines to be crossed and to eliminate hand crossing. An increase of the R-line may not require pollination by bees. Usually lines are planted on different dates so that these cages can be utilized for several increases each season. For small increases, hand crossing of the A-line with the B-line may be done. Hand pollination should be carried out in the morning on all days throughout the flowering period.

Field increases of breeder/foundation seed of the female (A) line involves planting the A and B lines using a ratio 1:1 or 2:1 (A:B) at low plant populations in rows spaced 75–90 cm apart. The production field is isolated at least 6–8 km from commercial fields or wild populations in countries such as USA where those are frequent. One hive of bees per hectare is placed in the field for pollination of the female parent.

An occasional problem in converting certain lines to cytoplasmic male sterility is the occurrence of fertile plants in the progeny of crosses between maintainer and male-sterile lines. The frequency of fertile plants was estimated 5.7% in a study involving 500 inbred lines (Vrânceanu and Stoenescu 1980). Because of potential problems with fertile plants in increasing foundation seed and in hybrid seed production, many breeders use the system of paired crosses (Vrânceanu 1974) in converting and maintaining A- and B-lines. In this system, the identity of individual A- and B-line plants is maintained and only those B-line plants that produce completely sterile progeny are used for further multiplication.

6.9.2 Commercial Hybrid Seed Production

6.9.2.1 Isolation

Similarly to the multiplication of parental lines, maintaining the recommended isolation from other sunflower crops or wild species is a crucial requirement in hybrid seed production to maintain genetically pure hybrid seed. Pollen from external sources will contaminate the crop, causing tall plants, reduced disease resistance, reduced yield potential, and in the case of wild sunflowers, multi-headed plants. Seed companies try to avoid this carrying out seed production in regions with no major commercial production to eliminate the problem of isolation from cultivated sunflower. In the USA, where wild sunflower is commonly present, wild plants must be removed from the area of seed production before flowering to avoid undesirable cross-pollination (Miller 1987). Considering the role of honeybees in pollination and their flight range, seed certifying agencies have established minimum isolation requirements of 1.6–4.8 km between seed production fields and those of commercial sunflower in order to maintain the genetic purity of parental lines or hybrids. Space isolation is the most important factor to be considered for the production of quality seed. When space isolation is not possible, time isolation of about 30 days is satisfactory. This means that the flowering stage of the parental lines in the seed production field should be at least 30 days earlier or later than that of other varieties grown within the area to avoid contamination by pollen.

6.9.2.2 Plant Population and Planting Methods

Optimum plant population for hybrid seed production depends on the characteristics of the female and male parents, environmental conditions, and desired seed size. For most of the oilseed type hybrids, plant population varies from 40,000 to 60,000 plants under irrigation. Optimum populations for confectionary type hybrids are higher when smaller seed is desired for the grower. However, when the market demands larger seeds, plant populations of 40,000 plants and wider spacing between rows are used. The ratio of female to male rows usually ranges from 2:1 to 6:1, depending on the pollination ability of the male parent, similarity in the flowering dates of the male and female parents, and number of row units used (Vrânceanu 1974). For example, if the restorer line incorporates the recessive branching trait, which allows the production of pollen during longer periods, the number of female rows can be increased. One important consideration in planting the seed production field is the staggered sowing of the parental lines in order to achieve flowering synchronization between the female and R-line to avoid problems in hybrid seed set.

6.9.2.3 Pollination

In the maintainer as well as in the hybrid seed production plots, pollination is a crucial aspect to be considered. Hives of honeybees are placed in the hybrid seed production field at the beginning of anthesis. The number of hives depends on the plant population and stage of flowering. Seed producers generally use one to four hives per hectare during the heaviest pollinating period. An adequate number of hives is important since placing too many may force bees to forage other sources of pollen and increase the percentage of outcrosses.

6.9.2.4 Roguing

Roguing is an essential practice in sunflower hybrid seed production for obtaining physical and genetic purity. The objective of roguing is to remove before anthesis the plants that do not meet the expected characteristics of the line (off-types). It has to be strictly carried out in all the stages of crop growth. A north–south orientation of the rows to improve its efficiency is recommended (Miller 1987). In breeder/foundation seed as well as in certified seed production, male-fertile plants within the male-sterile line are easily identified by dark anthers and pollen production. In addition, plants with other morphological deviations have to be removed in A- and B-lines before flowering. In breeder and foundation seed production of restorer lines as well as in certified seed when R-lines are involved, off-types have to be removed before anthesis. A row spacing of 76–90 cm facilitates walking between rows for observing plants. Twin rows, consisting of two rows of the female parent planted 60 cm apart and 90 cm from the next twin-row set, have also been used.

6.9.2.5 Harvesting and Processing

Processing hybrid seed from the field to the bag requires many operations. Harvesting seed production fields is a critical operation. It should not start until moisture has reached 11–13%, and much care must be taken to prevent excessive damage to the achenes. Rows of the male parent generally are removed before the female rows are harvested to avoid contamination. In some cases, seed producers remove rows of the male parent after pollination. After harvesting, the seed is transported to the seed processing plant where it is cleaned, graded, treated, and bagged. Quality control is required in order to maintain the seed quality certification standards. Seed is stored under adequate conditions of temperature and relative humidity to avoid deterioration of quality. Commercial seed lots are generally treated with a combination of insecticides and fungicides conferring protection against damage and stand loss by early season soil and foliar insects as well as early season diseases, particularly downy mildew.

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