



REVIEW ARTICLE

Harnessing heterosis in *Brassica juncea* (L.) Czern. & Coss.: Challenges in commercial hybrid production and future strategies

Chandan Kishore^{1*}, Khushboo Chandra¹, Shashikant², Ashutosh Kumar¹, Somnath Bhattacharyya³ & Prabir Kumar Bhattacharyya³

¹Department of Genetics and Plant Breeding, Bihar Agricultural University, Sabour, Bhagalpur 813 210, Bihar, India

²Department of Plant Physiology and Biochemistry, Bihar Agricultural University, Sabour, Bhagalpur 813 210, Bihar, India

³Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur 741 252, West Bengal, India

*Correspondence email - chandanpbg@gmail.com

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Abstract

India relies on imports to meet nearly 70 % of its edible oil demand, underscoring an urgent need to improve domestic oilseed productivity. Rapeseed-mustard (*Brassica juncea* (L.) Czern. & Cosson), the second-largest oilseed crop in India, has experienced a yield plateau due to the exhaustion of genetic variability in conventionally bred varieties. Heterosis breeding through hybrid development offers the most promising strategy to break this stagnation, yet commercialisation remains critically dependent on efficient pollination control systems, primarily cytoplasmic male sterility (CMS). A key challenge is the “biological penalty” imposed during CMS background conversion, wherein the combining ability and heterotic potential of superior genotypes may not remain intact, necessitating rigorous re-evaluation of converted lines. Public-sector brassica hybrids have largely failed to exceed the commercial heterosis threshold of 25–35 %. This review examines the fundamental requirements for hybrid development, the historical progression of breeding systems and the current status of major CMS systems (*Ogura*, *Moricaandia*, *Tournefortii* and others) deployed in India, along with the challenges hindering adoption. The regulatory approval of the genetically engineered hybrid DMH-11-utilising the Barnase-Barstar system marks a significant milestone, offering stable and precise pollination control independent of alien cytoplasm constraints with a demonstrated 28–37 % yield advantage. Future strategies must focus on broadening the genetic base through alien introgression, developing defined heterotic pools and integrating genomic selection, gene editing and marker-assisted selection (MAS) with conventional breeding to achieve self-sufficiency in edible oil production.

Keywords: *Brassica juncea*; cytoplasmic male sterility; hybrid; heterosis; barnase-barstar

Introduction

Rapeseed and mustard are major sources of oil and meal in the world. It comprises eight cultivated crops of the tribe *Brassicaceae* within the family *Cruciferae* (*Brassicaceae*). Traditionally grown species comprise Indian mustard [*Brassica juncea* (L.) Czern & Cosson, 2n (AABB) = 36], yellow sarson [*Brassica campestris* syn. *B. rapa* L. var. yellow sarson, 2n (AA) = 20], toria [*Brassica campestris* syn. *B. rapa* L. var. toria, 2n (AA) = 20], brown sarson [*Brassica campestris* syn. *B. rapa* L. var. brown sarson, 2n (AA) = 20], black mustard [*Brassica nigra* (L.) Koch, 2n (BB) = 16] and taramira [*Eruca sativa*/ *Vesicaria* Mill., 2n (EE) = 22]. Brown sarson has two ecotypes Lotni type (self-incompatible) and Tora type (self-compatible). In addition, three species are grown as non-traditional species viz. Gobhi sarson [*B. napus* L. ssp. *oleifera* DC var. *annua* L., 2n (AACC) = 38], white mustard [*Sinapis alba* L. 2n (SS) = 24] and Ethiopian mustard or Karan rai [*B. carinata* A. Braun, 2n (BBCC) = 34]. Indian mustard (*B. juncea*) is the most predominant oilseed crop among the rapeseed-mustard group and accounts for more than 90 % of the area grown in marginal or sub-marginal land either as pure crop or intercrop (1).

Current scenario of rapeseed-mustard production in India

Despite its importance, India faces a significant deficit in edible oil production. The annual domestic requirement is approximately 23–24 million tonnes (MT), while production hovers around 10–12 MT. This gap forces the nation to import 13–15 MT of edible oil annually, incurring a colossal cost of over INR 1.5 trillion (US\$18 billion) (2). Rapeseed-mustard is the second-largest contributor (~27 %) to India's total oilseed production after soybean (~34 %). However, the productivity of rapeseed-mustard in India (approximately 1320 kg/ha) lags significantly behind global leaders like Germany (~4500 kg/ha), highlighting a substantial yield gap and the immense potential for improvement through advanced breeding interventions illustrated in Fig. 1 (3).

Major production constraints in rapeseed and mustard

Preference for other staple crops: Small and marginal farmers in India primarily prioritise staple food crops like rice, wheat and maize to ensure food security for their households. As a result, mustard is often pushed to marginal or residual lands with limited inputs, reducing its production potential.

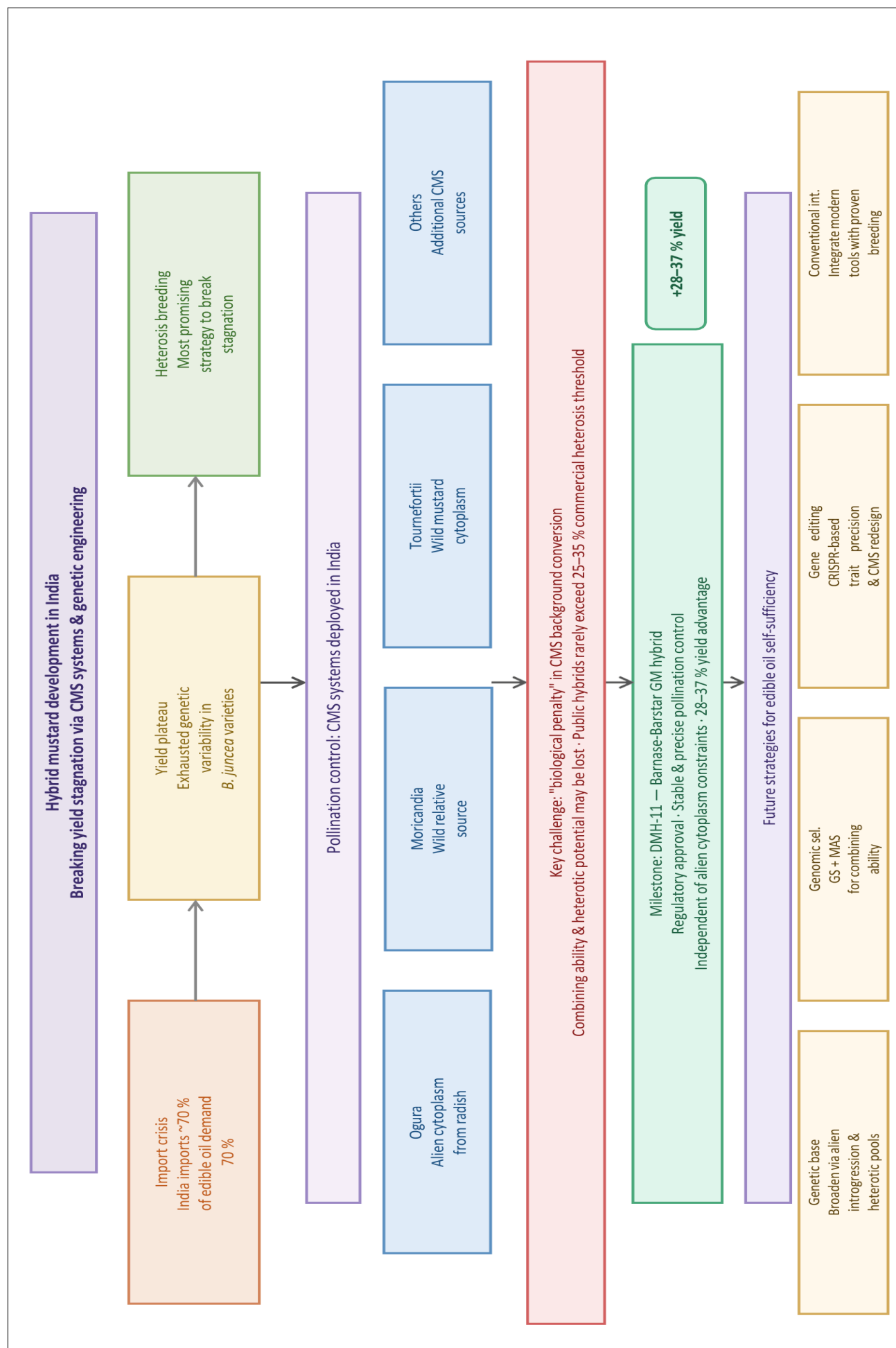


Fig. 1. Hybrid mustard development in India breaking yield stagnation via cytoplasmic male sterility systems and genetic engineering.

Inadequate availability of quality seeds: Farmers frequently suffer from poor access to certified and high-yielding mustard varieties. Limited seed production, distribution inefficiencies and lack of awareness often lead to use of farm-saved seeds, resulting in reduced yield and quality.

Low seed replacement rate: The low seed replacement rate (SRR) for mustard in India remains around 31 %, which is significantly lower than recommended levels. Continued use of old and genetically inferior seeds reduces crop performance and makes the crop more susceptible to biotic and abiotic stresses.

Lower profitability compared to competitive crops: Rapeseed and mustard generally offer lower economic returns compared to high-value rabi crops such as hybrid maize or vegetables. This discourages farmers from allocating prime land and adequate inputs to mustard cultivation, ultimately affecting overall productivity.

Slow adoption of improved technologies: Many farmers are still unaware or hesitant to adopt modern technologies, including improved varieties, advanced crop management practices and integrated pest management strategies. This technological gap keeps productivity stagnant.

Imbalanced fertiliser application: Excessive focus on nitrogenous fertilisers and neglect of secondary (sulphur (S)) and micronutrients (boron (B), zinc (Zn)) leads to nutrient deficiencies and poor plant health. As mustard has a high S requirement for oil synthesis, this imbalance significantly affects yield and oil quality.

Delayed sowing due to late rice harvesting: In states like Bihar, Jharkhand and Uttar Pradesh, mustard sowing is often delayed as the land remains occupied by the kharif rice crop until late in the season. Late sowing exposes mustard to terminal heat stress and aphid infestation, drastically reducing productivity.

Adverse and changing climatic conditions: Shorter winter periods and frequent warm spells during the flowering and pod formation stages negatively impact pollination, seed setting and oil accumulation. Unpredictable weather patterns associated with climate change pose a growing challenge to mustard cultivation.

Severe pest and disease pressure: Mustard is highly vulnerable to diseases like alternaria blight, stem rot and white rust and pests like aphids. High disease incidence and pest outbreaks lead to significant yield losses, especially in regions with limited access to plant protection measures.

Limited mechanisation in mustard farming: In many mustard-growing regions, sowing is still practiced using broadcasting methods and harvesting is done manually due to lack of appropriate machinery. This increases labour cost, reduces uniformity in plant stand and delays harvest operations.

Why are hybrids needed?

The persistent yield plateau in conventionally bred varieties of *B. juncea* is a major concern. This stagnation is largely attributed to the exhaustion of useful genetic variability within the cultivated gene pool. Heterosis breeding or the development of hybrids, offers the most promising strategy to break this yield barrier. The commercial impact of oilseed hybrids in India remains minimal. Their share is a meager ~4 % of the total hybrid seed market, generating revenue of only about ₹225 crores, in stark contrast to cotton hybrids, which command a 35 % share and generate ₹3650 crores (4). Within the oilseed sector, rapeseed-mustard hybrids

contribute 31 % to this small revenue. This low adoption underscores the challenges and the untapped potential in mustard hybrid technology. Cultivation of hybrids can boost yield significantly due to heterosis, while also incorporating inherent resistance to biotic (diseases, pests) and abiotic (drought, heat) stresses, alongside improved oil quality (Fig. 2). The recent upward trend in area (17 %), production (31 %) and productivity (13 %) of mustard from 2019–22 can be further accelerated by widespread adoption of high-yielding hybrids, moving India closer to self-sufficiency in edible oils (5). However, several challenges have hindered the widespread popularity of hybrids in *Brassica*. A significant issue is the negative effect of sterile cytoplasm, which in some hybrids like DMH-1 and NRCHB-506, results in the production of small seeds (half test weight i.e., ~3.5 g compared to ~7 g in varieties like RH-725). This leads to a perception issue, as farmers and brokers in the market disproportionately prefer bolder seeds, affecting adoption (6). Furthermore, maintaining purity in seed production can be challenging, with only around 85 % of seeds being pure hybrids in some systems due to male sterility breaks in female lines. Educating farmers and developing hybrids with improved seed size are crucial steps to overcome these market barriers.

Historical progress in hybrid breeding

The journey of hybrid breeding in *Brassica* can be divided into distinct phases:

The exploration period (1940–67): During this period heterosis in oleiferous spp. was recognised and attempts were made to exploit heterosis using sporophytic self-incompatibility (SI) but no significant achievement was reported.

Preparation period (1968–84): The CMS system such as Nap, Ogu, Korean, Diplotaxis, Juncea, Nigra, MS-4 were reported during this era and attempts were made to transfer SI genes from *B. campestris* to *B. napus*.

Utilisation period (1985 onwards): The first CMS hybrid of *B. napus* (Quinyou no. 2) was registered in China in 1985 and later more hybrids were released all in *B. napus*. Applications of biotechnology, such as protoplast fusion, were utilised to correct chlorophyll deficiency in CMS system and broadening of genetic bases by genetic engineering, gene editing, etc.

Fundamental requirements for commercial hybrid development

Efficient pollination control system

It is impossible to implement commercial hybrid seed production based on manual emasculation and pollination in *Brassica* and therefore it requires an efficient pollination control system. Natural pollination control system arises due to spontaneous mutation in mitochondrial DNA (mtDNA) that breaks the coordination between nuclear and organellar genes whereas alloplasmic substitution with widely differentiated wild spp. or cell fusion also results in CMS system (7). The CMS system is applicable to F_1 hybrid breeding in all *Brassicaceae* crops, provides an efficient, reliable and stable method of F_1 seed production without contamination by self-fertilised seeds.

Hybrid advantage

Hybrid advantage is not simply a function of heterosis. It depends upon:

Breeding method efficiency: Over the past few decades particularly in

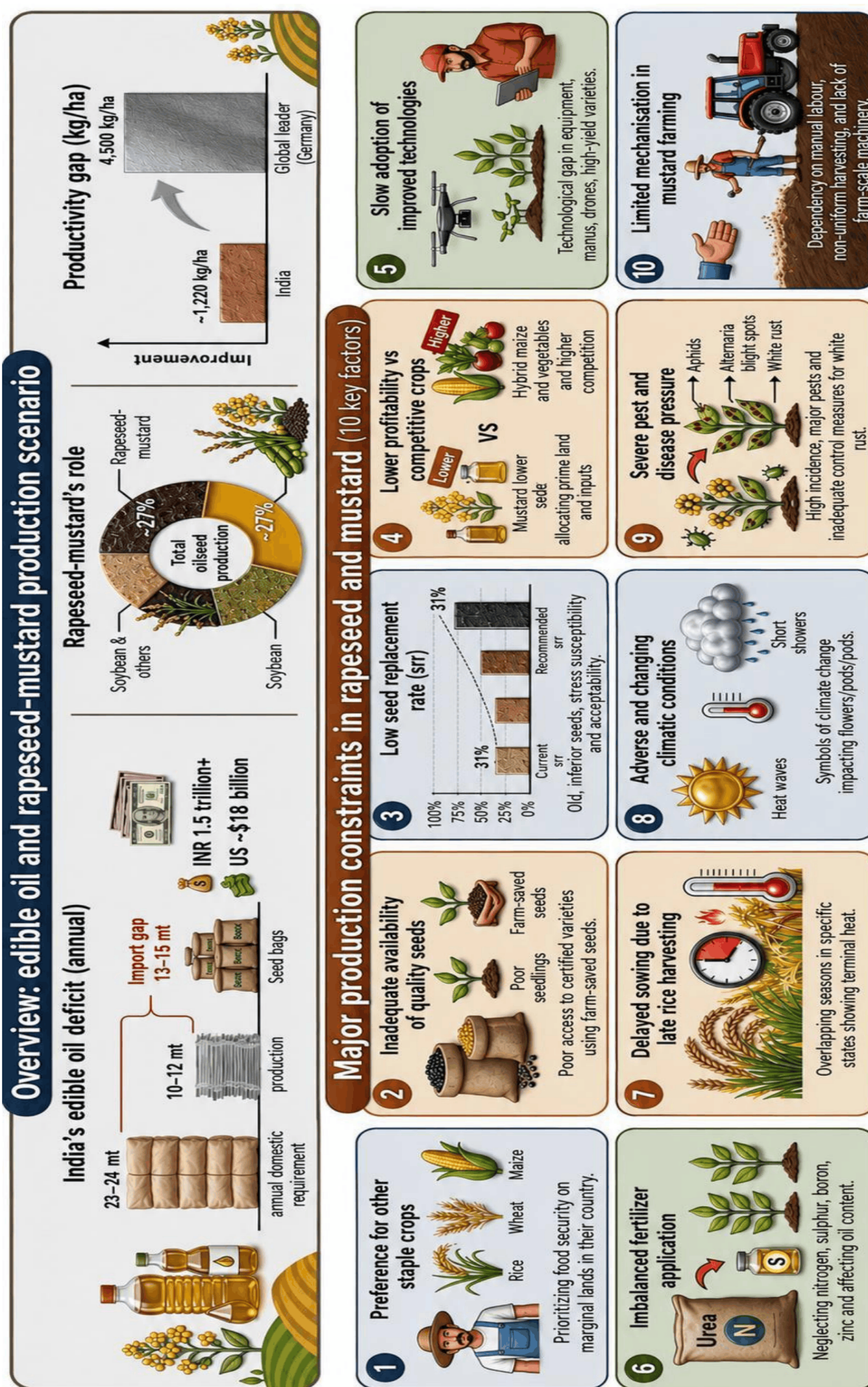


Fig. 2. Need of hybrid development in mustard.

India; the pure-line breeding method has been practiced tapping the genetic variability from the adapted gene pool of germplasm. The increase in productivity was insignificant. To realise significant gain in productivity new sources of variation may be utilised along with broadening of genetic base of existing varieties. But the most promising strategy which may substantially increase the productivity is heterosis breeding and development of hybrids. Heterosis breeding may utilise conventional methods like pedigree or backcross method or some biotechnological tools like marker assisted breeding and transgenic breeding. When exotic germplasm is utilised for transferring multiple traits simultaneously through pedigree or backcross methods; breeding efficiency is compromised due to poor adaptation of exotic germplasm and linkage drag during meiotic events. The marker assisted selection or transgenic approach are used to increase the breeding efficiency.

Effect of cyto-nuclear interaction: The CMS fertility restoration system may have negative or positive effects over yield and attributing traits used to produce the hybrid. In alloplasmic situation, the observed phenotype is often complex, resulting from the association of both chloroplast and mitochondrial genomes with an alien nuclear counterpart along with genetic background of recipient parent. This can lead to either a positive or a negative effect on yield and attributing traits. The pollen control mechanism though eliminates need of emasculation but adverse effects on agronomical traits can also be noticed which can be counted as biological penalty (difference in trait performance of euplasmic to that of alloplasmic lines > 10 %). Euplasmic lines are stable, fertile and naturally occurring. Alloplasmic lines, on the other hand, have foreign cytoplasm that causes CMS, which is necessary for making hybrid seeds in mustard. The percentage of biological penalty depends upon nuclear background of recipient of CMS and restorer genes which may be able to rectify and compensate for the defects. Thus, combining ability and heterosis of parental lines with native cytoplasm may or may not remain intact after their conversion into CMS background. There also exists possibility of finding crosses with negligible or no effect of cyto-nuclear interactions due to re-establishment of harmonious interaction.

The inherent heterosis: The majority of reports on heterosis documented over mean parental value or better parent value (8). Data based on superiority to commercial variety are rare. In countries like India with lower yield levels, a minimum of 25–35 % yield heterosis has been targeted for commercial value. In public sector maximum heterosis in brassica hybrids is observed to be <15 %. It has been reported that development of hybrids using lines of two known diverse gene pool i.e. east European gene pool and the Indian gene pool of *B. juncea* was heterotic for seed yield (9–11). However, in most cases European germplasm had reflected poor adaptation to Indian agro-climatic conditions and linkage drag may occur while adopting pedigree method or backcross breeding method during utilising this germplasm. If several characters are targeted for transfer through conventional method of breeding; it may disrupt the heterotic gene pool. The situation can be handled if one trait or quantitative trait locus (QTL) based precisely targeted traits are taken into consideration at one time using DNA markers or transgenic approaches.

Systems for exploiting heterosis in Brassica

Heterosis for economic yield in brassicas can be exploited through several pollination control mechanisms:

Cytoplasmic male sterility (CMS): This is the most widely used method

globally. It involves the use of sterile cytoplasm, which prevents pollen production. The system requires a stable male-sterile line (A-line), a maintainer line (B-line) and a restorer line (R-line), a three-line breeding system.

Self-incompatibility (SI): This is a natural genetic mechanism that prevents self-fertilisation. However, it is not used frequently in oilseed brassica due to self-contamination at higher temperatures or drought towards the end of flowering (12). It is mainly used in vegetable brassica by Japanese breeders. *Brassica juncea* does not express SI.

Genetic male sterility (GMS): This is controlled by nuclear genes. Economically, it is not viable as roguing of fertile plants from the male-sterile (ms) line requires extra labour and time. A major limitation is the lack of a linked seedling marker or pleiotropic effect to identify male fertile plants before flowering.

Chemical hybridising agents (CHA): These are chemicals that induce temporary male sterility. The identification of an ideal CHA remains difficult (13). It must cause only pollen abortion without affecting ovule fertility, have no mutagenic effects, be easy and economical to apply, have reproducible effects and pose no hazards to humans or the environment.

Genetically engineered male sterility: This is a modern approach that uses recombinant DNA technology to produce a precise pollination control mechanism. The first hybrid developed through this system in India is DMH-11, which has been approved by the Genetic Engineering Appraisal Committee (GEAC) for commercial cultivation.

Navigating the complexities of cytoplasmic male sterility development and genetic introgression

Development and introgression of cytoplasmic male sterility

Cytoplasmic male sterility systems can originate through intraspecific variations, induced mutations or most commonly alloplasmic substitution (cytoplasmic hybridisation), where the cytoplasm of a wild species is combined with the nucleus of a cultivated species (14). This creates an incompatibility between the alien mitochondria and the endogenous nucleus, leading to male sterility. Cytoplasm from several wild species, namely *Brassica oxyrrhina* (oxy), *Trachystoma balli* (trachy), *Moricandia arvensis* (mori), *Diplotaxis catholica* (cath), *D. sifolia* (sii), *Diplotaxis eruroides* (eru), *Diplotaxis berthautii* (berth), *Enarthrocarpus lyratus* (lyr), *Erucastrum canariense* (can) and *Raphanus sativus* (ogu), have been introgressed into *B. juncea* using sexual or somatic hybridisation (15).

Challenges and problems in cytoplasmic male sterility development

The development of a stable CMS system is a long and challenging process (6). Key problems include:

Difficulty in wide hybridisation: Transferring CMS from wild to cultivated species is extremely difficult due to crossability barriers, often requiring techniques like embryo rescue and tissue culture.

Physiological defects: Even after successful crossing, the resulting alloplasmic lines often exhibit physiological defects such as chlorosis (yellowing of leaves), deformed flowers that fail to attract pollinators or poor female fertility.

Post-development limitations: Even after a CMS system is established, limitations often persist. These can include chlorophyll deficiencies (e.g., in ogu, oxy, mori CMS), poor female fertility (e.g., in trachy, lyr CMS) and overall yield penalties, which are manifestations of the "biological penalty" (16).

Major cytoplasmic male sterility systems in *Brassica juncea*

Several CMS systems have been developed and studied extensively. The key characteristics, origins and challenges of the major CMS systems relevant to *B. juncea* are summarised in Table 1 (6, 14). This comparison provides a clear overview of the tools available to breeders and the specific issues associated with each system. The Ogura (ogu) system is the most stable and widely used, while others like Moricandia (mori) are important but come with complexities like gametophytic restoration (17) (Table 2). The table also includes newer systems like *B. fruticulosa*, which is still under development but shows promise for introducing disease resistance.

Shiga-Thomson (nap) system

Cytoplasmic male sterility in *B. napus* was first reported (17). The sterile plants were observed in F₂ generation of the cross between the collection of European spring and winter rape. Boronowski spring rape variety was used as male parent. Segregation for male sterility was not observed in reciprocal crosses and thus concluded that the male parent Boronowski contributes recessive genes for sterility but has normal cytoplasm. Later independently identified male sterility in the intervarietal crosses of Chisaya-natane and Hokuriku-23. Due to common restorer and mutual maintainers these two systems were considered identical. The schematic development of this first CMS system in *B. napus* is illustrated in Fig. 3 (18, 19). This was confirmed later through the restriction patterns of chloroplast DNA (cpDNA) and mtDNA in *B. napus* and revealed the alloplasmic nature of this CMS. This system probably exploits cytoplasmic diversity from *B. campestris* (20). Nuclear genes specific to this system do not cause sterility in maintainers (Bronowski and Isuzu-natane) because they have campestris cytoplasm but give rise to sterility in *B. napus* cytoplasm. The fertility restorer genes were widely distributed among both European and Japanese varieties of *B. napus* (21, 22). Inheritance of fertility restoration varied with the pollinator parent and one to four genes have been implicated in various studies (21, 23, 24). Unfortunately, the male sterility expression was found unstable and plants can revert to fertility at high temperature.

Raphanus or ogu system

Raphanus or ogu CMS system was discovered in Japanese radish (*Raphanus sativus* L.) of unknown cultivar by Ogura in 1968. Ogura observed that Japanese cultivars do not have fertility restorer (*Rf*) genes, but some European cultivars do have (25). The Ogura CMS was introduced into *B. napus* by European scientists (26, 27) using the backcrossing method. The process of this transfer is depicted in Fig. 4. The physiological defects like chlorosis or yellowing of leaves at low temperature reflected in alloplasmic line of *B. napus* due to functional incompatibility between nucleus of alloplasmic *napus* and chloroplast of *R. sativus*. It was corrected by cell fusion of alloplasmic *napus* lines and normal lines to substitute the chloroplast of *R. sativus* with that of *B. napus* (28–30). This CMS studied most extensively for its basic molecular mechanism and practical utility. It is used worldwide in F₁-breeding of *B. napus*, *B. juncea*, *B. oleracea* and *R. sativus*.

Genetic and breeding research on Ogura cytoplasmic male sterility

The chronological development of the Ogura CMS system from its basic discovery to breeding applications is summarised in Fig. 5 (14). Ogura CMS was first identified in Japanese radish, after which the sterility cytoplasm was transferred into European radish where nuclear restorer genes such as *Rf/Rfo* were detected. The same cytoplasm was then introduced into *B. napus*, initially through alloplasmic lines and later refined by protoplast fusion between male-sterile and normal *B. napus*, creating CMS systems suitable for commercial use. Subsequent molecular work identified the mitochondrial sterility gene *orf138* and characterised the nuclear restorer gene *Rfo* (also called *orf687*), clarifying the genetic basis of sterility and restoration. In parallel, breeders began using Ogura CMS for F₁ hybrid seed production first in European radish and then in *Brassica* vegetables and oilseed rape, where stable alloplasmic CMS lines and restorer lines enabled large-scale exploitation of heterosis. Finally, cloning of *orf138* and *Rfo* (as shown in Fig. 6) allowed development of DNA markers tightly linked to the restorer locus, so marker-assisted selection (MAS) could efficiently incorporate the restorer gene into new *Brassica* backgrounds.

Table 1. Major cytoplasmic male sterility (CMS) systems in *Brassica* and their status in *Brassica juncea*

CMS system	Origin	Key features/challenges	Status in <i>B. juncea</i>
Ogura (ogu)	<i>Raphanus sativus</i>	Stable, widely used; initial chlorosis corrected via protoplast fusion.	Most stable and popular
Moricandia (mori)	<i>Moricandia arvensis</i>	Gametophytic restoration; used in several Indian hybrids (e.g., NRCHB 506); restoration system can be complex.	Important, but has seed size issues
Tournefortii (tour)	<i>B. tournefortii</i>	Associated with chlorosis, poor female fertility and floral deformities.	Limited use
Polima (pol)	<i>B. napus</i>	Temperature-sensitive, unstable.	Limited use
126-1	Not specified	Used in DMH-1; constrained by the availability of complete maintainers.	Used in one released hybrid
<i>B. fruticulosa</i>	<i>B. fruticulosa</i>	New system; a valuable genetic resource for resistance to mustard aphids and Sclerotinia stem rot (69).	Under development

Table 2. Differences in organisation of Ogura and normal type mitochondrial genomes

Parameters	Ogura- type mitochondrial genome	Normal- type mitochondrial genome
Genome size	258426-bp circular sequence	244036-bp circular sequence
Genes	Ogura-type genomes contained same genes as of normal type and additional <i>atp9</i>	33 protein-coding and three rRNA genes
tRNAs	17 tRNAs and one additional trnFM	17 tRNAs
Homology	<i>orf138</i> was specific	No homology
Unique region	Four (2803 bp, 1601 bp, 451 bp and 15255 bp in size)	non-syntenic
Syteny	11 syntenic regions (length > 3 kb, similarity > 99.9 %) in radish mitochondrial genome	---

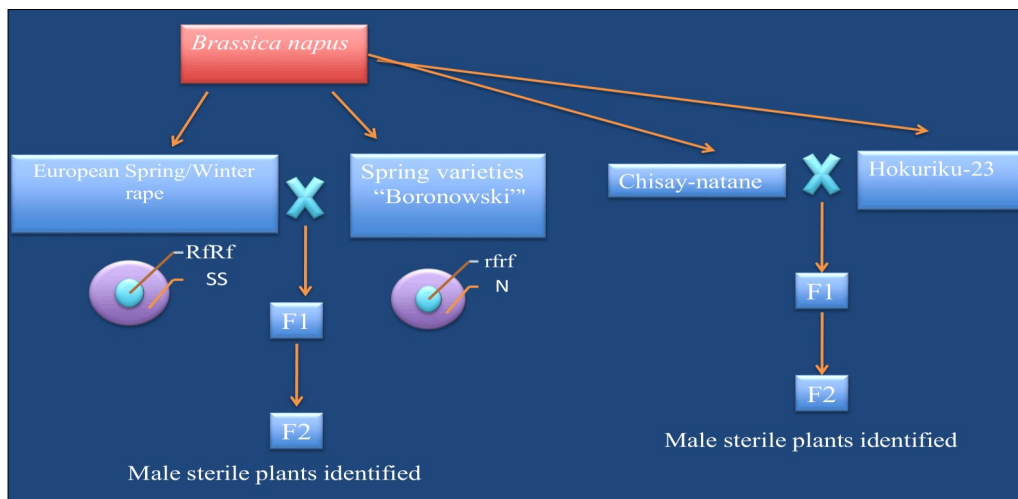


Fig. 3. Schematic diagram showing the development of first cytoplasmic male sterility in *B. napus*.

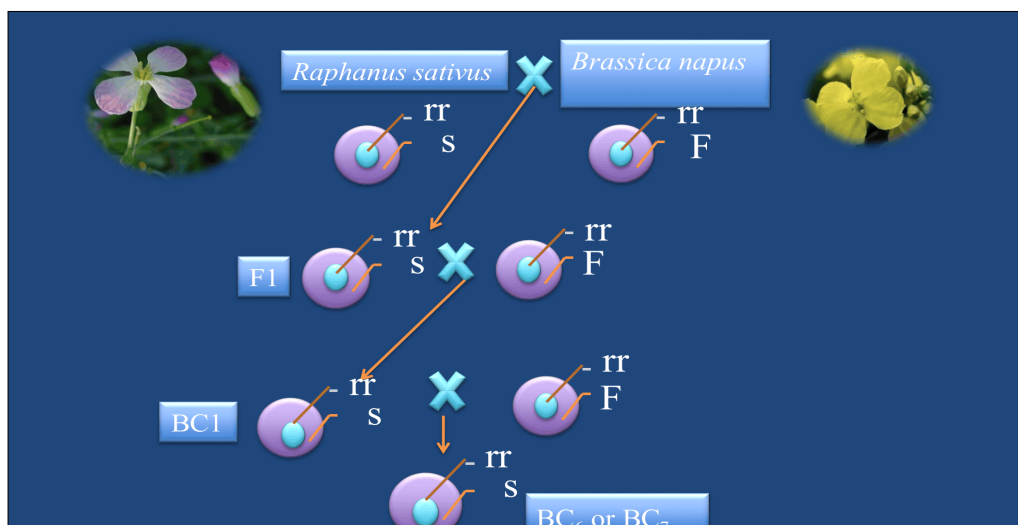


Fig. 4. Schematic diagram showing the transfer of Ogura cytoplasmic male sterility from *Raphanus sativus* to *B. napus*.

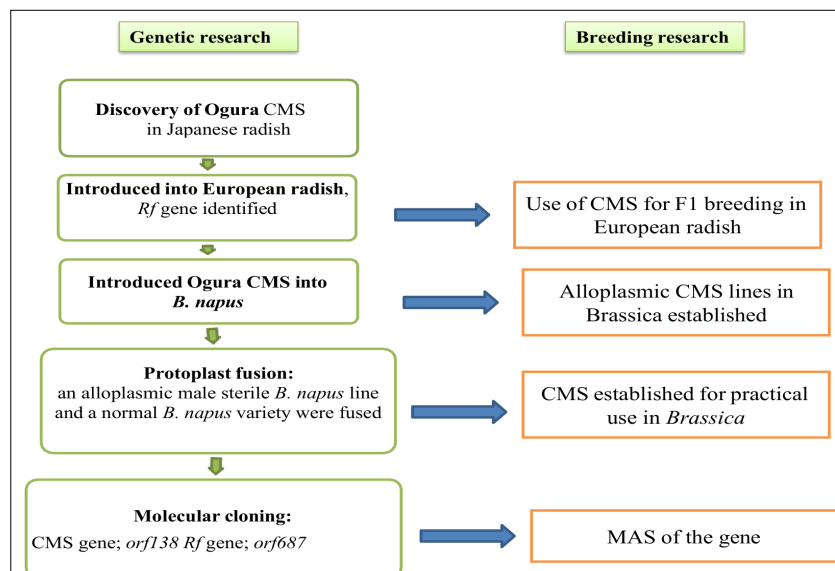


Fig. 5. Genetic and breeding research on Ogura cytoplasmic male sterility.

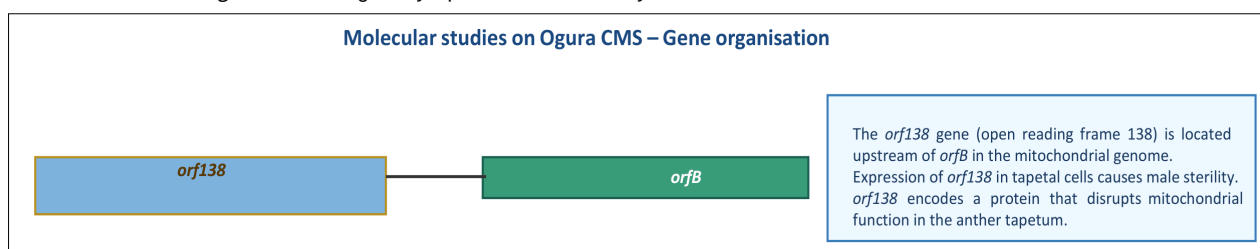


Fig. 6. Molecular studies on Ogura cytoplasmic male sterility.

Molecular studies on *Ogura* cytoplasmic male sterility

The male sterility inducing gene (*orf138*) comprises two open reading frames coding respectively 138- and 158-amino-acid (also called *orfB*) polypeptides which are co-transcribed. The following differences in organisation of *Ogura* and normal type mitochondrial genomes as shown in Table 2 (17).

Polima (pol) system: It is found spontaneously originated 19 male-sterile plants in the spring *B. napus* variety Polima. The causal gene for *Pol* CMS is *orf224*. The restoring gene is found in *B. napus*, *B. juncea* and *B. campestris* (31). This CMS is temperature sensitive and therefore its use in F_1 hybrid breeding is limited. These CMS lines are divided into 3 categories as per their sensitivity to temperature (32).

- High temperature CMS line
- Low temperature CMS line
- Stable CMS

Diplotaxis muralis (mur) system

This CMS system was developed by transferring the male sterility gene through manual crossing and backcrossing between wild brassica *D. muralis* and *Brassica campestris* cv. Yukina (33). *Rf* genes were found in *B. campestris*, whereas maintainer genes were transferred from *B. campestris* into well adapted *B. campestris* parents lacking maintainer genes (33, 34). The schematic diagram of this cytoplasmic transfer is presented in Fig. 7. Transfer of *muralis* cytoplasm into *B. juncea* led to partial sterility and not used frequently in *B. juncea*.

Mokopo or Korean system:- This system was found in the progenies of a *B. napus* varietal cross Tower x Isuzu (35, 36). The restorers with highest fertility restoring ability were identified from European napus varieties but no complete maintainer was identified in napus.

Tournefortii (tour) system:- The CMS plants in one collection of the alloplasmic *B. juncea* (37). Cytoplasm of this alloplasmic CMS line of *B. juncea* transferred to a number of *B. juncea* and *B. napus* cultivars and was found to be stable in different nuclear backgrounds (37). The fertility restorer genes were first identified in

the diploid species of *B. campestris* and *B. nigra* and transferred to *B. juncea*. However, fertility restoration appeared incomplete (38). Later on fertility restorer genes identified in both *B. napus* and *B. juncea*. The major drawback of this system is that the onset of long days causes reversion to sterility in restored plants of *B. juncea*. Fertility restoration in *B. napus*, however, is, complete and stable.

MS-4 system

MS-4 CMS system of *B. juncea* was observed in F_2 generation of cross between the cultivar RLM- 198 and EJ-33(24). The development of this system is schematically represented in Fig. 8. It is not in use due to the absence of complete maintainers for male sterility.

Chinese juncea (jun) system

This CMS system was observed during a cross combination of two *B. juncea* genotypes namely Xinpin youcai from China and European varieties (38, 39). They found the F_1 plants were not producing pollen or seeds. Fertility restorer genes are reported to widely distributed in the Yunnan province of China. Complete male sterility maintainer Xinpin B had identified in European *B. juncea* varieties. The schematic for the development of Chinese juncea CMS is shown in Fig. 9. This CMS system is being used for hybrid seed production in China.

Line-14 or campestris system:-

This CMS system was reported in *B. campestris* (40). The male sterile plants were identified in F_1 generation of a cross between New Zealand wild turnip *B. campestris* ssp. *rapifera* Line-14 and a Japanese cultivar of *B. campestris*. Restorers were identified in many subspecies of *B. campestris* (Fig. 10).

Nigra (nig) system:-

The male sterile cytoplasm from alloplasmic *B. nigra* to *B. oleracea* through repeated backcrossing and after 6–7 backcrosses he identified stable male sterile plants for utilisation in hybrid breeding (37, 41). Rousselle in 1985 used this converted CMS line of *B. oleracea* as a cytoplasm donor in an interspecific cross between *B. oleracea* and *B. napus* using 6-7 backcrossing method (42). The pathway of cytoplasm transfer is shown in Fig. 11. Complete male sterile plants were identified by Rousselle,

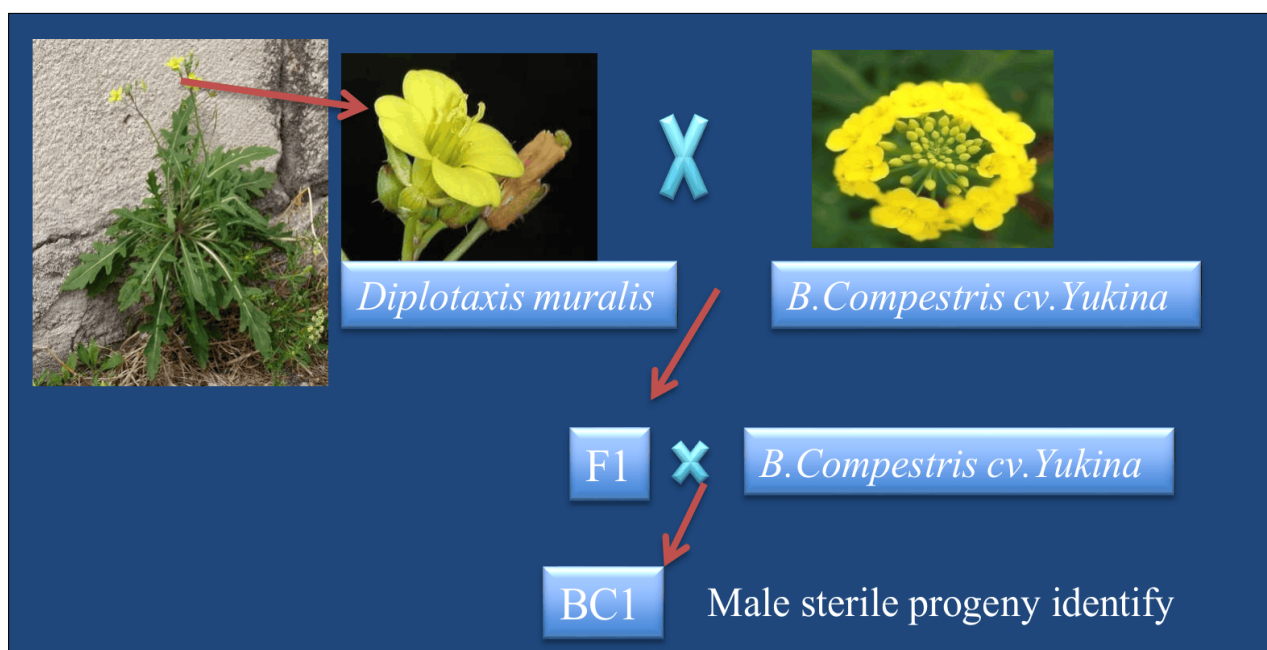


Fig. 7. Schematic diagram showing the transfer of *Diplotaxis muralis* to *B.campestris* cv. Yukina.

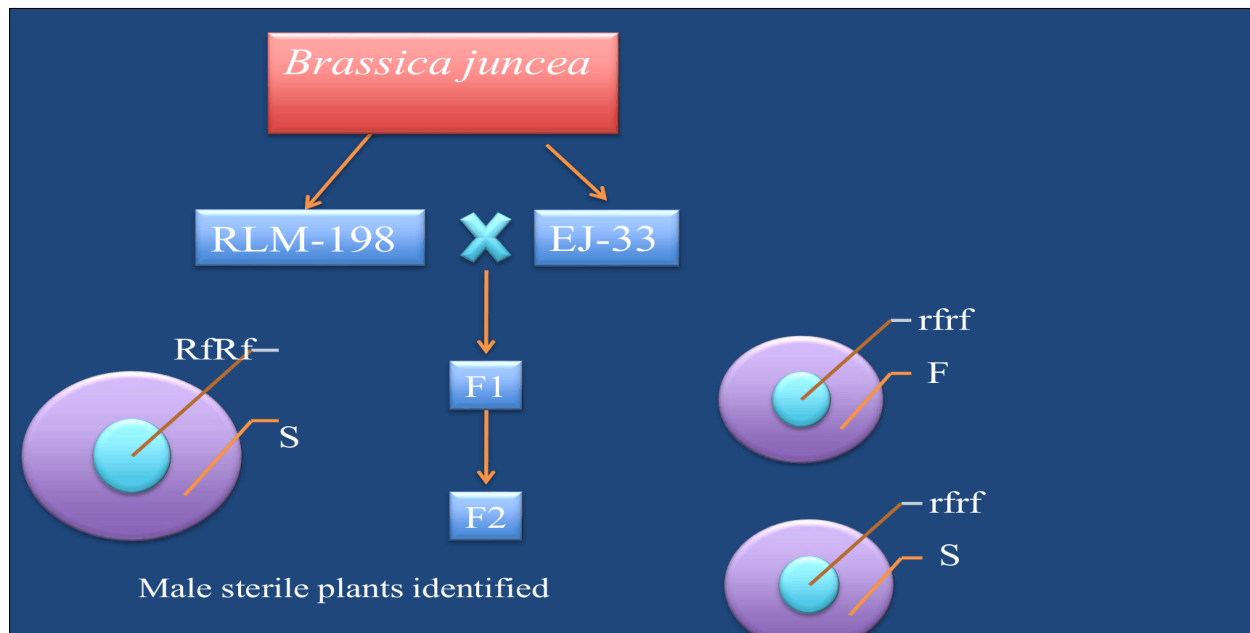


Fig. 8. Schematic diagram showing the development of MS-4 cytoplasmic male sterility (CMS) system in *Brassica juncea*.

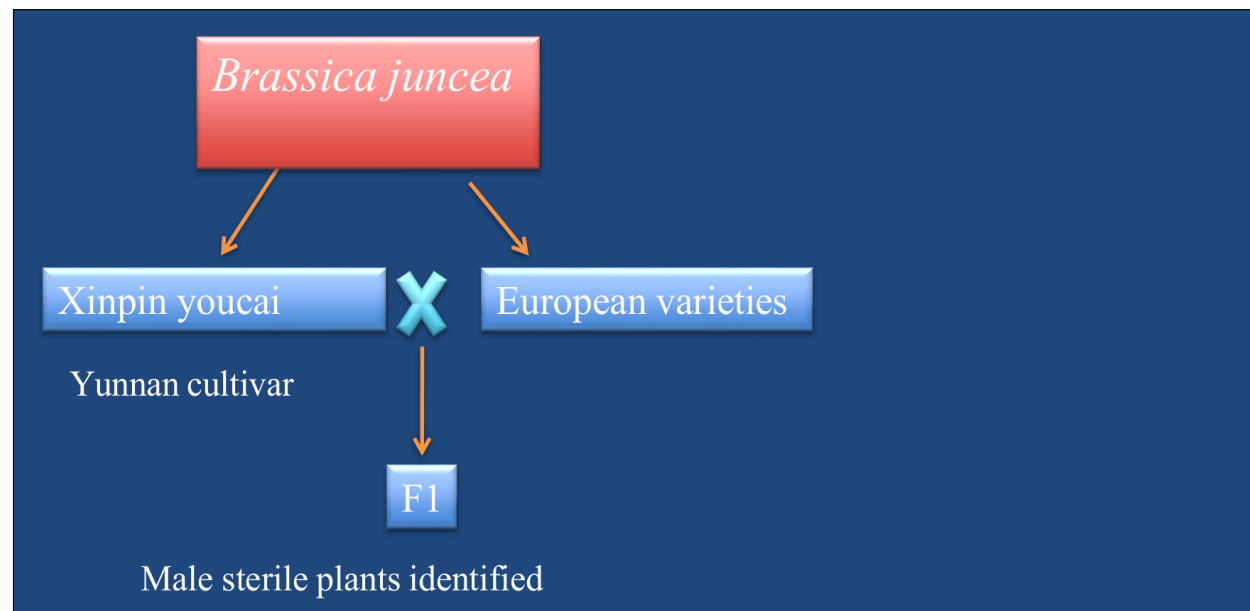


Fig. 9. Schematic diagram showing the development of Chinese *juncea* (*jun*) cytoplasmic male sterility system in *Brassica juncea*.

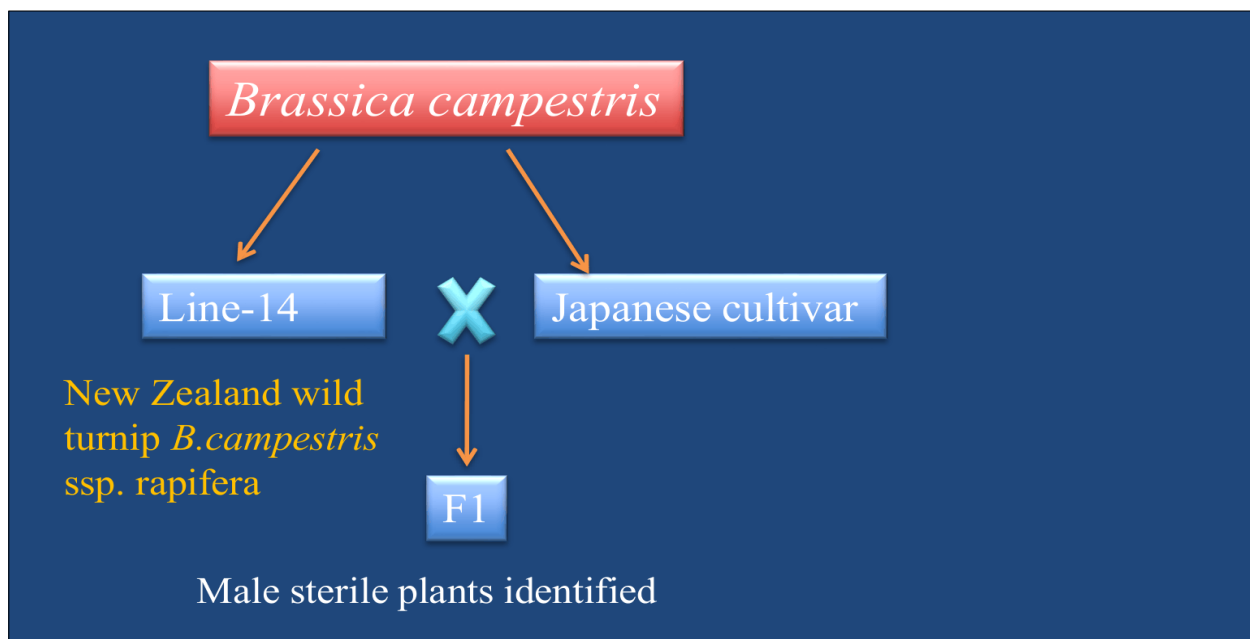


Fig. 10. Schematic diagram showing the development of Line-14 or cytoplasmic male sterility system.

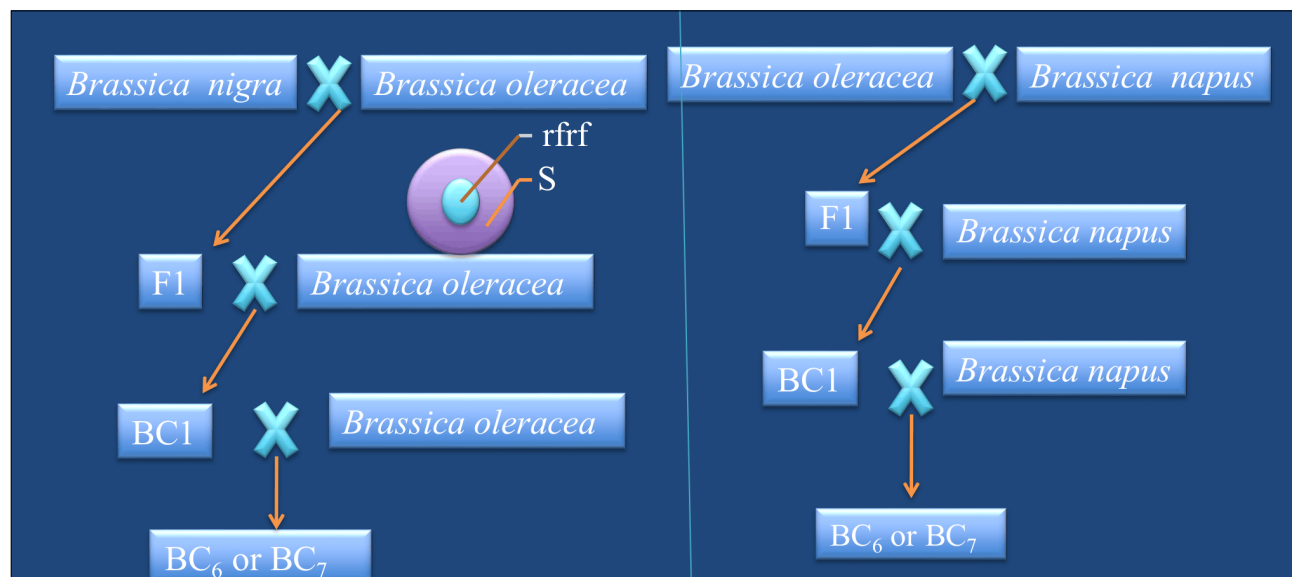


Fig. 11. Schematic diagram showing the development of Line-14 or cytoplasmic male sterility system.

but no maintainer was identified for revival of CMS lines.

Oxyrrhina (oxy) system

This CMS system was observed in a cross of *B. oxyrrhina* with repeated backcrossing in *B. campestris* and *B. juncea*. Male-sterile plants exhibit chlorophyll deficiency. No restoration has been found. The development of the Oxyrrhina CMS system is depicted in Fig. 12.

Cataloging non-conventional cytoplasmic male sterility systems in Brassica crops

In addition to the CMS systems described in detail above, several other systems have been developed and documented by researchers, expanding the genetic toolbox for hybrid breeding in *Brassica*. These systems, developed through various techniques including somatic hybridisation and protoplast fusion, provide a diverse array of cytoplasm that can be utilised to create new heterotic combinations and avoid genetic vulnerability. The key features, developers and restoration status of these additional systems are summarised in Table 3 (43–45).

Cyto-nuclear composition and cytoplasmic male sterility genes in Brassica juncea

The diverse cyto-nuclear sources that have been used to develop

CMS systems in *B. juncea* are depicted in Fig. 13 (14). As shown in the figure, the nuclear source is consistently *B. juncea*, while the cytoplasm sources are diverse. Male sterility system in *B. juncea* has been developed either through alloplasmic substitution of mitochondrial genes by wild species/genera or through cell fusion. Cytoplasm from two wild species (*B. oxyrrhina*, *B. tournefortii*) and six different genera (*D. berthautii*, *D. catholica*, *D. eruroides*, *D. siifolia*, *Enarthrocarpus lyratus* and *Erucastrium canariense*) cause CMS through alloplasmic substitution (Fig. 13). Additionally, CMS has been developed through cell fusion with three wild genera: *D. catholica*, *M. arvensis* and *T. ballii*.

The specific mitochondrial genes responsible for CMS that have been introgressed into *B. juncea* from various sources are detailed in Fig. 14 (14). The wild species/genera or cultivated genera of related *Brassica* cytoplasm have contributed different CMS-causing genes to *B. juncea* that originated in their mitochondrial genome. As shown in Fig. 14 (14), CMS gene *orf138* and *orf125* originated from *R. sativus* (Ogura) and *R. sativus* (Kosena) respectively. The CMS gene *orf108* came from three different species of *Diplotaxis* spp. through alloplasmic substitution in distant crosses and from *M. arvensis* through cell fusion. *orf263* gene was observed in *B. tournefortii* whereas *orf220* and *orf228* genes originated in *B. rapa* mitochondrial genome to cause CMS.

Table 3. Other documented cytoplasmic male sterility (CMS) systems in *Brassica*, their developers and key characteristics

Sl. No	CMS system	Brief description	Stability of CMS system	Restoration system	References
1	<i>Diplotaxis siifolia</i> (sif) system	Developed by substituting <i>B. juncea</i> nucleus in the cytoplasmic background of <i>Diplotaxis siifolia</i> .	Yes	No restoration	42
2	<i>Trachystoma ballii</i> (trachy) system	Discovered in <i>B. juncea</i> from the somatic hybrid <i>Trachystoma ballii</i> + <i>B. juncea</i> (2n = 52; TTAABB).	Yes	Single dominant gene for restoration	43
3	<i>Moricandia arvensis</i> (mori) system	Developed in <i>B. juncea</i> through somatic fusion between <i>Moricandia arvensis</i> and <i>B. juncea</i> (2n = 64; MMAABB).	Yes	Single dominant gene for restoration	44
4	<i>Diplotaxis catholica</i> (cath) system	Derived from somatic hybrid <i>D. catholica</i> + <i>B. juncea</i> (2n = 54, D ^c D ^c AABB).	Yes	No restoration	43
5	<i>Sinapis alba</i> (alba) system	Developed through cytoplasm substitution of <i>B. juncea</i> cytoplasm by <i>Sinapis alba</i> cytoplasm through protoplast fusion.	Yes	Fertility restoration reported in <i>B. napus</i>	44
6	<i>Enarthrocarpus lyratus</i> (lyr) system	Developed through introgression of gene for fertility restoration from <i>E. lyratus</i> to <i>B. juncea</i> .	Yes	Fertility restoration reported but not in use	45

CMS: cytoplasmic male sterility.

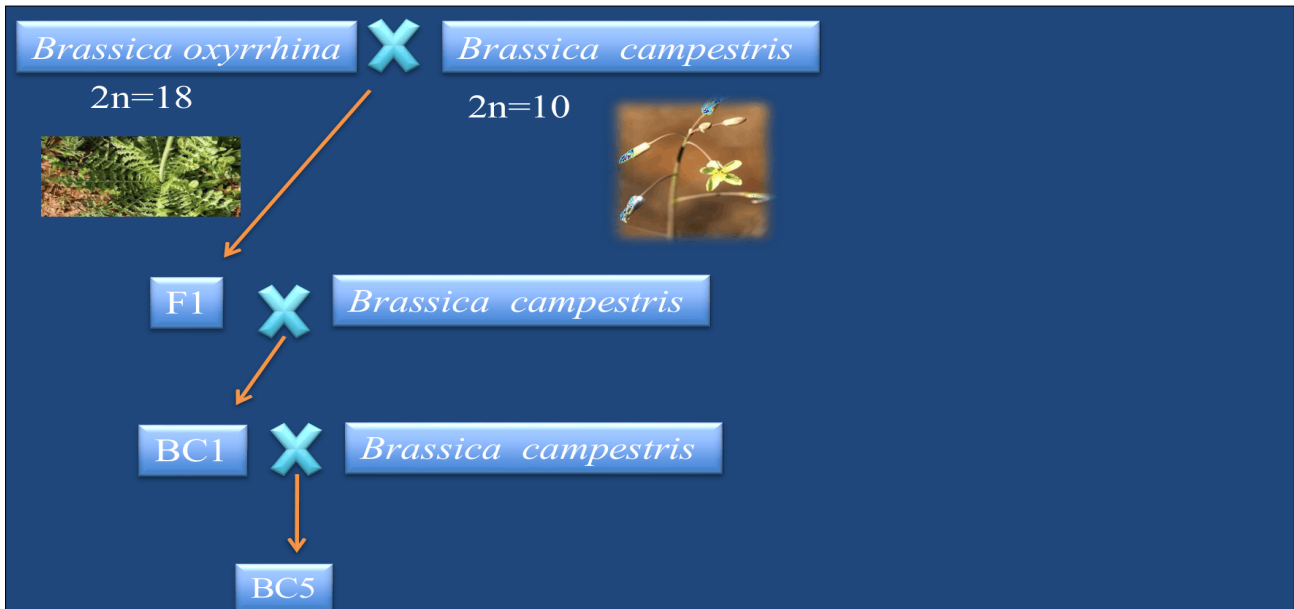


Fig. 12. Schematic diagram showing the development of Oxyrrhina (oxy) cytoplasmic male sterility system in *Brassica campestris*.

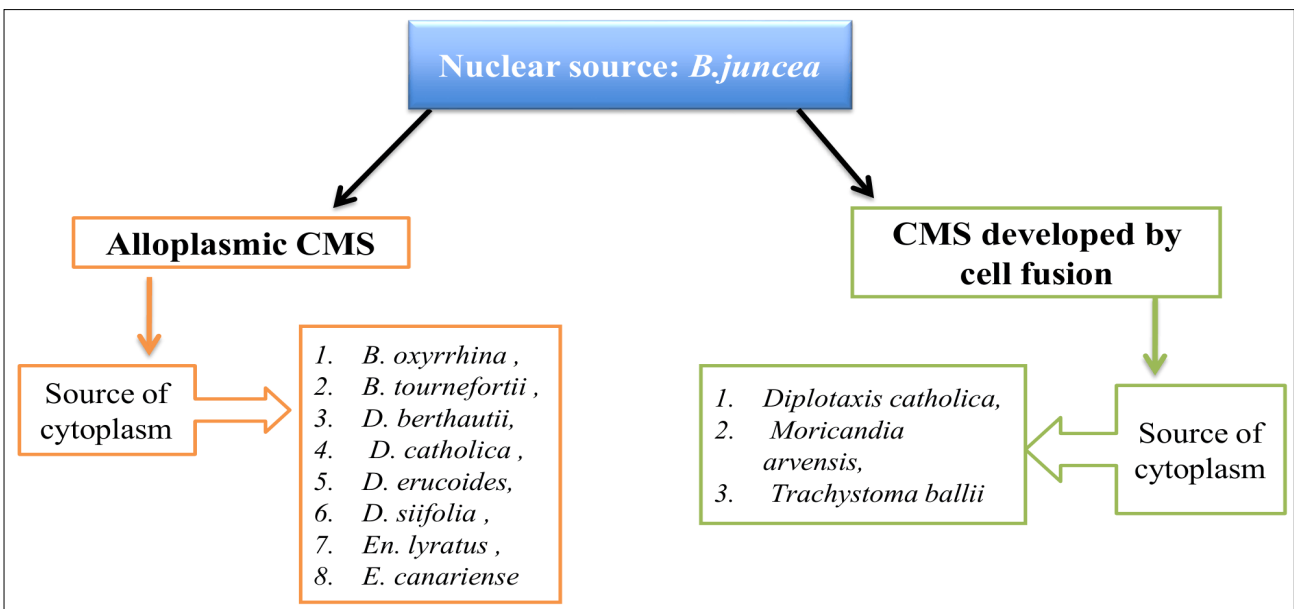


Fig. 13. Sources of cytoplasm and nucleus in cytoplasmic male sterility (CMS) system of *Brassica juncea*, showing alloplasmic and cell fusion origins.

Gene	Originated in the mitochondria of
<i>orf138</i>	<i>Raphanus sativus</i> (Ogura)
<i>orf125</i>	<i>Raphanus sativus</i> (Kosena)
<i>orf108</i>	<i>D. erucoides</i> , <i>D. berthautii</i> , <i>D. catholica</i> , <i>M. arvensis</i> (by cell fusion)
<i>orf263</i>	<i>Brassica tournefortii</i>
<i>orf220</i>	<i>Brassica rapa</i>
<i>orf288</i>	<i>Brassica rapa</i>

Fig. 14. Sources of specific cytoplasmic male sterility genes in *Brassica juncea* and their mitochondrial origins.

Fertility restoration (*Rf*) genes for the cytoplasmic male sterility systems

The discovery of *Rf* genes that confer male fertility to CMS plants is crucial for exploiting CMS for hybrid development. Intraspecific cytoplasmic variants are usually found among the germplasm lines of that species. For alloplasmic CMS lines derived from sexual or somatic hybridisation, *Rf* genes are not generally found in the recipient species and need to be introgressed from the species used as cytoplasm donors. *Rf* genes have been successfully transferred from other species into cultivated *Brassica* species for the CMS-inducing cytoplasm such as Ogura (27), *T. ballii* (46), *M. arvensis* (40), *Er. canariense* (15), *En. lyratus* (47) and *B. tournefortii* (48). *Rf* gene of *M. arvensis* restores fertility of three other cytoplasm namely *D. catholica*, *D. eruroides* and *D. berthautii* (49–51). Fertility restoration in most *Brassica* CMS lines is sporophytic (i.e. F1 hybrid plants are 100 % pollen-fertile). However, in CMS lines of *M. arvensis*, *D. eruroides* and *D. berthautii*, fertility restoration is gametophytic (50, 52), i.e., only *Rf* gene-carrying pollen is functional and thus F1 hybrid plants are 50 % pollen-fertile. Hence, the self-progeny of gametophytically restored plants do not segregate for male sterility and fertility.

What do *Rf* genes actually do?

The relationships between mitochondrial CMS genes and their counterpart *Rf* genes reflects the possible way of co-evolution of mitochondrial and nuclear genomes. The proteins encoded by pol CMS and nap CMS genes have similar structure. The *Rf* genes for nap (*Rfn*) and pol (*Rfp*) are allelic (54) and both affect mRNA processing of the CMS genes by inducing the removal of sequences from the 5'-ends of the transcripts (54–56).

Rf genes of different origin have a common function in alloplasmic *B. juncea* viz. four CMS lines *D. eruroides*, *D. berthautii*, *D. catholica*, *M. arvensis* have a common CMS gene (*orf108*) and share a common restorer gene. In the presence of the restorer, the bi-cistronic *orf108-atpA* transcript is cleaved just downstream of the start codon of *orf108*, leading to the production of monocistronic *atpA* transcript (57, 58). It indicates that the common genetic mechanisms of CMS and fertility restoration have evolved and are shared by different species and genera. For Ogura CMS, the *Rf* gene, *orf687* (59–61), does not affect transcription of the *orf138*, but regulates *orf138* mRNA translation. In Japanese wild radishes, where *Rf* genes for Ogura CMS are common, the frequency of plants with *orf687* is small (62). Another *Rf* gene (*Rft*), found in most Japanese wild radishes, regulates *orf138* mRNA processing (63). This finding indicates that *Rf* genes with different molecular mechanisms have evolved in radish to suppress the expression of *orf138*, the causal gene of Ogura CMS.

Effect of cyto-nuclear interactions on hybrid performance

Adverse effect of alien cytoplasm on hybrid performance is generally associated due to the incompatible plastid or mitochondrial genome. The negative effect of incompatible plastids causes leaf chlorosis in CMS *B. rapa*, *B. juncea* or *B. napus* lines with Ogura, *B. oxyrrhina*, *M. arvensis* cytoplasm (47, 62, 63). The CMS lines of *B. juncea* carrying Ogura, *D. catholica* or *T. ballii* cytoplasm show poor female fertility (64, 65). Generally mitochondrial gene that is associated with male sterility is focused under study. The expression of mitochondrial genes other than CMS gene which are also altered in the CMS lines; the effects of such changes and the role of *Rf* genes in correcting other defects have rarely been investigated. In the *T.*

ballii-based CMS system, a restorer gene could completely reverse the negative effect of cytoplasm on seed yield and yield-contributing traits, whereas in *D. catholica*-based CMS, a restorer gene was only partially effective. Another effect that could come into play in alien CMS systems is the linkage drag associated with the introgressed *Rf* gene. Introgression of a restorer gene for Ogura CMS from *R. sativus* into *B. napus* and further in *B. juncea* brought along an unwanted gene responsible for seed glucosinolate synthesis and it took considerable effort and time to break this linkage (66, 67).

Polymorphic floral phenotype induced by the same cytoplasm in different spp.

Specific mitochondrial gene causes male sterility, but diverse floral phenotypes may be induced by the same cytoplasm in different species. For example, *orf108* causes CMS in *B. juncea* lines carrying *D. catholica*, *D. berthautii*, *D. eruroides* or *M. arvensis* mitochondria and all these CMS lines are restored by a common restorer (51–53), *D. catholica* cytoplasm leads to a petaloid anther, multilocular silique and poor female fertility whereas others result only in gametophytic pollen sterility. Ogura cytoplasm leads to petaloid anthers in *B. juncea* but sterile anthers in *B. napus*. The fact that the same fertility restorer gene from *R. sativus* restores male fertility in CMS lines of *B. napus* and *B. juncea* carrying Ogura cytoplasm indicates that *orf138* causes male sterility in both the cases. Therefore, how *orf138* expression in *B. juncea* leads to petaloid anther remains unresolved.

The three-line hybrid breeding system in *Brassica juncea*

The hybrid seed production in *B. juncea* based on cytoplasmic genetic male sterility (CGMS) system involves three lines i.e. cytoplasmic genetic male sterile lines (A-line), maintainer line (B-line) and restorer line (R-line). It is also called three-line breeding. The A-line and the F1 produce through A × B produces non-viable pollen. B-line only maintains the sterility of A line. However, F1 produces through A × R line produces viable pollen and sets seed, which are used to plant commercial mustard crop. B and R line maintained through pure-line breeding. The schematic of this three-line system is presented in Fig. 15.

A-line breeding:

Development of A-line has three key components viz. per se performance of maintainer lines, Combining ability and Conversion of B-line into A-line. The schematic diagram showing the process of developing an A-line through repeated backcrossing is presented in Fig. 16.

Conversion process is achieved by 5–6 repeated backcrossing. The male sterile line is used as donor parent and the line to be converted into CMS is taken as recurrent pollen parent. In successive backcrossing, rigorous selection is done for plants with more matching character to male/ recurrent parent (B-line) having male sterility feature. Say for example if we have Varuna variety of Indian mustard and we want to obtain sterile version of Varuna then we go for repeated backcrossing with CMS line. The first crossing is done with donor CMS line acts as female and Varuna as pollen parent. Then selection for Varuna type having sterile anthers is done in F1. Now the main donor parent will not be used for crossing instead the F1 plants with traits like Varuna and having sterility will be used as female parent and Varuna variety will be repeatedly used further as recurrent parent. In BC1 generation same process will be adopted till 5–6 backcrossing. In the 5th or 6th backcrossing we get the sterile version of Varuna.

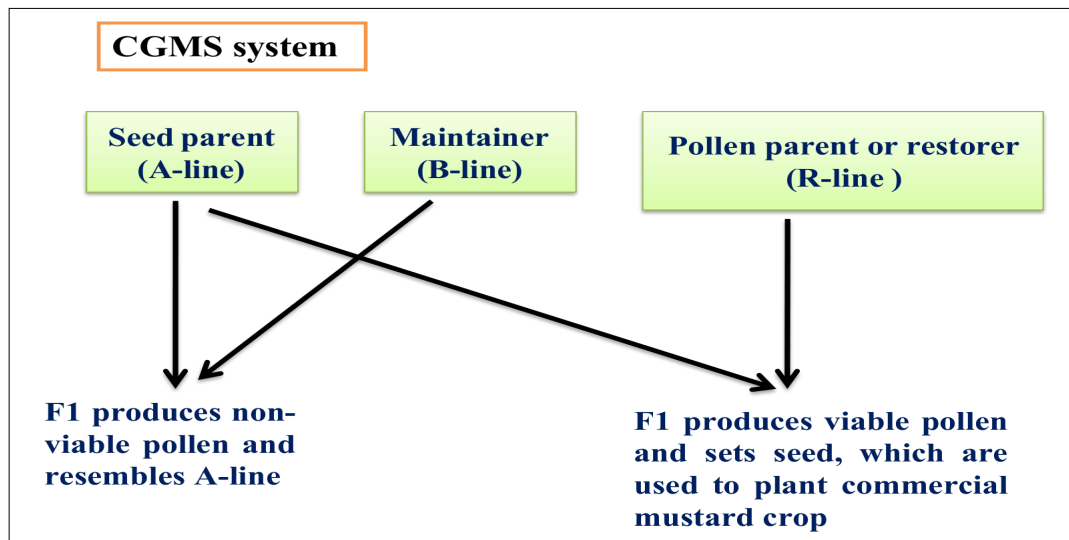


Fig. 15. The three-line hybrid breeding system using cytoplasmic genetic male sterility.

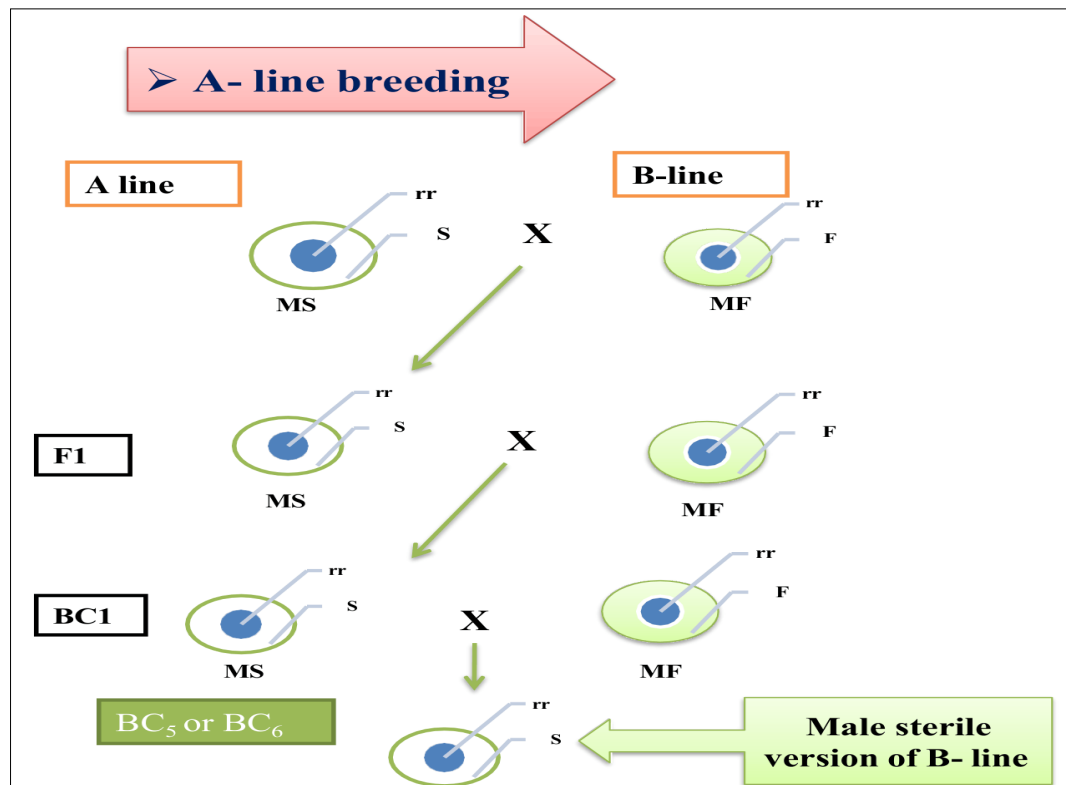


Fig. 16. Process for developing A-lines (cytoplasmic male sterility lines) through repeated backcrossing.

Ways other than cytoplasmic male sterility to employ yield heterosis in *Brassica*

While CMS is the predominant method, other systems have been explored for hybrid seed production in *Brassica*, each with its own set of advantages and limitations.

Self-incompatibility (SI): It is not used frequently due to self-contamination at higher temperatures or drought towards the end of flowering (68). It is mainly used in vegetable brassica by Japanese breeders. *Brassica juncea* does not express SI.

Genetic male sterility (GMS): Economically it is not viable as roguing of fertile plants from the male-sterile (ms) line requires extra labour and time. There is no linked seedling marker or pleiotropic effect to identify the male fertile plants before the initiation of flowering.

Chemical hybridising agent - (CHA) induced male sterility: An ideal chemical hybridising agent as suggested by Chopra in 1960 requires following criteria which is seldom fulfilled (13):

- Should cause only pollen abortion and not affect ovule fertility
- No mutagenic effect on treated materials
- Method of application should be easy and economical
- Effects should be reproducible means the CHA should be effective equally in every event of generation
- No undue hazards either to man or plant

Genetically engineered male sterility: The Barnase-Barstar system in Indian mustard

While CMS systems have been the mainstay for hybrid development, recombinant DNA technology has opened new avenues through genetically engineered (GE) male sterility. The most prominent example is the Barnase-Barstar system, which has been deployed in India's first transgenic mustard hybrid DMH-11, approved by the Genetic Engineering Appraisal Committee (GEAC) for commercial cultivation.

Molecular mechanism of Barnase-Barstar induced male sterility

The system utilises two genes from the soil bacterium *Bacillus amyloliquefaciens*:

Barnase: Codes for a ribonuclease (RNase) enzyme.

Barstar: Codes for a specific inhibitor that neutralises Barnase.

The fundamental mechanism is illustrated in Fig. 17, showing how male sterility is engineered.

Fertility restoration through Barstar expression

For practical hybrid seed production, the male sterility must be reversible in the F1 generation. This is achieved through the Barstar gene, as shown in Fig. 18.

Integrated system for hybrid development

The complete Barnase-Barstar system for hybrid development is summarised in Fig. 19, integrating both sterility induction and fertility restoration. The system shows how tapetum-specific Barnase expression causes male sterility and how Barstar introduction through crossing restores fertility in F1 hybrids, enabling commercial hybrid seed production.

Practical implementation and line maintenance

Unlike conventional CMS systems that require three distinct lines (A, B, R), the GE system offers a more streamlined approach. The practical implementation was illustrated in Fig. 20. The system utilises: (A-line)- female parent containing Barnase gene with linked herbicide resistance; (B-line)- maintainer without transgenes; (C-line) - restorer parent homozygous for Barstar. Herbicide application eliminates non-transgenic plants, ensuring purity of the male-sterile line. The F1 hybrid (A × C) contains both Barnase and Barstar, resulting in fertility restoration.

Advantages and applications

The Barnase-Barstar system offers several advantages:

Precision: Tapetum-specific expression ensures targeted male sterility without affecting female fertility.

Efficiency: Eliminates the need for complex CMS line development and maintenance.

Reliability: Provides stable male sterility across environments.

Selectability: Linked herbicide resistance enables efficient maintenance of male-sterile lines.

DMH-11, developed using this system by Professor Deepak Pental at the University of Delhi, has demonstrated a 28–37 % yield

advantage over conventional varieties in multi-location trials supervised by ICAR- Directorate of Rapeseed-Mustard Research (DRMR), Bharatpur, Rajasthan, India.

Current status of released hybrids in India

Persistent research efforts have culminated in the release of several rapeseed-mustard hybrids for cultivation in India. A comprehensive list of these hybrids, along with their species, year of release, developing institute and the CMS system used, is presented in Table 4. This overview helps in understanding the progress and the specific technologies that have been deployed commercially

As detailed in Table 4, the released hybrids are primarily based on different CMS systems, each with its own advantages and limitations. The early hybrids like PGSH-51 were developed in *B. napus*, while the focus later shifted to *B. juncea*, the predominant Indian species. The table highlights that the Moricandia CMS system has been the most frequently used in *B. juncea* hybrids from both public (e.g., NRCHB 506) and private (e.g., coral series) sectors. It also shows the recent advancement with the approval of the transgenic DMH-11 hybrid, which uses the Barnase/Barstar system, marking a significant milestone in India's hybrid mustard journey.

Challenges in mustard hybrid adoption and public perception

Despite the clear agronomic and economic potential of mustard hybrids, their widespread adoption in India has faced several significant hurdles. Understanding these challenges is crucial for formulating strategies to overcome them.

Agronomic and quality constraints

Several technical issues have been noted in existing hybrids that affect farmer acceptance:

Seed size issues: Hybrids like DMH-1 and NRCHB-506 produce smaller seeds (approximately 3.5 g test weight) compared to conventional varieties like RH-725 (approximately 7 g), primarily due to negative effects of sterile cytoplasm (6). Although seed size doesn't necessarily correlate with oil content, it creates a perception of inferior quality among farmers and traders.

Purity concerns: In some CMS systems, only around 85 % of seeds produced are pure hybrids due to male sterility breaks in female lines, raising concerns about genetic purity and performance consistency.

Table 4. Commercially released rapeseed-mustard hybrids in India.

Sl. No	Hybrid	Brassica sp.	Released year	Institute/Company	CMS system	Key features/issues
1.	PGSH-51	<i>B. napus</i>	1996	PAU, Ludhiana	<i>Tournefortii</i>	First hybrid of Gobhi sarson.
2.	Hyola 401	<i>B. napus</i>	1997	Advanta India Ltd.	-	Private sector hybrid.
3.	NRCHB 506	<i>B. juncea</i>	2008	DRMR, Bharatpur	<i>Moricandia</i>	Public sector hybrid; known for smaller seed size (6).
4.	NRCHB 101	<i>B. juncea</i>	2009	DRMR, Bharatpur	<i>Moricandia</i>	Public sector hybrid.
5.	Coral 432	<i>B. juncea</i>	2010	Advanta India Ltd.	<i>Moricandia</i>	Private sector hybrid.
6.	DMH-1	<i>B. juncea</i>	2010	Univ. of Delhi	"126-1"	Constrained by the availability of complete sterility maintainers
7.	Coral 437	<i>B. juncea</i>	2012	Advanta India Ltd.	<i>Moricandia</i>	Private sector hybrid.
8.	*DMH-11	<i>B. juncea</i>	2022* (Approved)	Univ. of Delhi	Barnase/Barstar	Transgenic hybrid; 28 % yield advantage over Varuna; awaiting final notification.

*DMH-11 has received regulatory approval from GEAC (2022) but its commercial cultivation is subject to final government notification.

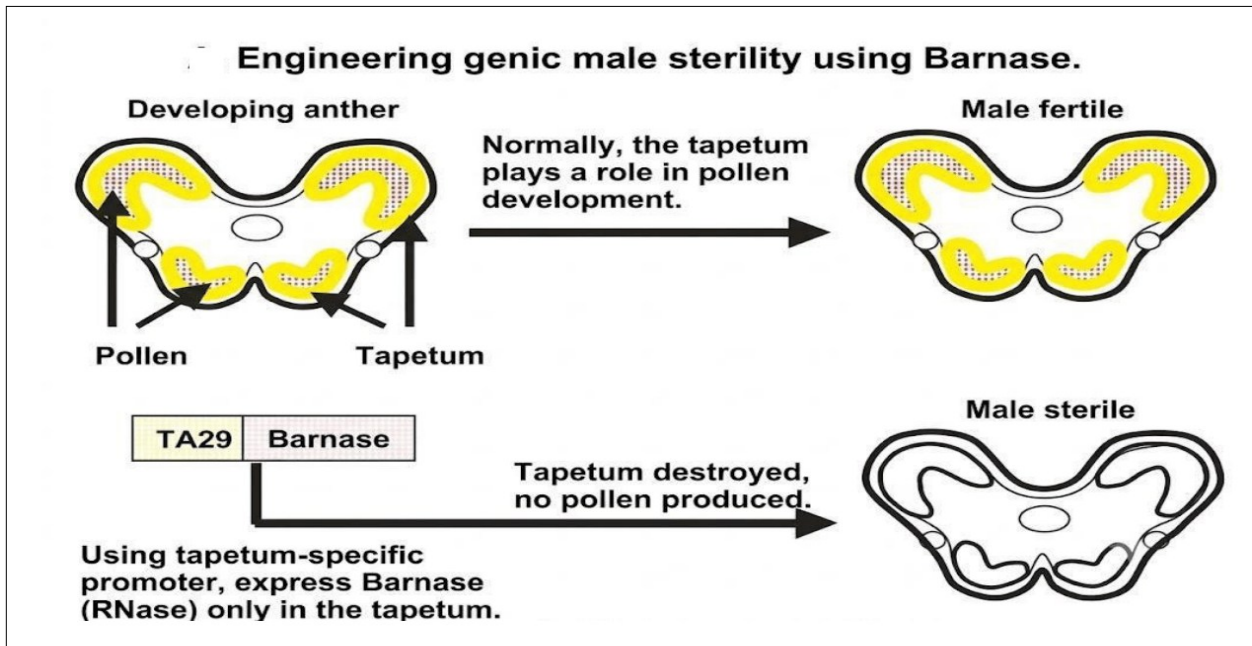


Fig. 17. Engineering male sterility using Barnase.

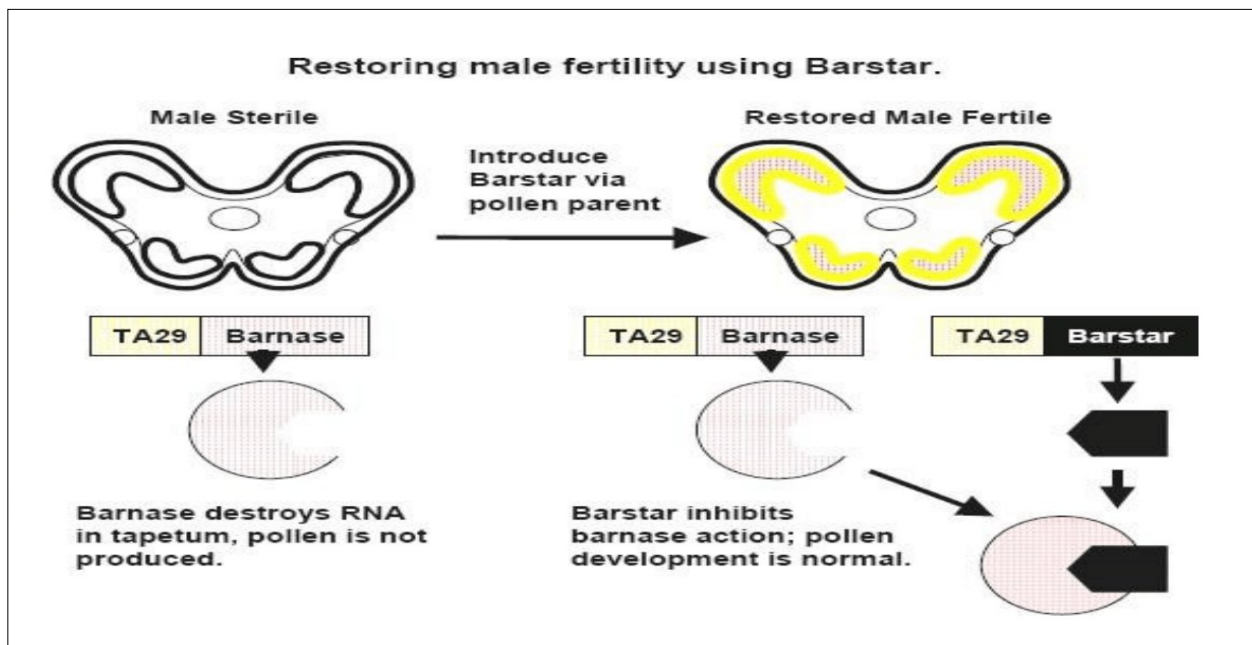


Fig. 18. Restoring male fertility using Barstar.

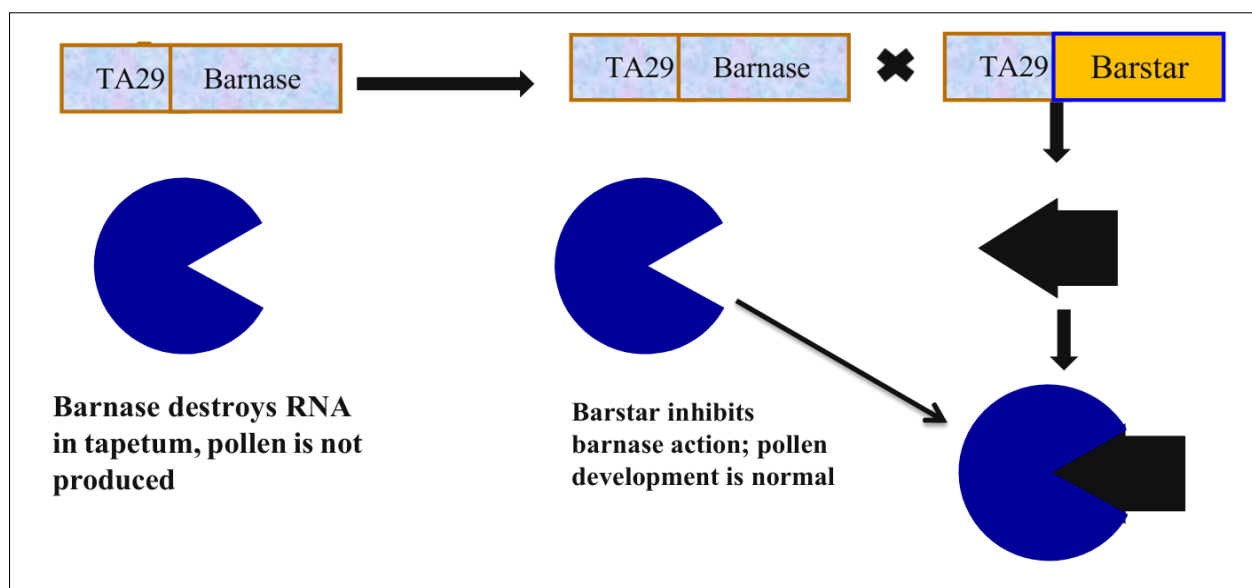


Fig. 19. Complete mechanism of genetically engineered male sterility using Barnase and fertility restoration using Barstar.

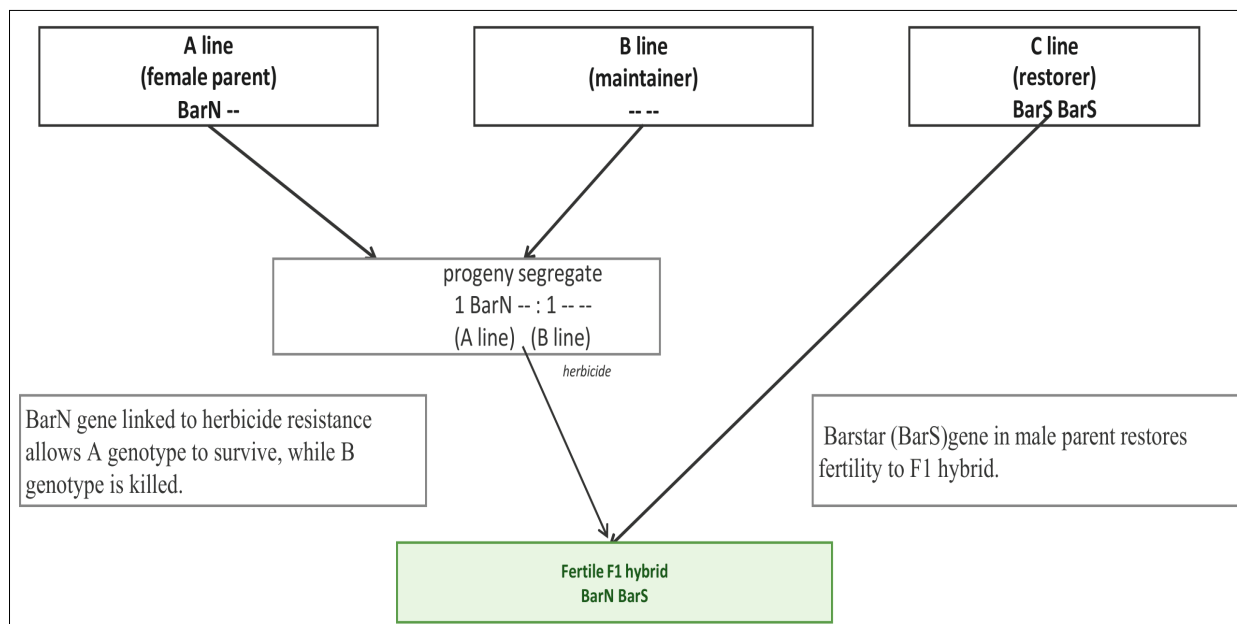


Fig. 20. Hybrid seed production scheme using the Barnase-Barstar system.

Market and perception barriers

Farmer preferences: There exists a persistent and irrational preference for bolder seeds in the market, despite evidence that smaller seeds can have comparable oil content and yield potential.

Supply chain inertia: Brokers and traders are accustomed to specific seed types, creating resistance in the marketing chain against hybrids with different physical characteristics.

The transgenic debate: Addressing public concerns

The approval of genetically modified (GM) mustard DMH-11 has generated significant scientific and public discourse. Based on comprehensive regulatory reviews, the following key concerns have been addressed.

Food and feed safety

Protein digestibility: All introduced proteins (Barnase, Barstar, PAT) are rapidly digested by pepsin in the mammalian stomach, similar to other dietary proteins.

Compositional analysis: Extensive studies have confirmed that GM mustard oil is compositionally identical to conventional mustard oil, containing no detectable novel proteins. The leaves for vegetable use show no alterations in nutrient profiles.

Animal feeding studies: Long-term animal feeding studies have demonstrated no adverse effects on livestock health or productivity.

Environmental biosafety

Gene flow assessment: Studies have shown negligible crossability with wild relatives, minimising environmental risks.

Pollinator safety: Comprehensive research supervised by regulatory authorities found no adverse effects on honey bees or other pollinator species.

Soil health: Investigations into soil microbial diversity around GM mustard cultivation sites revealed no significant alterations to soil ecosystem functioning.

Socio-economic considerations

Herbicide use: Concerns about increased herbicide use are mitigated by the fact that glufosinate is not subsidised and therefore unlikely to be used indiscriminately.

Labor impact: The technology aligns with national trends of agricultural labour scarcity and supports skill development initiatives by enabling more efficient farming practices.

Current status and regulatory framework

Progress in hybrid development: The persistent research efforts have led to the release of several rapeseed-mustard hybrids in India, as comprehensively documented in Table 1. The current portfolio includes hybrids based on multiple CMS systems (*Moricandia*, *Tournefortii*, 126-1) and the recently approved transgenic Barnase-Barstar system.

Regulatory milestones for genetically modified mustard: India has established a robust regulatory framework for genetically modified crops under the Environment Protection Act (1986). DMH-11 has successfully navigated this rigorous pathway:

Comprehensive assessment: The hybrid underwent extensive evaluation by the GEAC and its sub-committees.

Multi-location trials: Three years of supervised trials across eight locations demonstrated consistent yield advantages of 28–37 % over conventional checks.

Biosafety clearance: The Ministry of Environment, Forest and Climate Change granted environmental clearance in 2022, marking a significant milestone for agricultural biotechnology in India. Despite this comprehensive approval process, the final notification for commercial cultivation remains pending, highlighting the complex interplay between scientific evidence and public policy in agricultural biotechnology.

Future thrust areas

To break the yield plateau and achieve self-sufficiency in edible oils, a multi-pronged research strategy is essential. The future of hybrid development in *B. juncea* hinges on the following critical thrust areas.

Improvement of parental lines: Continuous refinement of both female (A and B lines) and male (R lines) parents is paramount. This involves enhancing them per se performance for core economic traits like yield potential and incorporating robust resistance to key biotic (aphids, white rust, alternaria blight) and abiotic (drought, heat, salinity) stresses.

Boosting the level of heterosis in cytoplasmic genetic male sterility-based hybrids: A primary goal is to elevate heterosis levels beyond the current ~15 % observed in public-sector hybrids to the commercially viable target of 25–35 %. This requires the identification and development of parental lines with higher inherent combining ability and the minimisation of the biological penalty associated with alloplasmic cytoplasms.

Development of defined heterotic pools: Moving from ad hoc crosses to a structured breeding strategy involving the creation of distinct heterotic pools (e.g., Indian gene pool vs. East European gene pool) will allow for more predictable and sustained exploitation of heterosis.

Introduction of novel genes from wild species: Broadening the genetic base through the introgression of valuable alleles from wild and alien species is crucial. Species like *B. fruticulosa* offer genes for resistance to mustard aphids and sclerotinia stem rot, which can be transferred into cultivated backgrounds to create more resilient hybrids.

Creation of new variability through re-synthesis and derived *B. juncea*:

Re-synthesis of *Brassica juncea*: This strategy involves recreating the allotetraploid *B. juncea* (AABB) by crossing its diploid progenitor species, *B. rapa* (AA) and *B. nigra* (BB). This taps into the enormous, untapped variability present in the primary gene pools, effectively broadening the genetic base of the cultivated species.

Development of derived *Brassica juncea* types: Creating new, high-photosynthesis capacity *B. juncea* lines (AABB) through interspecific hybridisation between *B. napus* (AACC) and *B. carinata* (BBCC) represents a promising avenue for breaking current yield barriers.

Leveraging transgenic technology and molecular tools: The success of the Barnase-Barstar system in DMH-11 paves the way for developing next-generation hybrids. Future efforts must include:

- Utilising this robust pollination control system to create higher-yielding and canola-quality hybrids.
- Employing MAS and genomic tools to pyramid multiple traits precisely and efficiently, overcoming linkage drag.
- Exploring gene editing to develop novel genetic variations for key traits.

Conclusion

India's annual expenditure of over INR 1.5 trillion on edible oil imports underscores the urgent need to boost domestic oilseed productivity through heterosis breeding. Hybrid development in *Brassica juncea* has advanced through distinct phases from the exploration of diverse cytoplasmic male sterility (CMS) systems (*Raphanus/ogu*, *Moricandia arvensis*, *Brassica tournefortii*) to genetically engineered approaches yet widespread adoption remains constrained by biological penalties, unstable sterility expression, complex restoration genetics and market barriers related to seed size. A persistent concern is that the combining ability and heterosis of parental lines may not be retained after CMS background conversion, necessitating rigorous re-evaluation of converted lines.

The regulatory approval of DMH-11 (Barnase-Barstar system) marks a landmark advancement, offering precise and stable pollination control independent of alien cytoplasm constraints, with a demonstrated 28–37 % yield advantage over conventional varieties. Achieving commercial self-sufficiency requires: (i) developing diverse and stable CMS systems with effective maintainers and restorers; (ii) elevating public-sector heterosis levels from the current ~15 % toward the 25–35 % commercial threshold through broadening of the genetic base via alien introgression and re-synthesis of *Brassica* digenomics; and (iii) integrating genomic selection, gene editing and MAS with conventional breeding to minimise linkage drag and pyramid key traits efficiently.

The successful commercialisation of high-yielding, resilient mustard hybrids underpinned by sound science and forward-looking policy is imperative to overcome the yield plateau. By harnessing heterosis to its fullest potential, India can stride confidently toward Atmanirbharta (self-reliance) in edible oil production, securing farmer prosperity and national food security.

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Authors' contributions

CK and KC conceptualised and wrote the manuscript. S and AK edited the manuscript. Proofreading was done by SB and PKB. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that AI-assisted writing tools (Paperpal and Grammarly) were used during the preparation of this manuscript for language editing, grammar correction and structural improvement purposes. All scientific content, data interpretation and conclusions are solely the responsibility of the authors. The use of AI tools was limited to language refinement and did not involve generation of scientific data, figures or references.

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