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Hybrids in Rapseed-mustard: Present status and future

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Introduction: Heterosis is a natural phenomenon whereby hybrid offspring from genetically diverse individuals show increased vigour relative to their parents. Heterosis in crop species can be visualized in terms of increases in growth rate, total biomass, stress resistances, seed yield and population fitness. Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is a premier oilseed crop of the Indian subcontinent. F_1 hybrid cultivars are now commercially available in Rapeseed-mustard (Table 1). These are currently based on *Mori*, *Ogura* and 126-I CMS systems. Mori CMS has a gametophytic fertility restorer system, and it is sometimes difficult to differentiate between CMS plants and fertile contaminants in the seed production plots. Although not much is known about the 126-I CMS system, it seems to be constrained by the availability of sterility maintainers. Ogura CMS system is now increasingly becoming popular in *B. juncea* due to the stability of the CMS expression. *Ogura* CMS system was first developed through backcross transfer of *Ogura* sterilizing cytoplasm from CMS *B. napus* to *B. juncea* but this CMS system have a problem of leaf chlorosis. Leaf chlorosis associated with this CMS was subsequently resolved through somatic hybridization (Kirti et al., 1995). Cytoplasms from several wild species, namely *Brassica oxyrrhina* (oxy), *Trachystoma balli* (trachy), *Moricandia arvensis* (Mori), *Diplotaxis catholica* (cath), *D. siifolia* (sii), *D. erucoides* (eru), *D.berthautii* (berth), *Enarthrocarpou lyratus* (lyr), *Erucastrum canariense* (can) and *Raphanus sativus* (ogu), have been introgressed into *B. juncea* using sexual or somatic hybridization (Prakash et al., 2010). Chloroplast substitutions or mitochondrial recombinations have helped improve deficiencies associated with many CMS systems (Kirti et al., 1995; Prakash et al., 2010). Backcross substitution of *Brassica juncea* (2n = 36; AABB) nucleus into the cytoplasm of a wild crucifer, *Brassica fruticulosa* helped in the development of a new cytoplasmic male sterility (CMS) system (*Atri et al., 2016*). *B. fruticulosa* is a valuable genetic resource for resistance to mustard aphids (Kumar *et al*. 2011) and Sclerotinia stem rot (Garg *et al*. 2010). On the other hand, genetic engineering ensures a precise and accurate technique for developing male sterility in plants by misfunctioning the tapetum cell layers of pollens rendering them non-functional.

Table 1: Released Rapeseed-Mustard hybrids at national level

S.No	Hybrid	*Brassica sp.*	Released year	Institute	CMS system
1.	PGSH-51	*B. napus*	1996	PAU, Ludhiana	*Tournefortii*
2.	Hyolla 401 (PAC 401)	*B. napus*	1997	Advanta, India Ltd.	-
3.	Coral 437 (PAC 437)	*B. juncea*	2012	Advanta, India Ltd.	*Moricandia*
4.	Coral 432 (PAC 432)	*B. juncea*	2010	Advanta, India Ltd.	*Moricandia*
5.	NRCHB 506	*B. juncea*	2008	DRMR, Bharatpur	*Moricandia*
6.	NRCHB 101	*B. juncea*	2009	DRMR, Bharatpur	*Moricandia*
7.	DMH-I	*B. juncea*	2010	Delhi University	"126-1"

(Kumar *et al.*, 2020)

Problems in CMS development

- Stable CMS system took a long time to develop because of its many challenges like

1) Transferring cytoplasmic male sterility from wild species to cultivated ones is extremely difficult because wild species don't cross easily with cultivated ones. Therefore, approaches such as embryo rescue and tissue culture had to be used. This was a long-running research program.
2) Even after they were crossed, the resulting male and female parents didn't always turn out perfect. Flowers on some would-be deformed and unable to attract bees for

pollination while the leaves on others would be discoloured. This stalled the development of CMS systems further.

3) However, limitations exists in CMS systems after development in the form of chlorophyll deficiencies (ogu, oxy, mori), poor female fertility (trachy, lyr) and the yield penalties (Kaur *et al*., 2004).

Viable solutions to problems in CMS development:

Transgenic techniques have the potential in developing male sterility and restorer systems. Chimaeric ribonuclease gene (barnase) expression within the anther selectively destroys the tapetal cell layer that surrounds the pollen sac, prevents pollen formation and leads to male sterility. A chimaeric tapetal cell-specific ribonuclease inhibitor gene (barstar) was used in male fertile plants to restore male fertility in F_1 progeny. The barnase-barstar system is so far one of the most exhaustively studied transgenic systems for the development of male sterile and restorer lines (Thakur *et al*., 2020). GEAC initially cleared the barnase-barstar use for commercial cultivation, however, they retracted their approval upon deciding that more tests and additional data concerning the effect of DMH - 11 on insect pollinators, in particular honeybees, and on soil microbial diversity was needed before commercialisation.

Features of Barnase-Barstar over other CMS systems

- Efficient fertility restorer system
- Easy maintenance of male sterile lines
- Easy elimination of male fertile plants from male sterile lines
- Lack of adverse effects on other traits
- Stable male-sterile phenotype over different environments
- Satisfactory performance of F_1- hybrids
- It does not depend on natural sources of male sterility.
- Other CMS systems are crop- and genotype-dependent but any genotype can be converted into a male sterile line in case of barnase/barstar system.

Why are hybrids not popular in Brassica?

- ✓ DMH-1 and NRCHB-506 produce small seeds (half test weight i.e. ~3.5g as compared to varieties like RH-725 ~ 7g) due to the negative effect of sterile cytoplasm.
- ✓ Only around 85% of the seeds produce are pure hybrid seeds due to male sterility break in female lines
- ✓ Perception issue (Even though the size of the seed does not affect oil content, farmers continued to irrationally favour bolder seeds)
- ✓ Brokers are used to buying a particular type of seed (**bold seed**) because of marketing issues
- ✓ It is not easy to change perception in the market." Unless farmers are educated about such fallacies, they may stay away from transgenic mustard as well.

What are the Solutions?

- Development of hybrids with bolder seeds using a transgenic approach because certain East European varieties of mustard do have this feature.
- Use of transgenic system (Barnase/Barstar) to make better hybrids than we have because transgenics saves the time of CMS lines development t.

Conclusion:

Different conventional and advanced breeding methods are under process to develop superior high yielding hybrids. Efforts to develop new CMS sources are thus continuing not only to improve agronomic performance of the F_1 hybrids but also to avoid genetic vulnerability associated with worldwide usage of single CMS in oilseed Brassica hybrids. Barnase/barstar based transgenic hybrid may be the future breakthrough in brassica.

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