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MALE STERILITY SYSTEMS IN OILSEED BRASSICA

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ABSTRACT

The several sources of male sterility systems are available in *Brassica napus*, *Brassica juncea* and *Brassica campestris* for commercially utilizing hybrid vigour. The different sources are described below:

- (a) **Raphanus based CMS System:** The naturally occurring male sterility in Japanese raphish (Ogura, 1968) was successfully transferred to *Brassica juncea* (Labana and Banga, 1989). However, a few obstacles were faced in handling the system. Some of the problems have been solved and CMS lines of *Brassica juncea* are now available.
- (b) **Tornefortii System:** Spontaneously occurring male sterile plants of *Brassica juncea* were initially identified by Rawat and Anand (1979). Later tests suggested that *Brassica tournefortii* was the most

likely donor of the cytoplasm, interacting with which the cultivated species like *Brassica juncea* developed male sterility. Several lines based on this cytoplasm are being tested in Punjab Agricultural University. The fertility restoration in *Brassica juncea* CMS is however low, work is in progress to break this barrier.

- (c) **Juncea System:** Male sterile plants were identified in one cross involving RLM 198. It was found that male sterility occurred against the cytoplasmic background of the said variety (Banga and Labana, 1984).
- (d) **Oxyrrhina System:** Alloplasmic combination of *Brassica oxyrrhina* cytoplasm with *Brassica campestris* and *Brassica juncea* nuclear genome results in production of cytoplasmic male sterility (Prakash and Chopra, 1988).
- (e) **Siifolia system:** Placement of *Brassica juncea* nucleus *Diplotaxis siifolia* cytoplasm results in production of cytoplasmic male sterility (Rao *et al.* 1994).

In *juncea* the problem of restoration of fertility is faced for which there has not been much progress in this crop towards development of F₁ hybrids (Salma and Hussain, 1991).

Besides, few other systems involving *Brassica juncea* with *Moricandia*, *Sinapsis alba*, *Diplotaxis catholica*, *Enarthrocarpus lyratus* and *Trachystoma balli* have also been developed (Prakash *et al.* 1995; Kirti *et al.* 1995). The systems described under (a), (b) and (c) are of more practical value.

Genetic male sterility: Generally two types of GMS, recessive and dominant have been recognized. Both these types are available in

oilseed *Brassicas*. GMS has its own advantages in rapeseed and mustard hybrid breeding. Firstly, the GMS are more stable than CMS and secondly, for a sterile line of recessive GMS, almost every variety is the restorer. This could be helpful for screening a large number of hybrid combinations to identify hybrids having much higher F₁ heterosis. Thirdly, it could be relatively easier to breed GMS lines by successive backcrosses than to breed CMS or SI lines.

Table-1: Genetic male sterility in Brassica.

Crop	Inheritance	Origin	Reference
<i>Brassica napus</i>	Monogenic recessive	Induced mutation	Koch and Peters (1953)
	Monogenic recessive	Induced mutation	Takagi (1970)
	Digenic recessive	Spontaneous	Heyn (1973)
	Monogenic dominant	Spontaneous	Mathias (1985)
	Digenic recessive	Spontaneous	Hou et al. (1990)
	Digenic recessive	Spontaneous	Li and Zhang (1983)
	Digenic dominant	Spontaneous	Li (1990); Li et al. (1988)
<i>Brassica juncea</i>	Monogenic	Spontaneous	Banga (1993)
	Digenic epistatic	Intervarietal cross	Banga and Labana (1983b)

Therefore, if a close linkage between a morphological marker and the GMS controlling gene is established, GMS will have excellent potential in hybrid breeding. So far such a marker system has not been in

these crops and hence GMS has not been considered useful for developing F₁ hybrids.

Cytoplasmic male sterility: Several sources of cytoplasmic male sterility (CMS) have been developed in *Brassica* oilseeds by substituting *Brassica* nucleus in alien cytoplasmic sources, using sexual or somatic hybridization techniques. Male sterility in all these CMS sources manifested in vestigial or petaloid anthers. The expression of the male sterile phenotype in the form of petaloid or vestigial anthers also depends on the genotype of the nuclear donor parents. Notable CMS systems developed and investigated in *brassica* oilseeds (Banga, 1994; Fu and Yang, 1998; Prakash, 2001; Banga *et al.*, 2003; Prakash *et al.*, 2009) have been summarized in Table-. Most of these systems are associated with imperfections like chlorosis (ogu, oxy, mon), impaired flower opening (tour, trachy, lyr) and almost universal absence of fertility restorers in the euplasmic mustard genotypes. Chlorosis has been corrected through protoplast fusion for ogu (Pelletier *et al.*, 1983; Kirti *et al.*, 1993), oxy and mori (Prakash *et al.*, 1995) CMS. Chlorosis associated with mori CMS system

Table-2: Alloplasmic CMS systems in Brassica oilseeds.

Cytoplasm donor	Code	Developed technique	Fertility restoration	Crops	References
<i>Raphanus sativus</i>	Ogu	Interspecific cross	Restorer gene introgressed	<i>B. napus</i>	Bannerot et al. (1974)
		Protoplast fusion		<i>B. juncea</i>	Kirti et al. (1995a)
<i>Brassica tournefortii</i>	Tour		Unstable, genotype specific partial restoration	<i>B. napus</i>	Banga et al. (1995)
				<i>B. juncea</i>	Rawat and Anand (1979)
<i>B. oxyrrhina</i>	Oxy	Interspecific cross Protoplast fusion	Not availability	<i>B. juncea</i>	Prakash and Chopra (1990)
<i>Diplotaxis siifolia</i>	Sif	Intergeneric cross	Not available	<i>B. juncea</i>	Rao et al. (1994)
<i>D. sietiana</i>	sie	Intergeneric cross	Not available	<i>B. juncea</i>	Prakash et al. (2001)
<i>D. catholica</i>	cath	Intergeneric cross	Restore gene being introgressed	<i>B. juncea</i>	Prakash (2001)
<i>Trachystoma ballii</i>	trachy	Protoplast fusion	Incomplete	<i>B. juncea</i>	Kirti et al. (1995b)
<i>Morichandia arvensis</i>	mori	Protoplast fusion	Restorer gene introgressed	<i>B. juncea</i>	Prakash et al. (1998)
<i>Enarthrocarpus lyratus</i>	lyr	Intergeneric cross	Restorer gene introgressed	<i>B. juncea</i>	Banga et al. (2003)
				<i>B. napus</i>	Janeja et al. (2003b)
<i>Erucastrum canariense</i>	can	Intergeneric cross	Restorer gene introgressed	<i>B. juncea</i>	Prakash et al. (2001)
				<i>B. napus</i>	Banga et al. (2003)



could also be improved by introgression of certain genes from cytoplasm donor species.

GENETICALLY ENGINEERED MALE STERILITY:

Barnase-Barstar system: TA29-Rnase : *Barnase* gene – ribonuclease gene with TA29 (tapetum specific promoter) to develop male sterility. *Barstar* gene inhibitor of ribonuclease encoded by *barnase* gene. Both genes are isolated from *Bacillus amyloliquefaciens*. When *barnase* and *barstar* both are present plant will be male fertile. *Barstar* is dominant over *barnase*. When male sterile (*barnase*-bb) plant is crossed with male fertile (*barstar* – BB), resulting F₁ will be (B/b) male fertile. The first success in developing genetically engineered male sterility in crop plants was by transforming tobacco and rapeseed plants with dominant gene *barnase* (bacterial RNase) driven by a tapetum-specific promoter TA 29 (Mariani *et al.*, 1990). About 92% of the TA29-*barnase* transformants failed to shed pollen and were completely male sterile whereas female fertility was normal. To restore fertility, the pollen parents were transformed with *barstar* gene from same bacterium (Mariani *et al.*, 1992). Upon crossing, *barnase* male sterile plants with transgenic fertile plants carrying TA29-*barstar* chimaeric gene, the F₁ progeny showed co expression of both the genes in the anthers of the male fertile plant. It was found that *barstar* gene is dominant to the *barnase* gene, and fertility restoration was due to the formation of tapetal cell-specific *barnase* and *barstar* complexes. Female fertility was not affected, and transformed plants had normal morphology. By coupling the TA29-*barnase* gene to a dominant herbicide resistance “bar” gene, uniform populations of male sterile plants could be produced. “Bar” gene confers

resistance to the herbicide bialophos (phosphinothricin or PPT) and is used as a marker for male sterility (Denis *et al.*, 1993). Transgenic male sterile plants of *brassica napus* variety Drakkar were linked to the “bar” gene coding for PPT acetyl transferase and were resistant to herbicide PPT. Maintenance progeny showed 50% male fertile susceptible plants. Application of PPT permitted the elimination of male fertile susceptible segregants in the field by herbicidal application and assured 100% production of hybrid seed on male sterile plants. Hybrids based on this male sterility system are now available in *B. Napus* and *B. Rapa* in Canada. Reversion to fertility from male sterile plants has been observed in some cases. Jagannath *et al.* (2001) could develop stable transgenics by using gene constructs having spacer DNA in between the *barnase* gene and the CaMV 35S promoter-driven bar gene. The newly developed male sterile lines, however, could not be restored by transgenics carrying wild type *barstar*. To restore fertility in such male sterile lines modified *barstar* constructs were developed (Jagannath *et al.*, 2002).

Hybrid seed production: Hybrid seed production based on CMS-FR system requires four isolations. These are : maintenance of CMS line (A X B), multiplication of maintainer (A) line, multiplication of restorer (R) line and hybrid seed (A X B) production. The production of hybrid seed by cross pollination is the most important factor affecting the bio-economics of seed set on female lines using various production techniques. Wind and insects have been reported to be the main agents for pollination, though only the pollen grains that are initially disturbed by insects may become truly air borne. The factors that can influence the extent of seed setting on female lines

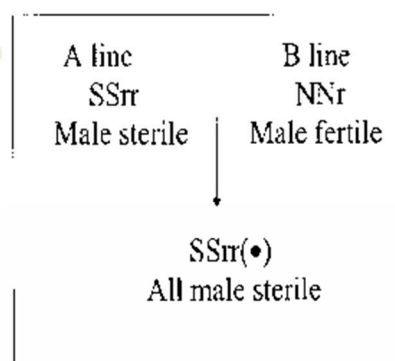


include ; male-female ratio, synchronized flowering of male and female parents, nector production and bee foraging, yielding ability of CMS lines, pollen production of male parents. A very wide variability in seed yield has been observed in different experiments. Genotype has been a major source of variation and selection of female parents suitable for cross-fertilization. Different kinds of row ratios for female vs. Male were found to be useful for commercial hybrid seed production. A study on Indian mustard (Banga *et al.*, 1995) has shown that the extent of hybrid seed set was maximum when the male rows were sown in higher frequency i.e., 2 : 4 > 1 : 2 > 1 : 3 > 1 : 4. There was significant reduction when the male : female ratio was changed from 1 : 3 to 1 : 4. In addition to the reduced seed set, a sharp decline occurred in the number of successful pods on the male sterile plants as was evident from an increased frequency of empty or aborted pods. While greater production of female rows is desirable to enhance the production of hybrid seed, care must be taken to ensure adequate pollen supply to facilitate hybrid seed set on female plants. Orientation of rows against the direction of wind has generally been found useful to maximize out-crossing on female plants. The block seed production method, using ogu CMS material has been found to be suitable in *B. Napus* (Hogarth *et al.*, 1995). Isolation distance depends upon several factors. These include location, wind direction, viability for air-borne pollen and extent of bee

population. Production of hybrid seed should be carried out in crossing fields isolated from other plants of related species with a minimum distance of 500 to 3000m depending upon the species. Crucial role of nector production for bee foraging and seed set on male sterile plants has been established in various crops. Keeping beehives in the seed production plots of rapeseed-mustard has been found to be useful. Another factor, which has profound influence on the economics of hybrid seed production, is the synchronized flowering of male and female parents. It must be realized that nicking is the best isolation. Nicking can be improved by cultural manipulations or by selective application of growth regulators to delay or advance flowering in male/female lines. In oilseed rape, detopping of alternate plants of male parent coupled with application of N @ 25 kg/ha between pollinator rows after flowering was found to be effective to delay flowering in male parent besides ensuring pollen supply for longer duration.

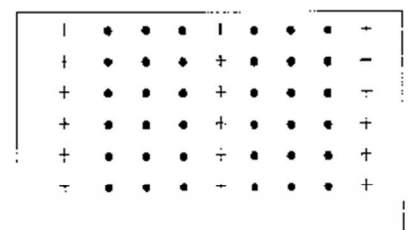
Fig.1- Hybrid seed production techniques in oilseed *Brassica* utilizing CMS system.

Male sterile seed multiplication



NN – Normal cytoplasm
 SS - Sterile cytoplasm
 r- Maintainer allele

Hybrid seed production field



Male parent (+)
 Male sterile plant (•)