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REVIEW ARTICLE

PRESENT STATUS AND FUTURE PROSPECTS FOR HETEROSIS BREEDING IN SUNFLOWER (*Helianthus annuus* L.)

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Sunflower (*Helianthus annuus* L.) is one of the major crops around the world, which is cultivated on a surface of 21 million hectares. It is one of the three crop species along with soybean and rapeseed which account for approximately 78% of the world vegetable oil. Heterosis of these crops has been exploited only over the past few decades. Hybrid sunflower became a reality with the discovery of cytoplasmic male sterility and effective male fertility restoration system during 1970. Hybrid vigor has been the main driving force for acceptance of this oilseed crop. Utilization of heterosis has allowed sunflower to become one of the major oilseed in many countries of Eastern and Western Europe, Russia and South America and is an important crop in the USA, Australia, South Africa, China, India and Turkey. Sunflower hybrid breeding has thus played a vital role in improvement of this crop. In this review, effort has been made to discuss the various approaches for hybrid breeding in sunflower and present status for development of hybrids in sunflower.

INTRODUCTION

The sunflower (*Helianthus annuus* L., 2n = 34) belongs to the family *compositae*. It was given its name, "helianthus" (Gr-*helios* = sun, *anthos* = flower), in Europe in the 18th century. Sunflower is one of the main crops around the world, cultivated on a surface of approximately 21 million hectares (Skoric et al., 2007). It is one of the three crop species along with soybean and rapeseed which account for approximately 78% of the world vegetable oil. Heterosis of these crops has been exploited only over the past few decades. Hybrid sunflower became a reality with the discovery of cytoplasmic male sterility and effective male fertility restoration system during 1970. Sunflower hybrid breeding was started economically in discovering CMS by Leclercq in 1960 and restorer genes by Kinman in 1970 (Miller and Fick, 1997). First sunflower hybrids were produced in US in 1972 and reached 80% of production in five years (Fick and Miller, 1997). Single-cross hybrids quickly became dominantly in sunflower cultivars in the world. Hybrids were preferred by farmers due to high yield and quality potential, homogeneity, same time maturing and easy possibility of cultural applications worldwide. Hybrid vigor has been the main driving force for acceptance of this oilseed crop. Utilization of heterosis has allowed sunflower to become one of the major oilseed in many countries of Eastern and Western Europe, Russia and South America and is an important crop in the USA, Australia, South Africa, China, India and Turkey. Of the approximately 16.5 million hectares of sunflower grown in the major producing countries, 11.5 million hectares are planted to hybrids.

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Utilization of heterosis has allowed sunflower to become one of the major oilseed in many countries of Eastern and Western Europe, Russia and South America and is an important crop in the USA, Australia, South Africa, China, India and Turkey. Of the approximately 16.5 million hectares of sunflower grown in the major producing countries, 11.5 million hectares are planted to hybrids (Miller, 1998). Present day sunflower cultivars contain more than 40% oil and 18-20% protein. Sunflower oil is of good quality as it contains high proportion of linoleic acid which is a polyunsaturated fatty acid. It is also a good source of calcium, phosphorus, nicotinic acid and vitamin E. There are a number of advantages of growing sunflower for oil compared with other cultivated species. No special machinery is needed to produce this crop. It can be grown as catch crop in many situations. Being drought resistant it is well suited for rainfed as well as irrigated areas. Sunflower seeds contain about 42 percent high quality edible oil. The crop duration is such that it can be sandwiched between two cotton, rice or potato crops. So it has a great potential to make the country self sufficient in edible oil. It is needed to improve the yield potential of sunflower varieties/hybrids to increase sunflower production of the country.

Hybrids due to their higher yield potential are being used throughout the world for increasing the productivity of almost all crops including sunflower. With an area of 2.1 million hectares and production of 1.25 million tons is one of the most important oilseed crops of India. India's share in total world production of sunflower is about 6%, accounting for 10.0% of world acreage. Sunflower is grown year round as a sole crop and it also fits well as an intercrop with legumes, in double cropping and in three-

Table 1. Sunflower genetic stocks registered

Sunflower Genetic Stocks Registered	Remarks
One Cytoplasmic Male-Sterile line cms RIGX-HA 89, two fertility restoration lines Rf RIGX-Luch, and Rf RIGX- RCMG1 **(Jan *et al* 2006)**	These were cooperatively developed and released by the USDA-ARS and the North Dakota Agricultural Experimentation Station Fargo, ND, in 2005. New sources of CMS and fertility restoration genes will help reduce the genetic vulnerability of commercial sunflower hybrids because of the current use of single cms cytoplasm ,PET1 (French) derived from *H petiolaris* Nutt and a few fertility restoration genes These new cms and corresponding fertility restoration lines will provide cytoplasmic diversity for hybrid sunflower production
Seven Cytoplasmic Male-Sterile lines, cms ANN14, cmsMUT7, cms MUT8, cms MUT9, cms MUT10, cms MUT11, and cms MUT12 and four corresponding fertility restoration lines Rf Armavir, Rf PI 432513, Rf VNIIMK and Rf P21**(Jan and Vick 2006)**	
Two Cytoplasmic Male-Sterile lines, cms ANN2-HA 89and cms ANN-3HA 89 and eight corresponding fertility restoration lines Rf ANN2-PI 413178, Rf ANN2-P21, Rf ANN2-RMAX1, Rf ANN3-PI 413180, Rf ANN3-P21, Rf ANN3-RHA 801, Rf ANN3-RPET2, and Rf ANN3-RHa 280 **(Jan 2006)**	

Table 2. Sunflower hybrids/varieties identified by ICAR for release based on yield and downy mildew resistance reaction

Sunflower hybrid/variety	Year	Remark
Sungene-85	1996	This variety was released during AICRP workshop held at JNKVV, Jabalpur, in April, 1996.
LS-11	1998	This variety was released for varietal identification at committee meeting held at TNAU, Coimbatore, in April, 1998.
MSFH-47	2000	This hybrid was highly resistant to downy mildew and it was released by ICAR for variety identification at committee meeting held at PAU, Ludhiana, in April, 2000
Pro-009 (Prosun-09)	2003	This hybrid was released for variety identification at committee meeting held at TANU, Coimbatore, in April, 2003.
SH-416	2003	This hybrid was identified for variety release at committee meeting held at TANU, Coimbatore, in April, 2003.
DRSF-108	2003	This variety was identified at ICAR'S variety release committee meeting held at TANU, Coimbatore, in April, 2003.
PCSH-243	2004	This hybrid was identified for release in the variety release committee meeting held at ANG Ranga Agric. University, Hyderabad, on May 18-20, 2004.
PRO-011	2004	This hybrid was identified for release at the variety release committee meeting held at ANG Ranga Agric. University, Hyderabad, on May, 18-20, 2004.
SCH-35 (Maruti)	2004	Released at Maharashtra State Variety release committee meeting held at Bombay in Feb. 2003.

Source : Shirshikar 2005

Table 3. Commercial sunflower hybrids (alphabetically ordered) from the Advanta Semillas multi-environment trials 1991–2005

Hybrid	Release	Year	Hybrid	Release	Year	Hybrid	Release	Year
ACA 864	2002	1	DK 3920	2003	2	Pararso 27	2003	1
ACA 872	1999	2	2 DK 4000 CL	2003	1	Pararso 30	1999	5
ACA 884	1991	9	DK 4030	1993	7	Pararso 33	2003	2
ACA 885	1999	2	DK 4040	1997	6	Pararso 35	2003	1
Agrobel 910	1993	1	DK 4050	1999	5	Pararso 40	2000	1
Agrobel 920	1995	1	DK 4200	2003	1	Pararso 50	2001	2
Aguara 4	1997	3	DK G100	1984	4	Pararso 101CL	2003	1
Asgrow 548	1989	3	DK G103	1987	6	Puelche	1991	3
Atue1	1999	2	MG 2	1997	6	SPS 3130	1986	7
CF 11	1997	3	MG 4	1999	1	Super 407	1988	1
CF 13	1999	3	MG 50	2002	3	Super 505	1991	2
CF 17	1997	3	Morgan 731	1984	4	T600	1998	9
CF 17 DMR	2004	10	Morgan 733	1988	2	TC2000	1991	5
CF 19	1997	11	Morgan 734	1989	5	TC2001	1994	
CF 21	1997	7	Morgan 742	1996	8	TC 3001	1991	6
CF 23CL	2005	1	Mycosol 2	1995	1	TC3002	1991	1
CF 25	1997	7	Olisun	2002	3	TC 3003	1992	4
CF 27	2004	3	Olisun 2	2005	1	VDH 370	2005	2
CF29	2004	4	P 6440	1998	4	VDH 475	1993	2
CF31	2005	2	P 64A41	1996	1	VDH480	1993	5
CF3 Negro	1997	3	P 6510	1989	5	VDH481	2003	3
Contiflor 15	1989	11	P 6520	1993	2	VDH483	1998	4
Contiflor 3	1983	10	Pararso	1990	5	VDH485	2000	4
Contiflor 7	1988	9	Pararso 2	1990	5	VDH487	2004	3
Contiflor 8	1985	7	Pararso 3	1993	5	VDH488	1999	7
Contiflor 9	1998	11	Pararso 4	1993	1	VDH93	1999	7
DK 3880 CL	2003	2	Pararso 5	1993	1	VDH 96	1994	4
DK 3881	1993	4	Pararso 6	1993	4	Zenith	1993	1
DK 3900	1997	4	Pararso 20	1997	6			
DK 3915	1997	6	Pararso 22	2003	2			

Source: De la Vega *et al* .2007

Table 4. Sunflower Hybrids from different seed companies for 2009-10

Seed Company	Hybrid	Remarks
Syngenta	3433 NS/DM	It is an early season NuSun (95-day relative maturity) with resistance to known races of downy mildew. It has an outstanding performance history, including being the top-yielding hybrid in the 2009 university trials at Hettinger, N.D., at 2,814 lbs/ac.
	3480 NS/CL/DM	An early season NuSun and Clearfield (95-day RM) with resistance to known races of downy mildew. One of the earliest Clearfield hybrids, it has excellent oil content and yield for maturity.
	3980 NS/CL	Medium-season (97-day RM) NuSun/Clearfield hybrid with excellent early season vigor. It is well adapted across North Dakota, South Dakota and the High Plains.
	4651 NS	Medium-season (97-day RM), NuSun hybrid with excellent yield potential for the Dakotas and the High Plains.
	3731 NS	Medium-season (100-day RM), elite NuSun hybrid with outstanding multi-year performance history and yield stability. It performs well from southern North Dakota across South Dakota and throughout the High Plains.
	3732 NS	Medium-season (100-day RM) elite NuSun hybrid with outstanding multi-year performance history and yield stability. A companion hybrid to 3731 NS, it performs well from southern North Dakota across South Dakota and throughout the High Plains.
	3875 NS	Medium-/full-season (102-day RM) Nusun hybrid. It generates excellent performance across South Dakota, where 3875 has been grown for many years. Performs well from southern North Dakota across South Dakota and throughout the High Plains.
	3845 HO	Medium-/full-season (105-day RM) high-oleic hybrid. Producing strong oleic levels desired for the high-oleic market, it is an attractive hybrid with strong performance from southern North Dakota across South Dakota and throughout the High Plains.
CHS Sunflower	Royal Hybrid 316 RT	CHS Sunflower is introducing Royal Hybrid 316 RT for 2010 planting. It is a single-cross, rust-resistant, "XL"-type confection hybrid.
	306 DMR,NS	It is an 88-day maturity mid-oleic hybrid that has been an excellent producer in trials from Canada to the High Plains. It is a NuSun hybrid with excellent yield potential and very good oil content. It also is resistant to all prevalent U.S. races of downy mildew. This hybrid is approximately two days earlier than 803 and has higher yield and oil content.
	460 E,NS	This is a 97-day NuSun hybrid with very strong roots and stalks. Given its combined oil content and yield potential, along with the new weed control practice, this is one hybrid you need to get on your farm. The 460 had similar oil per acre to 3080 in the past two years' trials
	555 CL,DMR,NS	A 94-day NuSun hybrid with outstanding oil content and yield potential. This is the first Clearfield hybrid in this maturity that is resistant to all prevalent U.S races of downy mildew. It also is resistant to the new downy mildew strain found in 2009. This hybrid has very good late-season plant health, with a distinct yellow color at maturity.
Dahlgren & Company	4416CL	An early maturing Clearfield NuSun variety with excellent uniformity and seedling vigor. This NuSun hulling variety gives growers additional marketing options. Very limited quantities are available for 2010.
	9579	A new single-cross XL confection variety best adapted to western North Dakota and South Dakota. This hybrid offers uniform plant height and bloom for easier insect control.
Mycogen Seeds'	8N358CLDM	It is a best oil content NuSun® hybrid in the Clearfield family; also carries genetic resistance to downy mildew. It is a mid-season, medium-height hybrid with good drydown characteristics. This hybrid is suitable for northern planting areas with shorter growing seasons.
	8N270CLDM	It carries genetic resistance to downy mildew. It will have very similar appearance, yield and plant health to 8N270. It tends to flower a day earlier than 8N270 and is 0.5 percentage unit higher in oil. Root and stalk lodging are similar to 8N270. This product will work very well in the NuSun oil, birdfood and hulling markets.
	8N433DM	It is a full-season downy mildew-resistant hybrid. It is medium-tall with an excellent tolerance to root lodging.

crop rotations. Despite the premier position the crop holds in the vegetable oil economy of the country, the average productivity level is low (629 kg/ha) as compared with the world's productivity (1240 kg/ ha). After the introduction of the crop in India in the early 1970s, a need for hybrids was recognized. Experimental hybrids were developed in 1974-75 using the *cms* and restorer lines introduced from USA.

The first hybrid from public sector was released for commercial cultivation in 1980. Since then the hybrid breeding program has been quite successful and 29 productive hybrids were developed by both public (18) and private sectors (11). These hybrids are intended for different agroproduction situations, which are occupying 95% of the crop-grown area. According to the website of the directorate of oilseeds research Hyderabad, India, eleven open pollinated and sixteen hybrids have been released under AICORPO. Despite these successes with the crop, the major problems threatening sunflower productivity in India are the stagnating and unstable yields and vulnerability to various biotic stresses. The major diseases attacking sunflower crop in India are *Alternaria* leaf spot (*Alternaria helianthi*), rust (*Puccinia helianthi* Schw.), downy mildew (*Plasmopara halstedii* (Farl.), *Rhizopus* head rot and a sunflower necrosis disease (a disease of recent origin). The crop is affected at all growth stages. Disease incidence is highly unpredictable and it tends to fluctuate from year to year, season to season and location to location, thus necessitating region-specific management strategies. Among the various approaches to manage these diseases, host plant resistance is the most reliable and economical to the end users. Plant breeding efforts to develop varieties/hybrids with inbuilt tolerance to the major diseases are constrained by the narrow genetic base of the cultivated sunflower.

Development of hybrids from Inbred Lines

Hybrids are also developed from inbred lines derived from the same narrow gene pool. A gene bank with about 1000

accessions is available in the country but it is characterized by unacceptable levels of intra accessional heterogeneity in many lines thus restricting the scope for utilization of the material in breeding programs. Most studies on breeding for disease resistance were confined to screening of the available cultivar germplasm against the diseases under natural conditions and hence, these sources could not be converted into usable forms. With the exception of downy mildew disease, the released cultivars rated as tolerant/resistant are based on their field reaction to the pathogen. However, none of these were bred from parents that were classified as genetically resistant to a particular disease.

Wild *Helianthus* species serve as potential sources of novel genetic variability and several desirable characteristics such as resistance to biotic and abiotic stresses, cytoplasmic male sterility, fertility restorer genes and oil quality have been successfully introgressed into cultivated sunflower (Seiler, 1992). The growing needs for additional genetic variability to improve the cultivated sunflower makes it necessary to collect, maintain, characterize, evaluate and utilize the wild sunflower germplasm. Concerted efforts are required to incorporate additional genetic variability from reliable sources by integrating modern biotechnological tools and conventional breeding methods (Sujatha, 2006). Recently, a study of genetic diversity among 177 public sunflower inbred lines was carried out. These inbred lines were developed and released by USDA –ARS from 1970-2005. Target region amplified polymorphism (TRAP) marker technique was found suitable for fingerprinting of sunflower inbred lines. These inbred lines will be useful for further hybrid breeding programme in sunflower (Yue et al., 2009).

Hybrid Selection

Selection of adaptable hybrids combined with use of recommended production practices are important factors for profitable sunflower production. Hybrids now are planted on over 99 percent of the USA acreage. Hybrids replaced open-pollinated varieties because of their increased yield, pest resistance, uniformity, stalk quality and self compatibility. Growers should use several criteria in hybrid selection. First, they should take an inventory of available hybrids being marketed in their area. Seed yield potential is an important trait to consider when looking at an available hybrid list. Yield trial results from university experiment stations and from commercial companies should identify a dozen or so consistently high yielding hybrids for a particular area. Results from strip tests or demonstration plots on or near growers' farms should be evaluated. Yield results from previous years on an individual's farm and information from neighbors is also valuable. The best producing hybrids in a region may produce approximately 2,000 pounds per acre with good soil fertility and favorable soil moisture, or up to 3,000 pounds per acre in the most favorable growing conditions. North Dakota's average yield ranges from 1,200 to 1,400 pounds per acre. An important trait to consider is pest resistance or tolerance. Hybrids are available with tolerance to rust, *Verticillium* wilt, certain races of downy mildew, and early ripening. Growers should check with their local seed dealer or sunflower seed company representative to obtain this information. Stalk quality, another trait to consider, is a stalk reaction to damage from several pests. Hybrids with good stalk quality are easier to harvest and field losses generally are reduced. Good stalk quality in sunflower also allows the crop to withstand damages and field losses due to high winds. Uniform stalk height at maturity is another important trait to consider.

Oil percentage should be a trait to consider in hybrid selection. Several environmental factors influence oil percentage, but the hybrid's genetic potential for oil percentage also is important. If the market price is influenced by oil percentage, then high oil hybrids should be considered. Current hybrids have oil percentages ranging from 38 to over 50 percent. Domestic sun oil crushers have been paying a premium for higher oilseed in recent years. Hybrids with oil percentages in the 40-45 range, on a 10 percent moisture basis, should be selected. Growers should examine physiological maturity ratings to take advantage of sunflower hybrids, especially when considering late plantings. Growers need a hybrid that will mature well within the average frost-free period. Compared to the mid-late 1970s, the maturity ratings of sunflower hybrids available today are much broader in range of maturity (earliest to full season). Test weight also is an important trait for consideration, especially for hybrids selected for late planting or replanting. A test weight of 25 pounds per bushel is required to make official USDA grade, but recently developed hybrids have test weights ranging from 28 to 32 pounds per bushel. Self compatibility, the ability of the sunflower plant to pollinate itself in the absence of insect pollinators, is another desirable trait in hybrids. Finally, the last item to consider is the purchase of the seed from a reputable seed company and dealer with a good technical service record if production problems do occur and assistance or consultation is required.

Cytoplasmic male sterility for developing Sunflower hybrids

The discovery of cytoplasmic male sterility (CMS) in sunflower by Leclereq (1969) and subsequent identification of genes for fertility restoration have resulted in the development of commercial hybrids since 1972. However, all the sunflower hybrids that are commercially grown have a single source of CMS discovered by Leclereq leading to homogeneity and potential risk that was evident in case of maize. Diversification of CMS sources is inevitable in any hybrid-breeding program. Fortunately in sunflower more than 62 new CMS sources of different origin have been reported (Serieys, 1999). The diversity of the new sources was assessed mainly based on cytoplasmic male sterility and fertility restoration systems. Only a few investigators have described interactions between cytoplasms and nuclear genes in the expression of several qualitative and quantitative characters and beneficial cytoplasmic nuclear interactions have been reported in various crops. In sunflower, a unique cytoplasmic nuclear interaction caused reduction in chlorophyll, photosynthetic rate and overall reduction in vigour and positive effect and oil content at present, the main aim of sunflower breeding is the obtaining of high yield commercial hybrids. Sunflower hybrids are object of breeding attention because of their agronomic and economic advantages over varieties (high

productivity, oil content, disease resistance, etc.). The central component of sunflower hybrid development is cytoplasmic male sterility (*cms*). The obtaining of hybrids with high heterosis effect became possible after the discovery of the first *cms* source by Leclercq, 1969 and detection of fertility restoration genes by Kinman, 1970.

Development of sterile *cms* analogues of lines used in sunflower breeding programs for commercial hybrid development is one of the practical applications of *cms* investigations. CMS PET-1 is a *cms* source which is widely used of in sunflower hybrid development. Such cytoplasmic uniformity presents a potential risk for hybrid sunflower production. The utilization of different cytoplasmic backgrounds in hybrid development will improve general variability of the sunflower and lessen the threat of epiphytotics. The different *cms* lines and genetic stocks registered are presented in Table 1. Thirteen new sterile *cms* analogues on the base of different sunflower *cms* sources have been obtained. The obtained backcrosses have been evaluated for main agronomic traits, including the resistance to the important sunflower pathogens. As a result of this study, the most prospective *cms* analogues were designated for future use in breeding programs. (Tavoljanskiy et al., 2004). Cytoplasmic male sterility (CMS) in commercial sunflower hybrids is thought to be derived from a related wild species, *Helianthus petiolaris*, yet CMS lines are known to carry the chloroplast DNA genotype of *H. annuus*. Commercial sunflower hybrids released from Argentina are listed in Table 3.To clarify the origin of sunflower CMS, polymerase chain reaction-based strategy was developed for detecting CMS in sunflower and surveyed more than 1,200 plants representing 55 accessions of *H. annuus* and 26 accessions of *H. petiolaris*. 160 progeny from three crosses were tested for strict maternal inheritance of organelle DNAs to determine if the apparent discrepancy in the species donor of the mitochondrial DNA and chloroplast DNA genotypes in CMS lines might result from low-frequency maternal or biparental inheritance of either organelle. No CMS cytotypes were observed in natural populations of either *H. annuus* or *H. petiolaris*, and strict maternal inheritance of organelle DNA was observed. Pollen scarcity has been studied in an Indian hybrid KBSH-1. In case of scarcity of pollen during pollination for hybrid seed production of KBSH-1 or when there is excess availability of pollen from restorer line-6D-1 due to lack of synchrony and to have better utility of available pollen, pollen grains may be stored either in refrigerator or in earthen pots filled with water and covered with wet cloth and utilized properly when male sterile line-CMS234A is receptive, to get increased yield (Sumathi et al., 2007). The sunflower hybrids for downey mildew resistance released by ICAR India are listed in Table 2. Private sector seed companies have also released many important hybrids for cultivation, some recent ones are listed in Table 4.

Future prospects

Contrary to conventional breeding methods, the F_1 hybrid method facilitates the genetic diversification of sunflower genotypes through a more efficient exploration of the *Helianthus* genus with its potential gene pools for superior quantitative and qualitative traits. The genetic progress already achieved will be further increased by modern methods of biotechnology and genetic engineering which will allow development of a new super productive type of sunflower. The yield advantage of F_1 hybrids compared with best open-pollinated varieties stands demonstrated. Unfortunately all presently available F_1 hybrids are based on the *petiolaris* CMS source. More efforts must be directed towards diversification of exploitable CMS sources. With the rapid pace that characterizes the development of the modern molecular genetics, the sunflower hybrid breeding can not progress correspondingly without the joint efforts of scientists and research institutes involved. Establishment of an FAO research network on sunflower was a step in the right direction The main current problems of sunflower hybrid breeding are the further improvement of productivity by increasing seed and oil yields and the reduction of genetic vulnerability to diseases and heat or water stress encountered in various ecological zones. (Vranceanu, 1998). New sources of CMS and fertility restoration genes will help reduce the genetic vulnerability of commercial sunflower hybrids because of the current use of single cms cytoplasm, PET1 (French) derived from *H petiolaris* Nutt and a few fertility restoration genes These new cms and corresponding fertility restoration lines will provide cytoplasmic diversity for hybrid sunflower production (Jan and Vick, 2006).

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