Sunflower Breeding

19

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Abstract

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops cultivated worldwide for its oil and confectionary purposes. Among the crops where heterosis has been successfully exploited, sunflower assumes importance as that of maize. Identification of the PET-1 cytoplasm along with the complementary fertility restoration system is a landmark achievement which paved the way for transformation of an ornamental crop to a commercial oil yielding crop. Breeding objectives are directed towards development of cultivars with high seed yield, early maturity, resistance to diseases (downy mildew, powdery mildew, rust, necrosis disease, *Alternariaster* leaf spot), insect pests (*Helicoverpa*, sucking pests) and tolerance to herbicides, besides improved content and quality of seed oil and protein. Genetic enhancement for widening the trait base exploited traditional breeding methods, mutation breeding and interspecific gene transfer. Interspecific hybridization was adopted as one of the key tools by various research groups due to the existence of a rich repertoire of genes in the wild *Helianthus* species, and several economically important traits such as cytoplasmic male sterility, resistance to biotic and abiotic stresses, herbicide tolerance and seed quality traits were successfully introgressed. The past two decades witnessed advancements in molecular marker technology and genomics which have been successfully used in marker-assisted breeding for simple inherited traits, while traits governed by quantitative trait loci still remain a challenge for the breeders. Despite the availability of genes in wild *Helianthus* species, successful transfer to cultivar germplasm is hampered by crossability between cultivated sunflower (annual diploid) and *Helianthus* species varying in habit (diploid perennial) and

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ploidy (tetraploids, hexaploids) warranting the use of genetic engineering approaches. This chapter presents a comprehensive account of the history, botany, the extent of genetic diversity in cultivated and wild *Helianthus* species. Strategies are adopted for development of inbreds and hybrids, seed production methodology and progress with regard to exploitation of molecular marker and genomic resources in marker-assisted breeding.

Keywords

Hybrids · Breeding techniques · Oil content · Breeding approaches · Genetic engineering · Genomics

19.1 Introduction

Sunflower is an important annual edible oilseed crop of Asteraceae family grown globally over an area of 27.36 m. ha with a production of 56 mt (FAOSTAT 2019) and world average yield of 2048 kg/ha. Because of its wide adaptability to diverse agro-climatic situation, it is grown in all the countries crossing climatic and geographical boundaries. Being photo-insensitive and day neutral, it can be grown throughout the year, i.e. *kharif*, *rabi* and *spring*/summer, and it is an ideal crop for contingency cropping plan. Being a short duration crop, it can fit into various inter- and sequence cropping systems. Leading producing countries are Ukraine, Russia and Argentina, while countries like Belarus, France, Hungary, Romania, Kazakhstan, Turkey, Tanzania, China and India produce relatively smaller quantity. The highest-yielding countries are Hungary (3025 kg/ha), China (2847 kg/ha), Turkey (2794 kg/ha), Romania (2782 kg/ha), Ukraine (2560 kg/ha), Bulgaria (2375 kg/ha), France (2149 kg/ha) and Argentina (2039 kg/ha) with an average yield of >2.0 t/ha as against the lowest-yielding countries like India and Myanmar with <1.0 t/ha. The crop has become one of the important oilseed crops in India with an area of 2.4 lakh ha and a production of 2.6 mt of seed (2019–2020).

In India, sunflower is mostly grown in the Southern and Central Peninsula comprising Karnataka, Andhra Pradesh, Telangana, Maharashtra and Tamil Nadu. The highest (>50%) area of sunflower is covered by only a single state (Karnataka) with productivity of 785 kg/ha. It has spread to non-traditional states like Punjab, Haryana and Uttar Pradesh as an important oilseed crop and is grown in West Bengal, Bihar and Odisha as well. The productivity (891 kg/ha) of sunflower in India at present is far lower than the world average (2048 kg/ha). Despite high productivity in Punjab (1950 kg/ha), Gujarat (1820 kg/ha), Haryana (1743 kg/ha) and Telangana (1698 kg/ha), area under sunflower has declined drastically due to unavailability of better hybrids that can give more than 3.0 t/ha seed yield, price fluctuations, shift in cropping pattern, profitability of other crops compared to sunflower, withdrawal of private players from sunflower research, bird damage and vulnerability to several biotic and abiotic stresses during various stages of crop growth.

19.2 Economic Importance

In pre-historic times, the North American Indians for the first time found that the seeds of wild sunflowers were a rich source of food and domesticated the plant. Different colour dyes like purple, black and yellow extracted from ray florets of wild sunflowers were used to dye basketry materials. The Hopi Indians obtained a purple dye from deep purple achenes (Heiser Jr 1976) to decorate their bodies. In the southwest, people used it as an antidote to snake bites and to cure rattlesnake bites by chewing the fresh or dried root followed by sucking the snake bite wound (Camazine and Bye 1980). The Europeans also used oil as a remedy for heart diseases, pulmonary infections, cold and whooping coughs (Heiser Jr 1976). It was cultivated as an ornamental or garden plant, where the blooms were cherished for their beauty and the seeds were eaten by both humans and wildlife. The oil is used for human consumption, salad oil, making paints, soaps, as biofuel and candles. Sunflower oil has potential applications in the cosmetic industry (Oliveira et al. 2019). Sunflower meal is an excellent source of protein for human consumption and as a supplementary diet which enhances the animal growth and milk production. Sunflower cake is used in South Africa and Tanzania as the main component of livestock feeds. Some species of sunflowers are grown for fodder or silage.

19.3 Origin and History

The genus *Helianthus* is derived from the Greek word *helios* meaning sun and *anthos* meaning flower and belongs to the subdivision Tubiflorae and tribe *Heliantheae*. It is a member of the Asteraceae (Compositae) family and has a typical composite flower. The cultivated sunflower originated in North America from a diploid ($2n = 34$) annual wild *H. annuus* species (Smith 2014). Sunflower is native to North America, but commercialization of the plant took place in Russia. The American Indians were the first to domesticate the multi-headed ornamental plant into a single-headed plant with a variety of seed colours.

The beginning of domestication and the first steps of sunflower breeding date back to the time when it was cultivated by native Americans over 4000 years ago (Seiler et al. 2017). It was used in food, to obtain oil, for medical purposes, and as an ornamental plant. From its wild weedy forms, the crop has spread to Europe during the sixteenth century and later to former USSR wherein the crop was domesticated. Sunflower as a cultivated plant was reintroduced to North America from Europe in the late nineteenth century. After the Second World War, the introduction of Russian varieties into Europe and America had a decisive impact on the development of sunflower as a commercial oilseed crop. Sunflower oil on commercial scale was first produced in Russia between 1830 and 1840.

19.4 Floral Biology

In sunflower, the inflorescence which is a capitulum or head is most prominent because of its large size and conspicuous yellow colour of the ray florets. The capitulum is made up of two distinct flower types: outer single row of ray florets which are male sterile and the inner disc florets which are hermaphrodite and fertile. The disc florets are arranged in arcs radiating from the centre of the head. Each disc floret is made up of inferior ovary, two pappi (modified sepals) and a tubular corolla formed by the fusion of five petals except at the tip. The five anthers are also fused to form a tube (syngenesious) with filaments attached independently at the base of the corolla tube. The style is inside the anther tube with stigma divided at the tip. When the flower is fully developed, the style elongates and the bifid stigma curls outward.

19.5 Pollination Mechanism

The unfolding of outer ray florets indicates the beginning of flowering in sunflower. Opening of disc florets follows from the periphery proceeding gradually towards the centre of the head at the rate of one to four whorls per day. The flowering in the capitulum is completed in 7–10 days depending upon the size of the capitulum and prevailing weather. Anthesis takes place in the morning between 6:00 and 8:00 AM on warm sunny days. Anthesis is delayed if weather is cool, cloudy or wet. The staminal filaments rapidly elongate and exert the anther tube from the corolla. Pollen is dehisced within the anther tube. The style elongates and forces the two lobed pubescent stigmas up the anther tube. The stigma is not receptive at this stage because the two lobes are held together covering the inner receptive surface. The stigma pushes the dehisced pollen through the upper end of the anther tube, and the lobes separate exposing inner receptive surface. The sunflower to a large extent is protandrous as anthesis takes place first, accompanied by a time lag of 10:00–12:00 h in the maturation of male and female elements. Because of this, cross-pollination is favoured as a rule, and insects, particularly bees, play an important role in pollination. The pollen is spiny and well adapted for transmission by insects mainly by honey bees. Pollination and fertilization occur when the spiny viable pollen is transferred to the stigmatic surface. The achene is the fruit of the sunflower that consists of outer pericarp (hull), thin and papery inner seed coat and embryo (kernel). The achene is attached by a funiculus, but the seed coat is free from the inner wall of the pericarp. Seed is nonendospermic, and the embryo is the major portion and is made up of mostly cotyledons. The endosperm is largely made up of a single layer of aleurone cells coalesced with the seed coat.

19.6 Genetic Resources

Availability of appropriate genetic resources with wide diversity is the key to any crop improvement programme. Several germplasm collections have been stored in different gene banks across the world. The highest number of collections are

maintained at USDA, USA (total 5217 accessions, of which 2616 are cultivated *H. annuus* accessions, 2597 are wild accessions including 1693 annual and 904 perennial and 4 are diverse CMS sources) (Terzić et al. 2020). The France collection at INRA maintains 3933 accessions, 2703 cultivated *H. annuus* accessions, 10 diverse CMS sources and 1214 wild accessions of which 804 accessions are annual and 410 are perennial species. In India, ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad gene bank maintains 3102 sunflower accessions. The collection includes exotic collection, augmented germplasm, inbreds, populations, genetic stocks, gene pools for high oil, high seed yield, autogamy, backcross-derived lines and wild species including their derivatives. Other countries that maintain sunflower germplasm accessions are Russia (1210), Argentina (922), Romania (681), Serbia (593), Germany (503) and Bulgaria (423). The lowest numbers of accessions are maintained in Spain (196). The collection may help improve economical traits related to yield and quality and also serve as the source for biotic and abiotic resistance genes. Further, the elite germplasm may help initiate sunflower breeding programmes in many countries. The details of germplasm collections in gene banks in different countries are presented in Table 19.1.

Table 19.1 Sunflower germplasm resources maintained in gene banks of different countries

S. no.	Country	No. of genetic resources	Type of material
1.	USA	5217	Cultivated lines, CMS, wild *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp. and other genera
2.	France	3933	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp., other genera
3.	India	3102	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp.
4.	Russia	1210	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp.
5.	Argentina	922	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp.
6.	Romania	681	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp.
7.	Serbia	593	Wild *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp.
8.	Germany	503	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp.
9.	Bulgaria	423	CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp., other genera
10.	Spain	196	Open-pollinated varieties and other populations

Source: Modified from Terzić et al. (2020)

19.7 Wild *Helianthus* Species

Cultivated sunflower is diploid with chromosome number 2n = 2x = 34. The genus *Helianthus* includes 53 species, of which 39 are perennials and 14 annuals being maintained at the USDA-ARS, North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA (Seiler and Jan 2014; Seiler et al. 2017). The 14 annual species are diploid (2n = 2*x* = 34), and the 39 perennial species include 26 diploids, three tetraploids (2n = 4*x* = 68), seven hexaploids (2n = 6*x* = 102), one mixoploid of either diploid or tetraploid and two mixoploids of tetraploid or hexaploid (Liu et al. 2017). Some species occur in dual ploidy series, such as *H. ciliaris* L., which displays both teraploid and hexaploid states, and *H. decapetalus* L., which exists in diploid and tetraploid forms (Atlagic 2004).

19.7.1 Utilization of *Helianthus* Species

The wild *Helianthus* species exhibit high variability for several agronomic attributes. Sunflower is one of the few crops where the use of wild *Helianthus* species in sunflower breeding programmes has produced significant results. Genes for disease (Seiler 2010) and insect resistance (Thompson et al. 1981), oil (Jovanka 2004) and protein content and quality (Miller et al. 1992), cytoplasmic male sterility (Horn 2002), herbicide tolerance (Al-Khatib et al. 1998; Al-Khatib and Miller 2000) or agronomic and physiological traits (Seiler et al. 2017) have been identified in wild *Helianthus* species and transferred into cultivated lines in the USSR, the USA and many East European countries. Interspecific hybridization paved the way for hybrid breeding in sunflower wherein several CMS and fertility restorer gene sources were derived from wild *Helianthus* species (Horn and Friedt 2006). The single most important breakthrough has been the discovery of cytoplasmic male sterility (CMS) via interspecific hybridization and *Rf* genes involving *H. petiolaris*, which allowed practical use of heterosis and development of hybrids worldwide (Leclercq 1969; Kinman 1970). In India, the sunflower variety LSF-8 with tolerance to downy mildew, rust and *Alternariaster* was derived from the cross involving *H. tuberosus*. The variety CO-5 (COSFV-5) with moderate resistance to *Alternariaster* leaf spot, rust and necrosis disease was derived from the gene pool of *H. annuus x H. praecox* (Sujatha et al. 2019). Using conventional methods of crossing, several prebred lines with altered plant architecture, high yield and oil content, maturity duration and inbuilt tolerance to major biotic stresses have been developed from crosses involving diploid annuals (Sujatha et al. 2008). Prebred lines derived from *H. annuus* × *H. argophyllus* crosses were found resistant to leafhopper (unpublished). Jacob et al. (2017) identified one sulfonylurea-based accession of *H. praecox* (PRA-1823) resistant to herbicides as well as to powdery mildew. Different diploid annual wild species like *H. debilis*, *H. petiolaris*, *H. argophyllus*, *H. praecox* and wild *H. annuus* are being utilized for broadening the genetic base of cultivated sunflower in India. Concerted efforts are warranted to overcome the deleterious effects of distant hybridization and combine desirable traits with high seed and oil

yield. Information on the global scenario of the progress made in augmentation and maintenance of wild *Helianthus* sources and utilization for introgression of gene (s) for resistance to biotic and abiotic stresses and oil content and quality is reviewed in the article of Seiler et al. (2017).

19.8 Evolution of High Oil Sunflower

Transformation of an ornamental sunflower into a high oil yielding crop occurred in the USSR by 1769 and by 1830, and the manufacture of sunflower oil was done on a commercial scale. During the early nineteenth century, the Soviet plant breeder, Vasilii Stepanovich Pustovoit, in 1860, initiated selection for high oil content from local varieties which contained 30–33% oil content, and concerted efforts at several agricultural stations resulted in the gradual stepping up of oil content in the cultivars from 33 to 50%. Consequently, the seed oil content increase was 21%. In 1927, V.S. Pustovoit bred a new sunflower variety with 35% oil content. High oil sunflower varieties such as VNIIMK-3519, VNIIMK-6540, VNIIMK-8931, Peredovik, Armavirsky-3497 and VNIIMK-309 developed by Pustovoit and his associates enabled the spread of sunflower as an oilseed crop (Pustovoit 1964). Much of the change was achieved by breeding for thin hulls surrounding the kernels. Following Pustovoit's method (Fig. 19.1), new early, broomrape-resistant, high oil content (45–48%) and high seed yielding varieties such as Krasnodarets, Armavirets, Chrnyanka-66 and Enissei were developed. These high oil selections found their way later on to all the continents, and most of the recently bred varieties owe their origin directly or indirectly to the materials bred in the USSR.

19.9 Inbreeding, Heterosis and Development of Hybrids

Inbreeding as a method for improving sunflower was used as early as 1922. Cardon (1922) described the available variation in sunflower varieties and attempted to isolate different types by self-pollination. Inbreeding was used in the Soviet Union during the 1920s to develop lines with improved oil percentage, strong single stems and resistance to diseases and insect pests (Calson et al. 1972; Jagodkin 1937; Voskoboinik and Soldatov 1974). Unrau and White (1944) recorded a 35% decline in seed yield after one generation of inbreeding in the cultivar Mennonite. Breeders soon realized that the value of inbreeding was to develop inbreds with certain desirable characteristics for subsequent crossing to produce synthetic cultivars or interline hybrids. Attia et al. (2014) observed 2.25% decrease in oil content due to inbreeding. Some of the results involving hybridization of inbred lines showed heterosis for plant height, head diameter, seed size and seed yield. To reduce inbreeding depression, Kovacik and Skaloud (1975) recommended sib cross in early inbred generation. Shvetsova (1979) observed no substantial differences between fertile lines and their male sterile counterparts for oil content in inbreeding. Schuster (1980) studied inbred lines for 25 generations and revealed mean

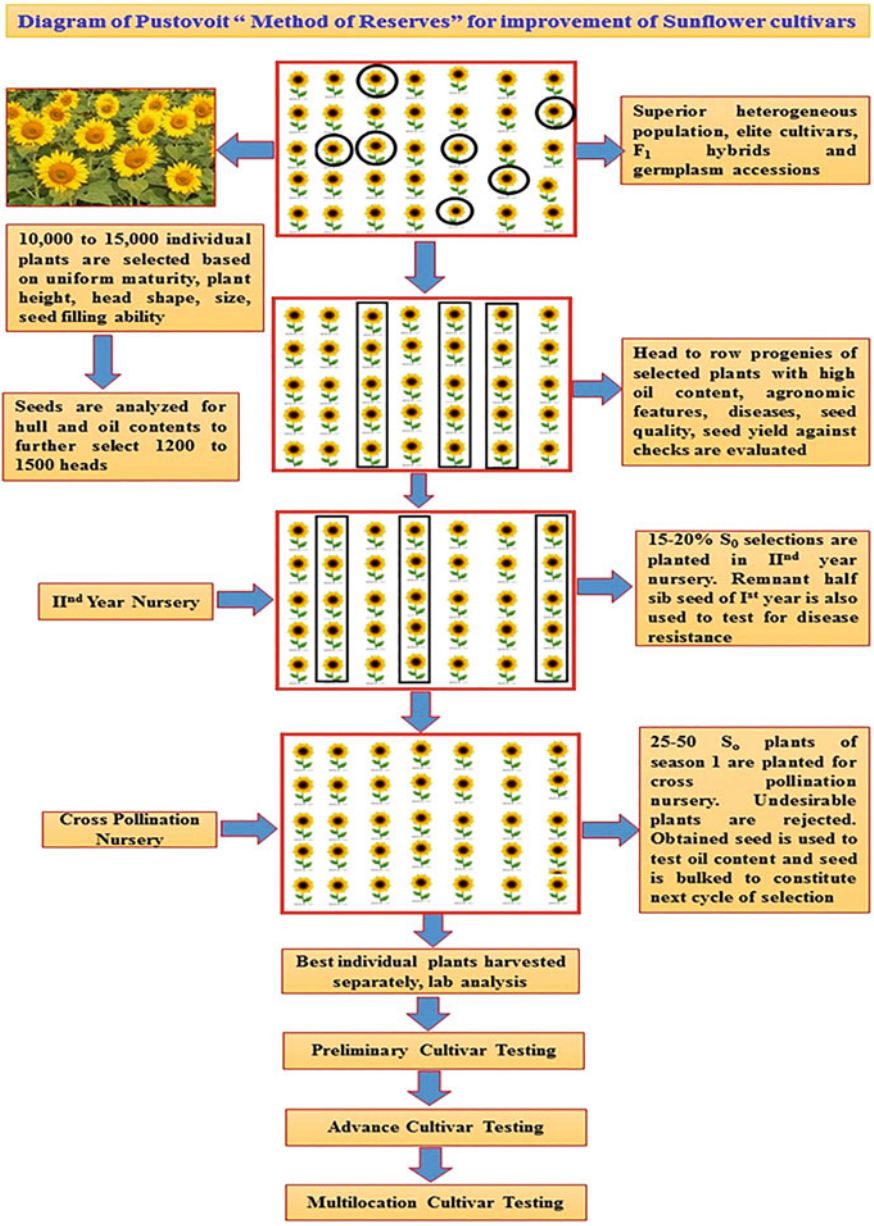

Fig. 19.1 Pustovoit method for developing high oil content material

inbreeding depressions for seed yield (61%), plant height (20%), capitulum diameter (21%), kernel/husk ratio (3%), crude oil content of achene (7%) and 1000 seed weight (22%). Tuberosa (1983) observed 41.7% decrease in achene yield, 4.2% in

oil content and 44% in oil yield in S_4 generation due to inbreeding. Gurev and Osipova (1986) studied a number of varieties and hybrids for the degree of inbreeding depression and found that the yield decreased by 33.0% in I_1, 53.5% in I_2, 46.5% in I_3 and 45% in I_4 generations.

As early as 1940, Putt in Canada carried out preliminary studies on heterosis breeding. Unrau and White (1944) reported 60.8% increase in seed yield and great uniformity in a hybrid compared to unimproved Mennonite. Heterosis studies carried out in sunflower have been presented for interline, inter-varietal and top-cross hybrids involving genetic and cytoplasmic male sterility. In a study of inter-varietal crosses, Kovacik (1959, 1960) observed good response with an increase in seed yield to the extent of 1 to 20% over the parents. While comparing progenies from selfed and crossed seeds, Kurnik and Zelles (1962) observed 18.7% more height, 4.2% higher seed yield, 10.8% higher 1000 seed weight and 3 days of delayed flowering in the plants from crossed seeds. Putt (1964) reported highly significant values of heterosis for seed yield and plant height in a diallel study involving ten inbred lines. In eight sunflower hybrids, Putt (1966) observed heterosis for plant height and seed yield. Reports from various countries indicate (Gundev 1966; Fick and Zimmer 1974; Putt and Dorrell 1975) heterosis to an extent of 61% in the crosses of inbred lines for seed yield and much greater uniformity in hybrids. Kolczowskii (1971) observed an average 10.4% heterosis in the F_1s from 20 crosses among varieties of diverse origin in comparison with the better parent. Stoyanova et al. (1971) studied heterosis in 140 F_1 hybrids obtained from crosses among 192 stabilised inbred lines and reported a heterosis of 25 to 29%. Ge (1971) observed heterosis of 15.9% for head diameter and 47.0% for a total number of seeds per head in certain sunflower hybrids. Heterosis of 12 to 21% for seed yield is reported by Klimov and Ermoshin (1977) in certain top-cross hybrids developed from 29 Soviet varieties and 3 testers. In India, Seetharam et al. (1977) studied the performance of hybrids produced by crossing four CMS and two fertility restorer lines and observed a significant positive heterosis for days to flowering, plant height, head diameter, test weight, oil content and seed yield. Sudhakar and Seetharam (1980) reported heterosis for seed yield up to 41.31% over the mid-parent and up to 5.24% over the better parent in 27 top-cross hybrids. Choudhary and Anand (1984) observed 62.8% heterosis for seed yield, 23.1% for oil content and negative heterosis (7.7%) for days to flowering over better parent. Singh et al. (1984) in a study on performance of variety × inbred crosses observed heterosis for yield to an extent of 47.0–206.0%. The studies of Shivaraju (1984) on ten F_1 hybrids indicated an average heterosis to an extent of 175.0% for seed yield, 129.0% for the number of filled seeds and 6.87% for oil content. Giriraj et al. (1986) observed 15.2% heterosis for head diameter, −7.7% for days to flowering and 37.7% for the number of filled achenes.

The advantages of maize and other crops stimulated sunflower researchers to work towards hybrid development in the crop. Introduction of male sterility using GA_3 tried on a large scale in many countries had inherent problems in exploiting heterosis satisfactorily in sunflower. Several workers (Luciano et al. 1965; Putt 1962) tried to develop hybrids based on the self-incompatibility mechanism. The occurrence of a higher proportion of selfs in hybrid seed plots came in the way of

large-scale seed production of hybrids based on this system. Before the discovery of cytoplasmic male sterility and the corresponding fertility restoration system, genetic male sterility discovered as early as 1934 was tried to produce hybrids (Leclercq 1966; Putt and Heiser 1966). This system was widely explored in France and Romania despite the major disadvantage of removal of 50% plants in seed production plots and higher costs associated with seed production.

The most significant development leading to the large-scale exploitation of heterosis was the discovery of cytoplasmic male sterility (CMS) and restorer system first developed by Leclercq (1969) working at the French National Institute for Agricultural Research (INRA) from the interspecific cross *H. petiolaris* Nutt. × *H. annuus* L. (the variety Armavirsky-9345) back crossed to *H. annuus* L. and identification of fertility restorer lines RHA-265 and RHA-266 derived from a composite cross by Kinman (1970) in the USA. After this discovery there has been a shift in the breeding emphasis from open-pollinated varieties (OPV) to development of single- and three-way cross hybrids. The first hybrid using CMS and genetic restoration system was available as early as 1972, and within a span of 5 years, the hybrids spread to many countries in Europe and America replacing the open-pollinated varieties. Today hybrids are predominantly cultivated worldwide, and > 95% of the area is covered by them.

19.10 Development of Sunflower Hybrids in India

The commercial cultivation of sunflower in India started in 1972 with the introduction of five Russian varieties [VNIIMK-8931 (EC-68413), Peredovik (EC-68414), Armavirskii-3497 (EC-68415), Armaverta (EC-69874) and Vashod (Sunrise)]. The value of hybrids and importance of heterosis breeding were recognized early with the inception of the All India Coordinated Research Project (AICRP) on Oilseeds in 1972–1973. A special project on 'Promotion of Research and Developmental Efforts on Hybrids in Selected Crops-Sunflower' was launched in 1989 as a thrust programme to develop hybrids for diverse situations. Experimental hybrids were developed at Bangalore in 1974–1975 using four CMS lines, namely, CMS-2A, CMS-124A, CMS-204A and CMS-234A, and two restorer lines, viz. RHA-266 and RHA-274, introduced from the USA. Based on seed yield, oil content, yield stability and synchronization of flowering in male and female parents, the first public sector hybrid BSH-1 (CMS-234A × RHA-274) was released for commercial cultivation from public sector in 1980 (Seetharam 1980). Since then, the hybrid base has been further widened in the country through AICRP centres. Till today, a total of 30 hybrids and 19 varieties were released in India from public sector (Sujatha et al. 2019). The salient features of hybrids released since 1980 are given in Table 19.2.

Table 19.2 Salient features of sunflower hybrids released from 1980 to 2020 in India (by public sector)

S. no.	Hybrids	Pedigree	Year of release	Maturity	Yield (kg/ha)	Oil content (%)	Salient features
1	BSH-1	CMS-234A × RHA-274	1980	85–90	1200–1300	41	Resistance to rust and downy mildew
2	LSH-1	CMS-338A × MRHA-II	1990	85–90	1000–1100	38	Resistance to downy mildew
3	LSH-3	CMS-207A × MRHA-1	1990	90–95	1200–1300	39	Resistance to downy mildew
4	KBSH-1	CMS-234A × RHA-6D1	1992	90–95	1300–1500	43	High yield with high oil content
5	PKVSH-27	CMS-2A × AK-1R	1996	85–90	1300–1400	39	Moderate resistance to downy mildew
6	DSH-1	DSF-15A × RHA-857	1997	85–88	1200–1300	38–40	Resistance to downy mildew
7	TCSH-1	CMS-234A × RHA-272	2000	–	–	–	–
8	KBSH-41	CMS-234A × RHA-95-C-1	2001	90–92	1300–1500 (R) 2500–3000 (I)	39–41	Tolerant to moisture stress
9	KBSH-42	CMS-851A × RHA-95-C-1	2001	90–92	1300–1500 (R) 2500–3000 (I)	38–41	Tolerant to moisture stress
10	NDSH-1	CMS-234A × RHA-859	2002	88–90	1400	40	Early hybrid
11	KBSH-44	CMS-17A × RHA-95-C-1	2002	95–98	1400–1600	36–38	High yield
12	LSFH-35	CMS-234A × RHA-1-1	2003	Medium	1400–1500	39–41	Resistance to downy mildew

(continued)

Table 19.2 (continued)

S. no.	Hybrids	Pedigree	Year of release	Maturity	Yield (kg/ha)	Oil content (%)	Salient features
13	PSFH-118	CMS-10A × P-61-R	2004	85–88	1400	40	Resistance to basal stem rot and head rot
14	HSFH-848	CMS-91A × RHA-298	2005	90–95	1800–2400	41–42	Resistance to *Alternaria* leaf spot and *Rhizoctonia*
15	DRSH-1 (PCSH-243)	ARM-243 × RHA-6D-1	2005	95–98	1300	43	High oil hybrid
16	TUNGA (RSFH-1)	CMS-103A × R-64-NB	2005	95–100	1300–1500	39–41	–
17	PSH-996	CMS-11A × P-93R	2012	96–100	1957	37–38	Suitable for late sown conditions
18	RSFH-130 (Bhadra)	CMS-104A × R-630	2008	95–100	1800–2000	39–42	Tolerant to necrosis
19	KBSH-53	CMS-335A × RHA-95C-1	2008	95–100	750–1250	38	Tolerant to powdery mildew
20	KSFH-437 (Phule Raviraj)	CMS-17A × R-437	2009	90–95	1800–2000	34	–
21	CO-2	COSF-1A × CSFI-99	2010	85–90	1900–2200	38–40	Early
22	LSFH-171	CMS-17A × RHA-1-1	2016	90–95	2000–2400	34–35	Resistance to downy mildew
23	PSH-1962	CMS-67A × P-93R	2015	99	2050	41.9	High yield and high oil content
24	RSFH-1887	CMS-38A × R-127-1	2016	95–100	1800–2500	38–40	Tolerant to necrosis and *Alternaria* leaf blight
25	NDSH-1012 (Prabhat)	NDCMS-30A × R-843	2016	90–95	2000–2500	40–41	Moderately resistant to downy mildew

26	PDKVSH-952	CMS-302A × AKSF-6R	2016	90	1800–2000	36.8	–
27	COH-3 (CSFH-12205)	COSF-6A × IR-6	2018	90–95	2200–2400	42	–
28	KBSH-78	CMS-1103A × RHA-92	2018	82–85	1700–2300	39–41	Short duration and medium height
29	DSFH-3	CMS-234A × RHA-IV-77	2018	90–95	2000–2500	37–39	High seed yield
30	PSH-2080	CMS-67A × P-160R	2019	97	2441	43.7	High seed and high oil content

Sources: Meena et al. (2013), Sujatha et al. (2019)

19.11 Major Breeding Objectives

19.11.1 Seed Yield

The main goal of plant breeding is to increase yield. Seed yield is a complex and polygenic controlled trait and strongly influenced by environmental conditions, and hence both additive (Sheriff and Appandurai 1985; Singh et al. 1989; Petakov 1992) and non-additive (Bajaj et al. 1997; Rether et al. 1998; Kumar et al. 1998; Goksoy et al. 2000; Cecconi et al. 2000) genetic effects play an important role in the inheritance of seed yield. Because of the importance of environmental effects, the heritability for seed yield is relatively low compared with other agronomic traits. Recurrent selection for general combining ability (gca), reciprocal recurrent selection, which capitalize on additive genetic variance, and recurrent selection for specific combining ability (sca), which capitalize on the non-additive portion of genetic variance, would be more effective breeding methods in improving seed yield. Seed yield consists of three main components: number of plants per hectare, seeds per plant and 1000 seed weight. Yield per unit area can be increased in a number of ways. One of the main approaches is to increase the seed number and seed size per head while maintaining or increasing plant number per unit area (Merrien 1992). To realize high seed yield in sunflower, parents that have good *gca* and *sca* for most yield components can be used in sunflower breeding (Tyagi 1988). Another most effective way of increasing the yield of sunflower is to exploit heterosis through hybrids. Sunflower seed yield may be increased significantly by breeding for seed size. According to Morozov (1947), the increase in 1000 seed mass by only 1.0 g brings an increase in seed yield of 40.0 kg/ha. To achieve high seed yield per unit area, many breeders consider it essential to develop a genotype capable of providing more than 1500 seeds per capitulum. A head size of 20 to 25 cm and flat shape are important in attaining this goal.

19.11.2 Oil Content and Quality

Sunflower seeds are mainly used for oil extraction, which is predominantly used for human nutrition. The oil concentration in sunflower may be reaching a plateau, but most breeders believe that selecting for higher oil content and oil quality in seed is still a very important and realistic objective when breeding for high oil and quality sunflower varieties and hybrids. According to Borodulina and Khachenko (1976), oil accumulation in sunflower seed begins on the first day after flowering and continues until physiological maturity. The period of most intensive oil accumulation takes place between the 15th and 22nd day after the beginning of flowering. It is a quantitatively inherited trait, and genetic variation is affected by additive genes. The heritability of this trait is relatively high to medium (Mokrani et al. 2002), and progress has been made in increasing oil content in sunflower. Hence, it can be improved through breeding methods like simple recurrent selection or through population improvement. Miller et al. (1987) reported that oleic acid was controlled

by a major gene with partially dominant gene action. Hence, it is clear that breeding for high oleic content is possible in sunflower. Wild *Helianthus* species may also be utilized for increasing oil, protein content and quality in cultivated sunflower. Thompson et al. (1978, 1981) reported that *H. niveus* (Benth.) and *H. salicifolius* are potential sources for oil content. 'Pervenets' breeding lines contain a dominant mutation, which increases oleic acid content to more than 89% in the sunflower oil. Several QTLs have been identified on various linkage groups for seed oil content and altered fatty acid composition of sunflower oil. These QTLs had additive to dominant effects and closely related to domesticated related traits in sunflower (Leon et al. 2001; Burke et al. 2005; Ameena et al. 2016).

19.11.3 Diversification of Cytosterility System

Although more than 72 diverse CMS systems have been reported in sunflower, until now only one system based on PET-1 cytoplasm has been exploited commercially due to the non-availability of proper restorers and maintainers for other CMS lines. Such type of dependency on a single source of male sterility could lead to a narrow genetic base leading to a potential risk and high degree of genetic vulnerability in hybrid sunflower cultivation which can predispose the crop for some unforeseen situations of biotic and abiotic stresses in future years. Sunflower yields have plateaued with the currently available genotypes. Hence, it is essential to diversify the parental material (CMS and restorer base) to develop hybrids and varieties with high yield potential. Development of newer CMS sources should be complemented by identification of suitable restorers for effective hybrid seed production. Among several strategies available to overcome this problem, diversification of CMS sources itself is possibly the inexpensive and most effective method.

19.11.4 Biotic Constraints

Biotic stresses cause significant losses in crop plants and management of biotic stresses (diseases and pests) not only increases the cost of production but also has implications on environment and ecology (Bainsla and Meena 2016). The important diseases that cause significant yield losses in sunflower are *Alternaria* leaf spot [*Alternariaster helianthi* (Hansf.) Tub. and Nish.] (Carson 1985), powdery mildew (*Golovinomyces orontii* (Castagne) V.P. Heluta), downy mildew [*Plasmopara halstedii* (Farl.) Berl. & De Toni] (Gulya et al. 2013), sunflower necrosis disease (SND) (Bhat and Reddy 2016) and rust (*Puccinia helianthi* Schwein.) (Shtienberg and Zohar 1992). Sunflower production in India is constrained by the susceptibility of the released varieties/hybrids to a wide array of biotic stresses. In India, the major diseases and insect pests prevalent in sunflower-growing areas are SND, *Alternariaster* leaf spot, powdery mildew, downy mildew, *Spodoptera litura* and leafhoppers (Basappa and Santhalakshmi Prasad 2005). Sunflower leaf curl virus (SuLCV) also has become severe in India, and there reports on resistance sources for

Table 19.3 Disease resistance genes identified in wild *Helianthus* species

S. no.	Biotic stresses	Resistant/tolerant wild species	References
1.	*lternariaster* leaf spot	*H. tuberosus*	Skoric (1988)
		H. maximiliani, *H. mollis*, *H. divaricatus*, *H. simulans*, *H. occidentalis*, *H. pauciflorus*, *H. decapetalus*, *H. resinosus*, *H. tuberosus*	Sujatha et al. (1997)
		H. occidentalis, *H. tuberosus*	Madhavi et al. (2005)
		H. tuberosus, *H. resinosus*	Sujatha and Prabakaran (2006)
		H. tuberosus, *H. maximiliani*, *H. strumosus*	Santhalakshmi Prasad et al. (2017)
2.	Rust	Wild *H. annuus*, *H. argophyllus*, *H. petiolaris*	Quresh et al. (1993)
		Wild *H. annuus*	Gong et al. (2012)
		Prebred (PS-1089) derived from *H. argophyllus* × cultivated sunflower	Sujatha et al. (2003)
3.	Downy mildew	AD-66 derived from wild *H. annuus*	Vranceanu and Stoenescu (1970)
		H. argophyllus	Qi et al. (2019)
4.	Powdery mildew	*H. tuberosus*, *H. praecox*, *H. bolanderi*	Acimovic (1998)
		H. decapetalus, *H. divaricatus*, *H. laevigatus*	Dedic et al. (2012)
5.	Sucking pests	*H. argophyllus*	Seetharam and Ravi Kumar (2003)
	Tobacco caterpillar	Backcross inbreds derived from *H. argophyllus*, *H. petiolaris*	Sujatha and Lakshminarayana (2006)
	Sunflower pests	*H. tuberosus*, *H. maximiliani*	Seetharam and Ravi Kumar (2003)
	Leafhoppers	Prebred lines derived from *H. argophyllus*	Unpublished

SuLCV from wild species are not available (Govindappa et al. 2011). Insect pests of sunflower are different in tropical and temperate countries. Crop losses due to insect pests in sunflower vary from place to place. Continuous cultivation of the crop subjected it to disease pressure with overlapping disease cycles. Host plant resistance is a major objective in most of the breeding programmes across the crop species including sunflower and is considered as one of the most viable options for enhancing the productivity. The level of resistance available in cultivated species/primary gene pool for some of the diseases and insect pests is rather low, and only a limited number of resistance sources are available. This limited resistance is inadequate to manage more virulent pathogenic strains of diseases that arise during intensive crop production. Therefore, the discovery and incorporation of new genes from wild species provide a means of complementing crop improvement programmes (Table 19.3). The chances of finding dominant genes containing resistance to economically important diseases are higher in wild species which if deployed

effectively using new breeding tools including molecular and biotechnology can be an effective means of combating virulent pathogens.

19.11.5 Abiotic Constraints

Sunflower is generally grown in marginal lands, often in semiarid conditions, where abiotic stresses always act as a major limiting factor for its production and productivity in many parts of the world (Škorić 1987). Therefore, development of a heat-resistant sunflower breeding population or hybrid or breeding for resistance to drought, high temperature and salinity assumes priority for sustainable yield under abiotic stress conditions.

Drought is considered as the single most devastating environmental stress, which decreases crop productivity more than any other environmental stresses (Lambers et al. 2008). Chimenti et al. (2002) reported 5–56% yield reduction in sunflower if drought occurs immediately prior to anthesis. An appropriate strategy and criteria of selection are essential in selection for drought resistance. Restorer gene pool lines RGP-46-P_3 and RGP-60-P_1 developed through simple recurrent selection at ICAR-IIOR, Hyderabad, recorded low drought susceptibility index and good seed yield under moisture stress and were found promising for drought breeding. The use of landraces, cultivated hybrids and varieties has produced some positive results, but not to the extent that would secure stable sunflower production under drought conditions. The best results in enhancing drought resistance of cultivated sunflower have been achieved using wild *Helianthus* species. Some wild sunflower species have been reported as drought-tolerant species, and the introgression of traits from these species is expected to increase drought tolerance in cultivated breeding lines (Saucă et al. 2014). Among the related species, *H. argophyllus* was identified as particularly drought tolerant (Belhassen et al. 1996; Jan et al. 2014; Saucă et al. 2014; Hussain et al. 2017).

Seiler (2012) indicated that *H. anomalus* Blake and *H. deserticola* Heiser are highly adapted to desert and sandy areas and could be used as germplasm source for heat stress studies. Sideli et al. (2013) reported *H. cusickii* to be a potential source for genes for drought resistance.

Salinity is also another factor reducing sunflower yield in many countries including India. Identification of resistance sources from cultivated sunflower is very important for improvement of salinity tolerance of sunflower. Based on plant growth and survival, hybrids CSFH-12205, CO-2, KBSH-44 and DRSH-1 and inbreds, viz. COSF-1A and CMS-103A, were found tolerant at Gangavathi, and CSFH-12205, CO-2, KBSH-44, COSF-6A and COSF-7A were found tolerant at Machilipatnam (Anonymous 2018). Seiler et al. (1981) and Rogers et al. (1982) suggested that *H. paradoxus* would be a likely candidate for salt-tolerant genes. Miller and Seiler (2003) transferred salt-tolerant genes from *H. paradoxus* into cultivated sunflower and released two salt-tolerant maintainer lines, HA-429 and HA-430. Hajjar and Hodgkin (2007) suggests that *H. paradoxus* has a great potential to breed more salt-tolerant cultivated sunflowers, with hybrids developed using this trait potentially

providing a 25% yield premium in saline soils. Shtereva et al. (2015) suggested that the diploid perennial species *H. mollis* could serve as an excellent candidate of salt-tolerant genes. Breeders should apply effective screening methods to identify the donor wild species that possess genes useful in breeding for abiotic stresses and equally effective breeding methods to transfer these genes into cultivated sunflower genotypes.

19.12 Breeding Approaches

19.12.1 Conventional Approaches

Sunflower is a highly cross-pollinated (allogamous) crop, and therefore breeding procedures suitable for cross-pollinated crops are utilized for improvement of sunflower. In general, introduction, mass selection, pedigree method, backcross breeding, recurrent selection and Pustovoit method of reserves and mutation breeding were used for sunflower improvement. The choice of the suitable breeding programme for the development of tolerant cultivars to a defined abiotic stress depends upon a number of factors: screening techniques, sources and mechanism (s) of tolerance, modes of gene action and heritability and their relationship to agronomic traits (Meena et al. 2016).

19.12.1.1 Introduction

Plant introduction consists of taking a genotype or a group of genotypes of plants into new environments where they were not grown earlier. In India, the first sunflower variety 'EC-68415' was developed in 1976 by AICRP (Sunflower) Centre, University of Agricultural Sciences, Bengaluru, and released for Karnataka state through an introduction. Another short-duration (80–82 days) popular variety 'Morden' was developed through introduction from Canada in 1978. It was released for all India cultivation. Morden was very popular among sunflower growers due to its short stature, short duration and high seed yield.

19.12.1.2 Mass Selection

Mass selection is a method of selection of desirable plants from a population based on their phenotypic characteristics. This method has been used for cultivar improvement in sunflower for many years and was effective in developing cultivars with early maturity, higher oil percentage and resistance to diseases. The efficiency of mass selection depends on gene effects of the selected traits, their heritability, sample size and genotype × environment interaction. Mass selection is effective for characters controlled by additive genes (Gowda and Seetharam 2008). Many varieties like Fuksinka-3, Fuksinka-10, Omskiy Skorospeliy, etc. were developed in the Soviet Union through this method (Skoric 1992). Varieties like Guayacan INTA, Cordobes INTA, Manfredi INTA, etc. were also developed using mass selection in Argentina (Luciano and Davreux 1967). In India, the variety Surya (PKV-SUF-72-37) was developed by mass selection from Latur bulk and released for Maharashtra

state in 1982. Still, this is one of the important breeding methods for sunflower improvement for many traits.

19.12.1.3 Pedigree Method

Pedigree selection is the most common procedure used to develop sunflower inbred lines. The procedure involves self-pollination of phenotypically desirable plants within existing cultivars, breeding populations or segregating generations of planned crosses. Fick and Swallers (1974) used this method to develop the first downy mildew-resistant restorer lines, viz. RHA-271, RHA-273 and RHA-274. Hulke et al. (2010) also developed new rust and downy mildew-resistant restorer line RHA-464 (Reg. No. GP-325; PI-655015; experimental '05187'), using pedigree method. In India, a large number of fertility restorer lines have been developed using the pedigree method (Meena et al. 2013). Jaffar et al. (2019) developed high oleic lines through pedigree method using PI-1806 × B-124 parents. In India, this is the only breeding method utilized by breeders for development of restorer lines and inbreds.

19.12.1.4 Backcross Method

Backcross selection refers to a form of breeding that uses a superior inbred that may nevertheless lack a particular trait. It is mainly used for the transfer of disease-resistant traits in superior inbred lines. But the fertility restorer lines are also developed by incorporating dominant restorer gene(s) by backcrossing using inbred lines of proven performance as the recurrent parent (Vranceanu and Stoenescu 1976). Backcross is also used for the transfer of disease resistance from related species to cultivated species. This method is also employed to transfer male sterility trait from a donor. New CMS lines are mostly developed in sunflower through the backcross method. High oleic acid (>90%) was identified in AOP-l and was used as a donor of this character in a backcross programme to incorporate the trait into commercial material. Liu et al. (2013) introgressed fertility restoration gene Rf_6 for CMS-514A from an interspecific amphiploid (Amp) of *H. angustifolius*/P-21 (2n = 68) into cultivated sunflower by backcross method.

19.12.1.5 Recurrent Selection

Recurrent selection refers to the method of selecting special gene traits from the best sunflower family. Presently, recurrent selection appears to be the most promising method of increasing the frequency of desirable genotypes in a source population, thus enhancing the chance of isolating superior inbred lines. It is a useful method in sunflower breeding, especially for the establishment of the gene pool for different purposes, primarily of increased productivity and resistance to diseases, insects, drought and other stresses. The recurrent selection method has not been exploited in breeding programmes in India even though considerable progress has been achieved using this method in other countries like the USA and France (Virupakshappa 1987). Miller et al. (1977) obtained an increment of 12.2% of oil content after three cycles of simple recurrent selection. Miller and Hammond (1985) reported an increase in seed yield by 6.3% after three cycles of selection. Resistance for *Sclerotinia*

sclerotiorum was obtained by Vear and De Labrouhe (1984) through three cycles of recurrent selection. Dr. Pustovoit in the USSR was a pioneer to practically utilize recurrent selection for oil improvement in which selection was based on progeny performance and subsequent cross-pollination is allowed only among superior progenies. Pustovoit (1964) was highly successful in improving oil content from about 30% in the early 1920s to over 50% in the present-day varieties. Shobha Rani and Ravikumar (2006) also improved partial resistance to *Alternariaster* leaf blight through sporophytic and gametophytic recurrent selection. Vear et al. (2007) reported significantly improved *Sclerotinia* head rot resistance after 15 cycles of recurrent selection. Harinarayana et al. (1980) noticed an improvement in seed yield, seed set and oil content through intra- and inter-population improvement. Powdery mildew-resistant restorer inbreds RGP-21-P_4-$S_{1–3}$ and RGP-50-P_2-S_1 were developed through population improvement after three cycles (Anonymous 2018). A wide range of variability was observed by Seneviratne et al. (2004) for plant height, days to maturity, head diameter, number of filled seeds and seed yield in C_3 cycle (Anonymous 2018). Emphasis has to be laid on using recurrent selection for population improvement to act as a source for development of new parental lines, and the improvement of existing parental lines should receive more attention to achieve a real breakthrough in yield.

19.12.1.6 Mutation Breeding

This method was mostly used in cultivated sunflower for creating genetic variability through irradiation with gamma rays or chemical mutagens. Seed treatment with gamma irradiation has been extensively used to increase variability for several characteristics, such as days to flowering, seed weight, seed coat colour and oil content (Giriraj et al. 1990; Jambhulkar and Joshua 1999; Encheva et al. 2003) in cultivated sunflower. Genetic variability for resistance to *Alternariaster* leaf spot disease was induced by radiation or chemical mutagens (De Oliveira et al. 2004). *Orobanche*-resistant lines were developed through mutation breeding by Encheva et al. (2008, 2012, 2014). Cvejić et al. (2014) found changes in fatty acid and tocopherol content and composition in sunflower oil. Lofgren and Ramaraje-Lers (1982) isolated plants with resistance to sunflower rust in M_2 and M_3. Herbicide-resistant sunflower mutants were generated by induced mutagenesis (Bervill et al. 1992; Sala et al. 2008). Drumeva (2012) developed 159 new downy mildew, *Phomopsis* and *Alternaria*-resistant fertility restorer lines with desirable breeding characteristics by using the gamma ray-induced parthenogenesis method. Jan and Rutger (1988) reported induced male sterility in M_1 heads of HA-89 treated with mitomycin C and streptomycin. Soldatov (1976) produced high-oleic sunflower variety 'Pervenets' by treating the seed of variety VNIIMK-8931 with 0.5% solution of dimethyl sulphate. In India, the sunflower variety Gujarat Sunflower-1 (GAUSUF-15) was developed through mutation breeding and released in 1993 all over India. Another variety CO-3 (TNAUSUF-10) was developed from CO-2 using 5KR gamma rays and released for Tamil Nadu state in 1995. The variety TAS-82 (TAS-82-8-1/3) was developed from Surya variety through mutation breeding in 2006 and recommended for Vidarbha region of Maharashtra state. Readers

interested in additional information on mutations can refer to the article of Vasko and Kyrychenko (2019) on induced mutagenesis for the creation of new starting material in sunflower breeding.

19.12.2 Hybrid Seed Production

19.12.2.1 Development of A, B and R Lines

Hybrids developed using the CMS fertility restorer system require three parental lines, viz. CMS or 'A' line, fertility maintainer or 'B' line and a restorer line or 'R' line (male line). In hybrid development programme, inbred lines are developed by selfing or sib mating from adapted varietal populations, gene pools, local varietal populations, inter-varietal hybrids, composites, synthetics, single-cross and multiple-cross hybrids and interspecific hybrids, etc., over the generations and essentially evaluated for combining ability by inter-crossing among the best by following standard genetic model. Once the best combinations are identified based on high combining ability and per se performance, the female line is converted into cytoplasmic male sterile line (A line) with the original inbred as the isogenic maintainer line (B), and the male inbred line is converted into restorer line (R line). CMS lines are developed by repeated backcrossing using known CMS lines as a non-recurrent parent (female parent). About five to six backcrosses are required to derive a new CMS line genetically similar to the recurrent parent. The inbred, which was used as a recurrent parent (male parent), serves as a maintainer (B line). Derived CMS line will be employed as the female in seed production of hybrids along with the selected male line based on the combining ability and productivity. Wherever the hybrids show 100% fertility in F_1, the 'R' lines may be used directly as the male parent. If the fertility restoration is partial or low in the F_1 plants, fertility restorer lines are developed by crossing the selected best combiner as a recurrent parent to a known restorer line of proven performance. By six to seven backcrosses, R gene(s) may be transferred to the selected male line.

19.12.2.2 Maintenance of Parental Lines

Two methods of planting, viz. row method and block method, are followed for maintenance of the 'A' line (Fig. 19.2). The A × B crosses are used to maintain the 'A' line following separate row and block method of sowing. To take up the breeder/foundation seed production of female (A) line, the planting ratio of 'A' and 'B' is 3:1. To facilitate better pollination, mark the first two rows and last two rows of the seed plot and subsequently every fourth row with a wooden peg to plant these lines with 'B'. The sowing of 'A' and 'B' lines should be taken up by engaging labourers separately. A and B lines in breeder/foundation stages are planted in 3:1 proportion in separate blocks. During anthesis, the pollen is collected from the 'B' line and pollinated on to 'A' line in respect of breeder/foundation seed production. This method ensures the production of high-quality hybrid seeds and meets the genetic standards.

Row method

BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB

Block method

Breeder/Foundation seed production	
Block-I	**Block-II**
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB

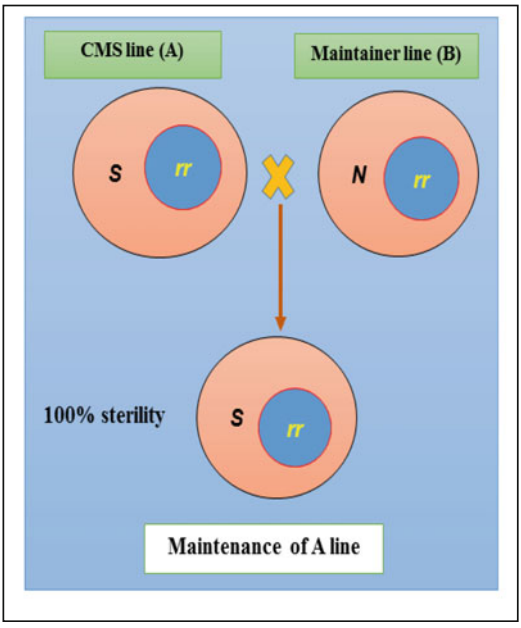

Fig. 19.2 Maintenance of 'A' line through row and block method

19.12.2.3 Certified Hybrid Seed Production

To realize high productivity levels in the commercial scale, the supply of quality hybrid seed assumes importance. The production of high-quality seed of parental lines and the certified seed of hybrid requires systematic planning and management on the part of the seed producers (Fig. 19.3). As in the production of 'A' line, two methods, viz. row method and block method, are being followed to produce hybrid seed in sunflower (Fig. 19.4). The proportion of female to male is 3:1, i.e. three rows of female (seed parent) to one row of male (pollen parent). In recent times, the seed production agencies have faced serious seed quality problems as the seed lots of hybrids containing a high amount of 'R' line plants are contaminated during harvesting/drying in addition to different stages of post-harvest operations followed in the above 3:1 method.

In the suggested block system, A and R lines are planted in separate bocks in 3:1 ratio. At the time of anthesis, the pollen is collected separately from R lines and pollinated on to 'A' line in respect of certified seed production. This method ensures the production of high-quality hybrid seed and meets the genetic standards.

19.12.3 Limitations of Conventional Breeding

Traditional approaches to breeding crop plants with improved abiotic stress tolerances have so far met with limited success (Richards 1996). This is due to several contributing factors including the following: (1) the focus has been on yield rather than on specific traits; (2) the difficulties in breeding for stress tolerance traits,

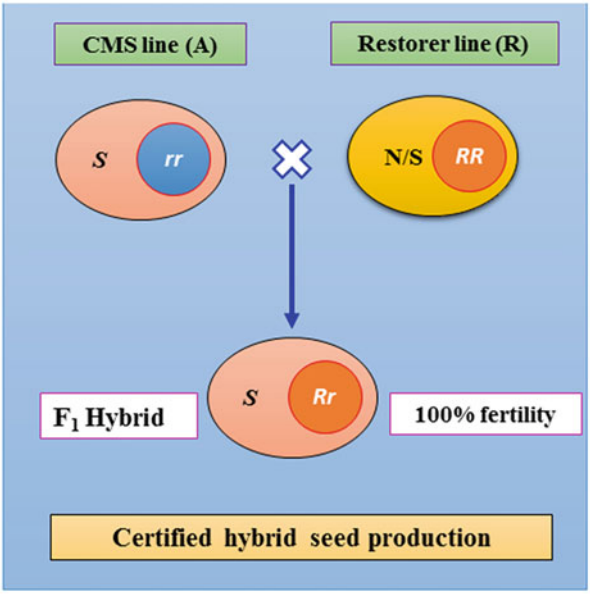

Fig. 19.3 Procedure of certified hybrid seed production

Breeder/Foundation seed production
Row method
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR

Certified seed production	
Block-I	Block-II
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR

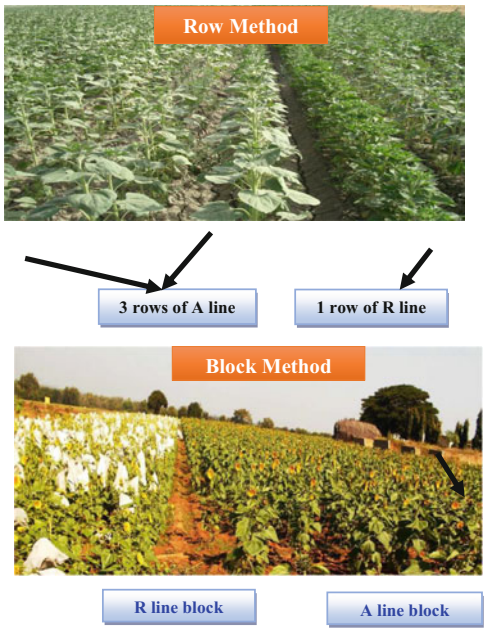

Fig. 19.4 Certified hybrid seed production through row and block method

which include complexities introduced by genotype by the environment, or G × E interactions and the relatively infrequent use of simple physiological traits as measures of tolerance; and (3) desired traits can only be introduced from closely related species.

19.12.4 Molecular Breeding

Despite the large genome size (2871–3189 Mbp) of cultivated sunflower, concerted efforts led to the genome assembly up to the chromosome level with a total length of 3010 (Mb), protein count of 75,695 and GC content of 38.8% (PRJNA396063). Molecular markers have played a significant role in accelerating sunflower breeding programmes through marker-assisted selection (MAS) by virtue of their abundance, technical ease and detection at any development stage of the plant besides being not affected by environmental factors. To facilitate MAS, a number of molecular marker systems, namely, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequence (CAPS), insertion/deletion (INDEL), single nucleotide polymorphism (SNP) and target region amplification polymorphism (TRAP) markers, have been developed and used for various molecular applications in sunflower (Berry et al. 1995; Gentzbittel et al. 1995, 1998; Jan

et al. 1998; Flores Berrios et al. 2000; Burke et al. 2002; Mokrani et al. 2002; Bert et al. 2003, 2004; Langar et al. 2003; Yu et al. 2003; Rachid Al-Chaarani et al. 2004; Lai et al. 2005; Hu et al. 2007; Poormohammad Kiani et al. 2007; Yue et al. 2008). These markers have been extensively used in several applications like assessment of genetic relationships among genotypes, construction of linkage maps, tagging and mapping genes/QTLs of agronomic interest, development of genomic and cDNA libraries, map-based cloning and marker-assisted selection.

In sunflower, the first map was developed using RAPD markers (Rieseberg et al. 1993), followed by RFLP markers (Berry et al. 1995; Gentzbittel et al. 1995; Jan et al. 1998), AFLP markers (Gedil et al. 2001), SSR markers (Tang et al. 2003; Yu et al. 2003) and SNP markers (Lai et al. 2005; Bowers et al. 2012; Talukder et al. 2014; Celik et al. 2016; Livaja et al. 2016) covering the 17 linkage groups. These maps were further enriched with gSSRs, EST-SSRs, INDELs, TRAPs and SNPs (Hu et al. 2007; Heesacker et al. 2008), and a consensus map with 10,080 loci was constructed (Bowers et al. 2012). Recently, studies on large-scale SNP detection and generation of large sets of markers through genotyping by sequencing have been undertaken by several groups (Baute et al. 2016; Celik et al. 2016; Talukder et al. 2016; Mangin et al. 2017; Ma et al. 2017; Qi et al. 2017;). For more information of molecular markers and marker-assisted breeding, readers may refer the articles of Dimitrijevic and Horn (2018).

Molecular markers for simple inherited traits like fertility restoration, herbicide tolerance, resistance to downy mildew, *Orobanche cumana*, rust, high stearic and oleic acid contents (β and γ tocopherols) have been successfully deployed in marker-assisted breeding programmes (Sujatha and Sujatha 2013). QTLs have been identified, but genome-wide association studies are required. However, for investigating complex traits controlled by polygenes, such as seed yield, oil content, resistance to *Sclerotinia* mid-stalk rot, black stem and abiotic stresses like drought, salinity and chilling. Of the various traits that have been improved through MAS, resistance to downy mildew was highly successful as the resistance was dominantly inherited (Dimitrijevic and Horn 2018).

With regard to the genetic resources, initial studies focused on development of biparental populations based on crosses involving elite breeding and introgressed lines (Berry et al. 1995; Horn et al. 2003; Livaja et al. 2016), land races (Kim and Rieseberg 1999), wild *Helianthus* species (Quillet et al. 1995; Ma et al. 2017) or the recombinant inbred lines (Tang et al. 2006; Talukder et al. 2016). These populations were used to map genes conferring resistance to downy mildew, QTLs governing *Sclerotinia* stalk rot resistance and seed quality traits, but owing to the disadvantages of the time and costs involved for deployment of individual populations, low resolution of mapping, mortality of the populations besides evaluation of only two alleles per locus alteration strategies were adopted. Understanding the need for assembling the association panels, researchers have directed their efforts towards this goal. Accordingly, association panels consisting of 433 cultivated accessions from the USA and Europe maintained at USDA and INRA, respectively (Mandel et al. 2011); 170 accessions representing Argentinian collections maintained at INTA (Filippi et al. 2015), which distinguished the maintainer and restorer gene

pools; and 196 Spanish confectionery accessions maintained at CRF-INIA (Velasco et al. 2014) were characterized which disclosed large genetic variation for seed oil content, test weight and seed quality traits.

19.12.5 Trait Improvement Through Genetic Engineering

Despite the successes with traditional breeding methods and interspecific gene transfer for several traits such as cytoplasmic male sterility, fertility restoration gene(s), seed quality traits, resistance to biotic and abiotic stresses, biotechnological interventions are required for introgression of beneficial traits into cultivated sunflower. Direct and callus-mediated shoot organogenesis from different tissues such as cotyledons, hypocotyls, petioles, leaves and immature embryos is reported (Witrzens et al. 1988; Knittel et al. 1991; Pugliesi et al. 1991; Sujatha et al. 2012a). Regeneration through somatic embryogenesis using immature zygotic embryos, mature seeds and seedling tissues is also reported (Finer 1987; Mc-Cann Wilcox et al. 1988; Espinasse and Lay 1989; Sujatha and Prabakaran 2001). The key factors favouring organogenesis include genotype, explant type, age and physiological state of the cultured tissue, exogenous growth regulator combinations and their balance with endogenous growth regulators, etc. Shoot regeneration and quality of shoots are reported to be enhanced through the use of ethylene inhibitors like cobaltous chloride ($CoCl_2$) and silver nitrate ($AgNO_3$) molecules (Chraibi et al. 1992), preconditioning of explants with 5 mg/l BAP (Zhang and Finer 2015) and short-pulse treatments coupled with micrografting (Zhang and Finer 2016). Precocious flowering, hyperhydricity, poor rooting and abnormal morphogenesis are few other problems encountered in sunflower shoot cultures (Lupi et al. 1987; Freyssinet and Freyssinet 1988). Even after four decades of research carried out at several laboratories, availability of a reliable, efficient, reproducible and genotype-independent tissue culture-based regeneration system still continues to be a major limitation for application of genetic engineering tools in sunflower.

Genetic transformation experiments were largely through *Agrobacterium*-mediated techniques using explants with pre-existing meristems as target tissues such as shoot tips, mature embryos, immature embryos and cotyledons from mature seeds. The initial studies were chiefly aimed at optimization of variables for enhancing the transformation efficiency and mostly confined to characterization of primary transformants and the T_1 generation plants. Owing to the amenability of sunflower to *Agrobacterium tumefaciens* infection, high frequency of transient expression is observed in most of the transformation experiments. However, conversion efficiency of transient to stable integration was rather limited besides leading to chimeras (Schrammeijer et al. 1990; Malone-Schoneberg et al. 1994; Grayburn and Vick 1995; Weber et al. 2003). Further, formation of chimeric shoots, low regeneration rate from transformed cells/calli, low *Agrobacterium* virulence, bacterial strain, extreme sensitivity to antibiotics, genotype dependence and lack of stable transmission of the introduced gene are major factors limiting the overall efficiency of the transformation systems reported so far. Attempts made at enhancing transformation

efficiency by imposing wounding treatments using glass beads (Grayburn and Vick 1995; Alibert et al. 1999); sonication at 50 MHz (Weber et al. 2003); vacuum infiltration (Hewezi et al. 2003); digestion with macerating enzymes like cellulose, pectinase and macerozyme (Alibert et al. 1999; Weber et al. 2003); incubation with acetosyringone (Laparra et al. 1995); co-transformation with cytokinin synthesis (*ipt*) gene (Molinier et al. 2002); and dehydration and rehydration of target tissues (Hewezi et al. 2002) met with limited success. *Among the commonly used reporter genes f*or selection of putative transformants (hygromycin, kanamycin and basta), kanamycin is used widely as the plant selection agent in sunflower due to ease of identification of green shoots (putatively transformed) from bleached shoots (untransformed). The problem of premature flowering has been overcome to some extent by incorporation of cytokinins like 2-isopentenyl adenine (2-iP) and kinetin, incubating the tissues at a temperature of 20 °C with 8/16 h light/dark photoperiod cycle or grafting of in vitro recovered putative transformants onto healthy root stocks (Weber et al. 2003; Sujatha et al. 2012b). Step-wise protocols for sunflower transformation are described by Lewi et al. (2006), Radonic et al. (2015) and Manavella and Chan (2009) for development of transformants and for transient expression analysis which can be used for gaining valuable insights of several biological processes through functional validation of genes.

While most of the genetic transformation studies undertaken in sunflower are aimed at establishment of the transformation system, transgenic development for agronomically desired traits was mainly for incorporation of resistance to biotic stresses (*Sclerotinia sclerotiorum*, *Alternariaster helianthi*, necrosis disease) and resistance to abiotic stresses (drought, salinity). *S. sclerotiorum* is an economically important disease in the temperate regions causing root rot, mid-stalk rot and head rot, and oxalic acid has been identified as the key component in *Sclerotinia* infection. Genetic engineering for enhanced resistance to *S. sclerotiorum* is aimed at strategies to degrade oxalic acid and was through deployment of candidate genes like wheat germin oxalate oxidase (OXO) (Lu et al. 2000; Scelonge et al. 2000); coumarin phytoalexins (ayapin and scopoletin) (Urdangarin et al. 1999); and antifungal genes like glucanase, chitinase, osmotin gene and a ribosome inhibitor protein (Radonic et al. 2008). For conferring resistance to *A. helianthi*, transgenic lines harbouring β-1,3-glucanase (Manoj Kumar et al. 2011) and *TVD1* gene (Sirisha et al. 2011) were developed. Sunflower necrosis disease (SND) incited by *Tobacco streak virus* of *Ilarvirus* group accounts for yield losses ranging from 10 to 80% in the tropics and sub-tropics (Jain et al. 2003). Deployment of *TSV-CP* gene in sense and antisense directions was undertaken for conferring resistance to SND (Pradeep et al. 2012; Singareddy et al. 2018; Sunderesha 2017).

Abiotic stresses like drought, salinity and heat have their effects not only on seed yield but also on the oil content. Candidate genes were deployed to increase tolerance to abiotic stresses such as suppression of proline dehydrogenase (*ProDH1*) gene for drought and salinity (Tishchenko et al. 2014). Studies at metabolic engineering towards improving oil stability and nutritional quality are targeted at development of high-oleic sunflower by knocking out delta-12-desaturase gene

which encodes linoleic acid and lines with increased linoleic acid using PTGS technology (Lacombe et al. 2009; Chen et al. 2010).

Genome editing through CRISPR/*cas9* technology is still in its infancy in case of sunflower. Innovative Genomics Institute, California, is at establishing tools for genome editing in sunflower (https://innovativegenomics.org/projects/establishing-tools-sunflower-genome-editing/).

19.13 Conclusions

One of the major challenges in sunflower would be to enhance productivity to the level of the world's average. To achieve this, populations and hybrids superior to the presently grown cultivars need to be bred which should combine high seed yield (>3.0 t/ha), high oil content (>42.0%) and resistance to major pests like leafhopper and diseases like *Alternariaster* leaf spot, powdery mildew, downy mildew, necrosis, rust, stem rot, etc. Another important area of breeding research would be to enhance tolerance/resistance to abiotic stresses. Intensive and monocropping of sunflower has been the major cause for outbursts of diseases like rust, leaf spots, powdery mildew, sunflower necrosis disease, leaf curl virus and downy mildew which require immediate attention. Root rots are becoming severe in some parts of the country. Hence, development of hybrids with multiple resistance(s) will have to be the major thrust areas in the years to come. Public-private partnership is also very important for area expansion and development of high heterotic hybrids. Utilization of information being generated through genomic resources and tools assumes importance in tackling problems such as abiotic stresses and identification of physiological traits contributing to seed yield through genomic selection and GWAS. Wild *Helianthus* species displayed extensive variability for various qualitative and quantitative traits including tolerance/resistance to biotic and abiotic stresses which can be incorporated in cultivated sunflower through introgressive breeding and novel breeding techniques (cisgenics, genome editing).

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