#### Sunflower

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Sunflower (Helianthus annuus L.) is an important oilseed crop which competes in the "world oilseed complex" with three other major oilseeds viz., soybean, groundnut and rapeseed. In 2010, global area under sunflower cultivation was about 23 million hectares and the production was about 30 million tonnes with an average productivity of 1322.6 kg/ ha (Table 1: FAOSTAT). An introduced crop in India in early 1970s, sunflower has become an important oilseed crop with 1 million hectares under its cultivation (Table 2). In 2000-01, Karnataka (45%), Maharashtra (31%) and Andhra Pradesh (18%) were the leading states, which covered almost 94 percent of total acreage of sunflower in India. In contrast, during 2009-10, Karnataka accounted for 54 percent followed by Andhra Pradesh with 24 percent (Table 3). A substantial reduction in sunflower area has occurred in Maharashtra between 2001 (31%) and 2009 (15%). The analysis of growth rates of sunflower during the past decade in the country indicates clear signals of deceleration in the area and production (Vision 2030, DOR; Nayak et al., 2010). The crop, hitherto confined to southern parts of India, has made inroads into non-traditional areas such as Punjab, Harvana, Uttar Pradesh as spring/summer crop during the last decade.

Sunflower is primarily used for extracting cooking oil but also finds use in other sectors such as an annual ornamental, confectionary, animal feed, cosmetic and pharmaceutical industry. The seeds contain 40–46

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percent edible oil. Traditional sunflower oil is composed of saturated - C16:0 palmitic (7%) and C18:0 stearic (4%), monounsaturated - C18:1 oleic (20%) and polyunsaturated - C18:2 linoleic (69%) fatty acids. Sunflower oil is valued as a vegetable oil because of high level of polyunsaturated linoleic acid, which can reduce the risk of

Table 1: Area, production and productivity of sunflower in major growing countries

Country	Area (m ha)	Production (m t)	Productivity (kg/ha)
Ukraine	4.526	6.771	1496
Russian Federation	5.575	5.345	958
Argentina	1.489	2.221	1491
France	0.695	1.633	2350
Bulgaria	0.700	1.596	2280
Turkey	0.641	1.320	2058
Romania	0.786	1.263	1606
China	0.970	1.710	1763
Hungary	0.501	0.970	1934
United States of America	0.758	1.241	1636
	0.698	0.887	1271
Spain	1.000	0.650	650
India World-Total	23.104	30.559	1323

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Table 2: Area, production and productivity of sunflower in India over the past five decades

Year	Area (m ha)	Production (m t)	Productivity (kg/ha)
2010	1.000	0.650	650.0
2000	1.074	0.646	602.0
1990	1.633	0.873	534.6
1980	0.119	0.066	555.3
1970	0.117	0.076	649.6

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Table 3: Area, production and productivity of sunflower in major growing states of India during 2009-10

State	Area (ha)	Production (t)	Productivity (kg/ha)
		304000	383
Karnataka Andhra Pradesh	794000 350000	270000	771
Maharashtra	210000	114000	520
Bihar	22600	31700	1403
Odisha	17700	16000	904
Tamil Nadu	14500	19000	1329

http://www.krishisewa.com/articles/2012/sunflower3.html

cardiovascular disease. In addition, the oil contains tocopherols, which are antioxidants that provide oxidative stability to the oil. More details can be found in Seiler and Jan (2010).

# Genetic Resources

Sunflower is domesticated from wild H. annuus in North America. It was introduced into Europe during early 16th century. Russia was the first country to recognize it as a source of oil. In 1969, sunflower was introduced as an oil seed crop in India. Taxonomically, sunflower belongs to the family Compositae (Asteraceae), subfamily Asteroideae, tribe Heliantheae, subtribe Helianthineae. As of now, 51 Helianthus species have been described which include 14 annuals and 37 perennials. Chromosome number (n) of annual species is 17 and perennials have n = 17, 34 or 51 (Seiler and Jan, 2010). The North Central Regional Plant Introduction Station, Ames, Iowa of the USDA-ARS maintains the largest sunflower germplasm collection in the world. USDA-GRIN database reports maintenance of 4155 accessions of which 2853 accessions belong to  $H.\ annuus$  collected from 59 countries (USDA-GRIN database as on 10 June 2012). Distribution of USDA germplasm has largely contributed to research programmes in Argentina, France, Italy, Spain, Germany, Bulgaria, Romania, Czech Republic, Hungary, Russia, Serbia, India, China and Mexico. In India, more than 2000 accessions are maintained at the Directorate of Oilseeds Research, Hyderabad and distributed through the All India Coordinated Research Programmes (AICRP) for sunflower improvement in the country. The Directorate of Oilseeds Research also maintains 43 wild Helianthus species for genetic enhancement of sunflower through interspecific gene transfer.

## Hybrids

Earlier, sunflower varieties were open pollinated populations. VNIMK 6540, VNIMK 8931 and Peredovik of Russia are the best known examples. However, the open pollinated varieties had problems of uniformity and therefore efforts were made to develop hybrids. Genic male sterility system (GMS) was first explored to develop hybrids. GMS-based hybrids include INRA6501 and Airelle, which were popular until 1980. Later on GMS based hybrids were abandoned due to the problems associated with maintenance and hybrid seed production. Leclercq (1969) discovered cytoplasmic male sterility (CMS) source, known as PET1, from a cross between *H. petiolaris* and *H. annuus*, which

spearheaded the development of commercial hybrids throughout the world. More than 70 CMS sources have been reported in sunflower (Horn  $et\ al.$ , 2002). Since introduction of the first sunflower hybrid BSH-1 (CMS234A × RHA274) in 1980, several varieties and hybrids from both public and private sectors have been released for cultivation in India (Tables 4, 5). More than 95% of the area under sunflower cultivation is

Table 4: Public sector varieties in India

Variety	Year of release	Releasing centre	Yield potential (kg/ha)	Oil content (%)	Recommended states/regions situations	Salient features/ traits
DRSF-108	2004	DOR, Hyderabad	900-1800	36-39	All India	High oil
CO-5	2005	TNAU Coimbatore	1000-1700	39-42	Tamil Nadu	
TAS-82	2005	PDKV Akola	800-1200	40-42	Maharashtra	Black seeded variety
LSF-8	2006	Latur, Mahara- shtra	100-1400	36-39	Maharashtra	Tolerant to downy mildew, rust and Alternaria
DRSF-113	2007	DOR, Hyderabad	1000-1500	36-39	All India	High yield
Phule Raviraj	2009	MPKV, Rahuri	1795	34	Western Maharashtra	Tolerant to necrosis, Alternaria and Capitulum borer

Source: Recent varieties and hybirds of annual oilseeds recommended for different states (2001-2010), Directorate of Oilseeds Research

occupied by hybrids. Of these, the hybrids from private sector dominate (@95%) of the market. Breeding methods such as backcross or recurrent selection or pedigree selection are commonly used to develop parental lines for the hybrid programmes (Vear, 2010).

#### MAJOR BREEDING OBJECTIVES AND ACCOMPLISHMENTS

Primary focus of both public and private sunflower breeding programs in India is oriented towards enhancing seed yield, improving oil content and introducing disease resistance (*Alternaria helianthi*, downy mildew, sunflower necrosis disease, powdery mildew). In other countries, breeding focus is on development of high oleic lines, resistance to major

diseases (*Sclerotinia sclerotiorum*, downy mildew, orobanche) and dual purpose types – as confectionery and oil-type hybrids. For spring cultivation in India, development of cultivars that combine short vegetative period with high productivity remains the main challenge.

Table 5: Public sector hybrids in India

	Year of release	Releasing centre	Yield potential (kg/ha)	Oil content (%)	Recommended states/regions situations	Salient features/ traits
KBSH-41	2002	UAS, Bangalore	1300-1500	39-41	Karnataka	Tolerant to moisture
KBSH-42	2002	UAS, Bangalore	1300-1500	38-41	Karnataka	stress Tolerant to moisture stress
PSFH-118	2002	PAU, Ludhiana	1400	40	Punjab	Resistant to stem and head rot
KBSH-44	2003	UAS, Dharwad	1400-1600	36-38	All India	Resistant to downy mildew
LSFH-35 (Maruti)	2003	Latur, Maha- rashtra	1400-1500	39-41	Maharashtra	Resistant to downy mildew
NDSH-1 (NDSH-15)	2003	ANGRAU, Nandyal	1400	40	Andhra Pradesh	Early maturing hybrid
RSFH-1	2004	UAS, Raichur	1300-1500	39-41	Karnataka (Zones 2 and 3)	High oleic hybrid
HSFH-848	2005	HAU, Hisar	1800-2400	41-42	Haryana	Dwarf hybrid
DRSH-1	2006	DOR, Hyderabad	1300-1600	42-44	All India	High oil
KBSH-53	2009	UAS, Bangalore	1700-2700	42-44	Karnataka	Resistant to powdery mildew
PSFH-569	2009	PAU Ludhiana	2232	40	Punjab	High oil, early hybrid

Source: Recent varieties and hybirds of annual oilseeds recommended for different states (2001-2010), Directorate of Oilseeds Research

#### Seed Yield

Seed yield *per se* is a low heritablity trait that is highly influenced by environment and is controlled by multiple genes. Number of seeds per head and weight of 1000 seeds are the primary traits that determine yield in sunflower. These traits may have higher heritability compared to yield *per se* but may be negatively correlated. Despite complications,

seed yield of sunflower hybrids has substantially improved over the past 40 years due to breeding efforts. For instance, in the year 2000, some of the sunflower varieties in France showed 140% gain as compared to during 1965-1975 (Vear, 2010).

### Oil Content and Quality

Oil content is considered to be a highly heritable trait. Therefore, selection can be made on individual plants from early generations of a breeding programme. But breeding for high oil content per se is complicated because seed yield and oil content are negatively correlated. There was no significant improvement in oil content per se but the gain for oil yield per hectare was 148% from 1970 to 2000, which may be attributed to the balanced improvements in seed yield and oil content (Vear, 2010). During the last 30 years, fatty acid components have been extensively modified in sunflower. A variety of specialty oil types, which have different levels and/or combinations of fatty acid components as well as tocopherols were bred. Genetics of fatty acid and tocopherol traits have been well worked out. Genes that control oil quality traits include Ol1 and several modifier genes for high levels of oleic acid content; P1, P2 and P3 for high palmitic acid; Es1, Es2, Es3 for high stearic acid content; Tph1 and Tph2 for altered tocopherol composition (Fernández-Martínez et al., 2007). Breeding for enhanced oil quality has been successful because of the smaller number of genes controlling the traits.

#### **Earliness**

Cultivars with different maturity periods are needed to suit the specific requirements of different regions. In general, hybrids that mature in less than 100 days from planting to maturity are classified as early, 100-120 days as medium and 120-140 days as late. Kaya *et al.* (2004) recommended a standard set of hybrids for different maturity groups to classify maturity of new lines and hybrids. Conventional breeding for earliness involves development of inbred lines with acceptable flowering onset for the region followed by selection among lines based on seed moisture content at harvest of the hybrids compared with their seed yield (Vear, 2010).

### **Lodging Resistance and Dwarfness**

Dwarf or semidwarf cultivars are needed to avoid yield loss due to lodging and stalk breakage caused by excessive growth. In India, Morden (Cerenianka-66) is the only dwarf (66–110 cm) and early maturing

variety available for cultivation. Other dwarf sources include DDR (90.3 cm), Donsky (65.5 cm), and Donskoi 47 (79.8 cm) and a mutant derived from Morden (35.7 cm) (Jagadeesan *et al.*, 2007).

#### **Resistance to Diseases**

Breeding for resistance (or at least tolerance) to pathogens is always an aim in sunflower breeding. Brief account of some of the important diseases is given below.

### Alternaria blight (Alternaria helianthi)

Lack of sources of resistance to A. helianthi has been a major constraint in sunflower breeding. Resistance appears to be quantitative; hence the usefulness of such levels of resistance may not be effective under epidemic conditions. However, resistance to A. helianthi has been reported in the perennial species like H. maximiliani, H. mollis, H. divaricatus, H. simulans and H. occidentalis (diploids), H. pauciflorus and H. decapetalus (tetraploids) and H. resinosus and H. tuberosus (hexaploids); subsequently, interspecific cross derivatives were developed (Sujatha et al., 1997; Sujatha and Prabakaran, 2006). Reddy et al. (2006) reported that RHA 587 had high level of resistance (1-5% infection) against Alternaria in both field and laboratory conditions, which may be a good source for breeding programmes.

#### Rust (Puccinia helianthi)

 $P.\ helianthi$  is a dynamic pathogen and has several races worldwide. Excellent sources of resistance to rust have been identified in cultivated sunflower and some of this resistance has been transferred into released germplasm (Miller and Gulya, 2001). Several genes conferring resistance to rust have also been identified in sunflower that include R1, R2, R3, R4, R5, R11, Pu6, and  $R_{adv}$  (Qi et~al., 2011). In India, Sujatha et~al. (2003) reported that interspecific cross derivatives PS 1089 (derived from  $H.\ argophyllus$  x cultivated sunflower) and PS 2011 and PS 2032 (derived from  $H.\ petiolaris$  x cultivated sunflower) were immune to rust isolate at DOR, Hyderabad.

### Powdery mildew (Golovinomyces cichoracearum)

Jan and Chandler (1985) incorporated powdery mildew resistance, which was incompletely dominant, into a cultivated background. This

was released as germplasm PM1 (Jan and Chandler, 1988). The species *H. argophyllus* and *H. debilis* were identified to possess at least two genes. The action of major genes in powdery mildew resistance from *H. debilis* ssp. *debilis* has been investigated (Jan and Chandler, 1985). In India, powdery mildew has become an important disease of sunflower only recently. The following sources of resistance to powdery mildew have been identified at DOR, Hyderabad - three annual wild species (*H. argophyllus*, *H. agrestis*, *H. debilis*), six perennials (*H. angustifolius*, *H. atrorubens*, *H. rigidus*, *H. salicifolius* Dietr., *H. pauciflorus* Nutt and *H. resinosus* Small), two interspecific derivatives (ID-16, ID-25), and two exotic lines with multiple resistance (TX16R, EC-537925) (Prathap Reddy *et al.*, 2013).

### Downy mildew (Plasmopara halstedii)

Downy mildew is also a very dynamic pathogen, which has several races around the world. At least 24 downy mildew resistance genes (*Pl1–16*, *Plv*, *Plw*, *Plx-z*, *Mw*, *Mx* and *PlArg*) have been identified so far in cultivated and wild sunflowers. Of these, only the *Pl8*, *PlArg* and *Pl15* genes are reported to be resistant to all *P. halstedii* races (Liu *et al.*, 2012). Genetic stocks/varieties with multiple resistance genes have been developed by backcross breeding programmes (Hulke *et al.*, 2010; Jocić *et al.*, 2010). In India, downy mildew was reported around 1986 in Marathwada region of Maharashtra State where sunflower is extensively grown. Hybrids resistant to downy mildew were released for commercial cultivation, which substantially reduced the downy mildew incidence in India (Shirshikar, 2005).

### Sunflower Necrosis Disease (SND)

SND was first observed in India during 1997 and caused significant yield losses. No reliable sources of resistance to SND in cultivated sunflower have been identified yet. Artificial screening experiments indicated that wild species, *H. occidentalis* and *H. maximiliani* show immune type of reaction against SND (M. Sujatha, unpublished). Transgenics conferring resistance to necrosis disease through deployment of the TSV coat protein gene have been developed at the Directorate of Oilseeds Research.

More information on the global scenario of the progress made in breeding for resistance to diseases in sunflower is available in the Proceedings of the International Symposium on "Sunflower Breeding on Resistance to Diseases" organized by All-Russia Research Institute of Oil Crops by V.S. Pustovoit (VNIIMK) and The International Sunflower Association (ISA) at Krasnodar, Russia June 23-24, 2010.

#### **Resistance to Insect Pests**

For sunflower in India, only a few insect species have become economically important for causing yield losses. These include *Helicoverpa armigera* and the tobacco caterpillar (*Spodoptera litura* (Fabr.). Genetic variability for resistance to *S. litura* is limited in the cultivar germplasm of sunflower. Sujatha and Lakshminarayana (2007) reported that *H. occidentalis* and *H. argophyllus* were immune with no leaf damage under field conditions. Laboratory bioassays confirmed resistance in eight species *viz.*, *H. occidentalis*, *H. argophyllus*, *H. tuberosus*, *H. maximiliani*, *H. mollis*, *H. simulans*, *H. divaricatus* and *H. hirsutus*.

#### **Tolerance to Abiotic Stresses**

Škorić (2009) reviewed the progress made in sunflower breeding for tolerance to abiotic stresses.

Sunflower is considered to be a moderately sensitive crop to drought stress. Developing high-yielding cultivars that flower and mature before soil water conditions become limiting may be a useful approach. The genetics of drought tolerance has not been studied thoroughly in sunflower. Sources of drought tolerance were found in *H. argophyllus* and *H. deserticola*. Breeding for stay green has been useful for drought tolerance. Some of the stay green inbred lines include HA-48, HA-22, CMS-1-50, PH-BC-2-91, PR-ST-3, RHA-SES and RHA-583. Use of *H. argophyllus* in breeding for drought tolerance has been challenging because it was difficult to preserve the original structure (pubescence) of *H. argophyllus* leaves in the backcrosses progenies. Other species such as *H. deserticola*, *H. hirsutus*, *H. maximiliani*, and *H. tuberosus* may also be explored (Škorić, 2009).

### Private Sector Hybrids in India

In India, private seed sector played a leading role in hybrid development and seed production in sunflower. In the year 1990-91, 22,000 tonnes of private hybrid seeds and about 4900 tonnes of varieties were marketed. No public bred hybrid seed was available (Gadwal, 2003). Now, more than 95% of hybrid seeds in the country are produced by

private sector as against only about 5% from the public sector (Seed Section, DOR).

### **Molecular Breeding**

#### Genomic resources

Genetic linkage maps of varying densities have been constructed in wild and cultivated sunflowers using RFLP, RAPD, AFLP and SSR markers. As of now, about 2,040 SSR markers have been developed (as reviewed by Sujatha and Sujatha, 2012). Lai et al. (2005) mined more than 67,000 ESTs in sunflower for SNP discovery and mapped 243 SNP markers. Bachlava et al. (2012) developed an array of 10,640 SNPs through transcriptome re-sequencing. Burke et al. (2012) developed a consensus genetic linkage map containing ca. 8500 SNPs along with ca. 1500 previously mapped SSRs. This SNP array was used to construct genetic linkage maps of the H. argophyllus and H. niveus spp. tephroides, and to association mapping within the sunflower gene pool. Through National Sunflower Association (NSA) funded initiative, a SNP chip consisting of 8,700 SNP markers has been developed and validated in a diverse panel of 1,200 sunflower accessions (Venkatramana et al., 2012). The NSA, in conjunction with the USDA and sunflower seed companies, has established the NSA Sunflower SNP Consortium to help sunflower breeders. Availability of fully sequenced sunflower genome would expedite large scale discovery of robust markers such as SSR and SNP for breeding applications. Significant progress has also been made towards developing a reference genome sequence in sunflower. A physical map that covers more than 85% of the genome has been developed (Kane et al., 2011). A TILLING population for high throughput identification of EMS-induced point mutations in sunflower genome has been developed, which may facilitate a better understanding of gene function in sunflower (Sabetta etal., 2011).

# Molecular genetic diversity analysis

Marker-based genetic diversity analysis in germplasm collections can be helpful for reliable classification of accessions and identification of core accessions for specific breeding purposes. In sunflower, molecular markers are widely used for identification of inbred lines, cultivars and wild species (Liu and Burke, 2006), for analysis of genetic diversity and relationships (Yue *et al.*, 2009), population structure (Mandel *et al.*, 2011) and linkage disequilibrium (Fusari *et al.*, 2008).

### Genetic mapping of agronomic traits

A wide range of molecular markers such as RFLP, AFLP, SSR and TRAP developed in sunflower have led to mapping of several major genes and QTLs for agronomically important traits. So far, progress has been made in mapping of major genes for the traits - resistance to downy mildew disease - Pl1, Pl2, Pl5, Pl6, Pl7, Pl8, Pl13, Pl16, PlARC; resistance to Orobanche - Or5, resistance to chlorotic mottle virus-Rcmo1, resistance to rust disease - R1, R2, R4, R5, R11 and Radv; tocopherol content -Tph1 (beta), Tph2 (gamma); stearic acid content - Es1, Es2 and Es3; fertility restoration - Rf1, Rf3, Rf4, Rf5; oleic acid - Ol; nuclear male sterility - Ms10, ms11; branching - b1; branching and pericarp pigment -Hyp; Lemon ray flower color *Yf*, *Yf1* and chlorophyll deficiency -Yl. QTLs have been reported for + traits such as photoperiod response and flowering, oil content, resistance to downy mildew, black stem, mid stalk rot and basal stem rot, drought, salinity or chilling stresses, early domestication, germination and seedling development, plant height, self-incompatibility, self-pollination and seed dormancy and seed quality (Schuppert et al., 2006; Davar et al., 2010; Wieckhorst et al., 2010; Yue et al., 2010; Lawson et al., 2011; Qi et al., 2011; Liu et al., 2012; Qi et al., 2012a: 2012b and as reviewed by Sujatha and Sujatha, 2012).

### Marker assisted selection (MAS)

A few examples highlight MAS having been successfully practiced in sunflower. Lawson  $et\ al$ . (1998) applied SCAR markers to select for rust resistance genes R1 and  $R_{adv}$ . Jociæ  $et\ al$ . (2010) applied marker-assisted backcrossing to incorporate Pl6 and Pl7 genes into commercial sunflower lines using co-dominant CAPS markers developed by Pankovic  $et\ al$ . (2007). Marker-assisted gene introgression or pyramiding of desirable genes appears to be very promising in sunflower given the diversity of genes that have been genetically mapped for various traits.

### Genetic seed purity testing

Seed quality is of great importance for enhancing productivity, especially in cross-pollinated crops like sunflower where most of its cultivated area is with hybrids. In sunflower, seed quality problems are often encountered due to spurious parental lines multiplication and production of certified hybrid seed. Research at various Centres under AICRP (Sunflower) resulted in development of appropriate seed production technologies for the maintenance of parental lines as well

as for certified seed production of hybrids/varieties. Systematic procedures with respect to season, row ratio of female and male plants, isolation distance for breeder, foundation and certified seed production were developed.

Molecular markers proved to be useful in circumventing the difficulties associated with traditional grow-out-test (GOT) for genetic purity testing of hybrid seed lots. In sunflower, SSR markers have been used to distinguish inbred lines and hybrids (Antonova *et al.*, 2006; Pallavi *et al.*, 2011), which in turn could be used for assessing the genetic purity in seed lots.

### In Vitro Techniques

Homozygous lines assume great importance in commercial hybrid production for fixing heterozygosity and also in development of dihaploid lines for mapping desirable traits. Anther and microspore cultures allow acceleration of breeding programmes by providing homozygous doubled haploids within a comparatively short time. In sunflower, anther culture still needs considerable improvement as the regeneration rates reported till date are very low (@ 10%) and the anther culture response is strongly influenced by physical, nutritional, physiological and genetic factors.

A prerequisite for genetic transformation is the availability of a reproducible and highly efficient protocol of shoot regeneration. During the past three decades, there have been several reports of plant regeneration from sunflower through direct and callus-mediated organogenesis and somatic embryogenesis from zygotic embryos and seedling tissues. The regeneration protocols described thus far are plagued with problems of genotype dependence, low rate of plant regeneration and reproducibility, precocious flowering, vitrification and poor rooting, lengthy culture time, abnormal morphogenesis, etc. A genotype-independent regeneration system through which transgenic plants could be produced would overcome a major bottleneck in sunflower. Owing to difficulties in adventitious shoot regeneration, most of the transformation experiments have relied on the use of shoot apices and cotyledonary nodes. Problems that need to be overcome include lack of an efficient adventitious shoot regeneration system, low Agrobacterium virulence, low transformation rates, lack of a stringent selection system for transformants, unusual sensitivity to antibiotics, genotype dependence of regeneration efficiency, gene instability and low expression levels of transgenes that lead to development of chimeras. Till date there are no GM varieties authorized for commercial use in sunflower.

# **Current Challenges in Sunflower Breeding**

A major challenge would be to develop superior hybrids and populations which can surpass the presently grown hybrids in both seed yield and oil content. These hybrids should combine high seed yield, high oil content, bold seeds with high 100 seed weight, resistance to *Alternaria* leaf spot, powdery mildew, necrosis and downy mildew. There is also an immediate need to develop early maturing hybrids of 75-80 days duration superior to Morden. Most of the sunflower breeding research in USA and European countries is supported by private sector and complemented with molecular breeding tools. There is a need to have such initiatives in Indian sunflower programmes to exploit the biotechnological tools for accelerating the breeding processes using marker assisted breeding and in transferring genes from alien sources through wide hybridization and transgenic approach.

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