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REVIEW ARTICLE

Heterosis Breeding in Oilseed Brassicas: An Indian Perspective

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ABSTRACT
Rapeseed- mustard is the second most important oil seed crop in India. To cope up with the increasing population, the yield potential of this oil seed crop needs to be improved. Heterosis breeding is suggested as a strategy to break the yield barrier in these predominately self-pollinated crops. Considerable hybrid vigour for seed yield has been reported by many researchers. Several CMS-FR systems, the most reliable method of pollination control, have been developed and rectified in Brassica oil seed crop. Apart from the mori CMS system, the recently developed eru and ber systems are stable and with almost no adverse effect in B. juncea backgrounds. Now the efforts need to be directed towards developing rapeseed- mustard hybrids with superior yield potential. A few glimpses of the significant research findings from the past few decades in hybrid breeding of rapeseed- mustard in an Indian context have been reviewed in this article.
Key words: *Heterosis breeding, Oilseed Brassicas, Pollination control systems, CMS system, Hybrid*

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INTRODUCTION

Brassicas are the second most important oilseed crop in the world after soybean. China, India, Canada, Japan and Germany are the major rapeseed-mustard growing countries. It is the second most important oilseed crop in India, next to soybean. India is one of the largest rapeseed-mustard growing country occupying first position in area and third position in production. Four oleiferous *Brassica* species *viz., Brassica juncea*, *B. napus*, *B. rapa* and *B. carinata*, commonly called rapeseed-mustard, are cultivated in about 6.07 million ha area and producing 7.92 million tons in India during 2016-17 (www.nfsm.gov.in). Out of these, Indian mustard (*B. juncea*) contributes more than 80 per cent to the total rapeseed-mustard production of the country. *B. juncea* has an enormous cultivation potential in semi-arid areas as it is known to be more drought tolerant and shattering resistant than *B. napus* and *B. rapa* [1]. However, the production and productivity of rapeseed- mustard in India is almost static during the last decade and productivity is hovering between 1 to 1.2 t/ha, which is much below the world's average of 1.9 t/ha. Cultivar development in mustard has been mostly undertaken by breeding methodologies for self-fertilized crops. Efforts in past were directed towards development of pureline varieties, which do not mobilizes sufficient genetic variations. Heterosis breeding has been suggested as one of the most effective means to break the yield barrier. Oilseed *Brassica* species are well suited for hybrid breeding because of their natural biological characteristics and higher heterotic response.

Heterosis in *Brassica* was first reported by Singh and Mehta in 1954 [2]; however, its commercial utilization has been demonstrated only during the past two decades. Fu and Yang [3] divided the progress of rapeseed-mustard hybrid breeding into three periods *viz*., the exploration period (1940-1967), the preparation period (1968-1984) and the utilization period. Heterosis was recognized during the exploration period. During the preparation period different CMS systems have been developed. In the utilization period first CMS hybrid Qinyou No.2 of *B. napus* has been released. Utilization of biotechnological tools to rapeseed-mustard breeding has also been started in this period. Rapeseed – mustard hybrid breeding programmes in India was started in 1989 when Indian Council of Agriculture Research (ICAR) started a special project on "promotion of research and development efforts on Hybrids

in selected crops" [4]. The present review summarizes salient past research findings and developments and attempts to prioritize the future research needs in heterosis breeding of Brassicas in India.

REQUIREMENTS FOR COMMERCIAL EXPLOITATION OF HETEROSIS

There are three main requirements for hybrid breeding: 1. Extent of outcrossing 2. Standard heterosis 3. Pollination control systems

Extent of outcrossing

Among rapeseed-mustard, B*. juncea* and *B. napus* are predominantly self-pollinating but *B. campestris* (now known as *B. rapa*) ecotypes except yellow sarson are cross-pollinated [5]. In Indian mustard outcrossing varied from 7.6 to 22% [6] whereas, in *B. napus* it is reported up to 21% [7]. Insect pollination by honey bees has observed as an important mechanism of outcrossing in *B. juncea* [8]

Heterosis

In many crops, such as maize, pearl millet, sorghum, cotton, rice etc. hybrid vigour has been commercially exploited. After the report of heterosis in Brassica by Singh and Mehta [2], various studies were carried out to estimate the heterosis for seed yield. The results indicated significant level of heterosis like 13 to 91% in *B. juncea* [9], 25 to 110% in *B. campestris* [10] and 10 to 72% in *B. napus* [11]. In India, different workers reported heterosis in Indian mustard for yield traits to the extent of 67.71% [12], 44.80% [10] and 80.97% [13] in the F_1 crosses. Most of these studies reported heterosis over better parent (heterobeltiosis). Singh *et al*. [14] evaluated 72 *B. juncea* hybrids, of which 13 displayed highly significant sca effect, heterobeltios>15% and higher per se performance for seed yield. Up to 43% mid-parent heterosis for seed yield was reported after the evaluation of *B. napus* hybrids under nutrient poor conditions. This suggested the existence of a strong heterotic effect on nutrient uptake efficiency [15]. Besides seed yield, hybrid vigour has also been observed for other characters such as water absorption & esterase enzyme activity during seed germination [16], total biomass [17] and yield components [18].

It has been demonstrated by many researchers that the use of resynthesized brassicas as one of the parents in hybrid development led to much higher level of heterosis. Szala *et al* (2019) [19] used semi – resynthesized *B. napus* lines as a parent for developing hybrid cultivars and found high heterosis effect for seed yield (4.56% to 90.17% heterobeltiosis) in the F_1 hybrids. A population of new type *B. juncea* was developed by Wei *et al*. [20] by combining the A^r subgenome from *B. rapa* and the B^c subgenome from *B. carinata* and observed a considerable potential for heterosis in inter-subgenomic hybrids between new-type *B. juncea* lines and traditional *B. juncea* accessions. It was found that the level of mid parent heterosis in hybrid of the inbred lines derived from *B. napus* × *B. oleracea* cross was twice greater than the level of heterosis found for the hybrids of inbred lines derived from spring × spring or winter × spring *B. napus* crosses [21].

Development of heterotic gene pool is an important approach in improving the level of heterosis in rapeseed-mustard hybrids [22]. Lefort-Buson *et al*. [23] conducted a study to investigate the relationship between heterosis and geographic distance in cultivars of *B. napus.* They established three groups of F_1 hybrids, i.e., the European group (E X E), the Asiatic group (A X A) and the mixed group (A X E), and observed that heterosis in the E X A group was higher than the E X E or A X A group. It was also observed that if the parents had originated from different geographic areas or had separate evolution over a long time or well adaptability to target areas, the heterosis would be better. Similar results were also reported in *B. juncea* by Jain *et al*. [24]. Different methods are available for selection of parents for their utilization in hybrid breeding. If small number of germplasm is available, it is possible to check the individual hybrid performance to select parents. Otherwise diallel /factorial crosses can be used to identify the better parental combinations. If large number of germplasm is available, considering the geographic origin of genotypes or polymorphism for molecular markers or using D^2 statistic, it is possible to determine the best parental combinations [25].

Pollination control systems

Commercial exploitation of heterosis is possible in brassicas with the availability of standard heterosis and efficient hybrid seed production systems such as self-incompatibility and male sterility systems. Sporophytic self-incompatibility (SI) and different male sterility systems such as genetic male sterility (GMS), cytoplasmic genetic male sterility- fertility restoration (CMS-FR) and genetically engineered male sterility systems are available in brassicas. Efforts were also made to identify gametocides which imparts male sterility [26]. Among these, CMS-FR is the most effective pollination control mechanism for the production of hybrid seed in many crop plants including *B. juncea* [27].

Genetic male sterility

Different genetic male sterility sources are available in *B. juncea* [28], *B. campestris* v. brown sarson [29], *B. campestris* v. yellow sarson [30] and *B. campestris* v. toria [31]. Most of them are spontaneous in origin

and exhibit monogenic inheritance. Use of genetic male sterility for hybrid seed production is not economically viable because rouging of fertile plants from the male sterile line plot require additional labour. Till now, any linked seedling markers or pleiotropic effect of the male sterility gene has not been reported for the easy identification of male fertile plants before the initiation of flowering [32].

Genetically engineered male sterility

A *B. napus* GMS called barnase/barstar system has been developed by engineering the chimeric ribonuclease gene which was characterized by its extreme cell specificity in the tapetal cells of immature anthers. The gene product destroys the tapetal cell layer, prevents pollen formation and results in male sterility [33]. It is also possible to restore fertility in genetically engineered male sterile plants by crossing with male plants that were transformed with a chimeric tapetal cell-specific ribonuclease inhibitor gene. The chimeric ribonuclease gene was linked to the phosphinothricin resistance gene, which act as dominant marker for sterility trait [33]. Similar system was developed in *B. juncea* by Jagannath and coworkers in 2002 [34].

Cytoplasmic male sterility-fertility restoration (CMS-FR) system

CMS-FR system has been identified as most potential method for hybrid seed production in brassicas. The male sterility inducing cytoplasm was first discovered in Japanese radish (*Raphanus sativus* L.) by Ogura in 1968 [35]. Later several male sterility systems, both autoplasmic and alloplasmic, have been developed in oilseed brassicas. Autoplasmic CMS are the result of mitochondrial genome mutations in a cultivated species while alloplasmic systems are produced through repeated backcrossing by transferring the nuclear background of a cultivated species into the cytoplasm of a wild species. The different autoplasmic and alloplasmic CMS systems reported in rapeseed-mustard [36] are given in table 1 and table 2**.**

Table 1: Autoplasmic CMS Systems reported in *Brassica* spp.

Code	Species	Restoration Status	Reference
Shiga-Thompson(nap) System	*B. napus*	Available	37 & 38
Polima (pol) system	*B. napus*	Available	39
Mokopo/ Korean system	*B. napus*	Available	40
MS-4 System	*B. juncea*	Available	28
Jun System	*B. juncea*	Available	41
Line-14/campestris System	*B. campestris*	Available	42
681A	*B. napus*	Available	43
hau	*B. juncea* *B. napus*	Available	44 & 45

Table 2: Alloplasmic CMS Systems reported in *Brassica* spp.

Cytoplasm donor	Code	Technique used	Restoration status	Species	References
Raphanus sativus	*ogu*	Inter specific cross, Protoplast fusion	Available	*B. napus* *B. juncea*	46 & 47
Diplotaxix siifolia	*sif*	Intergeneric cross	Not available	*B. juncea*	48
B. tournefortii	*tour*	?	Unstable, genotype specific partial restoration	*B. napus* *B. juncea*	49 & 50
B. oxyrrhina	*oxy*	Interspecific cross, protoplast fusion	Not available	*B. juncea*	51
Trychystoma ballii	*trachy*	Protoplast fusion	Incomplete	*B. juncea*	52
Moricandia arvensis	*mori*	Protoplast fusion	Available	*B. juncea*	53 & 54
D. sietiana	*sie*	Intergeneric cross	Not available	*B. juncea*	55
D. catholica	*cath*	Intergeneric cross	Available	*B. juncea*	55
Enarthrocarpus lyratus	*lyr*	Intergeneric cross	Available	*B. juncea* *B. napus*	56 & 57
Erucstrum canariense	*can*	Intergeneric cross	Available	*B. juncea* *B. napus*	58 & 59
Synthetic *B. napus*ISN-706	126-1	Interspecific cross	Available	*B. juncea*	60
D. erucoides	*Eru*	Intergeneric cross	Available	*B. juncea*	61 & 62
D. berthautii	*ber*	Intergeneric cross	Available	*B. juncea*	61 & 63
Isatis indigotica	Inap CMS	Protoplast fusion	Not available	*B. napus*	64

Among all these CMS systems reported, the commercially exploited systems for hybrid production are *tour*, *ogu, polima,* 126-1 and *mori*. In case of alloplasmic CMS, floral deformities, absence of nectaries,

chlorosis etc. are common drawbacks, however this can be rectified through somatic hybridization. The major limitation in alloplasmic CMS system is the absence of fertility restoring genotypes among euplasmic germplasm and the restorer gene need to be introgressed from the corresponding alien species itself. Among the different sterile cytoplasms *Moricandia arvensis* (*mori*), *Diplotaxis erucoides* (*eru*) & *Diplotaxis berthautii* (*ber*) cytoplasms are proved to be stable and with almost no adverse effects in *B. juncea* backgrounds [65, 66].

Chemical hybridizing agents

In rapeseed-mustard, a few reports on chemically induced male sterility are available. A study by Banga and Labana (1984) [8] showed that spraying 0.25% ethrel, twice before the emergence of the first flowering shoot, induced 90% male sterility in Indian mustard. Guan *et al*. (1990) [67] investigated the extent of male sterility induction in *B. napus* by 17 chemical male gametocides, of which three gametocides; MG1, MG2 and MG3 induced 60-80% male sterility when the gametocides were sprayed at the bud stage. *B. napus* hybrid Shuza No.2 produced by spraying MG1 was released in China. But the major limitations of this system are incomplete male sterility (could be affected by environmental factors and/or the differential developmental stage of pollens), reduced female fertility and environmental hazard imposed by usage of gametocides [68].

Diversification of cytoplasmic male sterility-fertility restoration (CMS-FR) systems

For a sustainable hybrid breeding programme diversification of CMS-FR system is required. Exploitation of a single cytoplasm may lead to disease and insect-pests epidemics [69]. This is evident from past experiences with maize and pearl millet crops. In maize the extensive use of Texas cytoplasm lead to the southern leaf blight epidemics caused by *Bipolaris maydis* race T in USA [70]. In India the pear lmillet hybrids based on Tift $23A_1$ cytoplasm succumbed to downy mildew infection in 1970's [71]. This has led to discovery of diverse cytoplasms in different crop plants such as maize (CMS-T, CMS-C & CMS-S), pearl millet (A_1, A_2, A_3,A_4& A_5), sorghum (A1, A2, A3, A4, A5, A6, 9E and KS) and rice (BT- CMS, HL- CMS, WA-CMS, LD- CMS and CW- CMS) etc. (72, 73, 74, 75, 76, 77). Similarly, in brassicas different alloplasmic and autoplasmic cytosterility sources were identified (table 2.1 and 2.2).

In case of alloplasmic CMS system development of restorer lines, which restore fertility in their respective sterile cytoplasms, is also important for diversaification of the CMS-FR systems. Fertility restorers for different cytoplasm in brassicas are not available in natural polulations, thus, need to be derived from their respective cytoplasm donors. The fertility restorers for different cytoplasm like *mori, ogura, cath, lyr, nap, pol* etc. are now available. Furthermore, a *B. juncea* line carrying fertility restorer (*Rf*) gene from *Moricandia arvensis*, restores fertility in *Diplotaxis catholica*, *Diplotaxis erucoides* and *Diplotaxis berthautii* cytoplasms and the fertility restoration is under gametophytic control [78, 62, 63]. In gametophytic fertility restoration system only *Rf* gene-carrying pollen is functional and F_1 hybrid plants produce 50% fertile and 50% sterile pollens [78]. This provides a great opportunity to diversify the cytosterility sources without investing in looking for the fertility restorer gene.

Rapeseed-Mustard Hybrids Released In India

Availability of effective means of hybrid seed production led to the development of six commercial hybrids in India through All India Coordinated Research Project on Rapeseed-mustard [79] as given below:

Name of hybrid	Developing institution	Year	CMS source
B. juncea			
NRCHB-506	NRCRM, Bharatpur	2008	mori
DMH-1	DUSC, Delhi	2008	126-1
PAC-432	Advanta, India	2010	ogu
PAC-437	Advanta, India	2011	ogu
44S01	Pioneer	2012	ogu
B. napus			
PGSH-51	PAU, Ludhiana	1996	tour

However, the level of yield gain achieved from these hybrids is marginal. As a result, the adoption rates of these hybrids are very less in the country as compared to pure line varieties [4]. Recently, a genetically modified mustard hybrid Dhara Mustard Hybrid 11 (DMH 11) was developed by Professor Deepak Pental and his team, Delhi University. It contains the bar, barnase and barstar gene system and claims to yield 25-30% more than the best standard Varuna in the country. But its commercial release is delayed due to the conflicting results from field trials and safety evaluations [80, 81]

Effects of genetic backgrounds on fertility restoration

Identification of heterotic combinations and then converting them to CMS and restorer lines are important steps in hybrid breeding programmes [14]. For diversification of restorer lines stability of fertility restoration in hybrids developed from A and R lines having different nuclear backgrounds is important. The fertility restoration was governed by two genes in *tournefortii (tour)* cytoplasmic male sterility (CMS) system in rapeseed (*B.napus*). The fertility restoration showed different segregation patterns such as 12:3:1 or 9:3:4 depending upon female parent genotypes and/or modified expression of the restorer gene(s) in different genetic backgrounds [82]. Vinu and coworkers [83] studied the effect of nuclear background on fertility restoration in *B. juncea* using *mori*, *eru* and *ber* cytoplasms and a common fertility restorer line. 108 single cross hybrids, 36 hybrids in each cytoplasm were evaluated and found that the per cent pollen fertility in hybrids was influenced by the genetic backgrounds of parents. However, this effect was not consistent for any nuclear background of parents.

Application of molecular markers in heterosis breeding

Currently molecular markers are considered as highly effective tools for genetic analyses and have applications in all areas of plant breeding. They have been used for predicting the heterosis and tagging or mapping of male sterility/fertility associated genes and heterotic loci. In Brassica many researchers used different molecular markers for assessing genetic diversity and to predict the heterosis from the genetic distance estimates. Becker and Engqvist [84] indicated the usefulness of RAPD-based genetic distance estimates to predict heterosis for leaf dry matter production in *B. napus*. Plieske and Struss [85] by using SSR markers suggested that they provide a reliable and effective means for predicting heterosis in Brassica. Shen *et al*. [86] found a positive association between hybrid seed yield and genetic distance estimated from AFLP in *B. napus*. In rapeseed, as well as in other species, a positive correlation has been found between genetic distances determined by molecular markers and heterosis [87]. A study on Ethiopian mustard (*B. carinata* A. Braun) conducted by Teklewold and Becker (2006) [88] compared the phenotypic distance and molecular distance to predict the heterosis and F_1 performance. Parental distances estimated from phenotypic traits better predicted heterosis, F_1 performance and GCA than distances estimated from RAPD markers. However, correlation between phenotypic and molecular distances was observed to be low. Diers *et al*. [89] using RFLP markers and Riaz *et al*. (2001) [90] using sequence-related amplified polymorphism (SRAP) in *B. napus* reported a strong association of heterosis with marker heterozygosity and recommended their use for predicting heterosis. In contrast to above observations, Knaak and Ecke [91], Girke *et al.* [92] and Yu *et al.* [93] in *B. napus* and Jain *et al*. [24] in *B. juncea* reported a low association of DNA markers with heterosis. Qian *et al*. [94, 95] in their studies, involving parents from different ecotypes, reported low correlation between parental genetic distance and hybrid performance. Similarly no significant association was found between genetic distance and hybrid performance in *B. juncea* after studying the relationship of parental distances estimated from phenotypic traits and SSR markers [96].

Ashutosh and co-workers [97] tested the utility of repetitive sequence based Polymerase Chain Reaction (rep-PCR) technique for distinguishing different CMS lines of *B. juncea*; and also identified a Sequence Characterized Amplified Region (SCAR) marker capable of distinguishing CMS *catholica* from other lines. Cleaved Amplified Polymorphic Sequence (CAPS) markers for the plastid gene psbB have identified for *D. berthautii* [63] & *D. erucoides* [62] cytoplasms, which could be useful for quick identification of these CMS lines. Ashuthosh *et al*. (2007) [98] used BC_1F_1 population and identified two AFLP and one SCAR markers linked to the male fertility restorer gene, derived from *Moricandia arvensis* in *B. juncea* nuclear background, with a map distance ranging from 0.6 to 2.9cM. Both the AFLP & SCAR markers are located on one side of the *Rf* locus. Being the dominant markers application of these markers in MAS is limited because they could not differentiate heterozygous and homozygous dominant plants in the breeding populations. This *Rf* gene from *M. arvensis* can restore the fertility of *D. berthautii* & *D. erucoides* CMS systems as well. Recently Bisht *et al*. [99] identified 13 SSRs and one STS marker flanking the *Rf* gene and confirmed its location on A09 linkage group of *B. juncea*. The two closest flanking markers reported, BjESSR06 and BjEST01 were at 0.6 and 1.4 cM apart from the *Rf* locus and can be used for maker assisted selection (MAS). Since the SSR markers are codominant and reproducible in nature they are capable of differentiating the dominant homozygous plants from the heterozygous plants, thus, highly suitable for converting maintainers into restorers through backcross breeding. Wei and coworkers [100] identified restorer lines for hau CMS in *B. napus* by extensive test crossing and mapped the restorer gene *Rfh* to a 94 kb candidate region on chromosome A03.

Advances in genomic studies can lead to better understanding of the molecular mechanisms of heterosis. Transcriptome analysis of interspecific hybrid between *B. napus* and *B. rapa* revealed the coexistence of multiple gene actions in the hybrid and delivered a list of candidate genes and pathways for heterosis

[101]. Genetic analysis of heterosis in rapeseed at the QTL level detected a total of 33 QTLs for four traits and concluded that epistasis together with all levels of dominance from partial to overdominance is responsible for the expression of heterosis in rapeseed [102].

EPILOGUE

Few hybrids released in India are not adopted in a large scale because of their marginal yield improvement over the ruling pureline varieties. Many improved pollination control systems such as *mori*, *eru*, *ber* etc. are available which aid the large scale hybrid seed production. Currently the main challenge in Brassica hybrid breeding is the identification of superior heterotic combinations. Advances in molecular genetics and genomic studies are leading to the better understanding of the molecular mechanisms of heterosis and identification of heterotic QTLs. This may open a way forward to screen a large number of parental combinations to identify commercially viable heterotic combinations in cost effective manner within a short period of time.

REFERENCES

1. Vinu, V., Singh, N., Sujata, V., Yadava, D. K., Kumar, S., Sugandh, N., Bhat, S.R. & Prabhu, K.V. (2013). Assessment of genetic diversity in *Brassica juncea* (*Brassicaceae*) genotypes using phenotypic differences and SSR markers. *Rev. biol.*, **61** (**4**): 1919-1934.
2. Singh, D. and Mehta, T.R. (1954). Studies in breeding of brown sarson I. comparison of F_1's and their parents. *Indian J. Genet. Plant Breed.*, **14**: 74-77.
3. Fu, T. D. and Yang, G. S. (1998). Rapeseed and Mustard. In: Hybrid Cultivar Development [Banga SS, Banga SK (eds)]. Narosa Publishing House, New Delhi, pp. 403–430.
4. Yadava, D. K. Vasudev, S., Singh, N., Mohapatra, T. and Prabhu, K. V. (2012). Breeding Major Oil Crops: Present Status and Future Research Needs. In: Technological innovations in major world oil crops, volume 1 Breeding [Gupta S.K. (Ed.)]. Springer ISBN: 978-1-4614-0355-5.
5. Labana, K.S., Banga, S.S. and Banga, S.K. (1992). Breeding Oilseed *Brassica*. Narosa Publishing House, New Delhi. pp.251.
6. Abraham, V. (1994). Rate of outcrossing in Indian mustard. *Cruciferae Newsletter*, **16**: 69-70.
7. Rakow, G. and Woods, D. L. (1987). Outcrossing in rape and mustard under Saskatchewan prairie conditions. *Cannadian J. Plant Sci.*, **67**: 141-151.
8. Banga, S. S. and Labana, K. S. (1984). Heterosis in Indian mustard. *Zeitscriftfiir Pflanzenjiichtung*, **92**: 61-70.
9. Verma, O.P., Singh, H. P. and Singh, V. P. (1998). Heterosis and inbreeding depression in Indian mustard. *Cruciferae Newsletter*, **20**: 75-76.
10. Vaghela, P. O., Thakkar, D. A., Bhadauria, H. S., Sutariya, D. A., Parmar, S. K. and Prajapati, D.V. (2011). Heterosis and combining ability for yield and its component traits in Indian mustard [*Brassica juncea*(L.)]. *J. oilseed Brassica*, **2**(**1**): 39-43.
11. Thakur, H. L. and Sagwal, J. C. (1997). Heterosis and combining ability in rapeseed *B. napus*. *Indian J. Genet. Plant Breed.*, **57**: 163-167.
12. Yadava, D. K., Singh, N., Vasudev, S., Singh, R., Singh, S., Giri, S. C., Dwivedi, V. K. and Prabhu, K. V. (2012). Combining Ability and Heterobeltiosis for Yield and Yield-Contributing Traits in Indian mustard (*Brassica juncea*). *Indian J. Agril. Sci.*, **82**(**7**): 563–567.
13. Verma, O.P., Yadav, R., Kumar, K., Singh, R., Maurya, K.N. and Ranjana. (2011). Combining ability and heterosis for seed yield and its components in Indian mustard (*Brassica juncea*). *Plant Archives*, **11**: 863–865.
14. Singh, N., Yadava, D. K., Sujata, V., Singh, R., Giri, S.C, Dass, B., Barun, S. and Prabhu, K. V. (2015). Combining ability and heterobeltiosis for yield and yield contributing traits in high quality oil Indian mustard (*Brassica juncea*) genotypes. *Indian J. Agrl. Sci.* **85** (**4**): 498–503.
15. Gehringer, A., Snowdon,R., Spiller, T., Basunanda, P. and Friedt, W. (2007). New oilseed Rape (*Brassica napus*) hybrids with high levels of heterosis for seed yield under nutrient-poor conditions. *Breed. Sci.*, **57**: 315–320.
16. Zang, S. F. (1991). Studies on the hybrid vigour and its physiological characters of *cms* in *Brassica napus*. *Annual Report on Rapeseed Research*, Huazong Agricultural University. Pp 66-68.
17. Sernyk, J.L. and Stefansson, B.R. (1983). Heterosis in summer rape (*Brassica napus* L.). *Cannadian J. Plant Sci.*, **63**: 407-413.
18. Fu, T. D, Yang, G. S. and Yang, X. (1990). Studies on "three line" Polima cytoplasmic male sterility developed in *Brassica napus. Plant Breed.,* **104**: 115–120.
19. Szała, L., Kaczmarek, Z., Popławska, W., Liersch, A., Wojtowicz, M., Matuszczak, M., Bilinski, Z.R., Sosnowska, K., Stefanowicz, M. and Cegielska-Taras, T. (2019). Estimation of seed yield in oilseed rape to identify the potential of semi- resynthesized parents for the development of new hybrid cultivars. *PLos One* |https://doi.org/10.1371/ journal.pone.0215661
20. Wei, Z., Wang, M., Chang, S., Wu, C., Liu, P., Meng, J. and Zou, J. (2016). Introgressing Subgenome Components from *Brassica rapa* and *B. Carinata* to *B. Juncea* for Broadening Its Genetic Base and Exploring Inter subgenomic Heterosis. *Fron. Plant Sci.,* doi: 10.3389/fpls.2016.01677
21. Rahman, H., Bennett, R. A. and Yang, R. C. (2016). Patterns of Heterosis in Three Distinct Inbred Populations of Spring *Brassica napus* Canola. *Crop Sci*., **56**:2536–2545.

22. Moll, R. H., Salhuana, W. S. and Robinson, H. F. (1962). Heterosis and genetic diversity in variety crosses of maize. *Crop Sci.*, **2**: 197–198.
23. Lefort-Buson, M., Guillot-Lemonie, B. and Bartkowiak-Broda, S. A study of rapeseed (*B. napus*) F1 hybrids, from lines of same and different geographic origins. (1987). Proceedings of 7th International Rapeseed Conference, Poznan, **1**: 63-68.
24. Jain, A., Bhatia, S., Banga, S. S., Prakash, S. and Laxmimurukan, M. (1994). Potential use of Random Amplified Polymorphic DNA (RAPD) technique to study genetic diversity in Indian mustard (*Brassica juncea*) and its relatedness to heterosis. *Theor. Appl. Genet.*, **88**: 116-122.
25. Banga, S. S. and Raman, R. (1998). Biotechnology and Hybrid Breeding. In: Hybrid Cultivar Development [Banga SS, Banga SK (eds)]. Narosa Publishing House, New Delhi, pp. 181-200.
26. Banga, S. S. and Labana, K. S.(1984b). Ethrel induced male sterility in Indian mustard. Zeitscriftfiir *Pflanzenjiichtung*, **92**: 229-233.
27. Yamagishi, H. and Bhat, S. R. (2014). Cytoplasmic male sterility in *Brassicaceae* crops. *Breeding Sci.*, **64**(**1**): 38–47.
28. Banga, S. S. and Labana, K. S. (1985). Male sterility in Indian mustard IV. Genetics of MS4. *Can. J. Genet. Cytol.*, **27**: 487-490.
29. Katiyar, R. K. (1983). Male sterility in turnip rape. *Current Sci.*, **52**(**22**): 1067-1068.
30. Gupta, S.K., Sharma, T.R., Singh, H. and Singh, H.(1997). Structural male sterility in *Brassica rapa*. *Cruciferae Newsletter*, **19**: 75-76.
31. Anonymous. (1997). Promotion of research and development efforts on hybrids in rapeseed mustard. Annual Report 1995-96. National Research Centre on Rapeseed-mustard, Bharatpur-321 -303, India.
32. Gupta, S. K., Rai, K. N., Govindaraj, M. and Rao, A. S. (2012). Genetics of Fertility Restoration of the A_4 Cytoplasmic Nuclear Male Sterility System in Pearl Millet. *Czech J. Genet. Plant Breed.*, **48** (**2**): 87–92.
33. Mariani, C., Beuckeleer, M. D., Truettner, J., Leemans, J. and Goldberg, B. 1990. Induction of male sterility in plants by a chimeric ribonuclear gene. *Nature*, **347**: 737-741.
34. Jagannath, A., Arumugam, N., Gupta, V., Pradhan, A., Burma, P. K. and Pental, D. 2002. Development of transgenic barstar lines and identification of a male sterile (barnase)/restorer (barstar) combination for heterosis breeding in Indian oilseed mustard (*Brassica juncea*). *Current Sci.*, (**82)**: 1- 10.
35. Ogura, H. (1968). Studies on new male sterility in Japanese radish with special reference to utilization of this sterility towards the practical raising of hybrid seeds. *Memoirs of Faculty of Agriculture Kagawa University*, **6**:39-78.
36. Prakash,S., Bhat, S.R. and Fu, T. (2009). Wild germplasm and male sterility. In: Biology and breeding of crucifers [S. K. Gupta (ed.)], CRC Press, pp. 113–127.
37. Thompson, K.F. (1972). Cytoplasmic male sterility in oil-seed rape. *Heredity*, **29**: 253-257.
38. Shiga, T. and Baba, S. (1973). Cytoplasmic male sterility in oilseed rape (*Brassica napus*) and its utilization to breeding. *Japan J. Breed.*, **23:** 187-193.
39. Fu, T. D. (1981). Production and research on rapeseed in the Peoples Republic in China. *Cruciferae Newsletter*, **6**: 6–7.
40. Lee, J.I., Toshic, S. and Know, B. S. (1976). Studies on heterosis breeding in rapeseed using cytoplasmic male sterility. 1. Heterosis of agronomic characters and fertility restoration in F_1 hybrids using CMS. *Korean J. Breed.*, **8**: 63-70.
41. Shi, H.Q., Gong, R.F., Zhuan, L. L. and Long, X. P. (1991). Studies on the utilization of heterosis in mustard (*Brassica juncea*), *ActaAgron. Sin.*,**17**: 32-41.
42. Ohkawa, Y. (1985). New cytoplasmic male sterility in *Brassica campestris ssp. rapifera* (Metzg and Sinsk) and its relationship to cytoplasmic male sterility in *Brassica napus* L. *Bull. Nat. agric. Sci. Series D*, **36**: 1-50.
43. Liu,Z., C.Guan, F.Zhao and S.Chen. (2005). Inheritance and mapping of a restorer gene for the rapeseed cytoplasmic male sterile line 681A. *Plant Breed*., **124**: 5–8.
44. Wan, Z., Jing, B., Tu, J., Ma, C., Shen, J., Yi, B., Wen, J., Huang, T., Wang, X. and Fu, T. 2008. Genetic characterization of a new cytoplasmic male sterility system (hau) in *Brassica juncea* and its transfer to *B. napus*. *Theor. Appl. Genet*. **116**: 355–362.
45. Wei, C., Wang, H., Heng, S., Wen, J., Yi, B., Ma, C., Tu, J., Shen, J. and Fu, T. 2019. Construction of restorer lines and molecular mapping for restorer gene of hau cytoplasmic male sterility in *Brassica napus*.*Theor. Appl. Genet*. **132**(**9**):2525-2539. doi: 10.1007/s00122-019-03368-3.
46. Bannerot, H., Boulidard, L., Cauderon, Y. and Temp, J. Transfer of cytoplasmic male sterility from *Raphanus sativus* to *Brassica oleracea*. 1974. Eucarpia Meeting Cruciferae, Dundee, Scotland, pp. 52– 54.
47. Kirti, P. B., Banga, S. S., Prakash, S. and Chopra, V. L. 1995a. Transfer of Ogu cytoplasmic male sterility to *Brassica juncea* and improvement of male sterile through somatic cell fusion. *Theor. Appl. Genet.*, **91**: 517–521.
48. Rao, G. U., Batra, V. S., Prakash, S. and Shivanna, K. R. 1994. Development of new cytoplasmic male sterile system in *Brassica juncea* through wide hybridization. *Plant Breed.*, **112**: 171-174.
49. Banga S. S., Banga S. K. and Sandha G. S. 1995. Development and characterization of *tournefortii* CMS system in *Brassica napus L*. Proc. 9th Intl Rapeseed congress, Cambridge, UK, **1**: 55-57.
50. Rawat, D.S. and Anand, I.J. 1979. Male sterility in Indian mustard. *Indian J. Genet. Plant Breed.*, **39**: 412-414.
51. Prakash, S. and Chopra, V. L. 1990. Male sterility caused by cytoplasm of *Brassica oxyrrhina* in *B. campestris* and *B. juncea*. *Theor. Appl. Genet.*, **79**: 285–287.

52. Kirti, P. B., Mohapatra, T., Prakash, S. and Chopra, V. L. (1995b). Development of a stable cytoplasmic male sterile line of *Brassica juncea* from somatic hybrid *Trachystoma ballii* + *Brassica juncea*. *Plant Breed.*, **114**: 434–438.
53. Prakash, S., Kirti, P. B., Bhat, S. R., Gaikwad, K., Kumar, V.D. and Chopra, V.L. (1998). *A Moricandia arvensis* based cytoplasmic male sterility and fertility restoration system in *Brassica juncea*. *Theor. Appl. Genet.*, **97**: 488–492.
54. Kirti, P. B., Prakash, S., Gaikwad, K., Bhat, S. R., Dinesh kumar, V. and Chopra, V. L. (1998). Chloroplast substitution overcomes leaf chlorosis in a *Moricandia arvensis* -based cytoplasmic male sterile *Brassica juncea*. *Theor. Appl. Genet.*, **97**: 1179–1182.
55. Prakash S. (2001a). Utilization of wild germplasm of Brassica allies in developing cytoplasmic male sterility-fertility restoration systems in Indian mustard *Brassica juncea*. Proceedings of International Symposium of Rapeseed Science, Science Press, New York, pp 63-68.
56. Banga, S. S., Deol, J. S. and Banga, S. K. 2003. Alloplasmic male-sterile *Brassica juncea* with *Enarthrocarpus lyratus* cytoplasm and the introgression of gene(s) for fertility restoration from cytoplasm donor species. *Theor. Appl. Genet.*, **106**: 1390–1395.
57. Janeja H. S., Banga S. K., Bhasker P. B. and Banga S. S. (2003). Alloplasmic male sterile *Brassica napus* with *Enarthrocarpus lyratus* cytoplasm: introgression and molecular mapping of an *E*. *lyratus* chromosome segment carrying a fertility restoring gene. *Genome*, **46**: 792-797.
58. Prakash, S., Ahuja, I., Uprety, H. C., Kumar, V. D., Bhat, S. R., Kirti, P. B. and Chopra, V. L. 2001. Expression of male sterility in alloplasmic *Brassica juncea* with *Erucastrum canariense* cytoplasm and the development of a fertility restoration system. *Plant Breed.*, **120**: 479–482.
59. Banga, S. S., Banga, S. K., Bhaskar, P. B., Ahuja, I. and Payal, B. (2003). Alloplasmic line of *Brassica napus* L. with *Erucastrum canariense* cytoplasm is male sterile. Proceedings of GCIRC 11th International Rapeseed Congress, **1**: 324-325.
60. Sodhi, Y. S. A., Chandra, A., Verm, J. K., Arumugam, N., Mukhopadhyay, A., Gupta, V., Pental, D. and Pradhan, A. K. (2006). A new cytoplasmic male sterility system for hybrid seed production in Indian oilseed mustard *Brassica juncea*. *Theor. Appl. Genet.*, **114**: 93–99.
61. Malik, M., Vyas, P., Rangaswamy, N. S. and Shivanna, K. R. (1999). Development of two new cytoplasmic male sterile lines of *Brassica juncea* through wide hybridization. *Plant Breed.*, **118**: 75–78.
62. Bhat, S. R., Vijayan, P., Ashutosh., Dwivedi, K. K. and Prakash, S. (2006). *Diplotaxis erucoides*-induced cytoplasmic male sterility in *Brassica juncea* is rescued by the *Moricandia arvensis* restorer: genetic and molecular analyses. *Plant Breed.*, **125**: 150-155.
63. Bhat, S.R., Kumar, P. and Prakash, S. (2008). An improved cytoplasmic male sterile (*Diplotaxis berthautii*) *Brassica juncea*: identification of restorer and molecular characterization. *Euphytica*, **159**: 145–152.
64. Kang, L., Li, P., Wang, A., Ge, X. and Li, Z. (2017). A Novel Cytoplasmic Male Sterility in *Brassica napus* (inap CMS) with Carpelloid Stamens via Protoplast Fusion with Chinese Woad. *Fron. Plant Sci*., doi: 10.3389/fpls.2017.00529
65. Kaur G., Banga S. K., Gogna, K. P. S., Joshi, S. and Banga, S. S. (2004). *Moricandia arvensis* cytoplasm based system of cytoplasmic male sterility in *Brassica juncea*: reappraisal of fertility restoration and agronomic potential. *Euphytica*, **138**: 271—276.
66. Chamola, R., Balyan H. S. and Bhat S. R. (2013). Effect of alien cytoplasm and fertility restorer genes on agronomic and physiological traits of *Brassica juncea* (L.) Czern. *Plant Breed.*, **132**: 681–687.
67. Guan, C. Y., Wang, G. H. and Li, S. (1990). Studies on chemical male gametocide mechanism and hybrids of rapeseed. *Crop Res.*, **4**: 13-19.
68. Mahajan, V. and Nagarajan, S. (1998). Opportunities in Hybrid Wheat- A Review. *Proc. Indian Natl. Sci. Acad.*, **64**(**1**): 51-58.
69. Gupta, S. K., Pratap., A., Gupta, D. S., Rajan, N. and Ganeshan, V. D. (2012). Hybrid technology. In: Technological innovations in major World Oil crops, volume 2: Perspectives [Gupta S.K. (Eds)]. Springer-Verlag, New York, pp 1-21.
70. Scheifele, G.L., Whitehead, W. and Rowe, C. (1970). Increased susceptibility of southern leaf spot (*Helminthosporiummaydis*) in inbred lines and hybrids of maize with Texas male sterile cytoplasm. *Plant Dis. Rep.*, **454**: 501–503.
71. Amiribehzadi, A. and Tara Satyavathi,C. (2012). Fertility restoration studies in different cytoplasms of pearl millet [*Pennisetumglaucum*(L.) R. BR.]. *Ann. Agric. Res. News Ser.*, **33** (**3**): 136-142.
72. Pring, D., Levings, C., Hu, W. and Timothy, D. (1977). Unique DNA associated with mitochondrial in the S type cytoplasm of male-sterile maize. *Proc.Indian Natl. Sci. Acad.*, **74**: 2904–2908.
73. Sofi, P. A., Rather, A.G. and Wani, S. A. (2007). Genetic and molecular basis of cytoplasmic male sterility in maize. *Communications in Biometry and Crop Science*, **2**(**1**): 49–60.
74. Burton, G.W. and Athwal, D.S. (1967). Two additional sources of cytoplasmic male sterility in pearl millet and their relationship to Tift 23A. *Crop Sci.e*, **7**: 209-211.
75. Hanna, W.W. (1989). Characteristics and stability of a new cytoplasmic nuclear male-sterile source in pearl millet. *Crop Sci.*, **29**:1457-1459.
76. Rai, K.N. (1995). A new cytoplasmic-nuclear male sterility system in pearl millet. *PlantBreed.*, 114: 445-447.
77. Ahmed, M.I. and Siddiq, E.A. (1998). Rice. In: Hybrid Cultivar Development [Banga SS, Banga SK (eds)]. Narosa Publishing House, New Delhi, pp. 221–256.

78. Bhat, S.R., Prakash, S.Kirti,P. B., Dineshkumar, V.and Chopra, V.L. (2005). A unique introgression from *Moricandiaarvensis*confersmale fertility upon two different cytoplasmic male-sterile lines of*Brassica juncea*. *Plant Breed.*,**124**: 117–120.
79. Kumar, A., Kumar, V. and Kandpal, B. K. Production barriers and technological options for sustainable production of rapeseed mustard in India. (2012). In:Souviner 1stNational Brassica Conference onProduction barriers and technological options in oilseed Brassica organizedby Society for Rapeseed Mustard Research, Bharatpur.
80. https://indianexpress.com/article/explained/gm-row-again-with-mustard-topping/
81. Pahwa, R. S., Banga, S. K., Gogna, K. P. S. and Banga, S. S. (2004). *Tournefortii* male sterile system in *Brassica napus*. Identification, expression and genetic characterization of male sterile restorers. *Plant Breed.*, **123**: 444–448.
82. Vinu, V, Singh, N., Yadava, D. K., Sujata, V., Saini, N., Bhat S. R. and Prabhu, K. V. (2017). Effect of cytoplasms and genetic backgrounds of parental lines on fertility restoration in *Brassica juncea* hybrids. *Indian J. Agrl. Sci*., **87** (**2**): 197–202.
83. Becker, H. C. and Engqvist, G. M. (1995). The potential of resynthesized rapeseed for hybrid breeding. In: Proceedings of 9th International Rapeseed Conference, Cambridge, UK 4–7 July 1995. Pp. 113–115.
84. Plieske, J. and Struss, D. (2001).Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in *Brassicaceae* species. *Theor. Appl. Genet*. **102**: 689–694.
85. Shen, J., Lu, G., Fu, T. and Yang, G. (2003). Relationship between heterosis and genetic distance based on AFLPs in *Brassica napus*. In: Proceedings of 11th International Rapeseed Conference, Copenhagen, Denmark, 6–10 July 2003, pp 343–346.
86. Shen, J., Lu, G., Fu, T. and Yang, G. (2004). Relationship between heterosis and genetic distance based AFLPs in *Brassica napus*. In: Proc. Of 11th International Rapeseed Congress, 6-10 July, Copenhagen, Denmark, pp. 343-346.
87. Teklewold, A. and Becker, H.C. (2006). Geographic pattern of genetic diversity among 43 Ethiopian mustard (*Brassica carinata* A. Braun) accessions as revealed by RAPD analysis. *Genet. Resour. Crop. Evol*. **53**: 1173-1185.
88. Diers, B.W., McVetty, P.B.E. and Osborn, T.C. (1996). Relationship between heterosis and genetic distance based on Restriction Fragment Length Polymorphism markers in oilseed rape (*Brassica napus* L.). *Ann Bot.***36**: 79–83.
89. Riaz, A., Li, G., Quresh, Z., Swati, M.S. and Quiros, C.F. (2001). Genetic diversity of oilseed *Brassica napus* inbred lines based on Sequence-Related Amplified Polymorphism and its relation to hybrid performance. *Plant Breed*.**120**: 411–415.
90. Knaak, C. and Ecke, W. 1995. Genetic diversity and F_1 performance in European winter oilseed rape (*Brassica napus* L.). In: Proceedings of 9th International Rapeseed Conference, Cambridge. **4–7**: 110–112.
91. Girke, A., Becker, H.C. and Engqvist, G.M. (2001). Predicting heterosis from genetic distances for RFLP markers in resynthesized oilseed rape. In: Quantitative genetics and breeding methods: the way ahead. Proceedings of the 11th Meeting of the Section Biometrics in Plant Breeding, Paris, France, 30.8. – 1.9.2000, pp 257–262.
92. Yu, C.Y., Hu, S.W., Zhao, H.X. and Guo, A.G. (2005). Genetic distances revealed by morphological characters, isozymes, protein and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). *Theor. Appl. Genet*. **110**: 511–518.
93. Qian, W., Sass, O., Meng, J., Li, M., Frauen, M. and Jung, C. (2007). I. Heterotic patterns in rapeseed (*Brassica napus* L.): Crosses between spring and Chinese semi-winter lines. *Theor. Appl. Genet*. **115**: 27–34.
94. Qian, W., Li, Q., Noack, J., Sass, O., Meng, J., Frauen, M. and Jung, C. (2009). Heterotic patterns in rapeseed (*Brassica napus* L.): II. Crosses between European winter and Chinese semi-winter lines. *Plant Breed*. **128**: 466-470.
95. Vinu, V., Singh, N., Sujata, V., giri, S.C., Singh, R., Dass, B. and Yadava. D.K. (2016). Relationship of molecular and phenotypic divergence with hybrid performance in Indian mustard (*Brassica juncea*). *Indian. J. Agrl. Sci.,* **86** (**9**): 1158–63.
96. Ashutosh., Dwivedia, K.K., Kumar, V. D., Prakash, S. and Bhat, S.R. (2005). rep- PCR helps to distinguish different alloplasmic cytoplasmic male sterile lines *of Brassica juncea. Plant Sci.*, **168**: 1083–1087.
97. Ashutosh., Sharma, P.C., Prakash, S. and Bhat, S.R. (2007). Identification of AFLP markers linked to the male fertility restorer gene of CMS (*Moricandia arvensis*) *Brassica juncea* and conversion to SCAR marker. *Theor. Appl. Genet*., **114**: 385–392.
98. Bisht, D. S., Chamola, R., Nath, M. and Bhat, S. R. (2015). Molecular mapping of fertility restorer geneof an alloplasmic CMS system in *Brassica juncea* containing *Moricandia arvensis* cytoplasm. *Mol. Breeding.*, **35**:14.
99. Wei, C., Wang, H., Heng, S., Wen, J., Yi, B., Ma C., Tu, J., Shen, J. and Fu, T. (2019). Construction of restorer lines and molecular mapping for restorer gene of hau cytoplasmic male sterility in Brassica napus. *Theor. Appl. Genet.,* 132, 2525–2539. https://doi.org/10.1007/s00122-019-03368-3
100. Zhang, J., Li, G., Li, H., Pu, X., Jiang, J., Chai, L., Zheng, B., Cui, C., Yang,Z., Zhu, Y. and Jiang, L. (2015). Transcriptome Analysis of Interspecific Hybrid between *Brassica napus* and *B. rapa* Reveals Heterosis for Oil Rape Improvement. *Int. J. Genomics*., http://dx.doi.org/10.1155/2015/230985
101. Radoev, M., Becker, H. C. and Ecke, W. (2008). Genetic Analysis of Heterosis for Yield and Yield Components in Rapeseed (*Brassica napus* L.) by Quantitative Trait Locus Mapping. *Genetics*, 1**79**: 1547–1558.

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