



Unlocking the Genetic Potential of Indian Mustard (*Brassica juncea* L.): A Review on Advances in Breeding Approaches

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10.18805/ag.D-6254

ABSTRACT

Indian mustard, *Brassica juncea* L., is one of the most important oilseed crops in India, requiring improved yield, quality and disease resistance. Breeding programs have been instrumental in upgrading its genetic potential. The progress being made in breeding Indian mustard, highlighting conventional and modern breeding approaches, achievements and in future directions. Recent advances in breeding approaches have provided tools to overcome conventional breeding limitations and accelerate genetic improvement. An overview of major advancements and strategies discussed in this review article. There is an urgent need to harmonize traditional practices with modern technologies to achieve sustainable growth in mustard production. This integrated approach focuses on overcoming challenges such as low yield, disease susceptibility and environmental stresses, while addressing the economic and nutritional needs of a growing population. This synergy aims to enhance food security and promote economic sustainability in the face of climate change and evolving agricultural demands by developing high-yielding, disease-resistant varieties with superior oil quality and adaptability.

Key words: Climate change, Disease resistance, Genetic improvement, Indian mustard, Quality, Yield.

Indian mustard (*Brassica juncea*), is a leading oilseed crop, plays a significant role in global agriculture and food security. As a robust source of edible oil and a key contributor to the livelihoods of millions of farmers in South Asia, especially India, its cultivation is critical to the agricultural economy. However, the rapidly changing climate scenario poses serious challenges to the sustainable production of Indian mustard. Rising global temperatures, erratic rainfall patterns, increased frequency of extreme weather events and growing soil salinity are already impacting crop productivity. In India, rapeseed-mustard is cultivated across a variety of agro-climatic zones, ranging from the North-East and North-West to Central and Southern states. It is grown under various conditions, including as a sole crop or mixed crop and across different planting times such as early, timely, or late. Additionally, it thrives in both rainfed and irrigated systems, as well as in saline or alkaline soils (Chauhan *et al.*, 2011). Climatic variations such as drought have high level of impingement on the yield of rain-fed crops (Kumar and Upadhyay, 2019).

Mustard, being sensitive to both heat and drought stress, often faces yield losses due to terminal heat during flowering and seed filling stages. Furthermore, climate-induced changes have intensified pest and disease pressures, which further threaten crop stability. Amidst these challenges, breeding Indian mustard for climate resilience has become an urgent priority. Advances in genomics, biotechnology and breeding strategies offer significant opportunities to develop varieties that can withstand abiotic stresses such as heat, drought and salinity, as well as biotic stresses like pests and diseases. Indian mustard can be adapted to thrive in the unpredictable environmental conditions brought on by climate change by

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How to cite this article: Anushree, Kumar, V. and Nagarjun, P. (2025). Unlocking the Genetic Potential of Indian Mustard (*Brassica juncea* L.): A Review on Advances in Breeding Approaches. Agricultural Science Digest. 1-9. doi: 10.18805/ag.D-6254.

Submitted: 26-11-2024 **Accepted:** 24-02-2025 **Online:** 23-04-2025

leveraging both traditional and modern breeding approaches. This pursuit is very crucial not only for the sustainability of the mustard crop but also for security in the supply chain of the oilseed and supporting farmer income, as it helps address the nutrition needs of a fast-growing population during an uncertain future climate.

Conventional breeding approaches

Traditional breeding methods continue to play a fundamental role in mustard improvement. Hybridization, for instance, involves crossing diverse genotypes to introduce variability, allowing the selection of desirable traits such as higher oil content, disease resistance and enhanced stress tolerance. Additionally, mass and pure line selection are key techniques used to maintain and stabilize high-yielding varieties, ensuring consistent

performance over time. Mutation breeding, another important traditional method, utilizes chemical or physical mutagens, such as gamma radiation, to induce genetic variations. This approach helps develop traits like early flowering and improved stress resistance, contributing to more robust and adaptable mustard varieties. These time-tested techniques remain essential in the ongoing effort to enhance mustard production.

Genomics-assisted breeding

Genomics-assisted breeding has significantly advanced the improvement of Indian mustard, driven by progress in molecular biology. Genome sequencing has played a pivotal role by providing a detailed understanding of the genetic architecture of Indian mustard, which enables precise gene targeting for desired traits. Marker-assisted selection (MAS) leverages molecular markers to efficiently select for important traits, such as resistance to diseases like *Alternaria* blight and *Sclerotinia* rot. Additionally, quantitative trait loci (QTL) mapping allows for the identification of specific genetic regions linked to yield-related traits and abiotic stress tolerance, facilitating more focused and effective breeding strategies. Genome-wide association studies (GWAS) further enhance breeding efforts by associating genetic markers with phenotypic traits, aiding the identification of candidate genes that can be targeted for improvement. These genomic tools are revolutionizing the way Indian mustard is bred, making it possible to develop more resilient and higher-yielding varieties. Advances in molecular biology have revolutionized Indian mustard breeding.

Biotechnological approaches

Genetic engineering

Insertion of genes such as *bar* or EPSPS for herbicide resistance.

Insertion of stress-tolerance genes, such as drought and salinity.

CRISPR-Cas9

A precise genome-editing tool used for removing unwanted traits or enhancing desirable ones like oil quality or pest resistance.

Transgenic approaches

Developing GM mustard (e.g., DMH-11) for higher yield and hybrid vigor.

Breeding for abiotic stress tolerance

Crop development for abiotic and biotic stress resistance is necessary for food security against climate change. Some of the most important traits that need to be targeted in order to provide drought tolerance include enhanced water use efficiency, a deeper root system and an osmotic adjustment to help maintain plant productivity even at low water availability. A drought is an extended period of months or years when region notes a deficiency in its water supply,

whether surface or underground water because of consistent below average precipitation. It is a global phenomenon which causes significant damage due to stochastic nature in occurrence and severity (Karthika *et al.*, 2017).

Approaches include phenotypic selection, focusing on screening for traits such as leaf wilting and chlorophyll retention under water stress, while QTL mapping and marker-assisted breeding identify genetic loci for root architecture and photosynthetic efficiency. Biotechnology plays a critical role by introducing genes such as DREB (Dehydration-Responsive Element-Binding protein) that enhance the plant's ability to cope with drought.

For heat tolerance, breeding efforts aim to sustain pollen viability, maintain photosynthetic efficiency and ensure stable yields under high temperatures. Germplasm screening for traits like early maturity and favourable canopy structure helps plants avoid peak heat stress periods. Advanced techniques such as CRISPR-Cas9 and transgenic approaches are employed to modify or introduce heat shock proteins (HSPs), which protect cellular functions during heat stress. Heat stress, exacerbated by human activities, has become a significant challenge for the growth and development of agricultural crops, including rapeseed-mustard. While early sowing of Indian mustard offers several benefits, as highlighted by Kaur *et al.*, 2009, elevated temperatures during the germination phase can severely impact plant emergence, leading to a weak plant stand. Late sowing of Indian mustard further aggravates the issue, as terminal heat stress markedly reduces its yield potential compared to timely sowing (Patidar *et al.*, 2020). Fluctuations in temperature during the growth period can damage the inter-molecular interactions needed for proper growth, thus impairing plant development and fruit set, which leads to significant yield loss with greater risks for future global food availability, food accessibility, food utilization and food systems stability (Tirkey *et al.*, 2022). Hot soil conditions during the emergence stage can result in considerable economic losses (Azharudheen *et al.*, 2013).

Ironically, the negative impact of high temperatures on yield can be minimized if such conditions occur during the early stages of pod formation. Among Brassica species, *B. rapa* exhibits higher sensitivity to heat stress, while *B. juncea* and *B. napus* show comparable susceptibility (Angadi *et al.*, 2000). Optimal growth temperatures vary among these species, with *B. napus* requiring lower temperatures than *B. juncea* and *B. rapa* (Young *et al.*, 2004). Elevated temperatures tend to increase the number of pods formed, though they simultaneously reduce seed weight. High temperatures directly interfere with the development of reproductive organs, further hampering productivity.

To address these challenges, research under controlled environments is crucial for identifying critical temperatures, stages of reproductive organ sensitivity,

genotypic variations and the relationship between source and sink under heat stress. Validation of such findings under natural conditions is essential to develop effective strategies for mitigating the adverse effects of high temperatures (Kumar *et al.*, 2013).

Several species exhibit genetic adaptations to tolerate abiotic stresses like drought, salt and freezing, through the expression of specific genes. In *Arabidopsis*, the DREB1A gene encodes a dehydration response element-binding protein that confers tolerance to drought, salt and freezing conditions (Kasuga *et al.* 1999). The SOS1 gene encodes a plasma membrane-bound Na⁺/H⁺ antiporter, enhancing salt tolerance (Shi *et al.*, 2000), while AtNHX1 and AtHKT1 function as Na⁺/H⁺ antiporters at the vacuolar level and Na⁺ transporters, respectively, contributing to salt tolerance (Zhang *et al.*, 2001 and Berthomieu *et al.*, 2004-14.). Other key genes include FTA, a farnesyltransferase involved in drought tolerance (Wang *et al.*, 2005) and AtFTB, the β -subunit of farnesyltransferase, which also aids in drought resistance (Wang *et al.*, 2009).

In *Arthrobacter globiformis*, the codA gene encodes choline oxidase, which enhances salt tolerance (Wang *et al.*, 2009). Among the *Brassica* species, *B. rapa* harbors BrERP4, an ethylene-responsive factor gene that provides resilience to drought and salt stress (Seo *et al.* 2010) and BrGI, which enhances salt tolerance by reducing GI expression (Kim *et al.*, 2016). In *B. napus*, genes like AtDWF4 improve defense gene expression for drought and heat tolerance (Maqbool *et al.* 2002), while BnNRT1 and BnLEA4-1 are salt-responsive genes with late embryogenesis-abundant (LEA) proteins (Agarwal *et al.*, 2006 and Dalal *et al.*, 2009). The BnLAS gene, a GRAS family transcriptional regulator, is drought-resistant (Yang *et al.*, 2011) and DREB improves general abiotic stress tolerance (Lata *et al.*, 2011). BnSIP1-1 plays a role in ABA synthesis and signalling, enhancing tolerance to salt and osmotic stress (Luo *et al.*, 2017), while AnnBn1 encodes Ca²⁺-binding membrane proteins that contribute to drought resilience (Xiao *et al.*, 2012).

In *B. oleracea* var. botrytis, the APX and SOD genes help mitigate oxidative stress, enhancing salt tolerance (Ali *et al.*, 2016). *B. juncea* cv. varuna expresses Glyoxalase I Lectin, which detoxifies methylglyoxal into d-lactate, aiding in drought and salt tolerance (Shinwari *et al.*, 1998). For *B. juncea*, notable genes include BrECS, encoding glutamylcysteine synthetase for salt stress (Bae *et al.*, 2013) and AtLEA4-1, producing LEA4 proteins (Saha *et al.*, 2016). Gly I also detoxifies methylglyoxal, enhancing salt resilience (Rajwanshi *et al.*, 2016). The AnnBj2 gene is associated with the upregulation of ABA-dependent and independent stress response genes, improving tolerance to salt stress (Ahmed *et al.*, 2017).

These genetic components have collectively provided insightful information on crop breeding programs, especially those involving crop improvement toward resilience under stressful environmental conditions.

Traits for salinity and waterlogging tolerance include ion homeostasis, reduced sodium uptake and anaerobic tolerance. Cross-species transfer of salt-tolerant traits from *Brassica napus* and *B. carinata* has been successful, while genomic selection focuses on genes like NHX1, which control the balance of sodium and potassium. These strategies enable crops to grow in saline soils and under waterlogged conditions.

Breeding for biotic stress tolerance

Biotic stress resistance involves combating diseases like white rust, *Alternaria* blight and *Sclerotinia* stem rot, as well as pests like aphids that are exacerbated by changing climates. Gene pyramiding combines multiple resistance genes for durable disease resistance, while novel genes from wild relatives or landraces can be introgressed into high-performing varieties. Genetic engineering introduces pathogen recognition (R) genes for enhanced disease resistance and QTL-based breeding is used to develop pest-resistant crops.

Various biotic stresses impact rapeseed-mustard cultivation in India, including critical diseases and insect pests. Among the major diseases are *Alternaria* blight (*Alternaria brassicae* and *A. brassicicola*), white rust (*Albugo candida*), stem rot (*Sclerotinia sclerotiorum*), Rhizoctonia rot and downy mildew (*Peronospora brassicae*). In addition, insect pests such as aphids (*Lipaphis erysimi*), mustard sawfly (*Athalia proxima*) and painted bug (*Bagrada hilaris*) exacerbate the problem. While pest and disease management strategies like the application of fungicides, pesticides, biological agents and non-chemical methods are practiced, developing resistant or tolerant varieties through conventional and molecular breeding remains the most efficient, sustainable and environmentally friendly solution.

Alternaria blight

Alternaria blight (*Alternaria brassicae*), one of the most prevalent diseases in *Brassica* spp., causes significant yield reductions. The pathogen infects plants at all growth stages, with disease severity peaking during the rainy season. Studies indicate that *B. juncea* and *B. rapa* are more prone to infection compared to *B. carinata* and *B. napus*. Resistance sources have been identified in cultivars like *B. juncea* cv. Divya and wild relatives such as *Sinapis alba*, *B. maurorum*, *Diplotaxis berthautii* and *D. erucoides* (Sharma *et al.* 2002). Traits like higher phenolic compound levels (polyphenol peroxidase, oxidase, catalase), reduced nitrogen content, increased leaf sugar concentration and greater wax deposition on leaves have been associated with disease resistance (Kumar *et al.* 2008). Despite these findings, the use of wild relatives is limited by pre- and post-fertilization barriers in hybridization programs. Notable tolerant *B. juncea* genotypes, including PHR 2, RC781, Divya, PAB 9534 and EC 399301, are being utilized in breeding for resistance (Chauhan *et al.*, 2011).

Sclerotinia rot

Sclerotinia rot, caused by *Sclerotinia sclerotiorum*, has become a severe threat in the cultivation of rapeseed-mustard, having shifted from being a minor to a major issue due to climate change. The disease causes premature ripening, affecting plant growth and productivity significantly. The wide host range of the pathogen presents a major challenge to the breeding for resistance (Chauhan et al., 2011). Thus, there is a need for innovative strategies combining advanced breeding techniques and effective management practices.

In conclusion, it is high time that through the development of resistant varieties, integrated pest management strategies, the biotic stresses be addressed, which are detrimental to sustaining rapeseed-mustard productivity in India. The breeding process must focus on harnessing genetic resources and novel means for long-term durability.

Genetic diversity from the wild relatives of Brassica species such as *Brassica nigra* and *Sinapis alba* offers a large pool of genes conferring resistance to stress factors. This also increases variability for traits like heat tolerance and water-use efficiency, so that a wide genetic base exists for further breeding. Short-duration and early-maturing varieties help crops avoid terminal heat and drought stress and the growth cycle of the crop aligns with the window of favourable climate. Genomics-assisted breeding, including GWAS and genomic selection, has accelerated the discovery of climate-resilient traits and the prediction of high-performing genotypes. Breeding strategies are also further refined using techniques such as transcriptomics and proteomics for stress-related pathways. Biotechnological interventions that include transgenic methods introduce DREB critical genes for drought, NHX1 for salinity and HSPs for heat. Using CRISPR-Cas9 genome editing precisely allows the elimination of deleterious genes and increasing those that are advantageous in the quest to develop stress-resistant plants.

High-throughput phenotyping technologies, including thermal and hyperspectral imaging, finally offer fast and highly accurate assessments of stress response traits, including canopy temperature and chlorophyll fluorescence. Stacking these strategies in breeding programs will effectively generate resilient varieties capable of performing under the non-optimal conditions of climate change.

Breeding achievements in Indian mustard in India

Indian mustard (*Brassica juncea* L.), one of India's most important oilseed crops, has witnessed significant advancements through systematic breeding programs to address productivity, stress tolerance and oil quality. Indian mustard is an important crop for edible oil, biofuel and livestock feed. Breeding programs aim to improve its yield, quality and disease resistance.

Key achievements

In the cultivation of mustard, various environmental conditions and stresses such as salinity, high temperature, drought and pest resistance play a crucial role in selecting the appropriate variety for optimal yield. For regions facing salinity, varieties like CS 234-4, Pusa Vijay and NRCDR 601 are recommended due to their ability to tolerate saline conditions. In areas with high temperatures, mustard varieties such as Pusa Mahak, Kanti and Pusa Agrani are preferred, as they are better suited to withstand heat stress. For those seeking high oil content and early maturing varieties, NRCDR 601, CS-52, Pusa Agrani and Pusa Mahak are notable options, providing high-quality oil with early harvest potential.

Varieties suitable for intercropping are PM 26 and PM 27, which are tolerant to grow well when sown along with other crops. In non-conventional growing regions, Pusa Agrani and RH-819 mustard varieties performed better in the less conventional environment. For late sown conditions, Pusa Agrani and Pusa Bold varieties have been recommended, as they can mature quickly if planted late. Frost tolerance is important in colder areas. Varieties such as Pusa Swarnim, Pusa Mahak and RH-781 have higher tolerance against frost damage. Narendra Rai-1, Pusa Agrani and Vardan varieties are best suited for drought-prone, rainfed conditions due to their tolerance to drought. For irrigated systems, varieties such as Pusa Jai Kisan and Gujarat Mustard 2 are ideal due to their compatibility with well-watered conditions. When considering mustard types with low erucic acid and glucosinolates, varieties like Pusa Mahak and Pusa Jai Kisan are favored for their lower content of these compounds, making them suitable for human consumption. Regarding disease resistance, varieties like Pusa Agrani, Pusa Vijay and Kanti exhibit resistance to white rust, while varieties such as RH-819, Vardan and Swarn Jyoti are more resistant to powdery mildew and Alternaria blight. Additionally, mustard varieties with wider adaptability, such as RH-781, GM1 and Pusa Bahar, are versatile and can be grown across a range of conditions.

For specific types of mustard, such as Indian mustard, varieties like CS-54, Pusa Vijay, NRCDR 2, CS 234-4, Pusa Agrani, Vardan, Narendra Swarna Rai 8 and RH-30 are highly recommended. Karan Rai (rapeseed) varieties like Pusa Aditya, DRMR 150-35 and Pusa Swarnim are suitable for cultivation in rapeseed systems, while Yellow Sarson is represented by varieties like NRCYS 05-01. For Gobhi Sarson (cauliflower mustard), varieties such as Hyola 401, GSC 5, GSC 6, NUDB 26-11 and Teri Uttam have shown good performance under diverse conditions.

This comprehensive list of recommended varieties is tailored to different growing conditions, stresses and production systems, ensuring that farmers can select the most suitable mustard variety to meet their specific needs.

Development of high-yielding varieties

Indian mustard, *Brassica juncea* L., is an important oilseed crop in India. The crop is used for edible oil, biofuel and livestock feed. Breeding programs have considerably improved crop productivity, stress tolerance, disease resistance and nutritional quality. Development of high-yielding varieties like Varuna, with its adaptability and higher seed yield under various conditions, is a landmark achievement. Other varieties like RH-749, Pusa Bold and Pusa Vijay are targeted at various agro-climatic zones, yielding above 40% oil content.

Disease and pest resistance

To address biotic stress, varieties like NRCDR-601 and NRCHB-101 have been bred for resistance against diseases such as white rust, *Alternaria* blight and *Sclerotinia* stem rot, along with tolerance to aphids, a common pest.

Development of hybrids

Hybrid breeding has resulted in superior hybrids like NRCHB-506, using CMS systems for better yield and oil content.

Transgenic systems such as barnase-barstar have further improved the production of hybrid seeds. Hybrids like DMH-1 and Coral 432 exhibit higher productivity and regional adaptability. For counteracting climate change, the breeders have come up with varieties which are climate-resilient, like DRMR 150-35 that shows tolerance for heat and salinity, thereby suitable for sowing during the early rains. Such efforts in terms of quality oil have given rise to varieties such as Pusa Mustard-30, having lesser erucic acid and glucosinolate, making the oil less toxic for humans and the meal value higher for animal consumption.

Climate-resilient varieties

India's extensive mustard germplasm collection, with over 14,700 accessions conserved at institutions like ICAR-DRMR, has been instrumental in introducing valuable traits for stress resistance and adaptability. Modern breeding approaches have leveraged molecular tools such as marker-assisted selection (MAS) to incorporate traits like disease resistance and improved oil quality. CRISPR-Cas9 genome editing and transgenic technologies have enabled precise genetic modifications, including the introduction of stress-tolerance genes like *DREB* for drought and herbicide resistance genes like *bar*. Double haploid technology, particularly through microspore culture, has shortened breeding cycles by producing homozygous lines in a single generation.

Significant advancements in genomics have further revolutionized Indian mustard breeding. Whole-genome sequencing has provided insights into the genetic architecture of the crop, enabling precise gene targeting. Techniques such as QTL mapping, GWAS and genomic selection have facilitated the identification and prediction of high-performing genotypes with yield and stress-tolerance traits. High-throughput phenotyping tools like

thermal imaging and chlorophyll fluorescence sensors have streamlined the evaluation of large populations.

Efforts to enhance nutritional quality focus on breeding varieties with traits like low erucic acid, high oleic acid and omega-3 fatty acids, employing both genetic engineering and MAS. Farmer participation in breeding programs ensures the development and adoption of varieties tailored to specific microclimates and local needs. To adapt to degraded soils and polluted environments, breeders are targeting climate-smart agronomic traits such as improved nitrogen assimilation, heavy metal tolerance and nutrient-use efficiency. Despite these achievements, challenges persist, including limited genetic diversity for stress tolerance, small seed size and low hybrid seed purity. The integration of advanced biotechnologies like CRISPR-Cas9 with traditional methods offers promising solutions, though regulatory hurdles for transgenic and genome-edited varieties need to be addressed. Continued innovations in breeding and agronomy are essential for ensuring the sustainable production of Indian mustard amidst changing climate scenarios.

Table 1 outlines the major CMS systems in rapeseed-mustard, highlighting their discovery, year of identification, fertility restoration status and references.

Varieties like DRMR 150-35 are tailored for early sowing under rainfed conditions and demonstrate heat tolerance and salinity tolerance. These developments are critical for addressing challenges posed by climate change.

Enhancing Nutritional quality and quality traits

Indian mustard (*Brassica juncea*) has been the subject of extensive breeding programs, both for improvement of economic traits and for enhancing nutritional values. Significant advancements have been achieved through wide hybridization techniques aided by embryo rescue. These efforts have led to the transfer of key traits such as high oil content, shattering tolerance, resistance/tolerance to fungal diseases like *Albugo candida* and *Alternaria brassicae* and seed quality improvements, including low erucic acid, high oleic acid, yellow seed coat colour and double low traits. Seven improved genotypes with enhanced quality have been registered with ICAR (Agnihotri *et al.*, 2004).

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Despite progress in improving mustard varieties, anti-nutritional factors in mustard seed meal continue to pose challenges. Substances like fibers, tannins, phytic acid, glucosinolates and sinapin reduce its overall feed value (Chauhan *et al.*, 2002). Among these, glucosinolates—a group of plant-derived thio-glucosides commonly found in the Cruciferae family—are particularly significant for determining the quality of seed meal. Over 120 types of glucosinolates have been identified (Fenwick *et al.*, 1983).

While the hydrolysis products of glucosinolates can be harmful-leading to reduced feed palatability, interference with iodine absorption by the thyroid and lower feed efficiency and weight gain in non-ruminants like pigs and poultry (Bell, 1984)-some glucosinolates have health-promoting properties. Notably, they have been linked to reduced cancer risk in humans (Zhang *et al.*, 1994; Fahey *et al.*, 2001; Shapiro *et al.*, 2001; Mithen *et al.*, 2003). Epidemiological research has highlighted a correlation between the consumption of cruciferous vegetables and a reduced risk of cancers, including those affecting the lung, stomach, colon and rectum (Conaway *et al.*, 2001).

Efforts to enhance the nutritional quality of mustard began in the 1970s, focusing on lowering glucosinolate content to under 30 $\mu\text{mol/g}$ in defatted seed meal and reducing erucic acid levels to less than 2%-standards that align with global benchmarks for oil and seed meal quality. These initiatives led to the development of the first low-erucic acid variety, *Pusa Karishma*, for Indian mustard and the first double-low variety, GSC 5, for gobhi sarson (*B. napus*), released in 2004 and 2005, respectively (Chauhan *et al.*, 2002). Since then, five low-erucic acid varieties of Indian mustard and five double-low varieties of gobhi sarson have been successfully introduced.

Ongoing breeding programs aim to integrate low erucic acid and glucosinolate traits in Indian mustard, while optimizing agronomic characteristics to enhance the yield potential of double-low gobhi sarson varieties. These efforts are expected to meet international quality standards, improve economic returns and make rapeseed-mustard crops more beneficial for both human and animal consumption.

To adapt to degraded soils and polluted environments, breeders are targeting climate-smart agronomic traits such as improved nitrogen assimilation, heavy metal tolerance and nutrient-use efficiency. These varieties are aimed at

improving oil quality for human consumption and enhancing meal quality for livestock feed.

Genetic resource utilization

Despite these successes, the challenges remain in the form of limited genetic diversity for stress tolerance, small seed size and low hybrid seed purity. The integration of advanced biotechnologies like CRISPR-Cas9 with traditional methods offers promising solutions, though regulatory hurdles for transgenic and genome-edited varieties need to be addressed. Continued innovations in breeding and agronomy are essential for ensuring the sustainable production of Indian mustard amidst changing climate scenarios.

India has one of the largest collections of mustard germplasm, with over 14,700 accessions conserved at institutions like the ICAR-Directorate of Rapeseed-Mustard Research (DRMR). This diversity has been instrumental in breeding efforts to introduce new traits like stress resistance.

Biotechnological interventions

The biotechnological tools that have impacted mustard breeding include the use of modern tools for precise genetic modification and accelerated improvement of traits. Some of the important techniques emerging today include transgenic approaches, CRISPR-Cas9 genome editing and marker-assisted selection.

Transgenic approaches for stress tolerance

Transgenic technologies have been employed to introduce specific stress-tolerance genes into mustard, providing robust mechanisms to counteract environmental challenges.

Drought tolerance

The *DREB* (Dehydration Responsive Element Binding) gene enhances the plant's ability to withstand water scarcity by regulating stress-responsive pathways.

Table 1: Major CMS source for hybrid seed production in rapeseed-mustard.

MS system	Discovered by	Year	Fertility restoration	References
Ogura	Ogura	1968	Restorer gene available in <i>B. juncea</i>	Ogura <i>et al.</i> (1968)
Tour	Rawat and Anand	1979	Available in <i>B. napus</i>	Rawat <i>et al.</i> (1979)
Oxyrrhina	Prakash and Chopra	1988	No restoration available	Prakash <i>et al.</i> (1988)
Siifolia	Rao and coworkers	1994	No restoration available	Rao <i>et al.</i> (1994)
Trachystoma	Kirti and coworkers	1995	Single dominant gene available for restoration	Kirti <i>et al.</i> (1995)
Moricandia	Prakash and coworkers	1995	Single dominant gene reported for restoration	Prakash <i>et al.</i> (1995)
Cotyledonary	Kirti and coworkers	1995	Reported but not in use	Kirti <i>et al.</i> (1995)
Alba	Prakash and coworkers	1995	Available in <i>B. napus</i>	Prakash <i>et al.</i> (1995)
Lyratus	Banga and Banga	1997	Reported but not in use	Banga <i>et al.</i> (1997)
Canariense	Prakash and coworkers	2001	Reported but not in use	Prakash <i>et al.</i> , 2001
Erucoides	Bhat and coworkers	2006	Reported but not in use	Bhat <i>et al.</i> , 2006
126-1	Sodhi and coworkers	2006	Reported in <i>B. napus</i>	Sodhi <i>et al.</i> , 2006
Barthauti	Bhat and coworkers	2008	Reported but not in use	Bhat <i>et al.</i> , 2008

Source: Rapeseed-mustard breeding in India: Scenario, achievements and research needs by Subhas Chand, Om Prakash, *et al.* (2021).

Salinity tolerance

The *NHX1* gene encodes a sodium/hydrogen antiporter that improves salt stress tolerance by maintaining ion homeostasis.

Heat tolerance

Heat Shock Proteins (HSPs) stabilize cellular proteins and membranes under high-temperature stress, ensuring sustained plant growth and development.

CRISPR-Cas9 genome editing for precision breeding

The advent of CRISPR-Cas9 technology has provided unparalleled precision in genome editing, enabling targeted modifications to enhance stress resilience and productivity.

Stress resilience

Deletion or silencing of deleterious genes that impair stress responses, alongside the enhancement of beneficial genes, has opened new avenues for developing stress-resilient mustard varieties.

Hybrid seed production

Systems like barnase-barstar, combined with CRISPR-Cas9, are being employed to create male sterility and fertility restoration systems, ensuring efficient and cost-effective hybrid seed production.

Marker-assisted selection (MAS) for targeted trait incorporation

Marker-assisted selection is an integral part of modern breeding programs, allowing precise incorporation of desirable traits into mustard varieties. MAS has been particularly effective in.

Disease resistance

Identifying and integrating resistance genes against major pathogens like *Alternaria brassicae* and *Albugo candida*.

Oil quality improvement

Enhancing traits like low erucic acid and glucosinolate content, thereby aligning mustard oil with global quality standards.

Integrative approaches for genetic improvement

The combination of transgenic technologies, CRISPR-Cas9 genome editing and MAS is transforming mustard breeding. For example, CRISPR-Cas9 is being integrated with marker-assisted approaches to fine-tune multiple traits simultaneously, optimizing both agronomic and nutritional qualities. The barnase-barstar system, supported by advanced genome editing, exemplifies the synergy between these technologies, significantly enhancing genetic improvement programs.

These advancements represent a paradigm shift in mustard breeding, enabling the development of resilient, high-yielding and nutritionally superior varieties. Future efforts will focus on integrating these technologies with traditional breeding practices to meet the growing demands for sustainable and climate-resilient agriculture.

High-throughput phenotyping for stress traits

Use of modern imaging techniques including thermal and hyperspectral imaging to obtain rapid measurements on stress response traits like canopy temperature and chlorophyll fluorescence.

Hybrid breeding challenges

Despite the achievements, challenges like small seed size, low hybrid seed purity and farmer preference for bold seeds persist. Ongoing research is focusing on developing hybrids with improved seed size and higher oil content through transgenic and conventional approaches.

KEY CHALLENGES AND FUTURE DIRECTIONS

Indian mustard does not have as much genetic diversity against stress tolerance traits in the domesticated germplasm, which proves to be challenging for breeding high-stress varieties. To overcome this, there is a growing focus on integrating advanced biotechnologies with traditional breeding methods. This approach aims to enhance the genetic base of mustard, improving its ability to withstand various environmental stresses. However, efforts to develop transgenic and genome-edited varieties must also address regulatory hurdles, which can slow down their adoption. Despite these challenges, breeding for climate resilience in Indian mustard shows great potential for ensuring sustainable production in the face of changing climate conditions. The combination of advanced breeding techniques and agronomic innovations will play a crucial role in achieving this goal, helping farmers adapt to new environmental challenges. Efforts to breed for climate resilience in Indian mustard hold promise for ensuring sustainable production under changing climate scenarios. Advanced breeding techniques, combined with agronomic innovations, will play a pivotal role in this endeavour.

CONCLUSION

Unlocking the genetic potential of Indian mustard requires an integrative approach combining traditional and modern breeding techniques. Advances in molecular biology, biotechnology and data-driven methodologies offer tremendous potential to achieve sustainable productivity gains. However, challenges such as regulatory approvals for GM crops and climate variability must be addressed alongside these innovations.

Conflict of interest

All authors declared that there is no conflict of interest.

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