

S.K. Gupta *Editor*

Technological Innovations in Major World Oil Crops, Volume 2

Perspectives

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Preface

Over the couple of decades, oilseed production of the major world oil crops has increased tremendously to become one of the most important world sources of vegetable oils. Several new and superior varieties have been continually evolved and released in major oil crops in different countries of the world. The advancements have necessitated adoption of newer technologies which have now made possible what till recently was considered improbable.

With the objective of providing major reference volume for those interested or involved with vegetable oil industry as well as undergraduate and graduate students, this volume presents general but essential information on the technology developed and a comprehensive review of the results that have led to the advancements in oilseed production, processing, and utilization. Advances in the technology of seed processing to produce oil and meal are well presented followed by hybrid technology, biotechnology, and meal quality for animal nutrition

In addition to edible uses, the potential for utilizing oil in developing biodiesel markets, fatty acid long chains, and their derivatives is reviewed. Realizing the need to update the knowledge of the recent improved technologies by all those interested in major oil crops, the editor approached the leading scientists of the world for their write-ups of the advances made in their respective areas of specialization during the last decade so that the same could be packed in one volume for the benefit of students, nutritionists, biotechnologists, and industrialists, as well and researchers engaged in the improvement of major oil crops.

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Jammu and Kashmir, India

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Chapter 1

Hybrid Technology

**Surinder K. Gupta, Aditya Pratap, Debjyoti Sen Gupta,
Neha Rajan, and Veena D. Ganeshan**

Abstract Development of commercial hybrids is one of the most preferred goals in any crop improvement programme since it offers an opportunity to harvest significantly better yields from a uniform crop due to the effects of heterosis. Heterosis is the superior performance of the heterozygous hybrid progeny over both the homozygous parents. Male sterility is an effective way of producing hybrid varieties since it avoids the need for labour-intensive hand emasculating and offers absolute male sterility, thereby avoiding the chances of selfing. While cytoplasmic male sterility (CMS) is maternally inherited and results in complete male sterility, the seed production plots of genetic male sterile females contain 50% female fertile plants which need to be rouged out before pollination takes place, and are hence an inferior option to CMS. In the major oilseed crops of the world, possibilities of development of hybrid varieties have been explored and extensive efforts have been made on identification of male sterility systems. While rapeseed-mustard is perhaps the most successful with regard to identification and utilization of male sterility systems, other crops such as sunflower, linseed and cottonseed, despite reporting a number of successful male sterility systems, have not been commercially exploited to that extent. In cottonseed, hybrid seed production is still largely dependent on hand emasculating and pollination, while the use of only one CMS source in sunflower has led to considerable reduction in variability, making the crop vulnerable to attacks

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by insects, pests and diseases. Commercial soybean hybrids have also not been successful, mainly due to the absence of stable male sterility-female fertility systems. This chapter elaborates the significant developments made in male sterility systems and hybrid seed production in major oilseed crops of the world and also discusses problems and prospects in this technology.

Keywords Cytoplasmic male sterility • Hybrid production • Heterosis • Rapeseed-mustard • Cottonseed • Linseed • Sunflower

1 Introduction

Hybrid vigour or heterosis is the superior performance of the heterozygous hybrid progeny over both the homozygous parents. Heterosis breeding could be used for enhancing productivity in many crops, including some oilseeds. Development of commercial hybrids is the one of the most preferred goals of breeding improved crop varieties, particularly self-pollinated crops, to exploit the non-additive gene action present in yield heterosis. While the concept of heterosis came half a century ago (Shull 1952) and slowly gained practical importance in many crops among oilseeds, it was successful only in a few crops.

Though most crops show hybrid vigour, commercial production of hybrids is only feasible if a reliable and cost-effective pollination control system is available (Perez-Prat and van Lookeren Compagne 2002). A strict control over self-pollination can be achieved through mechanical removal of anthers or male flowers, application of male gametocytes or the use of male sterility systems. Among these, male sterility – the inability of the plant to produce fertile pollen – provides one of the most efficient means of directed pollination control for large-scale production of hybrid seeds in crops (Prakash et al. 2009). Of the two types of male sterility viz., genic and cytoplasmic, the latter is more exploitable as it is maternally inherited. A combination of male sterility induced by cytoplasmic genes and restoration of fertility by nuclear genes is considered to be the most efficient. Cytoplasmic male sterility (CMS) is encoded in mitochondrial genome as evidenced by detailed molecular analyses of CMS systems in different crop species such as wheat, rice, maize, sunflower, *Petunia*, *Brassica*, etc. (Prakash et al. 2009).

The basic requirement for developing commercial hybrids in crops such as rapeseed is the availability of proven experimental hybrids, stable performing male sterile (A), maintainer (B), and fertility restoring (R) lines, good synchrony of flowering in seed and pollen parent, and adequate seed-setting on male sterile seed parent through natural cross pollination (Rai et al. 2007). CMS can be used effectively for hybrid seed production only if CMS mutants are available in a crop, nuclear fertility restorer genes are also present and the CMS is not associated with any kind of yield penalty. Despite these limitations of the CMS systems, commercial hybrids have been successful only in those crops where abundant CMS systems are available. In oilseeds, perhaps rapeseed-mustard is the only

crop which has seen the maximum success with commercial hybrid varieties. Efforts towards development of commercial hybrids in rapeseed-mustard gained momentum with the discovery of male sterility in Japanese radish (*Raphanus sativus*) by Ogura (1968), followed by a number of other CMS sources. Hybrid sunflower became a reality with the discovery of CMS (Leclercq 1969) and effective male fertility restoration systems (Kinman 1970). There has been a limited success in crops such as linseed and sunflower. At the same time, hybrid breeding programme has not been very successful in soybean owing to its highly self-pollinating nature, absence of stable male sterility-female fertility systems, lack of efficient pollen transfer mechanisms, low number of seed set per pod and poor natural crossing (Singh and Hymowitz 1999); Palmer et al. 2001). Still possibilities of development of hybrid varieties have been explored and efforts have been made towards identification of male sterility systems. This chapter discusses the development of hybrid technology in major oil crops of the world and their future prospects.

2 Rapeseed-Mustard

Oilseed rapes are the world's third most important source of vegetable oils after palm and soybean. They include number of crop species which have a range of breeding systems ranging from complete cross-pollination to a high level of self-pollination (Rai et al. 2007). Rapeseed production has witnessed a steady upward movement during the past 25 years and presently contributes about 14% of the global vegetable oils (Gupta and Pratap 2007). Six species of the genus *Brassica* are important for human use, among which *B. juncea* – Indian mustard – is the predominant oilseed species of the Indian subcontinent, while forms of *B. rapa* (var. *oleifera*) also serve as sources of oilseed.

Among the different *Brassica* species, *B. juncea*, *B. napus* and *B. carinata* are predominantly self-pollinating, while *B. campestris*, with the exception of yellow sarson, is cross-pollinated (Labana et al. 1992). Therefore, they are quite interesting source materials from the breeding point of view (Rai et al. 2007). Conventional breeding programmes have been pursued vigorously to increase seed yield in major oilseed *Brassica* species in many countries over the past 50 years. In India, *Brassica* hybrid development programme was initiated during 1989 under an ICAR-aided project, "Promotion of research and development efforts on hybrids in selected crops-subcrop brassica" (PIRCOM).

Since the first report of heterosis in brown sarson (Singh and Mehto 1954), many studies reported different levels of heterosis: 13–91% in *B. juncea* (Banga and Labana 1984a, b; Kumar et al. 1990; Rai 1995; Thakur and Bhateria 1993; Baishakh et al. 1994; Verma et al. 1998); 25–110% in *B. campestris* (Patnaik and Murty 1978; Verma et al. 1989; Dhillon et al. 1990; Varshney and Rao 1997; Yadav et al. 1998) and 10–72% in *B. napus* (Rai 1995; Dhillon et al. 1996; Thakur and Sagwal 1997). High level of heterosis for seed yield in both spring and winter

forms of *B. napus* has been reported. As much as 40% heterosis for yield has been reported in summer rape and 60–70% in its winter form (Grant and Beversdorf 1985; Lefort-Buson and Dattee 1982). So, these studies justify the efforts to develop CMS lines and search for usable fertility restorer lines for producing hybrids in oilseed brassicas. In many of the above studies, it was also observed that hybrids between genetically distant groups showed greater heterosis than within the group combinations. Seed heterosis has been reported in the range of 24–60% in *B. rapa* (Falk et al. 1994), 30–90% in *B. juncea* (Pradhan et al. 1993; Ghosh et al. 2002) and 30–80% in *B. napus* (Brandle and McVetty 1990). However, the majority of these studies report heterosis over mean or better parent which is generally based on small plot size. Further, the major characters which constitute the prime component of yield Heterosis, such as branch number, siliqua number, seed number and weight are the characters with poor heritability, and are severely affected by population density (Labana et al. 1978; Pradhan et al. 1993; Thakur and Sagwal 1997).

2.1 Genetic Male Sterility

A number of sources of genetic male sterility have been reported in *B. juncea* (Badwal and Labana 1983; Banga and Labana 1983, 1985), *B. campestris* v. brown sarson (Das and Pandey 1961; Chowdhury and Das 1966, 1967a, b, 1968; Katiyar 1983), *B. campestris* v. yellow sarson (Chowdhury and Das 1966; Singh et al. 1987, Bhajan et al. 1993; Gupta et al. 1997), and *B. campestris* var. *toria* (Singh et al. 1984; Anonymous 1997). Most of them are spontaneous in origin and exhibit monogenic inheritance. However, the exploitation of genetic male sterility has not been economically viable owing to the requirement of additional labour to rouge out the fertile plants from the MS lines well before anthesis. This problem could be overcome if some linked seedling markers for male sterility could be found or pleiotropic effect of the male sterility genes could be established. This could make it possible to identify the male fertile plants before the initiation of flowering. However, no major breakthrough has been achieved in this direction.

2.2 Cytoplasmic Male Sterility (CMS)

In oilseed Brassicas, a number of CMS sources, viz., *B. carinata* CMS, *B. juncea* CMS, *B. oxyrrhina* CMS, *B. tournefortii* CMS, *Raphanus*-based Ogura CMS, *B. napus*-based Polima CMS, Settiana CMS, Siifolia CMS, and so on, are now well known and some of them are being worked with rather intensively (Rai et al. 2007). Out of these CMS sources, fertility restoration has been identified in *Raphanus*-

based Ogura CMS and Polima CMS and it has been detected in the CMS-based crosses in *B. tournefortii*, *B. juncea* CMS, Polima CMS, and Siifolia CMS in India. Fertility restoration in *tournefortii* CMS has been observed to be female genotype specific and photoperiod dependent.

Realizing the importance of male sterility systems in *Brassicas*, serious investigations on CMS in *Brassica* for its utilization started with the discovery of male sterility in Japanese radish (*Raphanus sativus*) by Ogura (1968). The Ogu cytoplasm confers male sterility in both the *Raphanus* and *Brassica* species. To date, this has proved to be one of the extensively investigated CMS systems among crop plants. It was subsequently introgressed through backcrossing into *B. oleracea*, *B. napus* (Bannerot et al. 1974), *B. rapa* (Delourme et al. 1994) and *B. juncea* (Kirti et al. 1995a). However the major drawback with this system was that despite the male sterility being stable, the flowers had petaloid anthers and poorly developed or absent nectarines while the female fertility was poor (Rouselle 1982). These problems have been overcome in *B. napus* (Pelletier et al. 1983; Jarl and Bornman 1988), *B. oleracea* (Kao et al. 1992) and *B. juncea* (Kirti et al. 1995a). Fertility restorer nuclear genes for “Ogura” male sterility were reported to be located in several European *Raphanus* cultivars (Bonnet 1975) and these were introduced to *B. napus* through conventional breeding (Heyn 1976; Delourme and Renard 1988). The fertility restorer gene was also introgressed into *B. rapa* and *B. juncea* (Delourme et al. 1994). Genetical studies revealed that original Ogu CMS required several genes for restoration. However, the improved CMS obtained through protoplast fusion containing recombinant mitochondrial genomes requires only one dominant gene. Extensive investigations at INRA, France, resulted in double low R-lines with good female fertility and regular meiosis (Primard et al. 2005). Bartkowiak-Broda et al. (2003) also obtained double low winter rapeseed restorer lines. Bannerot et al. (1977) introduced the nuclear genes of *B. napus* into *Raphanus* cytoplasm to develop *B. napus* CMS source, which is stable under a wide range of environments, but restorer genes have not yet been identified. The Polima CMS is the most promising CMS in *B. napus*. Restorer genes are now available and fertility restoration in the hybrids is satisfactory.

The “*Moricandia arvensis* system” was obtained in *B. juncea* following back-crossings of somatic hybrid *Moricandia arvensis*+*B. juncea* ($2n=64$, MMAABB; cp and mt: *M. arvensis*; Prakash et al. 1998). The CMS plants show delayed flowering and chlorosis with absolute pollen sterility and normal female fertility (>96%). Chlorosis could be improved by protoplast fusion between sterile and fertile *B. juncea*. The resulting green plants have *Moricandia* chloroplasts substituted with those of *B. juncea* (Kirti et al. 1998). Transfer of fertility restoring gene(s) from *Moricandia* was facilitated by having a *Moricandia* chromosome addition line in *B. juncea*.

The *Diplotaxis catholica* system was developed in *B. juncea* through both sexual and somatic hybridizations, the former from *D. catholica* x *B. juncea* allopolyploid while the latter from *D. catholica*+*B. juncea* somatic hybrid ($2n=54$, DDAABB; cp: *D. catholica*, mt: recombined; Kirti et al. 1995b; Prakash et al. 2009). The CMS plants

of sexual origin have altered floral morphology leading to poor female fertility (<50%). CMS plants of somatic origin are green and vigorous, and late in flowering. Flowers are normal with slender anthers and needle-like short filaments. Female fertility is normal and is reported to be 97% (Pathania et al. 2007). Fertility restorer gene for CMS sexual was found in the BC₄-BC₅ progenies of somatic hybrid *D. catholica*+*B. juncea*. The fertility-restored plants have normal stamen development without any ovary abnormalities. Genetic study revealed that a single dominant gene controls male fertility restoration and is sporophytic in nature (Pathania et al. 2003).

The wild and related germplasm has also been successfully used to introduce male sterility in oilseed *Brassicac*s. Hinata and Konno (1979) obtained male sterile lines of alloplasmic origin by placing the nucleus of *B. rapa* var. *chinensis* in the cytoplasm of a related wild species, *Diplotaxis muralis*, followed by the repeated backcrossings of intergeneric hybrid *D. muralis* x *B. rapa*. Gulati et al. (1994) and Raut et al. (1996) observed that *B. oxyrrhina* CMS in *B. juncea* background could be exploited in the hybrid programme. Similarly, Gundimeda et al. (1992) demonstrated that *Enarthrocraprus lyratus* cytoplasm causes male sterility in oilseed rape while Banga and Banga (1997) developed *lyratus*-based CMS line in *B. napus*. High level of exploitable yield heterosis has also been reported in *B. campestris* hybrids (Das and Rai 1972 ; Hutchenson et al. 1981). In this species, CMS system has been developed by backcrossing *B. campestris* cv. "Yukina" into the *Diplotaxis muralis* cytoplasm (Hinata and Konno 1979). The *B. campestris* "Yukina" CMS was stable and restorer genes have been identified for this CMS source.

Alloplasmic *B. juncea* and *B. napus* have been obtained based on *B. oxyrrhina*, *Trachystoma balli*, *Moricondia arvensis*, *Diplotaxis siifolia* and *Sinapis alba* cytoplasm. In several reports, male sterility was found stable and was accompanied by high seed fertility (Prakash and Chopra 1988, 1990; Batra et al. 1990; Rao et al. 1994; Kirti et al. 1995a, b; Prakash et al. 1995, 1996; Rao and Shivanna 1996; Prakash and Kirti 1997).

In *B. juncea*, CMS was first reported by Rawat and Anand (1979) by isolating a male sterile plant. It was found later that this MS was due to the cytoplasm of *B. tournefortii* (Pradhan et al. 1991). This CMS probably originated from outcrossings of synthetic allopolyploids *B. tournefortii* x *B. nigra* synthesized earlier by Narain and Prakash (1972). The male sterility was stable over a wide range of environmental conditions. Subsequently, it was transferred to *B. carinata* (Anand 1987) and *B. campestris*. A limitation to this system is much reduced nectarines and the frequent occurrence of malformed flowers.

The back-cross-substitution of the *B. juncea* genome into the cytoplasmic background of *B. carinata* resulted in a stable male sterile line (Banga et al. 1983). Banga et al. (1988) reported that the breakdown of sterility at higher temperatures was a major limitation of the nap system. *Raphanus*-based CMS was transferred to *B. juncea* (Labana and Banga 1989). The resulting male sterility was highly thermostable, but three problems that arose in the utilization of this system were chlorophyll deficiency at low temperature (<13°C), low nectar production and lack of

fertility restoration. Banga and Gurjeet (1994) observed the thermosensitivity of sterility expression and yield penalty associated with the polima CMS system. ISN 126 and GLS 8909 acted as perfect maintainers for this system.

2.3 Fertility Restoration

Fertility-restoring nuclear gene(s) for alloplasmic CMS systems are generally not available in the natural population and have to be introgressed from the cytoplasm-donor species (Prakash et al. 2008). For the *Raphanus/Ogu* CMS, which was the first CMS to be exploited for commercial purpose, restorer genes were identified in European radish cultivars (Bonnet 1975) and were introgressed through synthetic *Raphanobrassica* by Heyn (1976) and Rouselle and Dosba (1985), respectively. Later, complete fertility restorers could be developed for polima, tournefortii and lyratus CMS sources in *B. napus* (Banga and Gurjeet 1994; Banga and Banga 1997). Later, restorers were successfully obtained for (*Raphanus*) *B. napus* (Paulman and Robbelen 1988), (*Tournefortii*) *B. napus* (Stiewe and Röbbelen 1994), (*Moricandia*) *B. juncea* (Prakash et al. 1998), (*Trachystoma*) *B. juncea* (Kirti et al. 1997), (*Canariense*) *B. juncea* (Prakash et al. 2001) and (*Enarthrocarpus*) *B. rapa*, *B. juncea* and *B. napus* (Deol et al. 2003; Banga et al. 2003).

2.4 Use of Male Specific Gametocides

Very few reports are available on the use of male gametocides in *Brassica* (Chopra et al. 1960; Banga and Labana 1984b; Banga et al. 1986). However, it was not found promising. Thus, the male gametocides do not seem to have immediate practical utility.

2.5 Commercial Utilization of CMS

With the advancement of male sterility–fertility restorer systems, it has become possible to produce commercial hybrids in *Brassica*. A number of hybrid varieties in *B. napus* based on improved Ogura-INRA CMS system developed by various seed companies have been cultivated in Europe, Canada, and Australia since 1994 and these occupy a large area. The first commercial hybrids based on Polima CMS in *gobhi sarson* (hybrid 10 and hybrid 2) were observed to have more than 90% Heterosis (Singh 1986). The Punjab Agricultural University, Ludhiana, in India recommended the release of the first CMS-based *gobhi sarson* hybrid “PGSH 51” for cultivation in Punjab in India (Banga et al. 1994). After this, much stress is now being laid on the development of commercial hybrids in mustard (*B. juncea*) in

India. In China too, a number of hybrids have been developed, such as Zhong You Zha 93–1, yielding about 10% higher than the double zero cultivars. Two new hybrids, viz., Hyola 42 (early maturing) and Hyola 51 (late maturing), were developed in Australia (Anonymous 1999). CMS *Moricandia*-based *B. juncea* hybrid varieties in India show 20–30% yield advantage (Prakash et al. 2009). Advanta India has recently marketed a *B. juncea* hybrid based on improved Ogura CMS. In the year 2007, INRA and SERASEM registered a new, restored, semi-dwarf winter hybrid rapeseed in the French official catalogue of species and varieties. Since efficient restorers are available for various CMS systems such as Ogu INRA system in *B. napus*, *Moricandia*, *Berthautii*, *Erucooides*, *Canariense* and Ogu IARI in *B. juncea*, these developments will accelerate greater use of CMS systems in developing heterotic hybrid varieties.

2.6 Biotechnological Interventions

The *Brassicacae* have proven to be commercially the most important crop plants to respond to biotechnological interventions. Techniques such as protoplast fusion have been deployed to overcome the incompatibility between mitochondrial and nuclear genomes by generating mitochondrial recombination (Kirti et al. 1991, 1992, 1995a, b). Further, somatic hybridization has helped in diversification of CMS systems (Kirti et al. 1995a, b; Arumugam et al. 1996; Gaikwad et al. 1996). The molecular basis of CMS has been studied by analysing mitochondrial DNA modification (Pradhan et al. 1991; Kirti et al. 1993, 1995a, b; Mohapatra et al. 1998; Rao et al. 1998). Molecular markers have also been deployed for characterization of the diversity of hybrids as well as their parental lines. For example, isozyme markers have been used by various workers (Sekhon and Gupta 1995; for this purpose.

3 Cottonseed

Cotton, though principally a fibre crop, is also an important source of edible oil throughout the world. Cottonseed carries around 18% oil and is estimated to contribute nearly a fifth of the global vegetable oil production. A few reports in the literature (Dani 1991; Khan 2003; Khan et al. 2007b) have determined that cotton genotypes differ in oil percentage. The global production of cottonseed was around 35 million tons in 2010 (FAOSTAT 2010). Cottonseed is a byproduct derived through process called ginning. Heterosis in cotton is important in improving its yield as well as fibre quality. The potential use of heterosis for increasing cotton yields has been an important objective of breeders in few regions of the world. Male sterility is an important means of producing hybrids in cotton since it avoids the use

of labour-intensive hand emasculation. Except in countries where a vast and cheap labour force is available to make emasculations and crosses by hand, essentially, no commercial use of heterosis currently exists in cotton (Chaudhry 1997; Khan et al. 2007a). Hybrid seed produced through hand emasculation and pollination techniques is expanding in China and India because of availability of cheaper workforce (Zhang and Pan 1999) and in China following the commercial exploitation of transgenic *Bacillus thuringiensis* (BT) cotton (Wang and Li 2000). In India, for hand emasculation, Doak's method with some modifications and pollination is still being used in conventional hybrid seed production programmes.

In India, at least 40% of the cotton produced is derived from intra-specific hybrids of *Gossypium hirsutum*, and 8% from inter-specific hybrids of *G. hirsutum* × *G. barbadense* (Chaudhry 1997). The yield increase through intra- and inter-specific heterosis over the better parent or best commercial cultivar (useful heterosis) has been documented by numerous authors (Yuan et al. 2002; Zhang et al. 2002; Khan et al. 2007a). The availability of male sterile plants has shown a way for possible commercial exploitation of hybridization in cotton using male sterility. Therefore, work has been undertaken to study hybrid performance by breeders (Khan et al. 1999). Basal and Turgut (2003) concluded that yield and fibre quality traits can be improved through applying three way crosses, or modified backcross and recurrent selection to genotypes having remarkable heterosis and combining ability. The F_2 hybrids having greater heterogeneity and genetic variation might result in a vast range of adaptations relative to their parents and F_1 hybrids (Meredith and Brown 1998; Wu et al. 2004). The F_2 s exhibited superiority over their better parents when grown under stress conditions and lower yielding sites. Meredith (1990) demonstrated that F_2 s can produce better combinations of yield and fibre quality than their parents. The F_2 s heterosis in cotton has also been mentioned in previous studies (Han and Liu 2002; Khan 2003; Khan et al. 2007a).

Nonetheless, F_2 hybrids with lower inbreeding depression in yield and superior performance than well-adapted cultivars have been found (Meredith 1990). Khan et al. (2007b) noticed moderate to high heritability for cotton seed traits and oil content in intra-hirsutum F_2 hybrids. The higher the heritability, the simpler the selection process and the greater the response to selection (Larik et al. 2000; Khan 2003). Numerous studies have been reported on seeds traits (seed number and index), but little work has been reported on the genetics and heterosis of cottonseed oil percentage in cotton breeding. The estimates of heterosis provided useful information with regard to the possibilities and extent of improvement in the characters of breeding material through selection.

3.1 Male Sterility Systems

Hybrid seed production based on GMS system is cheaper as compared to the conventional methods as it can reduce the cost of hybrid seed production at least by 40–50%. For genetic male sterility (GMS) in tetraploid cotton, a total of 11 loci

have been identified controlling GMS and ten of them are in *G. hirsutum* and one in *G. barbadense*. All *G. hirsutum* genotypes, which carry *Ms5* or *MS6* (or both genes), are restorers. Any *G. hirsutum* line can be converted into GMS system by repeated back-crossing with alternate selfing and selection. Maintenance of GMS lines involves sib mating between male sterile (*ms5ms5 ms6ms6*,) and fertile (*ms5ms5 MS6ms6* or *Ms5ms5 ms6ms6*) plants.

3.2 Cytoplasmic Genetic Male Sterility

Interaction between nucleus of *G. hirsutum* and cytoplasm of *G. arboreum*, *G. anomalum*, *G. harknessii*, *G. longicalyx*, *G. aridum* and *G. trilobum* has been reported to produce male sterility (Meyer 1973, 1975; Meshram et al. 1992). However, *G. harknessii* (D2 CMS), *G. trilobum* (D8 CMS) and *G. aridum* (D4 CMS) are sources of cytoplasm which induce stable male sterility for practical hybrid seed production. Most of the *G. hirsutum* lines are maintainers (B lines) for these CMS lines (A lines). Any B line can be converted into MS line by repeated backcrossing and selection. Restorer line can also be converted through backcross method by alternate selfing and selection with the background of sterile cytoplasm.

3.3 Genetic Male Sterility

Hybrid seed production based on GMS system is cheaper as compared to conventional method as it can reduce the cost of hybrid seed production at least by 40–50% (Bhatt 1995). GMS has enabled the exploitation of heterosis in diploids as well. In diploid cotton, up to 185% of useful heterosis has been reported by Rajput et al. (1998) in GMS-based hybrids. Superior performance of diploid hybrids over tetraploid by 10–102% was observed in the preliminary study conducted by Narayanan et al. (1989). Two sources of GMS have been reported in India, The Hissar source and the Akola source (Meshram and Wadodkar 1992). In Hissar source, the recessive GMS in *G. arboreum* cotton variety, DS 5 (GMS-1), has white small flowers with petal spot. The corolla is semi-closed which is drawn back. In Akola source, the GMS line GAK-423A is developed by transferring the genome of *G. arboretum* (AKH 4) into *G. anomalum* cytoplasm (Meshram and Wadodkar 1992). Sufficient seed set by using GMS has been obtained and seed production can be made economical. India is the first country to release a GMS-based hybrid (AAH-1) in diploids. The availability of genetic male sterility system (GMS) in diploid cotton developed by Singh and Kumar (1993) and Meshram et al. (1994) resulted in the release of GMS-based diploid hybrids viz., AAH-1, CISAA-2, AKDH-7 and G.cot MDH-11 (Patel et al. 2004) for commercial cultivation in India.

3.4 *Temperature-Sensitive Genetic Male Sterility*

In cotton, a spontaneous mutant of *G. arboreum* GMS line was observed showing sensitivity to temperature regimes (Khadi et al. 2001). Gradual alteration from sterility to fertility occurred when temperature was reduced to less than 180°C. These lines have been stabilized and are helpful to overcome the problem of crossing for the maintenance of male sterile line and hence no fertile segregants need to be rogued out.

4 Sunflower

Sunflower (*Helianthus annuus* L.) belongs to the genus *Helianthus*, family “Asteraceae”, tribe “Heliantheae”, subtribe Helianthinae, which includes 20 genera with 67 species. Sunflower, being a highly cross-pollinated crop, is ideally suited for exploitation of heterosis. Heterosis breeding in sunflower evolved successfully ever since the discovery of first CMS source by Leclercq (1969) and fertility restoration by Kinman (1970) that gave the required impetus to commercial hybrid seed production in the world. This was followed by the development of the first hybrid in the US in 1972. Single cross-hybrids quickly dominated sunflower cultivation of the world. Owing to huge success of the sunflower hybrids, it has now become the major oilseed crop of the world and is very popular in many countries of eastern and western Europe, Russia and South America, besides India, China and Turkey. The adoption of the hybrid sunflower was so fast that in 1997, of the 16.5 million hectares of sunflower area grown in major countries, 11.5 million hectares were under hybrid varieties alone (Miller 1998).

Most of the sunflower hybrids that are commercially grown are developed from a single CMS source developed by Leclercq (1969). CMS PET-I derived from *H. petiolaris* Nutt has been most widely used in hybrid development of this crop. This has led to homogeneity, making the crop vulnerable to epidemics. Fortunately, several other CMS sources have been reported to be available which could be used for diversification of CMS sources. Serieys (1999) reported 62 new CMS sources which are from different origins. Thirteen new sterile CMS analogues on the base of different sunflower CMS sources were developed, and the obtained crosses were evaluated for agronomic traits and resistance to sunflowers pathogens. Consequently, promising CMS analogues were obtained for future use in this crop (Tavoljanskiy et al. 2004). Jan et al. (2006) registered one CMS and two fertility restorer lines while Jan and Vick (2006) registered seven CMS lines and four corresponding fertility restoration lines. Jan (2006) also registered two more CMS lines and eight corresponding fertility restorers. These were cooperatively developed and released by the UDSA-ARS and the north-Dakota Agricultural Experimental Station in 2005.

Several workers have reported high heterosis for yield and yield component traits in the hybrids. Virupakshappa (1991) reported considerable average heterosis for all the characters studied in 24 F_1 hybrids. Highest heterosis was recorded for seed yield (172.08%). In the process of evaluating 24 F_1 single cross-hybrids over three seasons, Giriraj and Virupakshappa (1992) observed that the average heterotic effect was the highest for seed yield. The hybrids CMS 851 \times RHA 274 and CMS 234 \times NDRLOS 6 manifested high heterosis for seed yield over three seasons. Govindaraju et al. (1992), in their study consisting of 60 hybrids, reported Heterosis that ranged from -21.2 to 51.14 over mid parent, -17.28 to 49.92 over better parent and -27.63 to 9.38 over standard check for seed yield. The studies on heterosis conducted by Harini (1992) revealed that out of 72 cross combinations involved, heterosis occurred in only 11 hybrids registering significantly higher seed yield over the standard check BSH-1. Most of the hybrids showed positive heterosis for the characters except days to 50% flowering. Madrap and Makne (1993) synthesized 80 hybrids by using four lines and 20 testers and observed a higher magnitude of heterosis in crosses CMS 234A \times RHA 273 for filled seeds, CMS 338 \times CO 7-11 for oil content and CMS 338 \times KSP-1 for seed yield. Study conducted by Gangappa et al. (1997) revealed that majority of the hybrids involving CMS 207 and CMS 851 as female parents recorded highest heterotic effects for plant height, stem diameter, head diameter, 100 seed weight, seed filling, oil content and seed yield. Shekar et al. (1998) reported that the line CMS-335A produced early flowering hybrids in combination with RHA-274 and, on the other hand, CMS 234A produced late flowering hybrids with the same restorer. The cross-combination CMS 234A \times RHA 83 was superior to standard checks in both seed and oil yield.

Kumar et al. (2001) estimated the extent of heterosis in the hybrids synthesized using new CMS sources based on PET-1, GIG-1 and PET-2. They observed significant heterosis for days to 50% flowering and days to maturity over MFSH-17 in all the newly developed hybrids which was in the desired direction. Besides, the hybrid combination CMS-6 \times DRS-3 recorded 56.79% heterosis for seed yield per plant over KBSH-1.

In India, the experimental hybrids were first developed in 1974-1975 using the CMS lines and restorers introduced from USA. Later, the first hybrid from the public sector was released for commercial cultivation in 1980. The favourable characters of sunflower hybrids such as production stability, response to high input agriculture, high self-fertility, uniform growth and maturity shifted the focus towards heterosis breeding, leading to the release of the first ever sunflower hybrid BSH-1 in India (Seetharam 1981), which provided the required fillip to expand sunflower cultivation in the country. Since then, many hybrids have been released for commercial cultivation based on the cytoplasmic genetic male sterility system. The hybrid breeding programme has seen tremendous development in India also and consequently, more than 30 hybrids have been developed by both private and public sectors. The available variability in the parental material has been used for both sources.

5 Linseed

Linseed (*Linum usitatissimum*) oil is distinctive in terms of fatty acid composition of the triglyceride, which contains an unusually large amount of α -linolenic acid with unusual oxidation properties (Gill 1987). It is obtained from the dried mature seeds of the linseed plant by cold pressing, sometimes followed by solvent extraction. The success of hybridization programmes depends on the ability of the parents involved to yield desirable segregants/recombinants. Though hybrid varieties have been successful in many oil crops, there are no reports of stable male sterility systems in linseed. Therefore, commercial hybrid production still remains a distant dream for the breeders of this crop.

A few preliminary studies have been conducted in linseed to study the combining ability and level of heterosis. The ability of the parents to combine well depends on complex interactions between the genes, which cannot be judged by mere yield performance and the adaptation of the parents. A particular cross-producing transgressive segregants in a self-pollinated crop such as linseed would depend upon the precise estimates of various components, namely additive, dominance, inter-genic interactions, linkage disequilibrium among polygenes and genetic background in the parents of the cross, contributing to heterosis (Jinks 1983). Genetic diversity within linseed genotypes has been reported by various workers (Rowland 1991; Kurt and Evans 1996; Adugna and Labuschangne 2004). Combining ability analysis is an important tool for the selection of desirable parents, together with information on the nature and magnitude of gene effects controlling quantitative traits of economic importance. In addition, such information is more reliable when drawn from various environments (Patil and Chopde 1981; Verma and Mahto 1996; Yadav and Srivastava 2002). Heterosis and combining ability in linseed has been reported by various workers, based on different breeding materials (Shehata and Comstock 1971; Sood et al. 2007, 2011; Singh et al. 2009; Bhateria et al. 2006; Mishra and Rai 1993). Rai et al. (1993) investigated the direct and/or indirect effects of different factors, viz. protein content, iodine value, stearic acid, oleic acid, and linolenic acid content on the total oil content in linseed. It was reported that linolenic acid has high direct positive effect on the oil content, while protein and iodine values have direct negative effects, and other fatty acids such as stearic and oleic acids have negative effects which may be direct or indirect via other fatty acids.

6 Conclusions and Future Prospects

The development of hybrid crop varieties in oilseeds has led to increased productivity due to hybrid vigour, and has made mechanical harvesting possible due to increased uniformity. However, this gain has remained confined only to a limited number of oilseed crops and many others still have to enter into the era of hybrid

technology. A major hurdle to developing commercial heterotic hybrids in many oilseed crops is the non-availability of a reliable pollination control system, particularly for self-pollinated crops. A ray of hope lies in engineering CMS into such crops. Nuclear-coded male sterility has already been engineered successfully into important crops such as corn and *Brassica napus*. An alternative path could be the use of natural or induced mutations that cause male sterility or introduction of a gene or subset of genes which are able to alter the level of metabolites needed for the production of viable pollen. Male sterility could also be generated by bringing together two genes into the same plant, which then have a combined action by crossing two different lines, each expressing one of the genes. More concerted efforts are also needed towards searching effective chemical gametocides and also towards diversification of exploitable CMS sources and fertility restoration genes.

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