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Abstract	Sunflower is well known as an important oilseed crop and also consumed roasted, as a confectionary and bird feed. The plant has been subjected to the improvement by plant breeders resulting in the *yellow* revolution in many countries. Russian plant breeders have improved the oil content of sunflower seed that converted this crop from a roadside plant to a world famous oilseed crop. The cultivated germplasm retains 50% of genetic diversity present in crop wild relatives. This may be threatened due to worldwide hybrid cultivation which shares common parentage and a source of cytoplasmic male sterility. Therefore, there is a need to use the available genetic diversity within cultivated and wild germplasm to develop pre-breeding lines and elite breeding material with good combining quality. Sunflower breeding involves development of breeding lines suitable for hybrid breeding, diseases, abiotic stress and herbicide resistance. These objectives are fulfilled by recurrent selection for population improvement. Wide crosses were made to transfer cytoplasmic male sterility, diseases, abiotic and *Orobanche* resistance. Moreover, induced mutations were used to create new genetic variability for diseases and herbicide resistance and reduction of plant height. Marker-assisted selection has been validated for rust resistance, downy mildew resistance, and oleic acid content and fertility restorer genes. Transgenic sunflower development could be used to enhance oil content and quality. Sunflower breeding will be greatly facilitated by genomic tools such as CRISPR/Cas and whole genome association mapping.	
Keywords (separated by " - ")	Breeding objectives - Crop wild relatives - Combining ability - Cytoplasmic male sterility - Resistant genes - Stability	

Chapter 16
Breeding Strategies for Sunflower (*Helianthus annuus* L.) Genetic Improvement

Saeed Rauf

Abstract Sunflower is well known as an important oilseed crop and also consumed roasted, as a confectionary and bird feed. The plant has been subjected to the improvement by plant breeders resulting in the *yellow* revolution in many countries. Russian plant breeders have improved the oil content of sunflower seed that converted this crop from a roadside plant to a world famous oilseed crop. The cultivated germplasm retains 50% of genetic diversity present in crop wild relatives. This may be threatened due to worldwide hybrid cultivation which shares common parentage and a source of cytoplasmic male sterility. Therefore, there is a need to use the available genetic diversity within cultivated and wild germplasm to develop pre-breeding lines and elite breeding material with good combining quality. Sunflower breeding involves development of breeding lines suitable for hybrid breeding, diseases, abiotic stress and herbicide resistance. These objectives are fulfilled by recurrent selection for population improvement. Wide crosses were made to transfer cytoplasmic male sterility, diseases, abiotic and *Orobanche* resistance. Moreover, induced mutations were used to create new genetic variability for diseases and herbicide resistance and reduction of plant height. Marker-assisted selection has been validated for rust resistance, downy mildew resistance, and oleic acid content and fertility restorer genes. Transgenic sunflower development could be used to enhance oil content and quality. Sunflower breeding will be greatly facilitated by genomic tools such as CRISPR/Cas and whole genome association mapping.

Keywords Breeding objectives · Crop wild relatives · Combining ability · Cytoplasmic male sterility · Resistant genes · Stability

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16.1 Introduction

Sunflower is the fourth largest seed crop worldwide after the oil palm, soybean and rapeseed (Fig. 16.1). Total sunflower edible oil production was 15.84 million mt (FAO 2014) which represented 10% of the total edible oil production (Fig. 16.1). AUT Sunflower is grown in 72 countries with the Russia being the largest grower in terms of its harvested area followed by Ukraine and Argentina. These three countries contributed about 56% of the total harvested area. World average achene yield was 1806.7 kg ha^{-1}in 2014. Achene yield ha^{-1} showed a growth rate of 2.6% $year^{-1}$ (Fig. 16.2). Total trade value of the sunflower oil was USD 1864.24 billion while the trade value of sunflower seed was USD 705.76 billion in 2016. This represented 17 and 21% share of the total export and import values among the major oilseed crops, respectively, and was the second major oil crop after the palm oil in the total major edible oil trade of the world (FAO 2016).The world average sunflower oil annual per capita consumption of 152 nations was 2.56 kg. Kazakhstan had the highest per capita (17.98 kg) consumption of sunflower oil. Sunflower seed was consumed as snack food in 58 countries, and the consumption was the highest (2.2 kg per capita) in Tanzania (FAO 2013).

This chapter describes the importance of sunflower, germplasm resources, breeding achievements and objectives in sunflower breeding programs as well as breeding methodologies.

There are three types of sunflower i.e. oilseed, confectionary and bird food. The confectionary and bird food sunflower contain high protein content (>40%) and low oil content (≤30%). The confectionary and bird food sunflower are large seeded and

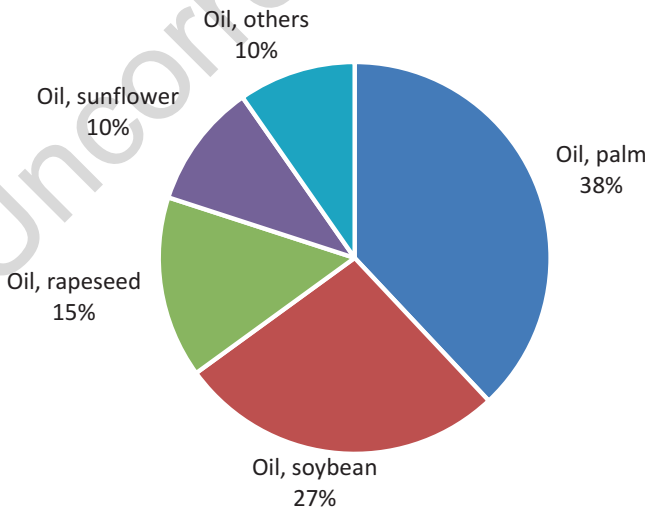


Fig. 16.1 Contribution of various oilseed crops to the total world edible oil production. (Data source FAO (2014). The figure was prepared from public data available at FAO (www.fao.org))

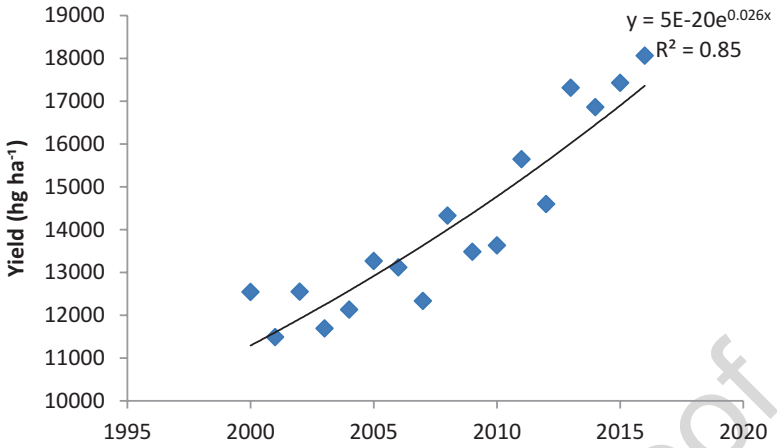


Fig. 16.2 Change in the sunflower achene yield (hg ha^{-1}) over the years. (Data Source: FAO (2016). The figure was prepared from public data available at FAO (www.fao.org))

striped with 100-seed weight greater than 10 g (Fig. 16.3). Oil content types are small seeded and black in color.

Cultivated sunflower plant is determinate with a single floral head called a *capitulum*. The head size range of 6–40 cm in diameter contains two types of flower discs and ray florets. Ray florets are sterile and generally yellow in color but exhibit different colors (crimson red to white) and shades (Fig. 16.4).The plant initiates a floral bud about 40–45 days after planting depending upon the cultivar and growth conditions (Fig. 16.5). The involcural bract (phyllaries) surrounds the floral bud. The number of disc florets range from 100–2000 per head arranged in various rows. Disc florets open from outside to inside rows. Two to four rows open daily and floral anthesis is completed within 5–8 days. Each disc floret is hermaphroditic, comprised of a single inferior ovary, tubular corolla form by the fusion of 5 petals, 5 anthers united to form tube with separate filaments, and upon fertilization, it bears a single large achene enclosing a kernel. The sunflower is a cross-pollinated species, and disc florets are protandrous i.e. male part matures ahead of the female part. The pollen is large, sticky and is carried by insect pollinators which are vital for hybrid seed production. Cross-contamination between breeding lines is prevented by enclosing the heads in net bags or net cages.

Sunflower seed kernels are a favorite snack food, consumed as a roasted and salted product in several countries. Dried seed kernels of sunflower contain 584 calories and 5% water per 100 g. They are a rich source of vitamin E, Vitamin B such as niacin and folate and also contain appreciable amount of magnesium, phosphorous, potassium, selenium and small amounts of iron and zinc. Dry sunflower seed kernels contain the highest amount of folate (227 µg10 0 g^{-1}) as compared with other popular snack nuts such as hazelnut, sesame, pistachio and almonds. Similarly, they are also rich in vitamin E (total tocopherol 36.74 mg10 0 g^{-1}), chlorogenic acid (antioxidant and purported anticarcinogenic 1003.7 mg 100 g^{-1}) and total choline

Fig. 16.3 Diversity in seed coat color of various types of sunflower: (**a**) Black seed oil type sunflower, (**b**) Light brown high seed coat, (**c**) Albino seed for confectionary and animal feed, (**d**) Striped sunflower for roasting. (Photos by Saeed Rauf)

(purported protection against heart diseases 55.1 mg 100 g^{-1}). The range of tocopherol content was 314.5–1024.5 mg kg^{-1} in seed kernels and 562.8–1872.8 mg kg^{-1} in oil (Velasco et al. 2002). Phytosterol content is known to lower low density lipid which ranged from 2100–4540 μg g^{-1} (Vlahakis and Hazebroek 2000). Breads (300 g) enriched with high oleic acid sunflower seed can provide 40% of the daily protein, 90% of copper, 20% of the zinc and 5 times the daily fiber requirement. A handful of sunflower seed consumed daily is reputed to appreciably improve the body requirements for fat, fiber, alpha tocopherols and linoleic and oleic acids.

Fig. 16.4 Diversity in ray and disc floret color of sunflower. (**a**) Crimson red ray floret with brown disc floret, (**b**) Yellow with crimson red pattern ray floret with purple disc floret, (**c**) Yellow wide ray floret with yellow disc floret, (**d**) Yellow narrow ray floret with green disc floret, (**e**) Yellow ray florets with purple disc floret, (**f**) Crimson red with yellow tip ray floret and purple disc florets. (Photos by Saeed Rauf)

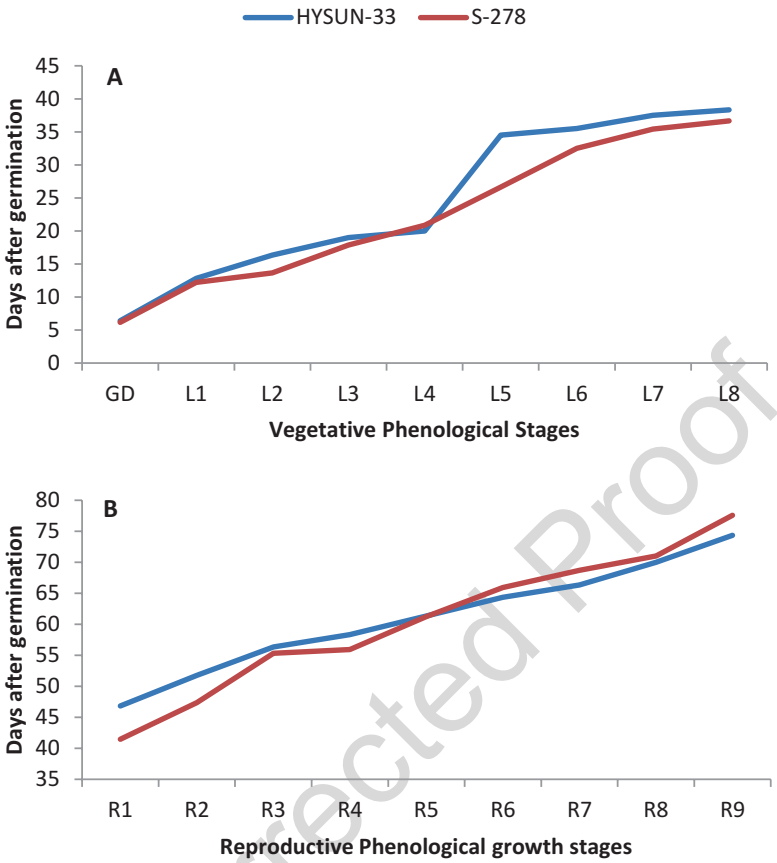


Fig. 16.5 Days to various phenological growth stages. Response during the vegetative stage (**a**) and reproductive stage (**b**) in two popular hybrids of sunflower (*Helianthus annuus* L.) grown at College of Agriculture, University of Sargodha, Pakistan in 2012–2013 growth season (Unpublished data) where *GD* days to germination, *L1–L8* day to 1st leaf to 8th leaf stages and *R1–R9* 1st to 9th reproductive stage. (Source: Saeed Rauf unpublished)

16.2 Germplasm Resources

Sunflower belongs to the genus *Helianthus* which is indigenous to North America. There are about 52 species and 19 subspecies which are widely distributed in central Mexico, the USA and southern Canada. These species have annual (18) and perennial (34) growth habits. The somatic chromosome number of diploid species is 2n = 2x = 34 (Tahara 1915). However, ploidy levels such as tetraploid (2n = 4x = 68) and hexaploid (2n = 6x = 102) have also been observed in various species of *Helianthus*. Among them, all annuals are diploid while polyploidy species are perennials. However, annual diploid species also exist within the genus. Some species occurs in dual ploidy levels such as *H. ciliaris* DC which has both tetraploids and hexaploid, whereas *H. decapetalus* L. has diploid and tetraploid forms.

16.2.1 Extent of Related Species Geographically

Crop wild relatives (CWR) are characterized on the basis of the extent their hybridization with related species. The primary germplasm is comprised of cultivated and wild species of *Helianthus annuus* and winter sunflower, *H. winterii* J.C. Stebbins, while secondary germplasm includes species such as *H. anomalus* S.F. Blake, *H. paradoxus* Heiser, *H. petiolaris* Nutt. and *H. deserticola* Heiser. Secondary species have undergone some degree of genetic differentiation from primary germplasm. Tertiary germplasm varies due to a high degree of genetic and cytological differentiation. The species considered tertiary germplasm are *H. hirsutus* Raf., *H. tuberosus* L. and *H. divericatus* L. These interspecific hybrids required specialized techniques such as embryo rescue for their recovery (Warburton et al. 2017). Differentiation can be accessed through molecular, cytological and morphological bases to characterize species.

The extent of wild species utilization depends on several factors such as ploidy level, growth habit and reproductive barrier. The reproductive barrier occurs through the evolution of any form of reproductive isolation such as prezygotic and postzygotic barriers. In plants, prezygotic barriers are more common than postzygotic, in contrast to animals where postzygotic barriers are more common and hybrid forms have selective disadvantages (Maheshwari and Barbash 2011). Prezygotic barriers include failure of pollen to germinate over the stigma, failure of the pollen tube to grow through the stylar tissue or failure of fertilization. These prezygotic barriers occur due to the divergence between species and adaptability to the particular habitat. Postzygotic barriers include embryo abortion after fertilization, hybrid unviability or sterility. Hybrid sterility is included due to negative interactions between loci. The speed of gene transfer from wild species depends on the ploidy level, the speediest transfer occurs at the diploid level followed by the tetraploid and hexaploid (Alix et al. 2017). The slow transfer of genes occurs due to time-consuming removal of extra chromosome through backcrosses (Jan et al. 2014). The speedy restoration of chromosome to 2n = 34 can also occur through the use of polyploid species as a male parent. The characterization of the wild species is poorly understood and many sister species are not well characterized (Vanzela et al. 2002).

Many *Helianthus* species originated from multiple homoploid hybrid speciation. Multiple homoploid events led to the origin of many species from common ancestral species due to chromosome sorting and rearrangement (Lai et al. 2005). Hybridization between *H. annuus* and *H. petiolaris* followed by the doubling of genome due to unreduced gametes, led to the evolution of three different diploid hybrid species (*H. anomalus*, *H. paradoxus*, *H. deserticola*) (Gross et al. 2003, Fig. 16.6). These species have undergone significant differentiation and adaptation to particular ecological conditions due to transgressive segregation of the chromosomes (Rosenthal et al. 2002). Transgressive segregation led to the appearance of extreme traits which led to the adaptation under extreme environment. For instance, *H. anomalus*, *H. paradoxus* and *H. deserticola* are adapted to sand dunes, salt marshes and high deserts, respectively (Rosenthal et al. 2002). It was noted that

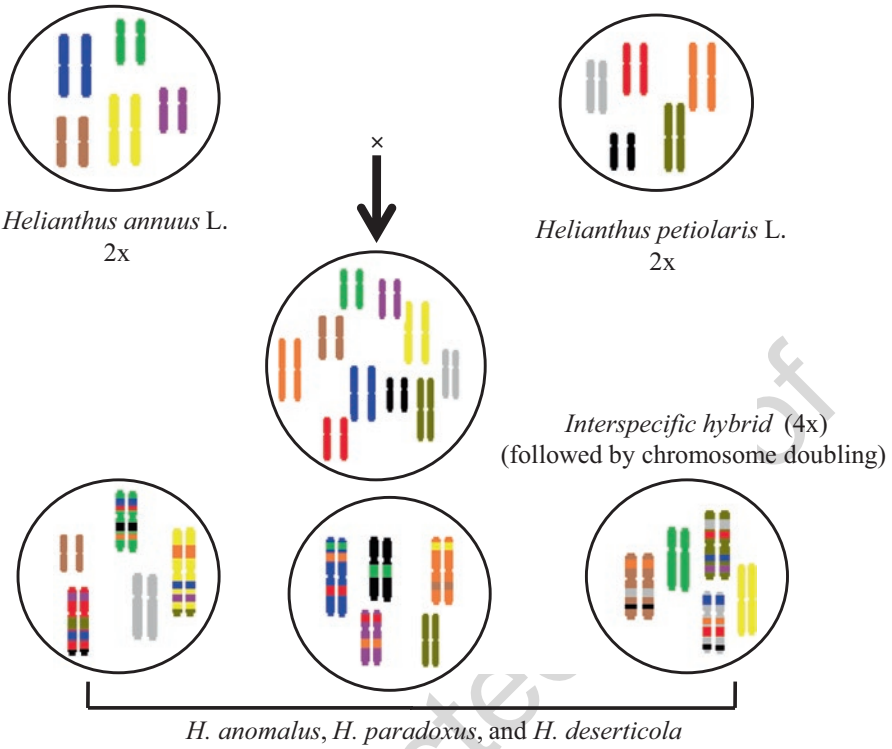


Fig. 16.6 Origin of three homoploid species i.e. *H. anomalus*, *H. paradoxus* and *H. deserticola* due to chromosome sorting and rearrangement. (Figure prepared Saeed Rauf)

these hybrid species were divergent in karyotype and gene order. Gene order differences were observed at 9 to 11 linkage groups due to chromosome sorting and de novo breakage and fusion cycle (Lai et al. 2005). The schematic origin of three species is shown in Fig. 16.6.

Sunflower species grow in a diverse range of habitats i.e. plains, deserts and salt marshes(Fig. 16.7). As a result of their adaptation to assorted ecological conditions, the wild species can be regarded as sanctuaries of new alleles to achieve diverse breeding goals (Kantar et al. 2015). The potential utilization of related species in breeding programs to provide various alleles is well documented (Seiler 1992, 2007a, b) and could be exploited for disease resistance breeding and diversified cytoplasmic sources, as well as drought, heat and salinity resistance. Moreover, these species could also be exploited for the modification of fatty acids and other industrial products. The monetary benefits to the sunflower industry due to wild crop relative contributions of economically-relevant traits have been estimated to be worth than USD 1 billion (Seiler and Marek 2011). The trait of greatest value was cytoplasmic male sterility (PET1) exploited by the sunflower hybrid seed industry from wild species of *Helianthus petiolaris*. Other important traits are disease and insect resistance genes which provide resilience and sustainability to the achene yield (Feng et al. 2009).

AU2

Fig. 16.7 Diversity in canopy and foliage color and adaptations among various species of sunflower. (**a**) *Helianthus petiolaris* (ex situ: Sargodha, Pakistan, Saeed Rauf), (**b**) *H. argophyllus* Torr. & A. Gray (ex situ: Sargodha, Pakistan, Saeed Rauf), (**c**) *H. anomolous* (San Juan County, Utah, SW of Cal Black Airport, Gerald Seiler, permission granted), (**d**) *H. debilis* Nutt. *(*in situ: Florida, USDA, Gerald Seiler, permission granted), (**e**) *H. paradoxus*(in situ: New Mexico, USDA, Gerald Seiler, permission granted), (**f**) *H. niveus* (Benth.) Brandeg. (in situ: California, USDA, Gerald Seiler, permission granted)

16.2.2 Germplasm Collections 158

Germplasm collections safeguard the genetic diversity within species in addition 159
to providing rare alleles for crop improvement. Several germplasm collections 160
have been created, for instance, the USDA-ARS collection of elite sunflower 161
germplasm at the National Plant Germplasm System, North Central Regional Plant 162
Introduction Station, Ames, Iowa in1948. On the other hand a sunflower wild 163
related species collection was established at the USDA-ARS Bushland, Texas sta- 164
tion in 1976. There were over 30 expeditions (Canada, USA, Australia), covering 165
175,000 km, carried out to collect wild sunflower diversity, which is considered as 166
the most updated collections in the world. The USDA collection contains 4087 167
accessions: 1886 cultivated *H. annuus* and 2201 wild, of which 1359 are annual 168
and 842 are perennial species (Seiler and Marek 2011). The collection may help to 169
improve economically-relevant traits related to the yield and quality and also 170

Table 16.1 Worldwide examples of sunflower germplasm resources collections

Institutes	Collection
USDA collection	4087 accessions: 1886 cultivated, 2201 wild accessions
French National Institute for Agricultural Research Toulouse, France	5576 cultivated accessions, >500 wild ecotypes
The Indian Institute of Oilseeds Research, India	3273 accessions: 1200 exotic lines, 97 genetic stocks, 360 inbred lines, 42 wild species, and 154 wild species derivatives, Dudhe and Sujatha (2016)
The Vavilov Research Institute of Plant Industry, Russia	2230 cultivated sunflowers and 550 wild accessions, Gavrilova et al.(2014)
The Oil Crop Research Institute of the Chinese Academy of Agriculture Science Wuhan, China	2813 accessions, predominantly cultivated, Gao et al. (2001)
The Institute of Field and Vegetable Crops Novi Sad, Serbia	Several thousand lines (S. Terzic, personal communication 2016), wild sunflower collection of just over 1000 accessions of 47 wild species, Atlagić and Terzić(2015)

supply biotic and abiotic resistance genes. Moreover, the elite germplasm may help to encourage creation of sunflower breeding programs in many countries. Germplasm collections are given in Table 16.1.

16.2.3 Diversity Present in Primary and Secondary Gene Pools

The genetic diversity in the primary gene pool (*Helianthus annuus*) has been characterized among 433 accessions collected from North America and Europe, along with the 24 wild populations (Mandel et al. 2011). The diversity index was 0.47, compared to the wild of 0.7, showing that cultivated germplasm retained two-thirds of the diversity. A core set of 288 accessions was sufficient to capture 90% of the allelic diversity, while only 12 accessions retained 50% of the allelic diversity showing a narrow base in the primary gene pool (Mandel et al. 2011). *Helianthus argophyllus*, *H. annuus*, *H. petiolaris* and *H. debilis* were crossed with cultivated sunflower to increase the diversity of the cultivated sunflower (Sujatha et al. 2008). Chromosomal pairing between the wild and cultivated sunflower led to the structural rearrangements in lines derived from *H. petiolaris*. The genetic distance of 40 diverse lines and 2 controls was measured using 118 simple sequence repeat (SSR) markers of known map location. A total of 204 alleles were identified and number of alleles per locus was 2–5. A total of 46 distinctive alleles were identified and number of distinctive alleles was highest in *H. petiolaris* derived lines, and the observed PIC value was 0.05–0.575. Pair-wise comparison value was 0.143–0.486 based on the dissimilarity estimate using molecular markers. Results suggest that the wild diploid species are a source of introgression of novel traits, especially from *H. petiolaris*.

Diploid annual and perennial species of sunflower do not cross easily due to the presence of different genomes in the species. Similarly, the genome of wild species is different from the cultivated species. However, annual species are mutually crossable and also with cultivated sunflower. However, sometimes techniques such as in vitro fertilization and embryo rescue are exploited to obtain viable seedlings of hybrids from perennial parental lines. It has been noted that annual wild species have shown karyotypic differences due to translocations and inversions. The diploid annuals have been shown to be susceptible to diseases and thus they are less exploited by the breeders for introgression in cultivated germplasm. However, they could be exploited for introgression of adaptability to abiotic stresses. On the other hand wild perennials have been exploited to introduce disease resistance genes (*Helianthus maximiliani* Schrad., *H. giganteus* L.), Liu et al. (2010) and morphological traits such as oil content (*H. salicifolius* A. Dietr.), Jovanka (2004) modification in cultivated sunflower. However, crossability between the species was poor and requires embryo rescue technique for the recovery of the hybrids.

Wild crop relative collections have contributed to the sunflower industry in several ways. The wild crop relatives have been extensively exploited in breeding programs as a source of resistance to major sunflower diseases i.e. rust, downy mildew, *Verticillium* wilt, powdery mildew, *Phomopsis* stem canker, *Sclerotinia* wilt, charcoal rot, *Phoma* black stem and the parasitic weed broomrape (Seiler 2010). Both horizontal and vertical resistance is known to exist in crop wild relatives. The resistance to all multiple races of rust was high in wild annuals while resistance for all races of powdery mildew was only present in two populations of *Helianthus argophyllus* and *H.debilis* (Jan and Chandler 1985). *Helianthus tuberosus* was useful for resistance to stem infecting disease i.e. *Phomopsis* stem canker, *Phoma* black stem and charcoal rot, while perennial species showed resistance to broomrape.

Germplasm evaluation showed that broomrape resistance and immunity was identified in 7 annuals and 32 perennials, providing breeders a broad genetic base for resistance to new races. (Cristov 2004; Petcu and and Pâcureanu 2011; Seiler and Jan 2014). Perennial species of genus *Helianthus* were resistant, but *H. divaricatus, H. maximiliani* and *H. pauciflorus* Nutt. showed susceptibility to the diseases. Annual wild species *H. anomalus* and *H. agrestis* Pollard were resistant, while *H. debilis* ssp. *cucumerifolius* and *H. exilis* A. Gray showed heterozygosity for the resistance (Fernandez-Martinez et al. 2000a, b).

Among the species, *Helianthus argophyllus* has developed specific phenotypes which help them to adapt under drought; is also known as silver sunflower due to it intense hairiness and thick leaves. The presence of high pubescence and smaller leaf area could help to reflect light and to protect the leaves from the transpiration losses. *Helianthus argophyllus* was the best source of stress resistance genes and used in interspecific hybridization. *Helianthus paradoxus* was utilized as a genetic source of salinity resistance (Skoric2009).

Development of perennial sunflower could benefit sustainable agriculture and remedy agriculture soil degradation. Perennial traits could be transferred to the cultivated type through introgression between the cultivated species *Helianthus tuberosus* and *H. annuus* L. (Kantar et al. 2014). The resulting selected transgres-

sive segregants could have tuber and have sustainable seed yield traits. Tuber traits are positively related with head diameter and seed traits.

16.3 Sunflower Breeding History

Sunflower was domesticated in North America about 4000–5000 years ago (Smith 2006). It was domesticated by Native Americans for its multiple uses such as food, body painting and folk medicine such as treating warts and snake bites, expelling worms and improving eyesight.

The sunflower plant has great aesthetic value and is famous for its peculiar heliotrope movements to track maximum radiation. Wild sunflower is characterized by multiple branches tipped by numerous flowers containing small achenes. Spanish travelers brought this plant to Europe during the fifteenth century from where it was introduced to Russia. Russian academician V.S. Pustovoitat VNIIMK made tremendous improvement in sunflower to make it one of the leading oilseed crops of the world during the first half of the nineteenth century. Sunflower oil content was improved through a modified recurrent selection method called *seed reserve* (Fig. 16.8). This method involves progeny testing and subsequent cross-pollination of selected superior progenies; as a result of this selection sunflower oil content increased from 33 to nearly 43% over three decades of selections (1913–1943). Cultivar Peredovick was released in 1958 with an oil content greater than 50% which was adopted by the rest of the world and became the source of global cultivation. The source of cytoplasmic male sterility in *Helianthus petiolaris* was discovered by P. Leclercq (1969) at INRA in France and a fertility restorer system was identified by Kinman at the USDA (Kinman 1970) which paved the way for exploitation of commercial heterosis which significantly improved the achene yield without sacrificing the seed oil content.

The first attempt to develop a hybrid was carried out by Putt (Vera 2016) at Morden Manitoba, Canada, without the presence of effective male sterility system, by crossing line S37.388RR with commercial cv. Sunrise to develop hybrid cv. Advent which was later discovered as a source of powdery mildew resistance by Vranceanu and Stoenescu (1969) at ICCPT, Romania. Canadian lines 953.88 and 953.102 (progenies from natural crosses with wild sunflower) were supplied to M. Kinman and he subsequently used them with French CMS line selected from the Russian population Cernianka (PI343765), which led to discovery of the restorer gene *Rf1* (Kinman 1970). The first USDA restorer RHA line was obtained by crossing (Peredovik × 953.102) and another line HA-61 came from the cross of (953.88 × Armavir3497). This line was a source of recessive branching and thus suppressed the branching in the sunflower hybrid. The Canadian sunflower team led by Putt also selected a line called CM-303 from Russian open pollinated cultivar which was further selected by USDA sunflower breeders and called HA-89.

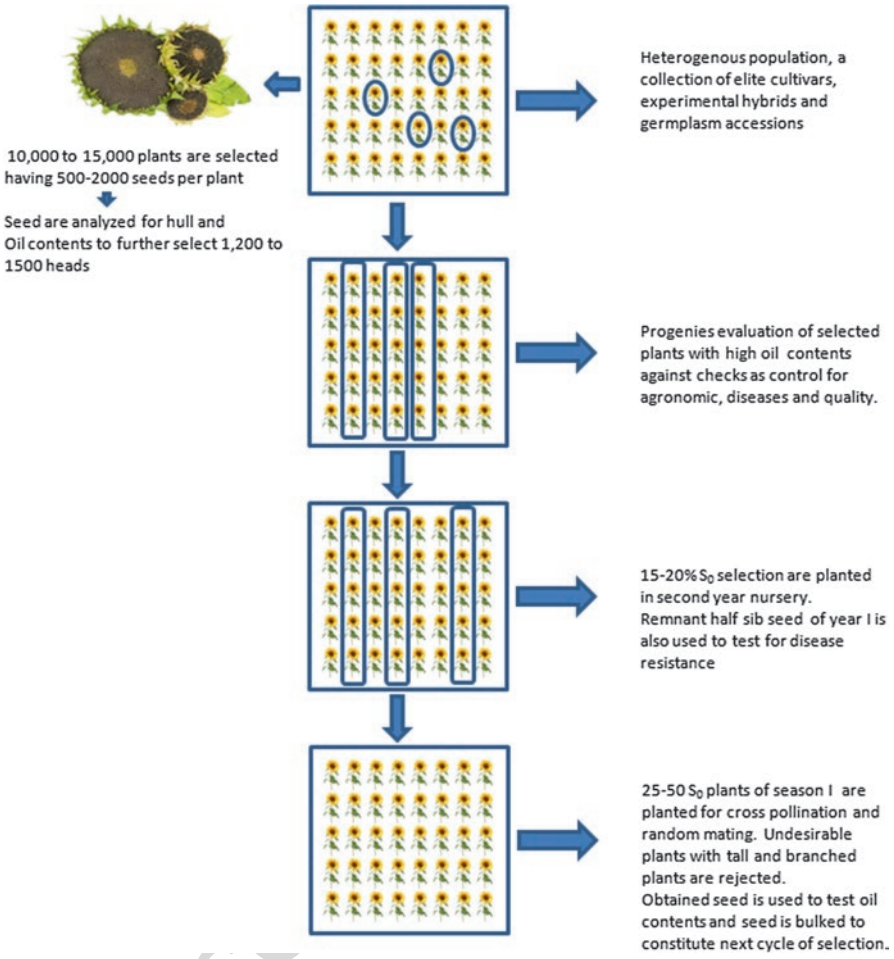


Fig. 16.8 Procedure for Pustovoit's famous *method of reserve* to develop high oil contents progenies. (Photo by Saeed Rauf)

Initially sunflower hybrids had little yield advantage over the open-pollinated cultivars, but hybrids were more uniform in maturity and harvesting. Commercial hybrids had a 30–40% yield advantage and were uniform in maturity. Today, sunflower is the second major field crop after maize being cultivated through hybrid seed. The cultivated hybrid sunflower is non-branching, a large leaf area with a single head (capitulum), which ceases to show heliotropic movement after the initiation of reproductive growth cycle.

16.4 CLEARFIELD Technology to Control Weeds

The sunflower plant is poor competitor with weeds during its early growth stages and broad leaf weeds are a major yield limiting factor due to their competition for light, soil nutrition and moisture. Moreover, weeds act as alternative hosts for the spread of insects and diseases (Pfenning et al. 2008). CLEARFIELD technology has been introduced to introgress imidazolinone herbicide resistance in elite sunflower hybrids. Imidazolinone is a post emergence herbicide for broad leaf weeds. It inhibits the enzyme acetohydroxyl acid synthase (AHAS). Conventional hybrids are sensitive to this herbicide while CLEARFIELD hybrids are known to carry a mutant form of AHAS gene which reduces their sensitivity to the broad leaf herbicide. The source of the resistance is a naturally-occurring mutation in the wild sunflower which was transferred to elite inbred lines by plant breeding methods (Pfenning et al. 2008). CLEARFIELD plus has been introduced to improve the effectiveness of the herbicide-resistant imidazolinone gene in sunflower. The gene was modified through mutation of *Ahas1* and was designated as CLHA-plus or *AhasII-3*. The beneficial effects of the genes were observed such as improved oil content, stability and reliability of herbicide tolerance due to better weed management (Weston et al. 2012).

16.5 Breeding Objectives

16.5.1 Hybrid Breeding

More than 90% of sunflowers are cultivated by hybrid seed. Hybrid crops were found to be superior to open-pollinated and synthetic cultivars due to higher yield potential and uniformity in maturity. The hybrid vigor and heterozygous genetic base of the hybrids allow them to show better yield than synthetic cultivars even under stress condition. The superior performance of hybrids is due to manifestation of heterosis, defined as the superiority of F_1 over the mid parent value or better performance of F_1 over the superior parent or best commercial cultivar. Heterosis is manifested due to genetic divergence of the parents and superior combining ability of both parents, causing the combination of diverse alleles in a single genotype. There is a positive relationship between genetic distance and best parent heterosis (Hladni et al. 2018). In contrast to open-pollinated varieties (OPVs), which only exploited additive gene action for the improvement of plant traits, the performance of a hybrid depends on both additive and non-additive gene action. Some plant traits such as oil content, day to maturity, biotic or abiotic are fixed during inbred line

development through pedigree selection and may be controlled through additive alleles. Allelic or nonallelic interaction may be broken to release additive alleles through recurrent selection. However, grain yield potential of a hybrid is based on over dominance, a genetic phenomenon, in which deviation from mid parents value greatly exceeds both parents.

A single cross hybrid has been developed in sunflower using a cytoplasmic male sterility system in A lines, maintaining it through B lines, and R fertility restorer lines are developed as male lines carrying fertility restorer genes. Morphological traits such as fertility of female lines, synchronization between A and R lines are vital for the development of hybrids. Hybrid seed production in sunflower is done by using cytoplasmic a male sterile female line and a fertility restorer male line at a ratio of 4:2; pollination is facilitated through honey bee hives (2 hives ha^{-1}), Green leaf and Kremen (2006).

16.5.2 Diversification of Cytoplasmic Male Sterility Source

Sunflower belongs to a genus of highly diverse species and thus cytoplasmic male sterility sources could be diversified using these species as a maternal parent (Table 16.1). Cytoplasmic male sterility is alloplasmic, meaning that it originates from a combination of interspecific and intergeneric crosses. The first sources of cytoplasmic male sterility were *Helianthus petiolaris* species which was transferred into sunflower lines through interspecific crossing. CMS-PET-1 has been commercially exploited for hybrid breeding. However, single use of a male sterility source could increase the vulnerability to diseases and insect pest due to a decrease in genetic diversity. Therefore, expansion of male sterility sources is one of the prime objectives of sunflower hybrid breeding. More than 70 male sterility sources have so far been exploited; however, very few of the sources have the potential to achieve commercial success. This may partly be due to the absence of male fertility restoration genes to completely overcome the cytoplasmic male sterility in hybrid breeding. New sources have been developed through mutagenesis, crossing with wild sunflower or its closely-related species and discovered spontaneously in sunflower fields (Christov 1999). Molecular characterization of 22 cytoplasmic male sterility sources of sunflower showed considerable similarity among the sources and could be differentiated in 10 mitochondrial types based on RFLP marker analyses (Horn 2002). Comparison of PET-1 and PET-2 showed variability and rearrangement in the mitochondrial sequences. PET-1 was characterized by the presence of atpA and orfH522 and 16KDA CMS specific proteins whereas PET-2 had two new open reading frames, orf288 and orf231. The orf encode protein of 11.1 KDa and 7.9 KDa, respectively (Horn et al. 2016) (Table 16.2).

Table 16.2 List of cytoplasmic sources for sunflower

Cytoplasm code	Species/Sources	References
CMS-PET-1, CMS-PET2, CMS-PET-4	*Helianthus petiolaris*	Leclercq (1969), Havekes et al. (1991), and Christov (1994)
CMS-Arg-1, Arg-2, Arg-3, Arg-4	*H. argophyllus*	Christov (1990) and Christov (1999)
CMS-GIG1/141,GIG1/477 GIG1/647, CMS-GIG2	*H. giganteus*	Jiuhuan et al. (2015)
ANN-1, ANN-2, ANN-3, ANN-5, ANN-10, ANN-11, ANN-12, ANN-13, ANN-14	Wild sunflower	Marinković and Miller (1995) and Christov (1999)
DEB1	*H. debilis*	Christov (1999)
ANT1/479, ANT1/ 645	*H. texanus*(= *H. annuus* ssp. *texanus*	Chepurnaya et al. (2003)
PRR1, PRH1	*H. praecox* Engelm. & A. Gray	Christov (1999)
PEF1	*H. fallax* (=*H. petiolaris* Nutt. ssp. *fallax* Heiser)	Miller (1996)
cmsMUT7, cmsMUT8, cmsMUT9, cmsMUT10, cmsMUT11, cmsMUT12	Mutagen, gamma rays 70 to 250 Gy	Christov (1999)
CMS 514A	*H. tuberosus*	Liu et al. (2013)
RIG1, RIG-2, RIG-L	*H. rigidus* (Cass.) Desf. (=*H. pauciflorus*)	Christov (1999) and Chepurnaya et al. (2003)
STR-1	*H. strumosus*L. M – 056	Christov (1999)
NEG-1	*H. neglectus* Heiser	Christov (1999)
EXI-2	*H. exilis*	Christov (1999)

16.5.3 Oil Content

Improvement in oil content is a major objective of sunflower breeding. Oil contents are quantitatively inherited traits and genetic variation is affected by additive genes. Oil content ranges from 30 to more than 50% and have shown tremendous improvement due to selection by plant breeders. Historically oil contents were subjected to improvement during the earlier part of nineteenth century through a method of seed reserve proposed by Pustovoit (Rauf et al. 2018) which led to the development of high oil contents lines. Seed morphological traits such as embryo size and testa thickness were important traits affecting oil content. Smaller seed size, with a larger embryo and a thin testa, give higher oil recovery (Rauf et al. 2017). Improvement in oil content occurred due to higher kernel to achene ratio (Pereira et al. 2000). Oil content accumulation is not constant during the grain filling period; the accumulation rate is slower after anthesis, but increases over the time and becomes constant at physiological maturity (Rondanini et al. 2003). Oil concentration is also dependent on the size and concentration of oil bodies. However, high oil content lines have a higher concentration of oil bodies rather than larger oil bodies. Oil bodies are the storehouse of triglycerides containing a single layer of phospholipid which is

kept intact through oleosin and caleosin proteins (Murphy 1990). The range of oil body diameter is 0.65–2.0 μm in various crop species including sunflower. The accumulation rate of oil bodies is negatively affected by protein content. However, oil content is significantly affected by the environment and medium heritability (0.57) of the trait was estimated over multiple environments (Mokrani et al. 2002). Several QTL have been identified on various linkage groups for seed oil content. These QTL have additive to dominant affects and are closely related to domesticated-related traits in sunflower (Burke et al. 2005; Leon et al. 2001).

16.5.4 Broomrape

Orobanche cumanais is an obligatory non-photosynthetic parasitic plant of sunflower. Yield losses occurring in Asia and Europe where its growth is unchecked can reach 80%. Infested plants have stunted plant height and reduced head diameter. Eight races of *O. cumana* (A to H) have been identified with races F, G and H more prevalent in various countries. Race F was identified in the middle 1990s and is prevalent in countries like Turkey, Spain, Romania and Bulgaria. Races G and H were identified in countries around the Black Sea (Martín-Sanz et al. 2016). Genetic factors such as recombination, mutations and high diversity in wild and parasitic populations led to the evolution of new races of broomrape. Resistance against broomrape infestation is the most efficient method to control this parasitic plant. Resistance is simply inherited with a single dominant gene. Wild species have been known to carry resistance genes which could be transferred through interspecific crossing. *Helianthus debilis* carries a dominant resistant gene for G race (Höniges et al. 2008). Dominant genes designated as *O1*, *O2*, *O3*, *O4* and *Or5* have been identified which confer resistance to each of the races, A to E, respectively (Louarn et al. 2016).

16.5.5 Rust Resistance

Rust is a disease caused by *Puccinia helianthi* Schwein and one of the major factors affecting yield and quality of sunflower seed. There are about four species of rust. It is found in the fields of Canada and the USA and can be identified on plants as cinnamon red pustules on the leaves and other parts of the plant. It causes reduction of green leaf area, reducing the overall assimilation of photosynthates and translocation. It also causes reduction of seed weight, size and oil content. Rust thrives in warm and humid conditions and fungicides can be used to control the diseases, but chemical control is expensive and difficult. Therefore incorporating rust resistance in inbred lines is a preferred method to control diseases. Several rust resistance genes (*R1*, *R2*, *R4u*, *R5*, *R12*, *R13a*, *RHAR6*) were identified in various breeding lines, cultivars and restorers. A single source of resistance is not sufficient due to

emergence of virulent races which may defeat single-source resistance in sunflower. Molecular markers have been developed to stake these genes in single genotypes which increases the durability and spectrum of resistance (Paniego et al. 2012). Two genes have been recently identified in HA-R6 and RHA-397 which provide resistance against all virulent races of rust. Pedigree and marker-assisted selection was carried out to develop two inbred lines (HA-R12, HA-R13) which contained multiple rust resistant genes. HA-R12 contained the rust resistance (*R*) genes, *R2* from MC29 (AUS) and *R13a* from HA-R6. HA-R13 contained *R5* from HA-R2 and *R13a* from HA-R6. Application of rust specific markers confirmed that HA-R12 and HA-R13 contained two rust resistance genes in the homozygous condition, with both lines showing high levels of resistance to rust races 336 and 777, which are the most predominant and virulent races (Ma et al. 2016).

16.5.6 Powdery Mildew

Powdery mildew, *Golovinomyces cichoracearum*, disease causes chlorosis, curling and death of leaves. Affected leaves have a lustrous white powdery growth on the upper surface. The disease can cause complete loss of sunflower yield in the absence of resistance or chemical control due to severe infestation. Powdery mildew occurs in the spring season and the infection increases under dry conditions. Intraspecific variability has been known to occur for resistance to this disease under artificial screening. Artificial infestation is generally carried out by spraying a suspension of inoculum along with 1% sucrose solution. A screening trial containing 120 accessions showed that only 2 restorer lines were resistant and 48 were medium resistant (Kulkarni et al. 2015). The screened accessions may be used to transfer resistant genes into the elite breeding lines with superior combining ability or may be directly exploited in hybrid breeding.

16.5.7 Downy Mildew

Downy mildew is one of the major diseases of sunflower caused by *Plasmopara halstedi* and found on all the habitable continents except Australia. A total of 36 pathotypes of downy mildew have been isolated in various studies, whereas more than 20 major genes (Pl_1–Pl_{21}) and (*PlArg*, Pl_{PMI}) have been identified as source of resistance. Thirteen genes (Pl_1, Pl_2, Pl_5 – Pl_8, Pl_{17}–$Pl_{21}$and Pl_{Arg}) have been mapped on various linkage groups (LG1, 2, 4, 8, 13) (Mirzahosein-Tabrizi 2017). Most of resistant genes were identified in wild sunflower, whereas *Pl5* was identified in *Helianthus tuberosus* and Pl_7in *H. praecox*. Resistant genes Pl_1 and Pl_2 have been extensively used in resistance breeding programs against races 100 and 300 of the

pathogen and are typical examples of vertical resistance. Resistant genes may not be effective due to the evolution of new races of pathogens; therefore, diversification of resistant sources is one of the major objectives of disease-resistant breeding. An example of genes Pl_6 and Pl_7 which were extensively used in French breeding lines, failed to provide resistance against a new race of pathogen (304) and disease infestation thereby increased to 88% in 2002, which had been less than 1% in 1989 (Mestries et al. 2004).The Pl_{Arg} and Pl_8 genes originate from *H. argophyllus* which is resistant against four tested races (Dussle et al. 2004). The Pl_{13} gene was found resistant to 13 races of downy mildew (Mulpuri et al. 2007). Gene pyramiding could be done to incorporate multiple resistant genes from various sources. A study showed that monoculture of a single hybrid with single resistant genes led to the loss of efficient resistance after 3 years and increased the vulnerability of the sunflower crop to downy mildew where a combination of different resistant genes and genes alternation provided long-term solution for the management and control of the downy mildew (De Labrouhe et al. 2010).

16.5.8 Drought

Drought is a major production constraint of sunflower, causing significant yield losses around the world. Drought is the phenomenon that occur sat the highest magnitude among all types of stresses (Rauf et al. 2016). Drought stress adversely effects the photosynthate assimilation and mobilization within plants due to closure of stomata, and a reduced photosynthesis process. Genetic variation among the breeding lines has been observed within sunflower germplasm (Khalil et al. 2016; Rauf 2008; Rauf and Sadaqat 2008; Rauf et al. 2009). Wild species are known to carry drought-resistant related traits. For instance, *Helianthu sargophyllus* has traits such as cuticular wax, intense leaf hairiness and small leaf area (Hussian et al. 2017, 2018). These traits increase the chances of survival of plants under drought stress and have higher water use efficiency due to lower water losses during transpiration. Moreover, intense hairiness is related to higher radiation reflection and repels sucking pests, thus plants have a higher stay-green trait. Cuticular wax has medium heritability and thus selection was effective in F_2 generation to develop drought resistant F_3 plant progenies. Silver canopy color marker was used to select plant with high cuticular wax and intense hairiness. Canopy temperature depression (CTD) was also effective in selecting plant progenies having higher transpiration under drought stress. Canopy temperature depression was an index of transpiration cooling and plants with higher ability for CTD with reference to air temperature had longer root length to explore water from deeper in the soil profile and such genotypes had better production under drought stress. CTD has practical utilization in a plant breeding program, and good heritability for selection in segregating generations for establishment of breeding lines (Rauf et al. 2016).

16.5.9 Heat Stress

Global warming due to emission of greenhouse gases into the atmosphere has caused a rapid rise in air temperatures (Kalyar et al. 2014). Higher temperatures causes increased leaf senescence, early maturity or premature phenological development due to higher accumulation of heat units in plants (Kalyar et al. 2013a, b). It causes higher gematophytic sterility and reduces grain filling. It also accelerates the impact of other stresses such as water and salinity. Sunflower showed the highest growth at 27 °C and temperatures higher than 30 °C was not known to induce temperature stress (Kalyar et al. 2014). Plant phenological traits such as downward head position and erect leaves reduced pollen sterility and lower canopy temperature. Both traits are selectable in segregating population and have medium realized heritability (Kalyar et al. 2013a, b).

16.5.10 Oil Quality Traits

16.5.10.1 Tocopherols and Sterols

Tocopherols are an important component of sunflower seed and human health as they impart antioxidant activity. They have a range of 314.5–1024.5 mg/kg in seed and 562.8–1872.8 mg/kg in sunflower oil (Velasco et al. 2002). Tocopherols have four derivatives, alpha-tocopherol being 90% of the four types (Fernández-Martínez et al. 2007). A variant of genes such as tph_1 (50% α- and 50% β-tocopherol), tph_2 (0%–5% α- and 95%–100% γ-tocopherol), and tph_1tph_2 (8%–40% α-, 0%–25% β-, 25%–84% γ- and 8%–50% δ-tocopherol) produces variable quantity of four derivative of tocopherols (Škoric et al. 2008). Tocopehrol content of sunflower lines was modified through mutation breeding; for example IAST-1 and IAST-540 had 95% of gamma tocopherol (95%). Genetic recombination and transgressive segregation produced breeding lines such as LG-15 (high 30–40% beta tocopherol) and LG-17 (> 90% high gamma tocopherol) (Velasco and Fernández-Martínez 2003).

Phytosterols are known for their antioxidant properties and role in reduction of low density lipids and thus their higher concentration is desirable for human health (Roche et al. 2010). Sunflower elite breeding material contains a high concentration of phytosterol content, which are concentrated in the embryo (72%) (Roche et al. 2010). Sunflower oil contains about 2100–4540 μg g^{-1} of phytosterol (Vlahakis and Hazebroek 2000). Wild germplasm contains1017–4308 mg per kg, while campesterol (5.1–16.3%), stigmasterol (3.1–23.9%), beta-sitosterol (35.1–72.3%), delta-5-avenasterol (1.9–20.5%), delta-7-stigmastenol (1.1–20.3%), and delta-7-avenasterol (0.3–10.6%) (Fernández-Cuesta et al. 2014). Selection for higher phytosterol contents led to the development of breeding line IASP-18 having a two-fold higher concentration than parental lines (Velasco et al. 2014). Both tocopherols and phytosterols have a polygenic mood of inheritance and are significantly affected by the environment (Merah et al. 2012).

16.5.10.2 High to Mid Oleic Acid Sunflower

Traditionally sunflower edible oil is rich in two major fatty acids: linoleic 18:2 andoleic acid 18:1). The concentration of linoleic acid ranges from 55–69% in traditional non-oleic acid types. Linoleic is a major polyunsaturated omega 6- fatty acid which is known to have health benefits by lowering serum cholesterol levels. However, sunflower oil rich in linoleic acid degrades under high temperature and produces toxic oxidants or radicals. Mutation breeding was used to produce mid to high oleic acid content sunflower genotypes. The oleic acid content in mutants range is 60–85%. The most useful source of high oleic acid content developed is through exposure to the chemical mutagen Pervenent (Soldatov 1976). However, a commercial cultivar named NUSUN was released in USA with mid oleic acid contents. Pervenent has been extensively used as a parent in backcross programs for development of high oleic acid content cultivars (León 2013a, b). However, high oleic acid cultivars have low yield potential and only express under a warm environment (Smith et al. 2007). High oleic acid content genotypes were dominant over low oleic acid content and are controlled by the genotype of the embryo (Fernandez-Martinez et al. 1989).

16.5.10.3 High Stearic Acid Sunflower

High stearic acid content is desirable for the production of margarine and vegetable fat for deep frying. Stearic acid (18:0) has neutral affects over the accumulation of low density lipids in comparison to palmitic acid which is known to induce cardiovascular diseases. Therefore, increasing stearic acid content at the expense of palmitic or linoleic acid is desirable for the production of high quality industrial oil. Mutagens such as X-ray have been used to modify the fatty acid profile of sunflower oil. Mutant line CAS-12 has been selected with 55% stearic acid contents and 5% linoleic acid contents (Fernández-Martínez et al. 1997). Similarly, lines such as CAS-29 and CAS-30 had 24.9% and 17.4% stearic acid, respectfully (Fernández-Moya et al. 2005). Two genes (*es1es1* and *es2es2*) were collectively known to produce high stearic acid content in sunflower (Fernández-Moya et al. 2005); medium stearic acid lines had a single recessive gene (Perez-Vich et al. 2004).

16.5.10.4 Sunflower Meal Quality

Sunflower seed is crushed to obtain oil; seed meal is a by-product which can be fed to animals, birds/poultry or may be directly consumed by humans in confectionary and baking products. The comparison of conventional sunflower hybrids between various species for seed meal shows that sunflower meal (20% crude protein) contained lower proportion of protein percentage when compared with other species such as cotton (42%) and soybean (50%). Therefore, there is a need to improve the

protein content at the expense of polysaccharides in sunflower seed, which may increase the value of hull contents. Ease of hulling by reducing the fiber contents not only enhances the protein content but may also have positive impact over oil crushing.

Studies have shown variation in protein and oil contents of sunflower elite germplasm. The maximum whole seed protein content identified in sunflower was 35–50% (Warburton et al. 2017). The variation in protein content was due to hull content. Thus protein content may be improved at the expense of reduction in fiber content and improvement of hull content. Moreover, variation within sunflower germplasm was observed for anti-nutritional components such as chlarogenic acid. High protein content was dependent over kernel-to-hull ratio and reduced fiber content. A decrease in fiber content increases the digestibility of the hull. Ease of hull removal is also an important criterion in the evaluation of confectionary sunflower. Both traits were known to be controlled by high heritability.

Improving the sunflower meal quality is one of the major breeding objectives, meal quality depends on reducing the fiber content, antinutritional factors such as chlorogenic acid, phytic acid and improving the protein content. Phytic acid is a chelating agent that binds with metallic ions and reduces the availability of the Ca++, Mg++ and Zn ions for animals. The total concentration of phytic acid in sunflower meal is 4.5%; it may be decreased to improve the meal quality. Phytic acid also makes complexes with amino acid making them unavailable to the non-ruminant animals. Improving the nutritional value of sunflower meal by enhancing the protein content is also an important breeding objective of sunflower. Sunflower meal carries all the essential amino acid contents except lysine, which may be improved in sunflower meal by exploiting the initial variation within elite or breeding lines. Phenolic compounds such as chlorogenic and caffeic acids interact with amino acids and denature the proteins and inhibit the functioning of enzymes in animals and their concentration needs to be reduced in sunflower.

16.6 Breeding Methods

16.6.1 Conventional Breeding Procedures

Sunflower is a cross-pollinated species and all breeding methods of cross-pollinated species such as recurrent selection methods are applicable for population improvement programs. However, the pure-line selection method is applicable for the development of inbred lines with an additional step of combining ability analysis of the inbred lines. The backcross method is used to introgress disease resistant or monogenictraits.

16.6.2 Inbred Line Development

Superior inbred lines are created by crossing appropriate breeding lines such as disease resistant or drought tolerant inbred lines with highly fertile, high oil content and early maturing lines. Disease resistant or drought tolerant lines are generally developed from interspecific crosses which have poor agronomic characteristics such as high plant height, low oil contents and late maturing (Hussain et al. 2017; Shehbaz et al. 2018). Therefore, they could not be directly exploited in hybrid breeding programs. Resistant genes may be transferred to elite lines through backcross methods or segregating populations may be developed for the pedigree selection in subsequent generations (Shehbaz et al. 2018). Pedigree selection is generally carried out for traits having high additive genetic variance (Kalyar et al. 2013a). Heritability in a narrow sense or realized heritability are indicators of selection response. Oil quality traits such as tocopherol and oleic acid contents have been transferred in elite germplasm through backcross schemes (Jonic et al. 2000). Traits such as oil content may be subjected to the pedigree selection for the development of high oil contents inbred lines. Generally, five to six rounds of selection (F2–F6) are sufficient for improvement and fixation of characteristics in inbred lines. Traits related to heat resistance such as downward head orientation at the time of anthesis have been used as a selection marker for the development of heat-resistant inbred lines (Kalyar et al. 2013a). These selections led to the development of some superior heat-resistant hybrids (Khan et al. 2017). Morphological traits such as reduced leaf area and cuticular wax have been used as marker traits for the selection of drought tolerant inbred lines in segregating generations (Hussain et al. 2017, 2018).

16.6.3 Combining Ability Analysis

Combining ability analysis is an important step in the evaluation of sunflower breeding lines for their performance in hybrid breeding. Combining ability is the ability of a breeding line to produce superior progeny upon crossing with testers. A tester may be low performing, if the aim is to uncover deleterious or recessive alleles carried by the female lines (Kalyar et al. 2013b). However, superior lines may be crossed to determine the best specific combiners, or cross combination, which can be used for the development of high-performing hybrids (Khan et al. 2018). Plants selected within F_2 and F_3 generations were selected on the basis of canopy temperature depression, canopy orientation and were crossed with randomly-selected plants to test their general combining ability (Kalayar et al. Kalyar et al. 2013a, b). A general combining ability test was also useful to uncover recessive lethal alleles within selected plants during early segregating generation (Kalyar et al. 2013a).Line × tester or diallel mating designs have been used to determine the general and specific combining ability of the inbred lines (Turkec and Goksoy 2006). Crossing of elite CMS lines with restorers led to the development of single cross hybrids and to the identification of superior combiners (Khan et al. 2018; Turkec and Goksoy 2006).

16.6.4 In Vitro Techniques

Sunflower inbred line development requires 6 cycles of self-pollination, and 3–4 years to achieve homozygosity. It takes an additional year to test combining ability of the developed homozygous lines. In vitro (anther or ovular culture) or in vivo (doubled haploid inducer lines) methods of haploid line development can reduce the time required to achieve homozygosity. Once the protocol for the development of haploid line is optimized, the required homozygosity can be achieved in a single year. The anthers collected between diad and tetrad stages were found more responsive for the haploid induction in sunflower. Anthers pretreated with high temperature treatment (35 °C) for 12 days were found more successful. Generally half-strength MS medium supplemented with Morel and Wetmore Vitamins with B-12 and a mixture of amino acids, 120 g L^{-1}glucose, pH 5.9 plus 0.5 g L^{-1} naphthalene acetic acid (NAA) and benzylaminopurine(BAP) were considered for haploid plant regeneration from anther culture (Mezzarobba and Jonard 1986).

A sunflower embryo rescue technique has been used to excise interspecific embryos and to culture them over the growth media after excision from the developing embryo. Seven-day-old embryos (*Helianthus annuus* × *H. mollis* Lam.) were excised from mother plants and inoculated over the Murashige and skoog (MS) media (Faure et al. 2002). In vitro multiplication through nodal culture of interspecific hybrid (*H. annuus* × *H. simulans* E. Watson) was done on MS media supplemented with 0.5 mg L^{-1}benzyladenine (Prabakaran and Sujatha 2004). Immature embryo culture was done to reduce the life cycle and subsequent generation advancement of the crosses. Embryos were excised after 10 days post pollination and inoculated over simple MS media. A majority of the cultured embryo were regenerated into vigorous seedlings with 3–6 leaves (Dagustu et al. 2010). In vitro screening for drought tolerance was carried out on one-half strength MS media supplemented with osmotica 5% polyethylene glycol (PEG-8000) to reduce the osmotic potential of the media (Khalil et al. 2016). Amphidiploid between cultivated and perennial wild sunflower was regenerated through somatic embryogenesis (Fu et al. 2017).

Somatic embryogenesis was optimized using various formulations and concentrations of media and growth regulators along with immature embryo size (Sujatha and Prabakaran 2001). The highest induction of somatic embryogenesis occurred at Gamborg basal salt media(120–210 g L^{-1}) sucrose, 0.8–1.0% agar, smaller-sized embryos (0.5–2 mm) and at an incubation temperature of 28–32 °C. Growth regulators such as 2,4-D promoted direct embryogenesis, BA+NAA facilitated formation of single/multiple shoots while there was no response on 2,4-D + kinetin supplemented medium (Sujatha and Prabakaran 2001).

16.6.5 Somatic Hybridization

Protoplast fusion is a novel technique of fusing genetically incompatible species to produced somatic hybrids. Species that differ in their ploidy levels, growth habit (perennial) and nonsynchronous due to photoperiod sensitivity can be combined through protoplast fusion. However, this technique is itself very delicate and requires a very high degree of expertise, technology and optimization of the protocols for the isolation, fusion of protoplast and finally regeneration of complete plantlets. There are two kinds of fusion (symmetrical, asymmetrical). Asymmetrical fusion induces few chromosomes from the donor species and alien addition lines are constituted as a result of protoplast fusion while symmetrical fusion induces complete sets of chromosomes from donor species (Binsfeld et al. 2000). Generally, callus is induced by invitro culturing over growth media. The obtained callus is plasmolysed and then cultured for enzymatic action to remove the cell wall. The obtained protoplast is filtered and centrifuged and then treated with a growth retardant to inhibit further cell division. The protoplast is fused by passing high voltage current or using polyethylene glycol. Fused protoplast is selected by markers and cultured on suitable media for regeneration.

There are several reports of the sunflower protoplast fusion between cultivated and wild species, and procedures may be followed in other species (Binsfeld et al. 2000; Krasnyanski and Menczel 1995). Protoplast of *Helianthus annuus* and *H. giganteus* was fused by using polyethylene glycol and treated with iodoacetic acid to inhibit the protoplast division before fusion (Krasnyanski and Menczel 1995). The fused protoplast was cultured over V-KM medium containing BAP and NAA acid and embryogenic calli was cultured over the MS media. Regenerated plants were intermediate between the two species and annual growth habit was dominant in hybrids (Krasnyanski and Menczel 1995). Asymmetric hybrids were obtained between cultivated and perennial species of sunflower through polyethylene glycol treatment (Binsfeld et al. 2000). Herbicides amiprophos-methyl or oryzalin were used to induce micronuclei of the perennial sunflower before fusion. The subdiploid microplast was isolated by centrifugation and filtration over nylon (Binsfeld et al. 2000). Molecular markers were used to identify hybrids and confirmed by chromosome counting. The asymmetric hybrids had 2–8 extra chromosome. Protoplast fusion of *H. annuus* × *H. maximiliani* was carried out to transfer *Sclerotinia sclerotiorum* resistance from the wild species due to poor crossability of the two species in the field. The fused protoplast was embedded in the agrose droplet, developed microcalli and was released from agrose and cultured over shoot regeneration media supplemented with 2.2 mg l^{-1} BAP and 0.01 mg l^{-1} NAA (Taski-Ajdukovic et al. 2006). Protoplast divisions i.e. total division or symmetric division was under genetic control and showed heritability of about 0.87 and 0.89, respectively. QTL analysis showed that important genes encoding traits such as somatic embryogenesis and protoplast divisions were located on linkage group I, XV and XVII (Berrios et al. 2000).

16.6.6 Mutation Breeding

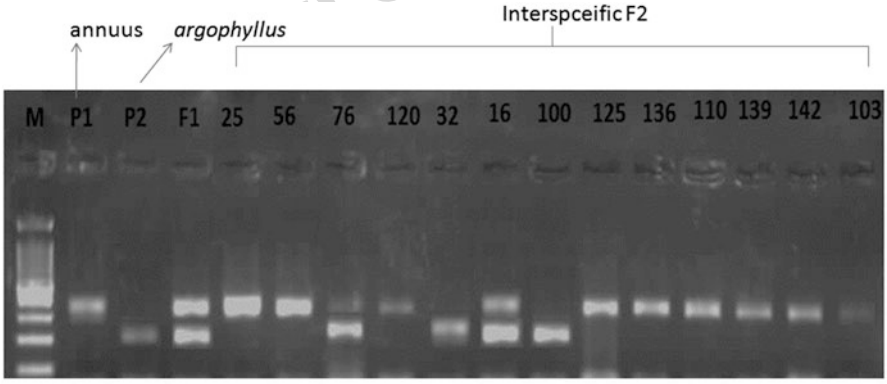
Induced mutation breeding has been used to improve sunflower for several economically-relevant traits such as oil content, nonbranching habit, altered fatty acids, dwarf growth habit, days to flowering, cytoplasmic male sterility, disease resistance and herbicide resistance (Cvejić et al. 2011; Dimitrijevic and Horn 2018). AU4 Herbicide tolerant sunflower CLEARFIELD PLUS was developed by selecting plant resistant to imidazolodine (post emergence herbicide) in the M2 population developed through the exposure to ethyl methane sulfonate (EMS). EMS was also used to produce high oleic acid plant genotype Pervenant. Similarly, high stearic acid and mid oleic acid lines were also developed by exposure to the chemical mutagen (Rauf et al. 2017).

Several cyctoplasmic male sterility sources were developed by gamma ray treatment doses 70–225 Gy (Christov 1999). Plants were irradiated with gamma rays (150–165 Gy) and EMS (0.015 mol dm^{-3}) to select resistant plant against *Alternaria* leaf spot (de Oliveira et al. 2004). Inbred lines were treated with physical and chemical mutagens and selection was carried out in M2 and M3 generations. Several mutant lines, e.g. M6, were developed for valuable traits such as high oil content, dwarf and nonbranching breeding lines (Cvejić et al. 2011). Change in ray petal color and dwarf plant size has been obtained when the plants were subjected to mutagens (Vasko and Kyrychenko 2016). A mutant line R 12003 having high oil contents, and resistant to *Orabanche*,was obtained by subjecting immature zygotic embryo to ultrasound treatment (Encheva et al. 2012). Mutation breeding was augmented by advanced molecular techniques such as (TILLING) targeted local lesion in the genome to identify key gene related to fatty acid biosynthesis (Sabetta et al. 2011). It was also used to identify single nucleotide polymorphism in genes such as *Fat4* and *SAD* (Kumar et al. 2013).

16.6.7 Marker-Assisted Selection

Marker-assisted selection (MAS) has been done to incorporate valuable traits in sunflower breeding such as disease resistance, herbicide resistance, oleic acid and male fertility restorer genes. Marker-assisted selection was used to reduce linkage drags and gene pyramiding of various resistant genes. Markers also provide a cost-effective way of selection in highly-laborious traits such as fatty acids i.e. oleic acid. Marker NI-3F/N2-IR was used to amplify the *A-12* gene (oleatedestaure) which has been validated under various genetic backgrounds (Nagrantha et al. 2011; Tilak et al. 2018). High oleic acid was dominant over high linoleic acid and was efficiently detected by the marker F4-R1 (Dimitrijević et al. 2017). Disease resistant genes of downy mildew and rust resistance have been assigned to their respective linkage groups. The marker related to resistant breeding and oleic acid content have been validated in various genetic backgrounds (Imerovski et al. 2013;

Mirzahosein-Tabrizi 2017; Mulpuri et al. 2009; Qi et al. 2011). The presence of closely-linked markers could help to increase the selection efficiency and development of the sunflower genotype with high oil content with betteroil quality (García-Moreno et al. 2006). Molecular markers have also been exploited to confirm interspecific crosses and to reduce linkage drags in segregating generations(Rauf 2018; Fig. 16.9).ORS-728 amplified two bands (250 and 350 bp in parents (P.I. 1806, *Helianthus argophyllus* species and B-124, *H. annuus*), respectively (Fig. 16.10). The band segregating ratios in F_2 plants showed that bands were present in 13 plants and 13 plants had B_{350} band while 24 plants showed heterozygous band A_{250}/B_{350}. The plants showing homozygous genotypes as depicted by marker ORS-728 were determined for oil and oleic acid contents. Multiple regression equations showed significant ($P \leq 0.05$) dependence of phenotypic (oil and oleic acid) and marker data. On the basis of marker ORS-728, plants were grouped into two types. The plants in one group were genotyped as A_{250} while the other group was genotyped as B_{350}. The oleic acid and oil contents of both group was averaged and is shown in Fig. 16.10. The grouping showed significant differences for oleic acid and oil content. The plants carrying marker A_{250} showed oleic acid (39.07%) and oil content (32.05%). The plants in group A showed about 18 and 13% increase in the oleic acid and oil content than plant in group B (Fig. 16.10). These results showed that marker ORS-728 was effective in selection for high oleic acid and oil content.



Primers	Repeat Motif	Forward	Reverse	Size
ORS 728	(AG)7	CTCCATAGCAACCACCTG AAA	CCAAACTCTGAATGAT ACTTGTGAC	317

Fig. 16.9 Polymorphism revealed by the marker ORS 728 in an interspecific population generated by crossing *Helianuthus argophyllus* and *H. annuus* . P1 = *H. argophyllus*P2 = *H. annuus*. (Source: Saeed Rauf (2018) unpublished data)

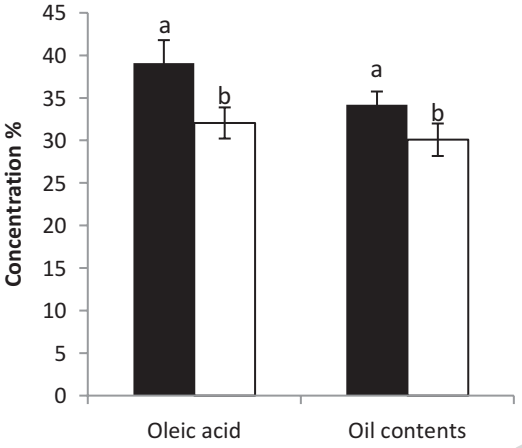


Fig. 16.10 Response of oleic acid and oil contents (%) selected on the basis of polymorphism generated by primer ORS-728, where bars showed two groups of the plants selected on the basis markers generated by primer ORS-728. The black bars indicated significant increase in oleic acid and oil contents due to selection through molecular markers. (Source: Saeed Rauf (2018) unpublished data)

16.6.8 Transgenic Sunflower

Improvement of sunflower through conventional methods is slow, while recombinant DNA technology offers novel and rapid ways to address crop issues such as herbicide tolerance, insect and disease resistance. However, sunflower has been known to be a highly recalcitrant species and difficult to regenerate after delivery of genes through *Agrobacetrium*-mediated transformation (Radonic et al. 2008). A protocol for the transformation of sunflower using microprojectile bombardment, in combination with *Agrobacterium tumeficians*, was optimized which regenerated 7% transgenic plants (Knittel et al. 1994).Insect resistance mediated by*Cry* genes has been experimentally introduced in sunflower (Cantamutto and Poverene 2007). Antifungal genes have been used to transform sunflower to induce disease resistance (Radonic et al. 2008). These genes encode cell wall degradation enzymes (glucanase, chitinase), osmotin and ribosome inhibitor proteins. The wheat oxalate oxidase gene was integrated in sunflower inbred lines and hybrids and is known to enhance *Sclerotinia* head rot resistance (Scelonge et al. 2000). A patent has been granted to genetically transform sunflower for latex production. The gene encoding cis-prenyltransferase was used to transform sunflower for production of latex (Hallan and Keiper-Hrynko 2007). There is also great potential to induce long chain fatty acids such as decosahexaenoic acid (DHA) and ecosapentaenoic acid (EPA) in edible oil of sunflower, which may increase its medicinal and industrial value (Rauf et al. 2017).

The release of transgenic sunflowers, especially for weedy traits such as resistance to herbicides, diseases and insects has ecological consequences. This is of concern particularly where wild populations grow in close proximity to cultivated fields, as natural gene flow occurs rapidly between both types of sunflower (Cantamutto and Poverene 2007; Gutierrez et al. 2010; Presotto et al. 2012).

16.7 Conclusions and Prospects

Sunflower is an important oilseed crop which is one of the diverse species of the genus *Helianthus*. Oil content was appreciably increased as a result of selection by plant breeders during first half of the previous century, which made it one of the popular oilseed crops for consumers. Modification of the fatty acids resulted in the development of mid to high oleic acid which is better suited for the deep frying. Moreover, development of high stearic sunflower lines has provided new opportunities for making margarine and to produce saturated vegetable fat without harmful industrial processing, such as transesterification. Biotic and abiotic stresses are major yield-limiting factors of sunflower. Genetic variation existing among elite and wild germplasm and introgression of resistant genes was successfully carried out in elite germplasm. Hybrid breeding is used to manipulate heterosis and to increase grain yield. Development of elite breeding lines with superior combining ability is one of the prime breeding objectives of sunflower. In order to expand genetic diversity, cytoplasmic male sterility sources have been expanded which could be used to develop hybrids from novel sources of cytoplasmic male sterility and fertility restorer lines. Mutation breeding has been extensively used to achieve breeding objectives such as dwarf breeding lines, herbicide resistance, oleic acid content, stearic acid content, tocopherols and phytosterols. Marker-assisted selection has been carried out for disease resistance, *Orobanche* resistance and oleic acid content and markers for these traits have been validated in various backgrounds. Transgenic development in sunflower is ecologically complicated by concerns of potential gene escape into closely-related wild sunflower species. Sunflower breeding will be greatly facilitated by new molecular techniques such as whole genome association mapping and genome editing through clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR/Cas9) technology. Whole genome association mapping could help to tag key genomic regions of breeding interest in wild and cultivated sunflower germplasm and their possible transfer in elite breeding lines with minimum linkage drags. Moreover, CRISPR/Cas9 would help in knocking down the undesirable genes such as disease susceptibility and late crop maturity. Seed and oil quality could be enhanced by knocking down the linoeleic acid and high fiber encoding genes.

Appendices

Appendix I: Research Institutes Relevant to Sunflower

Institution	Specialization and research activities	Website
All Russian Research Institute of Oil Crops, VNIIMK, Russia	Seed production, varietal development and testing, equipment for oil quality analyses, crop husbandry techniques	http://en.vniimk.ru/about/
Trakya Agricultural Research Institute, Turkey	Development of herbicide resistant hybrids	https://ttae.academia.edu/Departments/Sunflower/Documents
INRA Toulouse, France	Genetic resource of wild and cultivated sunflower, disease resistant breeding, genetic resistant against the broomrape	http://www.toulouse.inra.fr/en/contents/list/2519/inra_all/(word)/sunflower/(iLimit)/5/(type)/inra_actualite
Oilseed Research Institute, Faisalabad	Sunflower hybrid development for high yield and oil quality	https://aari.punjab.gov.pk/faqs_ori
Institute of Field and Vegetable Crops, Serbia	Development of sunflower hybrids for high yield potential, high oleic acid, herbicide resistance and confectionary purpose	http://www.nsseme.com/en/products/?opt=oilcrops&cat=products
National Agriculture Technology Institute	Genetic resource and wild species, resistance against biotic stress and herbicide	https://inta.gob.ar/documentos/argentina-national-institute-of-agricultural-technology-inta
Sunflower and Plant Biology Research, Fargo, North Dakota, USA	Genetic enhancement of yield and tolerance to biotic stress, novel weed management solutions	https://www.ars.usda.gov/plains-area/fargo-nd/rrvarc/sun/
Indian Institute of oilseed Research India	Seed production, varietal development and testing, crop husbandry techniques	http://www.icar-iior.org.in/index.php/aicrp-centres/sunflower#
Institute for sustainable agriculture	Development of high quality sunflower genotype, mutation breeding	http://www.ias.csic.es/en/
Directorate of oilseed Research India	Germplasm resources, tissue culture, molecular genetics, disease resistant	http://icar-iior.org.in/index.php/component/content/frontpage
Seed and Plant Improvement Institute, Iran	Genetic resources, molecular markers and disease resistance	https://www.gfar.net/organizations/seed-and-plant-improvement-institute
Oil crop research institute	Germplasm resource maintenance, breeding sunflower for various objectives	http://en.oilcrops.com.cn/

Appendix II: Sunflower Genetic Resources

Cultivar	Important traits	Cultivation location
Mas 88.OL Mas 83. R	High oleic acid Broom rape E tolerant	Maïsadour Semences SA, France – Europe
Parsun-3	High yield and stress tolerance	NARC, Islamabad, Pakistan
DRSH-1 (PCSH 243)	High yield	India
PHB 65A70	High yield, early maturity and resistant to disease	DUPONT, Pioneer, South Africa
7111	CLEARFIELD, Herbicide resistance	Syngenta, World wide
3080	NUSUN Mid oleic acid (55-75)	USA
Camaro II	NUSUN CLEARFIELD Mid oleic acid and herbicide resistance	USA
432E	DuPont ExpressSun (Herbicide resistant)	USA
E76437	High oleic acid, CLEARFIELD	USA
6946 DMR	Downey mildew resistant	Canada
Jaguar DMR	CLEARFIELD and Downey mildew resistant	Canada
PARAISO 1000	CLEARFIELD PLUS and disease resistant	Germany
VELEKA	Orobanche resistant hybrid	Germany
VNIIMK 6540 (k-1872), VNIIMK 8883 (k-1961), VNIIMK 8931 (k-1942), Armavirskii 1813 (k-1588), Armavirskii 3497 (k-1960)	High oil contents (47–51%)	Russia

References

Alix K, Gérard PR, Schwarzacher T, Heslop-Harrison JS (2017) Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. Ann Bot 120(2):183–194

Atlagić J, Terzić S (2015) The challenges of maintaining a collection of wild sunflower (*Helianthus*) species. Genet Resour Crop Evol 63:1–18

Berrios EF, Gentzbittel L, Mokrani L et al (2000) Genetic control of early events in protoplast division and regeneration pathways in sunflower. Theor Appl Genet 101(4):606–612

Binsfeld PC, Wingender R, Schnabl H (2000) Characterization and molecular analysis of transgenic plants obtained by microprotoplast fusion in sunflower. Theor Appl Genet 101(8):1250–1258

Burke JM, Knapp SJ, Rieseberg LH (2005) Genetic consequences of selection during the evolution of cultivated sunflower. Genet 171:1933–1940
Cantamutto M, Poverene M (2007) Genetically modified sunflower release: opportunities and risks. Field Crops Res 101(2):133–144
Chepurnaya AL, Sherstyuk SV, Tikhomirov VT (2003) CMS-Rf system for sunflower breeding/sistemascms-rf para la mejoragenética de girasol/systèmescms-rf pour la culture du tournesol. Helia 26(38):59–66
Christov M (1990) A new source of cytoplasmic male sterility in sunflower. Helia 13(13):55–61
Christov M (1999) Ways of production of new CMS sources in sunflower. Biotech Biotech Equip 13(1):25–32
Christov M, Kiryakov I, Shindrova P et al (2004) Evaluation of new interspecific and intergeneric sunflower hybrids for resistance to *Sclerotinia sclerotiorum*. In: Proceedings of16th international sunflower conference, Fargo, North Dakota, USA, International Sunflower Association, Paris, France, II, pp 693–698
Cvejić S, Jocić S, Prodanović S et al (2011) Creating new genetic variability in sunflower using induced mutations. Helia 34(55):47–54
Dagustu N, Sincik M, Bayram G, Bayraktaroglu M (2010) Regeneration of fertile plants from sunflower (*Helianthus annuus* L.) immature embryo. Helia 33(52):95–102
De Labrouhe DT, Bordat A, Tourvieille J et al (2010) Impact of major gene resistance management for sunflower on fitness of *Plasmopara halstedii* (downy mildew) populations. OCL 17(1):56–64
De Oliveira MF, TulmannNeto A, Leite RM et al (2004) Mutation breeding in sunflower for resistance to *Alternaria* leaf spot. Helia 27(41):41–50
Dimitrijević A, Horn R (2018) Sunflower hybrid breeding: from markers to genomic selection. Front Plant Sci 8:2238
Dimitrijević A, Imerovski I, Miladinović D et al (2017) Oleic acid variation and marker-assisted detection of Pervenets mutation in high- and low-oleic sunflower cross. Crop Breed Appl Biotech 17(3):235–241
Dudhe M, Sujatha M (2016) Four decades of sunflower genetic resources activities in India. In: Proceedings of the 19th international sunflower conference, Edirne, Turkey. International Sunflower Association, Paris, France
Dussle CM, Hahn V, Knapp SJ, Bauer E (2004) PlArg from *Helianthus argophyllus* is unlinked to other known downy mildew resistance genes in sunflower. Theor Appl Genet 109(5):1083–1086
Encheva J, Shindrova P, Encheva V, Valkova D (2012) Mutant sunflower line R 12003, produced through in vitro mutagenesis. Helia 35(56):19–30
Faure N, Serieys H, Kaan F, Berville A (2002) Partial hybridization in crosses between cultivated sunflower and the perennial *Helianthus mollis*: effect of in vitro culture compared to natural crosses. Plant Cell Rep 20(10):943–947
Feng J, Liu Z, Cai X et al (2009) Transferring *Sclerotinia*resistance genes from wild *Helianthus* into cultivated sunflower. In: Proceedings of the 31st sunflower research workshop, National Sunflower Association, January 13–14, 2009, Fargo, ND http://www.sunflowernsacom/research/research-workshop/documents/Feng_Genes_09pdf
Fernández-Cuesta A, Jan CC, Fernández-Martínez JM, Velasco L (2014) Variability for seed phytosterols in sunflower germplasm. Crop Sci 54:190–197
Fernández-Martínez J, Jimenez A, Dominguez J et al (1989) Genetic analysis of the high oleic acid content in cultivated sunflower (*Helianthus annuus* L). Euphytica 41:39–51
Fernández-Martínez J, Melero-Vara J, Muñoz-Ruz J et al (2000a) Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance of the gene. Crop Sci 40(2):550–555
Fernández-Martínez J, Melero-Vara J, Muñoz-Ruz J et al (2000b) Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance of the Or5 gene. Crop Sci 40(2):550–555

Fernández-Martínez JM, Pérez-Vich B, Velasco L, Domínguez J (2007) Breeding for specialty oil types in sunflower. Helia 30:75–84
Fernández-Moya V, Martínez-Force E, Garcés R (2005) Oils from improved high stearic acid sunflower seeds. J Agr Food Chem 53:5326–5330
Fu X, Qi L, Hulke B et al (2017) Somatic embryogenesis from corolla tubes of interspecific amphiploids between cultivated sunflower (*Helianthus annuus* L.) and its wild species. Helia 40(66):1–19
Gao W, Rao VR, Zhou M (2001) Plant genetic resources conservation and use in China. In: Proceedings of the national workshop on conservation and utilization of plant genetic resources, Beijing China, 25–27 October, pp 157–163
García-Moreno MJ, Vera-Ruiz EM, Fernández-Martínez JM et al (2006) Genetic and molecular analysis of high gamma-tocopherol content in sunflower. Crop Sci 46:2015–2021
Gavrilova VA, Rozhkova VT, Anisimova IN (2014) Sunflower genetic collection at the Vavilov Institute of Plant Industry. Helia 37:1–16
Greenleaf SS, Kremen C (2006) Wild bees enhance honeybees' pollination of hybrid sunflower. Proc Nat Acad Sci 103(37):13890–13895
Gross BL, Schwarzbach AE, Rieseberg LH (2003) Origin (s) of the diploid hybrid species *Helianthus deserticola* (Asteraceae). Am J Bot 90(12):1708–1719
Gutierrez A, Carrera A, Basualdo J et al (2010) Gene flow between cultivated sunflower and *Helianthus petiolaris* (Asteraceae). Euphytica 172(1):67–76
Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156(1–2):1–13
Hallahan D, Keiper-Hrynko N (2007) U.S. Patent Application No. 11/734,501
Havekes FWJ, Miller JF, Jan CC (1991) Diversity among sources of cytoplasmic male sterility in sunflower (*Helianthus annuus* L). Euphytica 55(2):125–129
Hladni N, Zorić M, Terzić S et al (2018) Comparison of methods for the estimation of best parent heterosis among lines developed from interspecific sunflower germplasm. Euphytica 214(7):108. https://doi.org/10.1007/s10681-018-2197-0
Höniges A, Wegmann K, Ardelean A (2008) Orobanche resistance in sunflower. Helia 31:1–12
Horn R (2002) Molecular diversity of male sterility inducing and male-fertile cytoplasms in the genus *Helianthus*. Theor Appl Genet 104(4):562–570
Horn R, Reddemann A, Drumeva M (2016) Comparison of cytoplasmic male sterility based on PET1 and PET2 cytoplasm in sunflower (*Helianthus annuus* L). In: proc 19th international sunflower conference, 2016, pp 620–629
Hussain MM, Rauf S, Riaz MA et al (2017) Determination of drought tolerance related traits in *Helianthus argophyllus*, *Helianthus annuus* and their hybrids. Breed Sci J 67(3):257–267
Hussain MM, Kausar M, Rauf S et al (2018) Selection for some functional markers for adaptability of *Helianthus argophyllus*× *Helianthus annuus* derived population under abiotic stress conditions. Helia 41(68):83–108
Imerovski I, Dimitrijevic A, Miladinovic D et al (2013) Identification of PCR markers linked to different or genes in sunflower. Plant Breed 132(1):115–120
Jan CC (2006) Registration of two cytoplasmic male-sterile and eight fertility restoration sunflower genetic stocks. Crop Sci 46(4):1835–1836
Jan CC, Vick BA (2006) Registration of seven cytoplasmic male-sterile and four fertility restoration sunflower germplasms. Crop Sci 46:1829–1830
Jan CC, Zhang TX, Miller JF, Fick GN (2002) Inheritance of fertility restoration for two cytoplasmic male sterility sources of *Helianthus pauciflorus* (*rigidus*) Nutt. Crop Sci 42(6):1873–1875
Jan CC, Miller JF, Seiler GJ, Fick GN (2006) Registration of one cytoplasmic male-sterile and two fertility restoration sunflower genetic stocks. Crop Sci 46:1835
Jan CC, Liu Z, Seiler GJ et al (2014) Broomrape (*Orobanche cumana* Wallr.) resistance breeding utilizing wild *Helianthus* species. Helia 37(61):141–150

Jiuhuan F, Liu Z, Seiler GJ, Jan CC (2015) Registration of cytoplasmic male–sterile oilseed sunflower genetic stocks, CMS GIG2 and CMS GIG2–RV, and fertility restoration lines, RF GIG2–MAX 1631 and RF GIG2–MAX 1631–RV. J Plant Reg 9:125–127

Jonic S, Skoric D, Lecic N, Molnar I (2000) Development of inbred lines of sunflower with various oil qualities. Actes Proceedings of the 15th international sunflower conference, Toulouse, France, pp 12–15

Jovanka A (2004) Roles of interspecific hybridization and cytogenetic studies in sunflower breeding. Helia 27(41):1–24

Kalyar T, Rauf S, Teixeira da Silva JA, Iqbal Z (2013a) Variation in leaf orientation and its related traits in sunflower (*Helianthus annuus* L.) breeding population under high temperature. Field Crop Res 150:91–98

Kalyar T, Rauf S, Teixeira da Silva JA, Iqbal Z (2013b) Utilization of leaf temperature for selection of leaf gas exchange traits for the induction of heat resistance in sunflower (*Helianthus annuus* L.). Photosynthesis 51(3):419–428

Kalyar T, Rauf S, Teixeira da Silva JA (2014) Handling sunflower (*Helianthus annuus L*) populations under heat stress. Arch Agron Soil Sci 60:655–672

Kantar MB, Betts K, Michno JM et al (2014) Evaluating an interspecific *Helianthus annuus*× *Helianthus tuberosus* population for use in a perennial sunflower breeding program. Field Crops Res 155:254–264

Khalil F, Rauf S, Monneveux P et al (2016) Genetic analysis of proline concentration under osmotic stress in sunflower (*Helianthus annuus* L.). Breed Sci J 66:463–470

Khan M, Rauf S, Munir H et al (2016) Evaluation of sunflower (*Helianthus annuus* L.) single cross hybrids under heat stress condition. Arch Agron Soil Sci 63(4):525–535

Kinman ML (1970) New developments in the USDA and state experiment station sunflower breeding programs. In: Proceedings of the 4th international sunflower conference Memphis, TN, USA, pp 181–183

Knittel N, Gruber V, Hahne G, Lénée P (1994) Transformation of sunflower (*Helianthus annuus* L.): a reliable protocol. Plant Cell Rep 14(2–3):81–86

Krasnyanski S, Menczel L (1995) Production of fertile somatic hybrid plants of sunflower and *Helianthus giganteus* L. by protoplast fusion. Plant Cell Rep 14(4):232–235

Kulkarni VV, Shankergoud I, Govindappa MR (2015) Identification of sunflower powdery mildew resistant sources under artificial screening. SABRAO J Breed Genet 47(4):502–509

Lai Z, Nakazato T, Salmaso M et al (2005) Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. Genet 171(1):291–303

Leclercq P (1969) Cytoplasmic male sterility in sunflower. Ann Amelior Plant 19:99–106

León AJ, Andrade FH, Lee M (2003) Genetic analysis of seed-oil concentration across generations and environments in sunflower. Crop Sci 43:135–140

León AJ, Zambelli AD, Reid RJ et al(2013a) Nucleotide sequences mutated by insertion that encode a truncated oleate desaturase protein, proteins, methods and uses. WIPO patent WO/2013/004281, Jan 10, 2013

León AJ, Zambelli AD, Reid RJ et al (2013b) Isolated mutated nucleotide sequences that encode a modified oleatedestaurase sunflower protein, modified protein, methods and uses. WIPO Patent WO/2013/004280, Jan 10, 2013

Liu Z, Wang D, Feng J et al (2013) Diversifying sunflower germplasm by integration and mapping of a novel male fertility restoration gene. Genet 193(3):727–737

Louarn J, Boniface MC, Pouilly N et al (2016) Sunflower resistance to broomrape (*Orobanche cumana*) is controlled by specific QTLs for different parasitism stages. Front Plant Sci 7:590

Ma GJ, Seiler GJ, Markell SG et al (2016) Registration of two double rust resistant germplasms, HA-R12 and HA-R13 for confection sunflower. J Plant Reg 10(1):69–74

Maheshwari S, Barbash DA (2011) The genetics of hybrid incompatibilities. Ann Rev Genet 45:331–355

Mandel JR, Dechaine JM, Marek LF, Burke JM (2011) Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annuus* L. Theor Appl Genet 123(5):693–704

Marinković R, Miller JF (1995) A new cytoplasmic male sterility source from wild *Helianthus annuus*. Euphytica 82(1):39–42

Martín-Sanz A, Malek J, Fernández-Martínez JM et al (2016). Increased virulence in sunflower broomrape (*Orobanche Cumana* Wallr.) populations from southern Spain is associated with greater genetic diversity. Front Plant Sci *7*:589

Merah O, Langlade N, Alignan M et al (2012) Genetic analysis of phytosterol content in sunflower seeds. Theor Appl Genet 125:1589–1601

Mestries E, Gillot L, Penaud A, Cetio M (2004) Sunflower downy mildew resistance gene pyramiding, alternation and mixture: first results comparing the effects of different varietal structures on changes in the pathogen. In: Proceedings of the 16th international sunflower conference, Fargo, ND, USA, 29 September, pp 111–116

Mezzarobba A, Jonard R (1986) Effect of the developmental stage and pretreatments on in vitro development of anthers isolated from cultivated sunflowers (*H. annuus* L.). Compt Rend AcadSci III Sciences de la Vie 303:181–186

Miller JF (1996) Inheritance of restoration of *Helianthus petiolaris* sp *fallax* (PEF1) cytoplasmic male sterility. Crop Sci 36:83–86

Mirzahosein-Tabrizi M (2017) Identification of downy mildew resistance loci in sunflower germplasm. Notulae Scient Biolog 9(4):515–519

Mokrani L, Gentzbittel L, Azanza F et al (2002) Mapping and analysis of quantitative trait loci for grain oil content and agronomic traits using AFLP and SSR in sunflower (*Helianthus annuus* L). Theor Appl Genet 106:149–156

Mulpuri S, Liu Z, Feng J et al (2009) Inheritance and molecular mapping of a downy mildew resistance gene, Pl 13 in cultivated sunflower (*Helianthus annuus* L). Theor Appl Genet 119(5):795–803

Murphy DJ (1990) Storage lipid bodies in plants and other organisms. Prog Lipid Res 29:299–324

Nagarathna TK, Shadakshari YG, Ramanappa TM (2011) Molecular analysis of sunflower (*Helianthus annuus* L) genotypes for high oleic acid using microsatellite markers. Helia 34(55):63–68

Paniego N, Bazzalo ME, Bulos M et al (2012) Genomics, mapping and marker assisted selection strategies for disease resistance. In: Proceedings of the 18th international sunflower conference, Mar del Plata, Argentina, pp 44–50

Perez-Vich B, Munoz-Ruz J, Fernandez-Martinez JM (2004) Developing midstearic acid sunflower lines from a high stearic acid mutant. Crop Sci 44:70–75

Petcu E, Pâcureanu JM (2011) Developing drought and broomrape resistant sunflower germplasm utilizing wild *Helianthus* species. Helia 34(54):1–8

Pfenning M, Palfay G, Guillet T (2008) The CLEARFIELD® technology a new broad-spectrum post-emergence weed control system for European sunflower growers. J Plant Dis Prot 21:649–654

Prabakaran AJ, Sujatha M (2004) Interspecific hybrid of *Helianthus annuus* × *H. simulans*: characterization and utilization in improvement of cultivated sunflower (*H. annuus* L.). Euphytica 135(3):275–282

Presotto A, Ureta MS, Cantamutto M, Poverene M (2012) Effects of gene flow from IMI resistant sunflower crop to wild *Helianthus annuus* populations. Agric Ecosys Environ 146(1):153–161

Qi L, Gulya T, Seiler GJ et al (2011) Identification of resistance to new virulent races of rust in sunflowers and validation of DNA markers in the gene pool. Phytopathology 101(2):241–249

Radonic LM, Zimmermann JM, Zavallo D et al (2008) Introduction of antifungal genes in sunflower via agrobacterium. Electron J Biotechnol 11(5):8–9

Rauf S (2008) Breeding sunflower (*Helianthus annuus* L) for drought tolerance. Commun Biomet Crop Sci 3(1):29–44

Rauf S, Sadaqat HA (2008) Identification of physiological traits and genotypes combined to high achene yield. Aust J Crop Sci 1(1):23–30
Rauf S, Sadaqat HA, Khan IA, Ahmed R (2009) Genetic analysis of leaf hydraulics in sunflower (*Helianthus annuus*L) under drought stress. Plant Soil Environ 55(2):62–69
Rauf S, Al-Khayri JM, Zaharieva M et al (2016) Breeding strategies to enhance drought tolerance in crops. In: Al-Khayri JM, Jain SM, Johnson DV (eds) Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. Springer, Dordrecht, pp 397–445
Rauf S, Jamil N, Tariq SA et al (2017) Progress in modification of sunflower oil to expand its industrial value. J Sci Food Agric 97:1997–2006
Roche J, Alignan M, Bouniols A et al (2010) Sterol content in sunflower seeds (*Helianthus annuus* L.) as affected by genotypes and environmental conditions. Food Chemist 121:990–995
Rondanini D, Savin R, Hall AJ (2003) Dynamics of fruit growth and oil quality of sunflower (*Helianthus annuus* L) exposed to brief intervals of high temperature during grain filling. Field Crops Res 83(1):79–90
Rosenthal DM, Schwarzbach AE, Donovan LA et al (2002) Phenotypic differentiation between three ancient hybrid taxa and their parental species. Int J Plant Sci 163(3):387–398
Scelonge C, Wang L, Bidney D et al (2000) Transgenic *Sclerotinia* resistance in sunflower (*Helianthus annuus* L.). In: Proceedings of 15th international sunflower conference. Toulouse, France, 12–15 June, pp 1–5
Seiler GJ (1992) Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crops Res 30(3):195–230
Seiler GJ (2007a) The potential of wild sunflower species for industrial uses. Helia 30(46):175–198
Seiler GJ (2007b) Wild annual *Helianthus anomalus* and *H deserticola* for improving oil content and quality in sunflower. Indust Crops Prod 25(1):95–100
Seiler GJ (2010) Utilization of wild *Helianthus* species in breeding for disease resistance. In: Proceedings of the International Sunflower Association (ISA) symposium sunflower breeding on resistance to diseases, 2010, pp 36–50
Seiler GJ, Jan CC (2014) Wild sunflower species as a genetic resource for resistance to sunflower broomrape (*Oroban checumana* Wallr). Helia 37(61):129–139
Seiler G, Marek LF (2011) Germplasm resources for increasing the genetic diversity of global cultivated sunflower. Helia 34(55):1–20
Shehbaz M, Rauf S, Al-Sadi AM et al (2018) Introgression and inheritance of charcoal rot (*Macrophomina phaseolina*) resistance from silver sunflower (*Helianthus argophyllus* Torr. & A. Gray) into cultivated sunflower (*Helianthus annuus* L.). Aust Plant Path 47(4):413–420
Škorić D (2009) Sunflower breeding for resistance to abiotic stresses. Helia 32(50):1–16
Smith BD (2006) Eastern North America as an independent center of plant domestication. Proc Nat Acad Sci 103(33):12223–12228
Smith SA, King RE, Min DB (2007) Oxidative and thermal stabilities of genetically modified high oleic sunflower oil. Food Chemist 102(4):1208–1213
Soldatov KI (1976) Chemical mutagenesis in sunflower breeding. In: Proceedings of the 7th international sunflower conference. International Sunflower Association, Vlaardingen, pp 352–357
Sujatha M, Prabakaran AJ (2001) High frequency embryogenesis in immature zygotic embryos of sunflower. Plant Cell Tissue Org Cult 65(1):23–29
Sujatha M, Prabakaran AJ, Dwivedi SL, Chandra S (2008) Cytomorphological and molecular diversity in backcross-derived inbred lines of sunflower (*Helianthus annuus* L). Genome 51(4):282–293
Tahara M (1915) Cytological investigation on the root tips of *Helianthus annuus*. Bot Magaz Tokyo 29:1–5
Taski-Ajdukovic K, Vasic D, Nagl N (2006) Regeneration of interspecific somatic hybrids between *Helianthus annuus* L. and *Helianthus maximiliani* (Schrader) via protoplast electrofusion. Plant Cell Rep 25(7):698–704

Tilak IS, Kisan B, Goud IS et al (2018) Biochemical and molecular characterization of parents and its crosses for high oleic acid content in sunflower (*Helianthus annuus* L). Int J Curr Microbiol App Sci 7(4):2000–2020
Turkec A, Goksoy AT (2006) Identification of inbred lines with superior combining ability for hybrid sunflower (*Helianthus annuus*) production in Turkey. New Zealand J Crop Hort Sci 34(1):7–10
Vanzela AL, Ruas CF, Oliveira MF, Ruas PM (2002) Characterization of diploid, tetraploid and hexaploid *Helianthus* species by chromosome banding and FISH with 45S rDNA probe. Genetics 114(2):105–111
Vasko V, Kyrychenko V (2016) Variability of valuable economic traits in M1 and M2 sunflower generations influenced by dimethyl sulfate and γ–rays. Žemėsūkiomokslai 23(4):142–159
Vear F (2016) Changes in sunflower breeding over the last fifty years. OCL 23(2):D202
Velasco L, Fernández-Martínez JM (2003) Identification and genetic characterization of new sources of beta- and gamma-tocopherol in sunflower germplasm. Helia 26:17–23
Velasco L, Fernández-Cuesta Á, Fernández-Martínez JM (2014) New sunflower seeds with high contents of phytosterols. OCL 21:D604
Vlahakis C, Hazebroek J (2000) Phytosterol accumulation in canola, sunflower, and soybean oils:effects of genetics, planting location, and temperature. J Am Oil Chem Soc 77:49–53
Vranceanu VA, Stoenescu FM (1969) Pollen fertility restorer gene from cultivated sunflower (*Helianthus annuus* L). Euphytica 20(4):536–541
Warburton ML, Rauf S, Marek L et al (2017) The use of crop wild relatives for crop improvement. Crop Sci 57:1–14
Weston B, McNevin G, Carlson D (2012) Clearfield® plus technology in sunflowers. In: Proceedings of the XVIII Sunflower Conference, Mar del Plata-Balcarce, Argentina, pp 149–154
Whelan ED (1981) Cytoplasmic male sterility in *Helianthus giganteus* L× *H. annuus* L interspecific hybrids. Crop Sci 21(6):855–858

Author Queries

Chapter No.: 16 482814_1_En_16_Chapter

Queries	Details Required	Author's Response
AU1	References FAO (2013, 2014, 2016), Velasco et al. (2002), Kantar et al. (2015), Liu et al. (2010), Jan and Chandler (1985), Cristov (2004), Christov (1994), Vera (2016), Rauf et al. (2018), Rauf (2018), Pereira et al. (2000), Mulpuri et al. (2007), Leon et al. (2001), Hussian et al. (2017, 2018), Škoric et al. (2008), Fernandez-Martinez et al. (1997), Khan et al. (2017, 2018), Sabetta et al. (2011), Kumar et al. (2013), Nagrantha et al. (2011), Hallan and Keiper-Hrynko (2007) or should we delete from the reference list if applicable.	
AU2	Reference citations Seiler (2007), Kalyar et al. (2013), Martinez et al. (2000) have been changed to Seiler (2007a, b), Fernández-Martínez et al. (2000a, b) as per the reference list. Please check if okay.	
AU3	Please confirm the inserted call-out for Table 16.2.	
AU4	Reference citations Cveji et al. (2013), Petcu et al. (2011), Seiler et al. (2014), Vranceanu and Stoenescu (1966), Murphy (2001), Velasco et al. (2003), Fernández-Cuesta et al. (2011) have been changed to Cvejić et al. (2011), Petcu and Pâcureanu (2011), Seiler and Jan (2014), Vranceanu and Stoenescu (1969), Murphy (1990), Velasco and Fernández-Martínez (2003), Fernández-Cuesta et al. (2014) as per the reference list. Please check if okay.	
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