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Review Article

Achievements and bottlenecks of heterosis breeding of sunflower (*Helianthus annuus* L.) in India

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Abstract

Heterosis breeding has been commercially exploited in sunflower and is expected to enhance productivity further. This is the only crop which has more than 80% sunflower growing area under the hybrids. The first hybrid (BSH-1) of sunflower 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). Inspite of these successes, problems in the form of stagnating and unstable yields, genetic vulnerability and susceptibility to various diseases are threatening sunflower productivity in India. One of the major challenges would be to develop newer hybrids for diverse situation and superior to those presently grown in terms of seed and oil yield in order to sustain the competitive ability of sunflower *vis-a-vis* other crops. There is a need for new CMS lines with diverse cytoplasm having several desirable attributes like dwarfness and early maturity. The diverse CMS lines are expected to contribute towards the development of superior hybrids producing high seed yield and oil content with better heterosis and stability. The CMS base exploited in the sunflower breeding programmes is narrow and there is an urgent need for widening the CMS and restorer (R) line base for enhancing the productivity and production of sunflower.

Key words: Achievements, bottlenecks, heterosis, sunflower hybrids

Introduction

Due to ever increasing supply demand gap for edible oilseeds, heterosis breeding is being viewed as a main driving force for productivity enhancement and has been commercially exploited in sunflower (*Helianthus annuus* L.). Present day sunflower breeding is totally dependent upon the development of F_1 hybrids. Hybrids are now planted on over 12 million ha worldwide. Sunflower was introduced to India in late 1960s. Although commercial production of four introduced Russian varieties began in 1972, its emergence as an important oilseed crop becomes perceptible only in 1980s. Karnataka, Andhra Pradesh and Maharashtra are the major sunflower producing states accounting for 92.3% area and 80.8% of production [1]. Of late, it has spread to non traditional states like as an important oilseeds crop in Punjab, Haryana and Uttar Pradesh [2]. The value of hybrids and the importance of heterosis breeding was recognized early with the inception of All India Coordinated Research Project on Oilseeds in 1972-73. Hybrid sunflower became a reality with the discovery of cytoplasmic male sterility [3] and effective male fertility restoration system [4] during 1970 [5]. The importance of hybrid cultivars in sunflower has increased recently because of their higher seed yield compared to cross-pollinated varieties in India. Today researchers are interested to develop hybrids in sunflower because hybrids have more production stability, approximately 20 percent greater yield potential, response to high input agriculture, high self fertility, which reduces the need for bees for cross pollination and are more uniform in height and maturity facilitating harvest and easy possibility of cultural applications worldwide [6, 7] compare to open pollinated varieties. Despite the

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premier position the crop holds in the vegetable oil economy of the country, the average productivity level is low (542 kg/ha) as compared with the world's productivity (1424 kg/ ha) [8] (Table 1). One of the major challenges would be to develop newer hybrids for diverse situations and superior to those presently grown in terms of seed and oil yield to sustain the competitive ability of sunflower *vis-a-vis* other crops. At present, much of the plant breeding research in AICRP is directed to exploitation of heterosis utilizing Cytoplasmic Male Sterility (CMS) system. Because all the sunflower hybrids that are commercially grown have a single source (PET-1) leading to homogeneity and potential risk that was evident in case of maize [9, 10]. Diversification of CMS (A-line) sources is inevitable in any hybrid-breeding program and it is true for sunflower also. Fortunately in sunflower more than 72 new CMS sources of different origin have been reported. However, these sources have not been exploited commercially in breeding programme because of their instability and ineffective restoration sources. In this article we discuss about achievements and bottlenecks of sunflower breeding and some alternatives to overcome these bottlenecks to increase the average productivity of sunflower.

Heterosis in sunflower

Sunflower is a highly cross pollinated crop. Since the species is allogamous and entomophilous, a foreign pollen grain germinates on the stigma much faster than a pollen grain coming from the same plant, irrespective of variety or hybrid. In sunflower high heterosis for yield and its components have been reported by many previous researchers [11-13]. However, heterosis does not appear in all hybrid combinations of the F_1 generation [14]. Therefore, achieving success in hybrid breeding is quite difficult and it takes some time. Haldani *et al*. [14] also reported that the occurrence of heterosis in sunflower hybrids is highly correlated with genetic distance between the parental lines. At present much of the plant breeding research in AICRP is directed at exploitation of heterosis utilizing a single source of CMS. All centres are maintaining the same CMS sources from 1980 to till date and the exchange of the same CMS sources between AICRP centres.

Table 1. Productivity of sunflower in major sunflower growing countries (2010-11)

S. No.	Country	Kg/ha
1	France	2360
2	Serbia	2350
3	Italy	2270
4	Argentina	2110
5	Turkey	2060
6	Hungary	1980
7	Bulgaria	1802
8	U.S.A	1640
9	Pakistan	1520
10	South Africa	1340
11	Canada	1330
12	Spain	1210
13	Australia	1160
14	Russia	960
15	India	860
16	World	1440

Breakthrough in hybrid development

The hybrid development programme of sunflower had their beginning in 1970's at different research centres in India. Earlier researchers focused more on development of open pollinated varieties in sunflower but after identification of stable CMS (PET-1) source and highly cross pollinated nature of the crop made it ideal for exploitation of heterosis. This has resulted in the development and release of several hybrids suitable for different agroclimatic situations in India as well as in the world. During 1974-75, four CMS lines (CMS-2, CMS-124, CMS-204 and CMS-234) and two fertility restorer lines (RHA-266 and RHA-274) were introduced from USA. Based on seed yield, oil content, yield stability and synchronization of flowering in male and female parents, the hybrid BSH-1 (CMS-234A x RHA-274) was released for commercial cultivation in 1980 [15] inface gave a fillip and renewed the interest again in the crop; as there was a set back in sunflower area and production from 1977-78 onwards. Since then, many hybrids have been released for commercial cultivation based on cytoplasmic genetic male sterility system to suit different agro-production situations, including spring season for northern parts of the country. The salient features of hybrids released since 1980 are given in Table 3.

Bottlenecks and alternatives for overcoming bottlenecks of sunflower hybrids

In India very limited numbers of CMS lines are available for national hybrid breeding programme. From 1970, in India all the AICRP sunflower centres maintaining same CMS lines (Table 2) and very limited number of germplasm. Large number of scientist are

Table 2. Salient features of released hybrids of sunflower in India

S. No.	Hybrids	Pedigree	Year of release	Maturity (days)	Yield (kg/ha)	Oil content (%)	Salient Features
1	BSH-1	CMS-234A x RHA-274	1980	85-90	1200-1300	41	Resistance to rust and downy mildew
2	LSH-1	CMS-338A x MRHA-II	1990	85-90	1000-1100	38	Resistance to downy mildew
3	LSH-3	CMS-207A x MRHA-1	1990	90-95	1200-1300	39	Resistance to downy mildew
4	KBSH-1	CMS-234A x RHA-6D1	1992	90-95	1300-1500	43	High yield with high oil content
5	PKVSH-27	CMS-2A x AK-1R	1996	85-90	1300-1400	39	Moderate resistance to downy mildew
6	DSH-1	DSF-15A x RHA-857	1997	85-88	1200-1300	38-40	Resistance to downy mildew
7	TCSH-1	CMS-234A x RHA-272	2000	-	-	-	-
8	KBSH-41	CMS-234A x RHA-95-C-1	2001	90-92	1300-1500 ®2500-3000(I)	39-41	Tolerant to moisture stress
9	KBSH-42	CMS-851A x RHA-95-C-1	2001	90-92	1300-1500 ®2500-3000(I)	38-41	Tolerant to moisture stress
10	NDSH-1	CMS-234A x RHA-859	2002	88-90	1400	40	Early hybrid
11	KBSH-44	CMS-17A x RHA-95-C-1	2002	95-98	1400-1600	36-38	Early maturity, resistant to downy mildew
12	LSFH-35	CMS-234A x RHA-1-1	2003	Medium	1400-1500	39-41	Resistance to downy mildew
13	PSFH-118	CMS-10A x P-61-R	2004	85-88	1400	40	Resistance to basal stem rot and head rot
14	HSFH-848	CMS-91A x RHA-298	2005	90-95	1800-2400	41-42	Resistance to *Alternaria helianthi* & *Rhizoctonia*
15	DRSH-1	ARM-243 x RHA-6D-1	2005	95-98	1300	43	High oil hybrid
16	TUNGA(RSFH-1)	CMS-103A x R-64-NB	2005	95-100	1300-1500	39-41	-
17	PSH-966		2012	96-100	1957	37-38	-
18	KBSH-53	CMS-335A x RHA-95C-1	2008	95-100	750-1250	38	Tolerant to powdery mildew

- Data not available

working on development of inbred lines and conversion of inbred lines into CMS through back cross method but it is a time and labour consuming method and at the same time converted lines have many inherent defects. Most of the time converted CMS lines do not perform better than the original sources. Another problem is that there is no exchange of good material across the centres working on hybrid breeding.

Alternatives

A very few introductions of CMS and transfer line have been made. In the situation described above there is a good scope for importing the good CMS lines from US and European countries because these countries are engaged in developing large number of good CMS lines every year. More than 300 maintainer, CMS, and restorer lines have been developed by the USDA-ARS programs or other public researchers [16].

Single source of CMS

There is an apprehension in some quarters that at present in heterosis breeding the CMS base is very narrow. From 1972 [18] to-date, PET-1 CMS discovered by Leclercq and few restorer lines are being utilized for commercial hybrid seed production in sunflower around the world. This narrow genetic base may limit the progress in sunflower breeding programmes. In India, all the developed sunflower hybrids are utilizing a single source of male sterility (PET-1) because of its stability and availability of fertility restorers and maintainers easily. Nevertheless, the large scale use of same sterilizing cytoplasms increases the genetic vulnerability to biotic and abiotic stresses.

Alternatives

The boom of hybrid sunflower that is being witnessed all over the world during the last two decades is largely due to cytoplasmic male sterility source contributed by wild annual *H. petiolaris*. There is an urgent need for diversification of cytosterility system through interspecific hybridization. More efforts are needed in future to overcome the deleterious effects of distant hybridization and combine desirable traits with seed and oil yield. The wild species can also be used to alter sunflower plant structure by developing cultivated sunflower genotypes with shorter internodes and much shorter petioles (*H. mollis*). This may pave the way towards a significant increase in the number of plants per unit area and hence may enhance the productivity of the future hybrids.

Instability of new CMS sources

Recently, several new sources of CMS have been reported but these alternate CMS sources not being utilized effectively because of their instability. Sreetharan and Virupakshappa [17] reported that only CMS PF and CMS I showed total sterility and stability, while CMS GIG1 and CMS MAX1 showed complete fertility. Kumar et al. [18] reported that CMSRF and CMSL showed total sterility and they were considered as stable, while CMS G1G1 and CMS MAX1 exhibited complete fertility and hence rejected. Rajanna *et al*. [19] studied stability of male sterility in three diverse CMS lines of sunflower under planting dates covering *kharif, rabi* and *summer* seasons. The results revealed that CMS I was unstable and produced fertile pollen when planted in summer months (Feb. to April). In contrast, CMS F and CMS PF sources were stable in all sowing dates throughout the year. The breeding value of some of these new sources is yet be assessed, as many of these new sources may be unstable and lack complementary restorer systems. It can be inferred that a need for more elaborate investigations on new CMS systems are needed before they are considered for utilization in hybrid breeding.

Alternatives

Major lacunae in the present Indian breeding programme has been the lack of strong approach to

Table 3. CMS and restorer lines developed at different AICRP centres

Centre	CMS lines	Restorer lines
Akola	CMS-2A, CMS-21A, CMS-59A, IB-79A, IB-297A, CMS-103A, CMS-240, CMS-148A and ID 145-2/5	PKV-101 to PKV-110, IBK-1, IB-174/2-2, ID-4005, ID-4036, ID-4070, ID-5004-3, ID-2039
Bangalore	CMS-1A, CMS-M1A, CMS-17A, CMS-42A, CMS-47A, CMS-53A and CMS-62A	RHA-95-C-1, RHA-95-C-2, GKVK-1 and GKVK-2, RHA-6D-1, RHA C-1, RHA C-2, RHA-M17A, HAM167, 168, 169, 187, 188, 194, 195, 1R, 8R, 9R, 16R and17R
Coimbatore	TNAU CMS-1A, TNAU CMS-2A, COSF-1A, CMS-7A, 8A, 9A and 10A	MH-1 to MH-14 and MH-20, MH-22
DOR, Hyderabad	DCMS-1A to DCMS-45A	DRS-1 to DRS-14
Latur	CMS-207A, CMS-71106, CMS-338423 (DMM resistance), DSF-2 A , KSP-11, CMS-207A (DS) and CMS-M-5A	RNS-341, RLW-5, RLP-1, RLY-1, RNDL-2095, LRR-3, LRR-6, LRR-14, LRR-15, LRR-73, LRR-80, PSM-20, MRH-1, DMLTY-1, NDR-1, J-6, IB-43R, NDRR-9, DMLT-5
Ludhiana	CMS-3A, CMS-7A, CMS-10A, CMS-13A, CMS-18A, CMS-31A, CMS-32A, CMS-33A, CMS-34, CMS-35A, CMS-36A,	P111R, P114R, P116R, P120R, P122R to P129R, LR-O6-3, LR-06-12, LTR-08-9, R-27, NDR-6D, NDR-93-17 CMS-37A, CMS-38, CMS-44A, CMS-304A and CMS-395A
Raichur	CMS CFA-1A to CMS CFA-4, CMS-34A,	CFR-1 to CFR-6 and R-630 CMS-29A, CMS-38A
Nandyal	NDCMS-1A, NDCMS-2A, NDCMS-3A, NDCMS-4A, NDCMS-5A	26HAP

Source: Anonymous [20] (from1980 to 2011) develop large number of inbred lines and evaluate them for their nicking ability and convert the promising ones into CMS. Sooner than later the Directorate of Oil Seed Research will have parental lines developed for hybrid breeding. Hybrids developed from such lines may have better adaptability under different agro-climatic conditions.

Ineffective fertility restorers for diverse CMS sources

The narrow genetic base of the cytoplasmic male sterility (CMS) in sunflower has resulted in the development of several alternate cytoplasmic sources namely, CMS GIG1, CMS PF, CMS I, CMS MAX1, CMS PET-2 by different workers but they could not be exploited commercially due to lack of effective fertility restorers [21]. Several sources of CMS have negative effects on seed yield, and other plant and seed characteristics due to nuclear cytoplasmic interaction. Our observations indicate that majority of the inbreds being tested, behaved as maintainers for the new CMS sources. The restorer for one CMS source behaved as maintainer for other CMS sources and vice versa, confirming the diversity among the CMS sources. It was also observed that there is a lack of effective good restorers for diverse CMS sources. Most of the lines restored fertility for classical petiolaris cytoplasm PET-1. Only a few could restore fertility in new CMS sources [22-23]. Similar observations have earlier been reported in sunflower [24-26].

Alternatives

Due to narrow genetic base of available facility restorer (R) lines, a number of new diverse restorer lines with high oil content were developed from the polycross method (CMS line x pollen mixture of R lines). The restorer RHA 6D-1 which is the male parent for hybrid KBSH-1 and DRSH-1 is derived from the poly cross of a male sterile plant of the line 3Cr-37 pollinated with a pollen mixture of RHA-273, RHA-274, RHA-266 and Krasnodarates in 1977 in Bangalore.

Subsequently, efforts were made at different AICRP centres to diversify the restorer base in sunflower. To develop new restorer lines, several crosses were made among different best R lines (R x R) and segregating material was selected to identify R lines (mono and multi headed) for new CMS sources at different AICRP centres. Also, this restorer combination can be seen as a new potent restorer line. It is likely that the fertility restoration genes are accumulated through the recombination in these crosses. Such crosses can be used for the development of new "R" lines in sunflower. The restorer lines *viz.,* RHA-95C-1, MRHA-1, P-61R, RHA-1-1, R-64NB, AK-1 and MRHA-II were utilized in developing different hybrids developed by different AICRP centres suitable for different agro-climatic conditions (Table 4). Fertility restorer lines are also being developed by backcrossing using inbred lines of proven performance as the recurrent parent. The problem with this method is the identification of genotypes in the backcross progenies that carry genes for fertility restoration. Such identification involves test crossing of selected plants to a male sterile line to determine whether they carry the restorer gene(s). The F_1 plants of these test crosses will be fully male fertile if the selected plants carry gene for fertility restoration.

Table 4. Recently used CMS and Restorer lines in different countries

Country	CMS Line	R Line
Argentina	HA89 (CMS PET1)	RHA-271
	HA89 (CMS MAX1)	RHA-801
	CMS RES1	
Russia	Lg-21	VK-66-1
	Lg-24	VK-66-2
	Lg-25	VK-66-3
Turkey	CMS-7658-A	7887-1-R
	CMS-7674-A	7887-2-R
	CMS-7675-A	7887-3-R
	CMS-7682-A	7887-5-R
	CMS-7710-A	7961-R
	CMS-7751-A	7915-R
	CMS-6388-A	7794-R
	CMS-6397-A	7818-R
	CMS-6398-A	7820-R
	CMS-6522-A	7821-R
	CMS-6626-A	6973-R
	CMS-6535-A	70351-R
	CMS-65371-A	70352-R
	CMS-65373-A	7069-R
	CMS-6545-A	7487-R
	CMS-66241-A	48315-R
	CMS-66244-A	48312-R
	CMSHA-89 (PET2)	HA-R6
	CMSHA-89(GIG1)	RHA-271
	CMSHA-89(MAX1)	RHA-801
	CMSHA-89(PEF1)	
USA	CMSHA-851	
	CMSHA-852	
	CMSHA-853	

Another method to develop new restorers

involved crosses of R lines to a cytoplasmic male sterile line (A line x R). From such crosses, fertility restorer lines coupled with other agronomic characters are isolated by continuous selfing and selection. The widely used restorers *viz*., RHA-271, RHA-273 and RHA-274 were developed using this procedure. The ideal method to develop R lines is through having parallel programmes for developing inbreds separately from restorer gene pools maintained at the centre.

Conversion of inbred lines into CMS without testing combining ability

The availability of good combiner inbred lines still remained a serious handicap in large scale production of high yielding hybrids. Hybrids are being developed from the inbred lines derived from the same narrow gene pool. An excessive use of a few inbreds in most of the hybrid breeding programmes calls for some system of continuous upgradation of such indispensible inbreds. With respect to the ultimate use of inbreds in hybrid, inbreds should command the following three basic characteristics; i) they should be productive by themselves i.e., high *per se* productivity so as to produce a sufficient quantity of their own seeds and that of hybrid seeds ii) they should be agronomically suitable, disease resistant and standability so that hybrids produced by them are sound and disease free, and iii) they should be high combiners with high SCA for hybrid seed production. The male sterile lines must have a high GCA, so that it can combine well with an array of R-lines to produce a large number of hybrids. Before conversion of an inbred to CMS lines, inbreds must be evaluated preferably under more than one environment for their breeding value for utilization in hybrid breeding programs in order to develope hybrids with better heterosis and resistance to diseases and insect pests. The *petiolaris* cytoplasm controlling male sterility has no apparent adverse effects on agronomic or seed oil characteristics when incorporated into inbred lines but, it is unknown in comparison to other available CMS sources. An occasional problem in converting certain lines to CMS is the occurrence of fertile plants in the progeny of crosses between maintainer and sterile lines.

Alternatives

There are many methods to develop inbred lines but all are having some advantages and disadvantages. An innovative method of developing diverse inbred lines is doubled haploid (DH) technique. It is accelerating for inbred line development of sunflower in USA. The technique of DH production requires four steps: (1) induction of haploid, (2) identifying the achene with haploid embryos, (3) doubling haploid chromosomes by colchicines or any chromosome doubling agent(s) and (4) self-pollinating doubled haploid plants.

Doubled-haploid breeding is an important tool that enables the refinement of breeding of most crops. But it requires costly laboratory and growth chamber facilities, as well as highly skilled technicians. It has been observed that the DH technique works better with some sunflower genotypes than with others. For induction of haploidy to produce DH, the selection of suitable genotypes carrying the genes for disease resistance, herbicide tolerance or high-oleic acid, for example is, however, a difficult exercise. The production of DH in sunflower, the model of maize can be considered which has several advantages :

(i) Desired inbreds can be developed much faster than through conventional methods, (ii) these inbreds possess 100% genetic purity, (iii) maximum additive variance available, and (iv) reduction of costs for nursery and maintenance breeding work. However, some disadvantages have also been listed in maize. They are :

(i) The expensive high-tech laboratory facilities and skilled personnel needed for this technology and (ii) inspite of quick development of inbreds, they have to be evaluated for desirable traits.

Poor maintenance breeding

The goal of plant breeding research is to see that high quality seed, generally identical to the variety released by the breeder is produced in successive years. Maintenance of genetic purity of the parental lines of the released hybrids is one of the most difficult aspects and this has not received adequate attention till now. Earlier it was reported that the hybrids after their identification (and some times even before release) show a rapid genetic deterioration thereby defeating the purpose of plant breeding research. Many reasons could be attributed to genetic deterioration.

Alternatives

As mentioned above that maintenance of genetic purity of the parental lines of hybrids is not being followed meticulously. Today DNA marker technology offer an efficient alternative to this approach which is based on sequence variation of specific genomic regions, provide powerful tools for cultivar identification and seed quality control in various crops with advantages of time-saving, less labour consumption and more efficiency [28].

Other issues

There are many CMS sources available for commercial utilization in hybrid breeding programme in United State, European and other countries. Seventy CMS sources have been identified from progenies of crosses between wild *Helianthus* accessions and cultivated lines, from wild accessions grown in observation nurseries, or from induced mutation. Fertility restoration genes have been reported for 34 CMS sources, and detailed inheritance studies have been conducted for 19 out of the 34 sources [29].

Future prospects

In India, the development and adoption of hybrids has helped in increasing the area and productivity. This was facilitated by excellent CMS system, ease of fertility restoration and efficient seed production. Inspite of these successes, problems in the form of stagnating and unstable yields, genetic vulnerability and susceptibility to various diseases are threatening sunflower productivity. One of the major challenges would be to push up the productivity to the level of world average. The future programme to achieve self sufficiency in the crop, the following approaches *viz.,* increase the genetic diversity, heterosis breeding to develop better hybrids (high seed yield and oil content, more autogamy and resistance to major pests and diseases), diversification of CMS sources and synthesis of diverse and heterotic gene pools and identification of good maintainers and restorer lines remain the need of the hour. The new CMS lines with several desirable attributes like dwarfness, early maturity, good seed yield and oil content coupled with diverse cytoplasm are expected to make a significant dent in development of superior hybrids with better heterosis and stability there by enhancing the productivity and production of sunflower. The reluctance is presumably due to a lack of superior CMS-restorer combinations, as well as the time consuming conversion programs of CMS and incorporating fertility restoration genes into inbred lines. There is an urgent need to import of diverse stable CMS and restorer lines and evaluate them for their nicking ability under Indian conditions.

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