



REVIEW ARTICLE

Integrative approaches for mustard improvement: Bridging conventional breeding, genetics and biotechnological advances

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Abstract

Mustard is globally an oilseed crop that provides essential edible oil and industrial raw materials, particularly in regions like South Asia, Europe, and Canada, where it plays a critical role in agricultural economies and food industries. However, worldwide biotic and abiotic stresses pose major challenges to mustard production. Advances in conventional breeding techniques, genetics, and biotechnological tools hold immense potential for developing improved mustard varieties. This review elucidates the evolution of mustard breeding, moving from conventional approaches to advanced molecular tools that allow for precise genetic modifications, enhancing mustard resilience and yield. It highlights the roles of phenotypic and genotypic selection, molecular markers, transgenics, and genomics-assisted breeding in augmenting mustard improvement endeavours. The promise of emerging technologies like genome editing and systems biology is discussed for mustard genetic enhancement and climate-resilient varietal development. The review emphasizes the need of collaboration among research institutions, public-private partnerships, and international networks to accelerate, sustainable mustard improvement efforts.

Keywords

abiotic stress tolerance; agronomic traits; climate resilience; genomics-assisted breeding; molecular markers; mustard; transgenics

Introduction

Amidst rising demand for edible oils, mustard plays a pivotal role in India's efforts to reduce reliance on imports and bolster domestic production. This situation is exacerbated by the slow growth in domestic oilseed production and the high income elasticity of demand for edible oils. To address this challenge, policymakers are prioritizing the enhancement of domestic edible oil availability to reduce reliance on imports. In India's agricultural economy, oilseed ranks second only to cereals in importance. They are not only a crucial component of the human diet but also serve as essential inputs for various industrial products, including paints, coatings, hydrogenated oils, soaps, detergents, lubricants, and fragrances. It plays a significant role in India's edible oil production. This crop is extensively cultivated across diverse agroclimatic zones, ranging from the northern plains to the southern region, as well as in the north eastern and north western parts of the country. The broad adaptability of rapeseed-mustard underscores its importance

in India's agricultural landscape. Over the past few decades, this crop has significantly bolstered the domestic supply of edible oils. From 1980-1981 to 2021-2022, rapeseed-mustard productivity increased from 560 kg/ha to 1,558 kg/ha, and production rose from 2.3 million tonnes to approximately 10.75 million tonnes. This upward trend continued up to 2022-2023, with the productivity reaching 1,570 kg/ha and output climbing to about 10.95 million tonnes.

Rapeseed-mustard cultivation is not confined to India; it spans a wide range of agroclimatic conditions worldwide, including in China, Canada, the United Kingdom, France, Australia, Poland, and the United States. Rapeseed-mustard is extensively cultivated worldwide, underscoring its global significance in edible oil production. Mustard seeds, which are classified into three primary species—*Brassica nigra* (black or dark brown mustard), *Brassica juncea* (brown or oriental mustard), and *Sinapis alba* (light yellow or white mustard) are valued for their diverse uses and agronomic traits. *Brassica nigra* is primarily grown for its spice value, with its pungent seeds being a key ingredient in traditional condiments and culinary applications, especially in Indian cuisine. *Brassica juncea*, on the other hand, is known for its adaptability to various climates and high oil content, making it a widely cultivated species for oil extraction. The oil derived from it is used both for cooking and in industrial applications, making it a crucial global oilseed crop. *Sinapis alba* is primarily cultivated for its milder seeds, which are used in condiments such as yellow mustard, and it also plays a role in cover cropping and bio fumigation due to its pest control benefits in soil. Beyond their culinary and industrial uses, these mustard species are important for their agronomic traits and biochemical properties. These characteristics are crucial for breeding programs and biotechnological applications. In India, mustard is also grown as a salad crop, green vegetable, green fertilizer, and fodder crop, and it is a major source of industrial oil. Mustard oil is notable for its high erucic acid content, ranging from 40 to 50%. Additionally, it contains various antioxidants, oleic acid, gamma-linolenic acid, and glucosinolates, which contribute to both its nutritional value and industrial utility (1). One of the largest producers of edible oil in *Brassica*, oilseed *B. juncea*, is mostly found in Northwest China and the Indian subcontinent (2, 3).

Abiotic stresses such as water scarcity, salinity, and temperature fluctuations significantly affect mustard productivity. Abiotic stresses that impact the productivity of *Brassica* sp. (mustard) crops include excessive or insufficient water availability, high levels of salt in irrigation water and soil, and very high or low temperatures (4). Climate models project that global temperatures are likely to increase by approximately 1.0 to 3.0°C by the mid-century and by 2.0 to 4.0°C by the end of the twenty-first century. These temperature rises are expected to have a significant impact on agricultural systems and crop performance (5). India is a major global player in rapeseed-mustard cultivation, leading the world in terms of area under cultivation. Despite this significant acreage, the country ranks second

in production, with China holding the top position. This discrepancy highlights a gap in production efficiency. Climate models predict a rise in global temperatures, which could exacerbate these challenges.

In addition to abiotic pressures, Indian mustard crops face severe threats from biotic factors, particularly insect pests. The productivity of Indian rapeseed-mustard is hindered by various biological constraints, notably insect pests, which pose substantial risks throughout the crop's development stages, from seedling to maturity. Addressing these challenges is crucial for enhancing production and ensuring the sustainability of this important oilseed crop. Mustard crops in India encounter substantial challenges from a wide range of insect pests, with over 50 species documented. Among these, aphids such as *Lipaphis erysimi*, *Brevicoryne brassicae*, and *Myzus persicae* are particularly detrimental. *Lipaphis erysimi*, the most detrimental pest species, has led to substantial yield losses and reductions in oil content. Addressing these pest issues is crucial for enhancing mustard production (6, 7).

Optimizing rapeseed-mustard cultivation: nutritional, industrial and biofuel applications

Effective crop management techniques, such as precise irrigation strategies and integrated pest control, along with genetic improvements through biotechnology, are key to maximizing mustard yield and ensuring resilience in diverse agroclimatic conditions. These practices enhance productivity and sustainability in edible oilseed production. Optimizing rapeseed-mustard cultivation is crucial, as it accounts for over 80% of Rabi oilseed production and plays a vital role in both the edible oil industry and renewable energy sectors. Improving the yield and efficiency of these crops is vital not only for agricultural productivity but also for the broader edible oil sector, which impacts various related industries. *Brassica* oil stands out among edible oils for its superior nutritional profile, featuring a low level of saturated fatty acids and a high content of health-promoting mono and polyunsaturated fatty acids, as detailed in (Table 1) (8). Beyond its importance in human nutrition, *Brassica* seed oil's versatility extends to industrial applications, including the petrochemical industry, while its potential as a renewable energy source in biofuel production highlights its growing significance across multiple sectors. *Brassica* oils are also rich in essential vitamins (A, C, and E), phenolics, dietary fibres, and potassium. Additionally, they contain compounds with potential cancer preventive properties and other health benefits of mustard (*Brassica* spp.) (9). Widely cultivated for both its seeds and greens, mustard has gained attention for its rich content of health-promoting compounds, particularly glucosinolates. These sulfur-containing compounds are especially notable for their role in cancer prevention. Upon plant tissue damage or consumption, glucosinolates undergo enzymatic hydrolysis by myrosinase, yielding various breakdown products, including *isothiocyanates*, which exhibit significant chemopreventive properties. A well-known glucosinolate in mustard is *sinigrin*, which is hydrolyzed into *allyl isothiocyanate* (AITC), a compound with documented anticancer potential. AITC has

Table 1. Key nutritional parameters of *Brassica* oil and their health and industrial uses

Component	Typical Content	Range (%)	Health Uses	Industrial Uses
Saturated Fatty Acids	Low levels, e.g., Palmitic Acid	5-7%	Reduces the risk of cardiovascular diseases by promoting better lipid profiles.	Enhances stability and shelf-life in food processing; provides structural integrity in products.
Mono-unsaturated Fatty Acids (MUFA)	High levels, e.g., Oleic Acid	55-60%	Supports heart health by lowering LDL cholesterol and may reduce the risk of heart disease.	Used in biodiesel production for its high energy content and stability.
Poly-unsaturated Fatty Acids (PUFA)	High levels, e.g., Alpha-linolenic Acid	15-20%	Anti-inflammatory properties, supports brain health, and may improve mood and cognitive function.	Applicable in the production of high-performance lubricants and other industrial chemicals.
Palmitic Acid	Typically low in <i>Brassica</i> oil	5-7%	While not directly associated with health benefits, it is important in maintaining cell membrane integrity.	Used in various industrial applications, including the production of soaps and detergents.
Oleic Acid	Typically high in <i>Brassica</i> oil	55-60%	Enhances skin health, possesses anti-inflammatory properties, and may reduce the risk of chronic diseases.	Utilized in cosmetics and personal care products for its emollient and stabilizing effects.
Alpha-linolenic Acid	Typically high in <i>Brassica</i> oil	15-20%	Supports cardiovascular health, possesses anti-inflammatory properties, and may improve overall well-being.	Employed in biofuels for its high energy content and in industrial applications requiring high-quality oils.

been shown to inhibit the growth of cancer cells through mechanisms such as the induction of apoptosis, inhibition of cell cycle progression, and disruption of pathways involved in carcinogen activation. The efficacy of these compounds has been demonstrated in various experimental studies, highlighting mustard as a functional food with potential health benefits. Furthermore, one of the most extensively studied isothiocyanates, *sulforaphane*, particularly abundant in broccoli and mustard seeds, exhibits potent anti-cancer properties by modulating key detoxification enzymes and signalling pathways that control cancer initiation and progression. Sulforaphane and related compounds have been shown to arrest cell cycles in cancer cells and trigger cellular detoxification processes, contributing to reduced cancer risks (10). Research indicates that the developmental stage of the plant, as well as its genotype, significantly influences glucosinolate concentrations and, consequently, the formation of isothiocyanates. For example, young mustard sprouts tend to have higher concentrations of glucosinolates compared to mature plants, resulting in greater bioavailability of these cancer-preventive compounds (11). This underscores the importance of improving rapeseed-mustard cultivation, which offers widespread benefits across agriculture, industry, and energy sectors. Furthermore, Brassica crops, such as *B. carinata*, provide substantial sustainability benefits as alternative biofuel sources, particularly by reducing dependence on fossil fuels. These crops help cut greenhouse gas emissions compared to traditional fuels, with second-generation biofuels offering considerable potential, when land-use changes are minimized (12). Moreover, *B. carinata* can be grown in crop rotation systems, enhancing soil health and avoiding the food production competition often linked with biofuels (13). Life cycle assessments demonstrate that biofuels derived from Brassica reduce both fossil fuel consumption and greenhouse gas emissions, while their co-products, such as glycerine and meal, further boost sustainability (14). Therefore, Brassica-based biofuel production can significantly help reduce environmental impacts while also enhancing energy security.

Brassica oils are particularly advantageous for biodiesel production due to their high levels of monounsaturated fatty acids (MUFAs), such as oleic acid. These MUFAs contribute to better oxidative stability in biodiesel, making it less susceptible to degradation over time compared to oils that are high in polyunsaturated fatty acids, which tend to oxidize more rapidly. This enhanced stability not only improves the storage of biodiesel but also elevates its overall fuel quality. Moreover, MUFAs enhance the cold flow properties of biodiesel, enabling it to perform more effectively in lower temperatures—a vital factor for operational reliability in various climates (15). Collectively, these attributes, including strong oxidative stability and improved cold flow, position Brassica-derived biodiesel as a promising alternative to fossil fuels. Parameters like water content, cold filter plugging point, ester percentage, flash point, acid number, iodine value, and glyceride content all align with the standards outlined in TS EN 14214 for biodiesel (16). This compliance highlights the viability of *Brassica* oil as a reliable and efficient source for renewable energy.

Genetic and agronomic advances in enhancing biodiesel and oil quality in brassica species

Analysis of yield and quality metrics reveals that lines of *B. juncea* and *B. rapa* show promising potential as raw materials for biodiesel production. Research has consistently highlighted the biodiesel capabilities of several *Brassica* species, including *B. rapa*, *B. napus*, *B. juncea*, and *B. carinata*. Notably, biodiesel derived from mustard plants has been linked to improved engine performance and lower emissions of harmful substances, such as hydrocarbons (HC) and carbon monoxide (CO), as well as reduced operational noise (17). Specifically, mustard-based biodiesel can decrease HC emissions by 24-42% and CO emissions by 19-40%, along with a reduction in operational noise by 2-7% compared to conventional diesel (18). These benefits position mustard biodiesel as a promising alternative fuel, offering both environmental and performance advantages that outstrip those of other biofuels in critical areas. For example, when compared to soybean biodiesel, mustard

biodiesel demonstrates greater reductions in HC and CO emissions, underscoring its potential as a cleaner and more efficient biofuel (19). This underscores the importance of ongoing genetic and agronomic advancements in optimizing *Brassica* crops for biodiesel applications and improving their overall oil quality. Oilseed crops, such as *Camelina*, flax, *B. rapa*, canola, and oriental mustard, have been identified as having high potential for biodiesel production due to the ease with which their oils can be converted (20).

The genetic control of fatty acid biosynthesis in *Brassica* species, regulated by genes such as *FATTY ACID ELONGATION1* (*fae1*), *FATTY ACID ELONGATION2* (*fae2*), *FATTY ACID DESATURASE1* (*FAD1*), and *FATTY ACID DESATURASE2* (*FAD2*), has been extensively characterized. Studies on the silencing and overexpression of these genes have revealed the potential of metabolic engineering to enhance fatty acid profiles for biodiesel applications (21). Indian varieties of *B. juncea* and *B. rapa* are notable for their high content of erucic acid and other fatty acids, which make them advantageous for biodiesel production. The elevated levels of these fatty acids enhance their suitability as feedstocks for biodiesel, offering potential improvements in fuel properties and engine performance. This emphasizes the role of these *Brassica* species in advancing biodiesel technology and highlights the need for further research to optimize their oil composition for energy applications. Some research highlighted mustard oil as a potential alternative for biodiesel production (22). However, the high viscosity of mustard oil, attributed to long chain fatty acids, poses a challenge for direct use in diesel based engines. To address this, a study (23) explored genetic modifications by introducing the diacylglycerol acetyltransferase (*EaDAcT*) gene from *Euonymus alatus* into Indian mustard using the glycinin promoter. This modification aimed to produce triacylglycerols with acetate at the sn-3 position, reducing oil viscosity and removing the need for transesterification before use in engines. These genetic advancements are crucial for improving mustard oil as a biodiesel feedstock. Despite these advances, practical applications are limited by the presence of two major antinutritional factors: erucic acid, a long chain unsaturated fatty acid, and glucosinolates, sulfur-containing compounds. Addressing these limitations is essential for optimizing mustard oil's use in biodiesel production. Some researchers emphasized that an oil's fatty acid composition determines its quality, and the intended use of the oil affects the desired profile (5).

The quality of edible oils from mustard and rapeseed has improved significantly due to reductions in erucic and eicosenoic acid content. Typically, *B. juncea* cultivars exhibit high glucosinolate concentrations, ranging from 80 to 120 $\mu\text{mol/g}$ of dry seed meal, which are considered antinutritional and limit their use in poultry feed (24). Quality breeding programs aim to reduce glucosinolate content to 30 $\mu\text{mol/g}$ of dry seed weight (DSW) (22).

The term "Canola" refers to a genetically modified form of rapeseed that meets specific quality standards: an oil erucic acid content of less than 2% and glucosinolate

levels below 30 $\mu\text{mol/g}$ of deoiled meal. This designation is a trademark of the Canadian Oil Association and signifies quality rather than biological classification. "Double low" or "00" rapeseed varieties (low-erucic varieties ("0") and low-erucic, low-glucosinolate varieties ("00", double-zero, double low, canola), also known as Low Erucic Acid Rapeseed (LEAR), meet these criteria. The development of Canola cultivars, initiated in Canada in the 1970s, has become widespread in countries such as Australia, Japan, Canada, and several European nations. Breeding efforts in India to develop "00" type canola cultivars began in 1970, focusing on varieties with less than 2% erucic acid and glucosinolate levels below 30 $\mu\text{mol/g}$ in defatted meal (5). Notable achievements include the introduction of GSC 5 in 2005, the first "00" variety of *B. napus* (Gobhi Sarson) (25), and Pusa Karishma (LES-39), which marked the start of low erucic acid variants in Indian mustard. Subsequently, IARI, New Delhi, has released five additional low erucic acid types, including Pusa Double Zero Mustard (PDZ-1), the first "00" variety of *B. juncea* introduced in 2017 for Zone-II (5) (Eastern Himalayan Region: Assam, Sikkim, West Bengal and all North-Eastern states), using a pedigree selection method. Glucosinolates, which are nitrogen and sulfur-rich secondary metabolites in Brassicales, undergo hydrolysis into various compounds like isothiocyanates and nitriles when plant tissues are damaged. Breeding efforts focus on increasing seed oil content, though the genetic factors controlling this trait are not fully understood. Recent research (26) using POLY4 (multi-nutrient fertiliser containing K, S, Mg, Ca, and micronutrients) on Indian mustard has demonstrated an increase in oil content and improvements in oil quality, particularly in linolenic acid levels in Indian mustard. Specific nutrients such as sulfur and magnesium are essential for boosting linolenic acid levels and enhancing oil quality in mustard (*Brassica juncea*). Sulfur contributes to the production of unsaturated fatty acids and elevates oil content, whereas magnesium improves photosynthesis and activates enzymes necessary for fatty acid synthesis. Studies have indicated that the application of multi-nutrient fertilizers like POLY4 leads to significant improvements in both oil content and linolenic acid levels, through enhanced nutrient absorption in mustard crops. This research provides valuable insights into ongoing efforts to improve mustard for biodiesel applications.

Advances in conventional breeding techniques

Conventional breeding techniques have been vital in developing superior mustard varieties, addressing key traits and challenges in the crop. Mass selection targets traits such as yield and disease resistance by allowing breeders to select the best-performing plants from a segregating population. This approach effectively enhances overall yield potential while improving the crop's resilience to diseases like downy mildew and white rust. Pure-line selection focuses on establishing genetically uniform lines from diverse germplasm, which is crucial for maintaining traits like oil content and quality, seed viability, and adaptability to different growing conditions (27). These strategies remain relevant but have been significantly enhanced

by modern advancements. Mass selection and pure line selection are fundamental for self-pollinated species, involving the selection of the best individuals based on the desirable traits to establish pure lines, which helps maintain genetic purity and achieve steady improvements in yield and resistance (28). Recurrent selection, including half-sib (HS) and full-sib (FS) reciprocal recurrent selection, has evolved to leverage both additive and non-additive gene effects, improving selection cycles for traits such as drought and saline tolerance (29).

Backcross breeding has been instrumental in introducing disease resistance, such as resistance to *Leptosphaeria maculans*, while minimizing undesirable traits through marker-assisted selection, which helps in recovering the recurrent parent genome. Recent advancements in marker-assisted selection (MAS) have accelerated this process by allowing breeders to monitor the recovery of the recurrent parent genome early, making the selection process more accurate and efficient (30). In addition to these conventional methods, new advancements such as speed breeding and fast generation advancement are also being explored to shorten the breeding cycles (31, 32). While speed breeding significantly reduces generation times, it often requires controlled environments and high resource input, which may limit its scalability in traditional mustard breeding programs.

Molecular diversity analysis, made possible by genomics-driven breeding, has greatly improved the selection of parent plants for crossbreeding (33). However, phenotypic selection remains critical for traits like heat and drought resistance, which are difficult to select using genotypic data alone. Although phenotypic selection can be resource-intensive and influenced by genotype-environment interactions, breeding for drought tolerance in mustard (*Brassica* species) is complex due to these interactions and the polygenic nature of the trait. Drought tolerance in mustard is influenced by various factors, including root depth, water use efficiency, and osmotic adjustment, complicating phenotypic selection in variable environments. Nevertheless, advancements in genomic tools, such as marker-assisted selection (MAS) and genomic selection (GS), provide more efficient methods for managing G×E interactions and enhancing drought tolerance (34). High-throughput phenotyping (HTP) platforms facilitate the rapid and precise screening of drought-adaptive traits across large populations, helping to identify mustard genotypes with improved tolerance. These tools, along with quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS), hold promise for increasing mustard yield under drought conditions (35). Recent developments in genomics, including next-generation sequencing and genomic selection models, can support the introgression of drought-resilient traits into elite mustard cultivars (36). Molecular markers provide a more reliable, cost-effective, and high-throughput approach for selecting desirable genotypes (37). To address the limitations of relying solely on molecular markers, current breeding ideally combines genotypic information with phenotypic data across the targeted habitats. Emerging

phenomics technologies are being developed for precision phenotyping, further enhancing the efficiency of breeding programs (38). Integrating cutting-edge molecular technologies with traditional selection techniques allows for the significant acceleration in mustard improvement, optimizing the breeding process and enhancing overall program effectiveness (39). Emerging techniques such as genomic selection (GS) and CRISPR/Cas9 gene editing, while not strictly conventional, are increasingly being incorporated into crop improvement programs. Genomic selection enables breeders to make early and accurate predictions about an individual's performance based on genome-wide marker data, improving the efficiency of selecting complex traits like yield, drought tolerance, and disease resistance. Meanwhile, CRISPR/Cas9 offers precise genome editing capabilities, allowing for targeted modifications in specific genes to enhance traits such as oil content, pest resistance, or abiotic stress tolerance (40, 41). These technologies, when used alongside conventional methods like mass selection, pure-line selection, and backcross breeding, provide powerful tools to accelerate the development of high-performing mustard varieties that meet the growing demands of modern agriculture.

Genomic tools and resources

The genetic diversity within the *Brassica* species has been systematically categorized into diploids and amphidiploids through basic genetic analysis. The diploid species include *B. rapa* (AA genome, $2n = 20$), *B. nigra* (BB genome, $2n = 16$), and *B. oleracea* (CC genome, $2n = 18$) (42). Through natural hybridization and genomic recombination, three major amphidiploids have emerged: *B. juncea* (AABB, $2n = 36$), *B. carinata* (BBCC, $2n = 34$), and *B. napus* (AACC, $2n = 38$) (43). This genetic diversity forms the foundation for advanced breeding programs aimed at improving mustard crops.

Advances in Whole-Genome Sequencing and Trait Mapping

The whole-genome sequencing of the mustard variety 'Varuna' in 2017 (44) marked a pivotal advancement in the field of mustard genetics. This milestone provided a comprehensive reference genome that has enabled high-resolution trait mapping, which is critical for genomics-driven breeding programs (45). The availability of this reference genome has facilitated the identification of key quantitative trait loci (QTLs), enhancing mustard breeding efforts. QTL mapping and Genome-Wide Association Studies (GWAS) are widely used genomic tools in mustard breeding to identify regions of the genome associated with specific agronomic traits, such as seed yield, oil content, and disease resistance. QTL mapping involves crossing two parental mustard lines with contrasting traits and genotyping their progeny to identify markers linked to quantitative traits. For example, QTL mapping has been used to localize QTLs for oleic acid content in *B. juncea*, facilitating the selection of high-oil-content mustard varieties (46). GWAS, on the other hand, relies on natural genetic variation within a large, diverse population. It uses high-density markers to identify associations between specific genetic

variants and traits. This method has been applied to identify stable QTLs for seed oil content in mustard, combining data from diverse populations and different environments to enhance the precision of trait selection (47). By integrating QTL mapping and GWAS, breeders can develop more efficient marker-assisted selection (MAS) programs, accelerating the development of improved mustard varieties with enhanced productivity and quality traits (48). This combined approach has been shown to be particularly effective in mapping complex traits like yield and oil content under different genetic backgrounds (49). Resequencing studies of wild and cultivated mustard germplasm have uncovered extensive genetic variation, including millions of single nucleotide polymorphisms (SNPs). These SNPs are invaluable resources for genomic selection, fine mapping of genes and QTLs, and developing high-density genetic maps (3). Single nucleotide polymorphisms (SNPs) are crucial genetic markers in plant breeding, including *B. juncea*, enhancing the precision and speed of developing improved varieties. SNPs represent genetic variations at single nucleotide positions in the DNA sequence, allowing breeders to link these variations to traits like yield, oil content, or disease resistance. By identifying and mapping SNPs across the genome, breeders can locate quantitative trait loci (QTLs) responsible for these traits, improving their ability to select plants with desired characteristics. Incorporating SNPs into genomic selection models enables the prediction of the breeding value of plants without full phenotyping. High-density SNP arrays, which genotype thousands of markers simultaneously, help identify associations between specific SNPs and traits. These SNPs are then integrated into genomic selection models, such as genomic best linear unbiased prediction (GBLUP) or Bayesian models, predicting plant performance based on their SNP profiles. By creating genomic prediction equations from a "training population" (plants with both genotype and phenotype data), breeders can predict the genetic potential of genotyped plants, significantly speeding up the selection process (50). SNPs linked to QTLs (Quantitative Trait Loci) controlling oil content and fatty acid composition are crucial for selecting mustard varieties with higher-quality oil. These genetic markers are also associated with disease resistance, particularly against common fungal infections, helping breeders enhance the resilience of crops. Additionally, SNPs target yield-related traits like plant height, pod number, and seed size, allowing breeders to identify genome regions associated with higher yields. By incorporating SNP markers into breeding programs, marker-assisted selection (MAS) and genomic selection enable breeders to make early, data-driven decisions, reducing dependence on lengthy field trials. This accelerates the development of mustard varieties that are high-yielding, disease-resistant, and rich in oil content—advancing agricultural productivity in regions like India, where mustard is a critical oilseed crop. These efforts have greatly enhanced the understanding of the genetic architecture of mustard and provide the tools necessary for marker-assisted selection (MAS) in breeding programs. Despite the progress made in mustard genomics, challenges remain, such as the high cost of sequencing and the

complexity of polyploid genomes, which complicate the identification of trait-linked markers.

Integration of Genomic and Physical Mapping

The integration of genomic and physical mapping techniques has significantly advanced the positional cloning of trait-regulating genes in mustard. In mustard (*Brassica* species), the integration of physical mapping techniques such as BAC (Bacterial Artificial Chromosome) libraries and Fluorescence In Situ Hybridization (FISH) has become indispensable for positional cloning and gene identification of key traits. These methods complement genomic tools such as SNP markers by providing structural insights into the genome, thereby enhancing the precision of genetic research and breeding programs. BAC libraries are vital genomic resources in mustard research. They allow for the isolation of large DNA fragments essential for mapping chromosomal regions containing trait-related genes. These libraries enable researchers to physically localize and anchor specific genetic markers, particularly in regions where traditional genetic mapping approaches fall short. This targeted approach is crucial for cloning key genes related to disease resistance or yield improvement. For example, a study demonstrated the broader applicability of BAC-FISH techniques in chromosome cataloguing and positional cloning in other species (51). FISH further refines gene localization resolution by visualizing BAC clones on chromosomes. This technique is particularly valuable in mustard for aligning genetic and physical maps, leading to more accurate gene identification. By saturating chromosomal regions of interest with markers, FISH helps pinpoint key loci, especially in complex genome regions with limited recombination. The integration of physical mapping techniques with SNP markers is pivotal for mustard breeding. SNP markers provide dense genetic data, but BAC-FISH techniques bridge the gap by linking these markers to specific chromosomal regions, particularly in areas with low recombination rates (52). This combination of genomic markers and physical maps accelerates the identification of candidate genes for essential traits. In mustard, these techniques are crucial for discovering genes associated with stress resistance, oil content, and yield. By refining marker-assisted selection, mustard breeding programs can achieve more precise outcomes. Studies that developed chromosome-specific BAC clones for cotton serve as a useful comparison for applying these techniques in *Brassica* species (53). Together, BAC libraries, FISH, and SNP markers form a powerful toolkit for modern mustard research, offering unprecedented insights into genome structure and facilitating the discovery of key trait-regulating genes. This integration advances mustard breeding by enhancing the accuracy and efficiency of genetic selection. Whole-genome sequencing of diverse mustard genotypes has revealed crucial structural variations, such as insertions, deletions, and copy number variations, which are instrumental in introducing fresh allelic diversity into breeding lines. These advancements have propelled the genomic selection-based breeding methods as a promising alternative to traditional pedigree-based methods, offering higher precision and efficiency in

cultivar development. Despite progress, aligning physical and genomic maps remains challenging due to the complexity of polyploid genomes in mustard, necessitating further refinement of mapping techniques.

Molecular studies conducted on *B. juncea*, have demonstrated the conservation of genomes from parental species, further validating the robustness of these genomic tools (54). The advent of next-generation sequencing (NGS) technologies has revolutionized breeding programs in mustard (*Brassica* species) by accelerating the discovery of genomic markers and improving the precision of trait mapping. NGS facilitates rapid SNP discovery, enabling the construction of high-density linkage maps crucial for marker-assisted selection (MAS). These dense maps allow for more accurate genomic predictions, enhancing the selection of traits such as oil content, drought tolerance, and disease resistance (55). By integrating NGS with physical mapping techniques like BAC libraries and FISH, breeders can pinpoint genes with greater precision, expediting the development of improved varieties. NGS also streamlines breeding cycles by enabling early selection through genomic selection (GS), reducing the reliance on time-consuming field trials. The ability to genotype large populations at lower costs further accelerates the breeding process, making NGS a key driver in the enhancement of mustard breeding efficiency. Through these advances, NGS has become indispensable for developing high-yielding, stress-tolerant, and disease-resistant mustard varieties. NGS has also facilitated a deeper understanding of how abiotic and biotic stresses can induce modifications in genetic structures, with transcriptome analysis offering insights into the genetic control mechanisms underlying these changes (43). Moving forward, the integration of genomic mapping with CRISPR-based gene editing holds promise for rapidly improving mustard varieties, while phenomics can enhance the precision of selecting desirable traits in the field.

Association Genetics and Mutation Breeding

Association genetics has emerged as a powerful tool for trait mapping and gene identification, particularly for complex phenotypes. Unlike traditional QTL mapping, which relies on segregating populations from bi-parental crosses, association genetics leverages the existing genetic diversity in germplasm collections, wild populations, and selection lines. This approach allows for the identification of genes responsible for traits such as yield, oil quality, and disease resistance.

However, crop domestication has led to a narrowing of genetic diversity (56). This reduction in diversity has necessitated the development of advanced genetic tools, such as mutation induction, to introduce artificial variation into breeding programs. Early methods involved the use of X-rays as a mutagen, but modern techniques have evolved to include gamma radiation and chemical mutagens, which effectively induce physiological and biochemical changes in plants (57). For example, exposing mustard seeds to gamma radiation (25-50 Gy) has been shown to increase dry weight, likely due to enhanced RNA activation

and subsequent protein synthesis during early germination stages (58, 59). By integrating association genetics with mutation breeding, breeders can capitalize on both natural and artificially induced genetic diversity, allowing for the development of mustard varieties that are both high yielding and resilient to environmental stresses.

Recent Technological Developments and Bioinformatics

Recent advancements in sequencing technologies and gene editing tools, such as CRISPR/Cas9, have propelled mustard species to the forefront of comparative genomic research. Specific genes targeted using CRISPR in mustard include glucosinolate transporter genes to develop low-seed, high-leaf glucosinolate plants, and genes like *MYB28*, responsible for pungency, to create low-pungent mustard varieties. These innovations have significantly enhanced traits such as pest resistance, oil content, and consumer acceptability in mustard. These technologies have provided deep insights into the exploration of phenotypic diversity and have revealed how evolutionary processes shape genetic variation within the *Brassica* genus (3). These advancements are crucial for the development of mustard varieties with enhanced traits, such as increased oil content, improved stress tolerance, and resistance to pests and diseases. These insights into evolutionary divergence within the *Brassica* genus have helped identify genes conserved for stress tolerance across species, aiding in cross-species trait selection.

The rapid expansion of genetic resources has created a pressing need for advanced bioinformatics infrastructure capable of managing, analysing, and integrating vast amounts of genomic data. High-throughput omics systems, combined with bioinformatics tools, facilitate the integration of multi-omics datasets with phenotypic data, enabling the identification of markers linked to complex traits and the establishment of genotype-phenotype correlations (60). In mustard breeding, the integration of multi-omics data, including genomics, transcriptomics, proteomics, and metabolomics, has led to significant advancements. This integration allows breeders to better understand complex traits like yield, disease resistance, and environmental stress tolerance. Bioinformatics tools enable the correlation of genotype with phenotype, accelerating the identification of key genetic markers linked to these traits. Platforms such as the Mustard Genome Portal and databases like MusBase provide open-access resources that combine genomic, genetic, and breeding data, enhancing knowledge discovery and application in mustard improvement (3). Cloud computing platforms, further empower decentralized analysis of large genomic datasets, enabling researchers to collaborate and share data more effectively. Cloud computing platforms significantly enhance mustard breeding efforts by providing scalable data storage and computational power for managing extensive genomic and phenotypic datasets. Researchers can leverage cloud-based tools for data analysis, allowing for real-time collaboration across geographical boundaries, which fosters interdisciplinary teamwork in breeding programs. With access to advanced bioinformatics software and machine learning algorithms, cloud computing

facilitates the identification of genetic markers and traits associated with desirable characteristics, accelerating the breeding process. Additionally, cloud platforms offer automated workflows that streamline data processing, enabling researchers to focus on interpretation and decision-making. Ultimately, cloud computing empowers mustard breeding by enhancing efficiency, collaboration, and data-driven insights, paving the way for improved varieties with higher yields and better disease resistance.

Despite advancements in mustard breeding through genomic research and bioinformatics, several challenges remain. Key concerns include data security and privacy, as the sharing of sensitive genomic data on cloud platforms raises intellectual property issues, necessitating robust security measures and regulatory compliance. Additionally, the integration of diverse bioinformatics tools is complicated by varying data formats, highlighting the need for standardized protocols to enhance interoperability. Ongoing costs for data storage and computational resources can also accumulate, particularly for large breeding programs, making cost-effective solutions essential. Furthermore, the rapid evolution of genomic technologies may lead to skill gaps among researchers, underscoring the need for adequate training and resources. Effective data management strategies are crucial for ensuring the quality and consistency of the vast amounts of genomic and phenotypic data generated. Collaborative efforts among researchers, institutions, and technology providers are essential to address these challenges, enabling the mustard breeding field to fully leverage advancements in genomics and bioinformatics for improved varieties that meet global agricultural demands.

Role of biotechnology in mustard improvement

The natural hybridization of *B. oleracea* and *B. rapa* has long been regarded as the foundational explanation for the origin of rapeseed (*B. napus*), a crop widely cultivated in temperate regions. Protoplast fusion between *B. oleracea* and *B. rapa* has successfully enhanced genetic diversity in *B. napus*, resulting in increased allelic variation and improved adaptability to diverse environmental conditions. Additionally, this technique has enabled modifications in oil content, leading to alterations in fatty acid composition and increased oil yields, thus improving the economic viability of mustard cultivation. Biotechnology has played a crucial role in transferring desired traits from closely related species or even unrelated crops to mustard, thereby increasing genetic variability. This approach has significantly boosted the crop's yield potential while addressing the limitations of traditional breeding techniques (61).

However, challenges in mustard oil quality, particularly in Indian mustard, remain prominent. Table 2 outlines several genetically engineered mustard lines that have been developed to combat both biotic and abiotic stresses, enhancing the crop's resilience and oil quality.

(62) highlighted the issue of high erucic acid content (35-50%) in Indian mustard oil, which, despite having lower levels of polyunsaturated and saturated fatty acids compared to other edible oils, poses health concerns. It has been emphasized that the reliance on palm, soybean, and rapeseed oils for biodiesel production, advocates for a shift to non-edible plants (63). Such a transition would necessitate improvements in oil yield, quality, and stress tolerance. In efforts to enhance the oil quality of *Brassica* species, transgenic approaches have been employed.

Table 2. Details on biotechnological interventions in oilseed *Brassica* for stress tolerance, gene discovery, and metabolic engineering

Sl.No	Crop	Gene	Traits	References
1	<i>Brassica juncea</i>	<i>Cod A</i>	Tolerance to salinity stress, enhanced growth	(88)
2	<i>Brassica rapa</i>	<i>Lea</i>	Enhanced resilience to both salinity and drought	(89)
3	<i>Brassica juncea</i>	<i>PgNHX1</i>	Maintained growth with improved salinity tolerance	(90)
Biotic stress				
4	<i>Brassica juncea</i>	Tomato glucanase gene	Reduced infection and growth arrest of <i>Alternaria Brassicae</i>	(91)
5	<i>Brassica juncea</i>	α -tocopherol	Enhanced tolerance to salt, heavy metals, and osmotic stress	(92)
6	<i>Brassica juncea</i>	Chickpea Lectin gene	Improved tolerance to salt and drought stress	(93)
7	<i>Brassica juncea</i>	<i>MsrA1</i>	Resistance to <i>Alternaria Brassicae</i> and <i>Sclerotinia sclerotiorum</i>	(94)
8	<i>Brassica juncea</i>	Chickpea Lectin gene	Resistance to <i>Alternaria Brassicae</i>	(93)
9	<i>Brassica juncea</i>	<i>BjNPR1</i>	Increased resistance to <i>Alternaria Brassicae</i> and <i>Erysiphe cruciferarum</i>	(95)
Gene Discovery & Metabolic Engineering				
10	<i>Brassica juncea</i>	<i>PiD6</i>	Increased β -Linolenic acid production	(96)
11	<i>Brassica juncea</i>	<i>fad2</i>	High oleic acid and low linolenic acid	
12	<i>Brassica rapa</i>	<i>BrTT8</i>	Yellow seed coat color due to insertion mutation	(97)
13	<i>Brassica juncea</i>	ORF288	Male sterility caused by aborted pollen development	(98)
14	<i>Brassica juncea</i>	<i>BjMYB28</i>	Low glucosinolate levels through gene silencing	(24)
15	<i>Brassica juncea</i>	<i>GSLALK</i>	High levels of glucoraphanin, an anticancer glucosinolate	(83)
Male Sterility & Heterotic Hybrid Production				
16	<i>Brassica juncea</i>	<i>Cre/lox</i> , <i>lox</i> linked with ALS gene	Marker-free transgenics with insect (<i>Lipaphis erysimi</i>) resistance	(99)
17	<i>Brassica juncea</i>	Silencing of <i>SGT</i> and <i>SCT</i> genes	Reduction in sinabin content in seeds	(100)

Some researchers introduced the $\Delta 12$ -desaturase transgene from the fungus *Mortierella alpine*, leading to increased linolenic acid levels (64). However, the impact on yield might be limited or neutral. Genetic modifications targeting oil quality usually focus on altering the biochemical pathways that affect fatty acid composition without directly influencing traits like seed yield or overall biomass production. Any potential yield effects would depend on the specific regulatory pathways involved and whether they interfere with or promote other growth or reproductive processes in the plant. Another significant focus of biotechnological interventions has been the reduction of glucosinolate content. Another experiment employed RNA interference (RNAi) to inhibit the *BjMYB28* transcription factor gene, involved in aliphatic glucosinolate production, resulting in transgenic Indian mustard lines with significantly lower glucosinolate levels (24).

Genetic engineering has also been utilized to enhance nutrient uptake and overall plant health. For instance, overexpressing the tomato *LeST 1.1* gene in *B. juncea* var. Pusa Jaikisan increased sulfur uptake, which in turn improved nitrogen assimilation and soluble protein content (65). In order to produce male sterility, (66) in mustard by introducing the *Arachis diogeni* cysteine protease (*AdCP*) gene under the TA29 tapetum-specific promoter. This male sterility trait offers promising applications for future mustard improvement through hybrid breeding. Quantitative Trait Loci (QTL) mapping has identified several regions associated with oil content regulation in rapeseed. It was discovered that 7–14 QTLs per study, each contributing less than 10% to the total variance in oil content, underscoring the polygenic nature of this trait (67). Marker-assisted selection has also been employed to develop low erucic acid genotypes in *B. rapa* and *B. juncea*, based on coding sequence (CDS) variants identified by (68) and (62).

The advent of CRISPR/Cas genome editing technology has opened new avenues for precision breeding in mustard. It has also been emphasized that the importance of developing novel breeding technologies to increase agricultural output and to enhance the availability of nutrient-dense foods (69). CRISPR/Cas9 has proven to be a reliable and efficient tool for functional genomics research in *Brassica*, with potential applications in developing cultivars resistant to emerging pests and abiotic stresses. The use of a DNA-free protocol, involving the delivery of the CRISPR/Cas system *via* Ribonucleoprotein (RNP), ensures that no foreign DNA is integrated into the host genome, thereby offering more control over gene editing and minimizing unintended effects (70). Moreover, recent efforts have focused on reducing erucic acid content in high seed oil content (SOC) germplasms of *B. napus*. By employing gene editing to knockout the *BnaFAE1* genes, researchers successfully obtained mutants with reduced erucic acid content and a substantial increase in oleic acid (C18:1) levels, exceeding 66%. This research provides valuable low erucic acid germplasm resources for future breeding programs aimed at improving *B. napus* (64). In the realm of herbicide resistance, significant progress has been made

in *B. napus* through the identification of genes that confer resistance to specific herbicides or enable the detoxification of herbicides *via* metabolic pathways. Despite these advancements, herbicide-resistant rapeseed has not yet been made available to Indian farmers. To address this, scientists have developed glyphosate tolerance through targeted amino acid replacements, using a CRISPR/Cas9-based system that includes a guide RNA cassette and donor fragments, along with a geminiviral replicon system. This modular system can be adapted for different objectives and, through genetic segregation, eventually resulted in the creation of genetically modified plants (71).

Another innovative approach involves the use of a Cytosine Base Editor (CBE) in conjunction with a uracil glycosylase inhibitor and cytidine deaminase APOBEC1, which introduces specific cytosine-to-thymine transition mutations near the PAM region of *BnAHAS1* and *BnAHAS3* (72). Transformation processes using *Agrobacterium tumefaciens* have also been successfully applied to the semi-winter OSR cultivar Zhongshuang 6, resulting in transgenic plants with desired gene edits.

Further advancements have been made in improving stress tolerance in mustard. The introduction of the dehydration-responsive element-binding (DREB) gene in *B. napus* var. Wester has demonstrated significant benefits. DREB is a key transcription factor that regulates the expression of genes responsive to abiotic stress. Another research reported that transgenic canola with the DREB gene exhibited increased proline content under high NaCl concentrations, aiding in osmoregulation under challenging conditions (73). Additionally, (74) highlighted the critical role of the DREB gene in cold stress response, further emphasizing its importance in enhancing stress tolerance. These biotechnological interventions represent significant strides in mustard improvement, offering solutions to current challenges and paving the way for future innovations. By integrating conventional breeding techniques with advanced genetic and biotechnological approaches, researchers are poised to make substantial contributions to the sustainable development of mustard crops.

Climate-smart breeding strategies

India's agricultural sector is profoundly influenced by monsoon variability, which plays a critical role in determining the country's food grain production. Mustard and rapeseed are the vital crops in this landscape, especially in rainfed regions where they thrive due to their low water requirements. Approximately 35% of the area under rapeseed and mustard cultivation in India is rainfed, with states like Assam (84.2%), Jharkhand (38%) and Madhya Pradesh (33.5%) heavily dependent on natural rainfall (75). This reliance on rainfall makes mustard cultivation particularly vulnerable to moisture stress, especially drought, which can cause yield reductions ranging from 17 to 94 per cent (25).

The development of climate-resilient mustard cultivars is essential. Key physiological traits that contribute to drought tolerance in mustard, such as enhanced transpiration efficiency, osmotic adjustment, and canopy tempera-

ture regulation, have been identified (25). These traits are critical for maintaining productivity under water stress. Moreover, short-duration mustard cultivars is advantageous in multiple cropping systems, as they avoid terminal phase temperature stress, which is detrimental during crucial stages like pod development and oil filling (76). For example, 'Pusa Vijay' and 'RH 749' are well-known drought-tolerant mustard cultivars that exhibit high water-use efficiency and osmotic adjustment, ensuring stable yields under water-deficit conditions. Additionally, 'NRCDR 2' is a short-duration cultivar widely adopted in regions with multiple cropping systems. Its early maturity helps it escape high temperatures during the terminal stages, thus protecting the yield and oil quality during critical growth phases. These cultivars demonstrate the importance of breeding strategies focused on both drought tolerance and shorter crop duration to address climate challenges in mustard production.

Brassica crops, including mustard, exhibit resilience against various environmental stresses, such as pests, temperature extremes, and water scarcity. These crops adapt by enhancing stress-related compounds, adjusting leaf traits, and modifying stomatal characteristics to ensure productivity under adverse conditions (77). Among the major abiotic stresses, drought causes major damage to crop, leading to reduced seed weight, seed yield, oil yield, and overall seed oil content in mustard cultivars (78). The extensive cultivation of mustard in dry and semi-arid regions are especially prone to drought (79).

To address these challenges, climate-smart breeding strategies that enhance mustard's resilience are imperative. Recent advancements in plant breeding, including genomics-aided selection and high-throughput genome sequencing, have opened new avenues to overcome the limitations of traditional breeding methods (80). Genetic engineering techniques, such as interspecific and intergeneric crossings, facilitate the transfer of alleles that enhance crop resistance to pests, diseases, and adverse environmental conditions. For instance, the development of salt-tolerant mustard cultivars through rigorous breeding efforts and the introduction of transgenic lines with improved osmolyte accumulation and ion homeostasis capabilities offer promising solutions to rising soil salinity (81). The inclusion of Crop Wild Relatives (CWRs) in breeding programs presents another strategy for enhancing mustard's resilience to climate change. As progenitors of cultivated crops, CWRs provide a rich source of genetic diversity that can be harnessed to develop varieties better suited to changing agricultural environments (82). Research has demonstrated that transgenic *B. juncea* plants with foreign genes exhibit improved tolerance to cold, drought, and salinity stress, highlighting the importance of genetic diversity in breeding climate-resilient crops (83).

Advanced genetic tools like Quantitative Trait Loci (QTL) mapping and association mapping have become indispensable in identifying the genes responsible for stress tolerance. Several mustard cultivars have benefited from QTL mapping to enhance stress tolerance traits effectively. For instance, in Indian mustard (*B. juncea*), the culti-

var Pusa Jai Kisan has been improved for drought tolerance through the identification of specific QTLs associated with this trait. Similarly, Pusa Bold has undergone QTL studies that revealed loci linked to disease resistance, particularly against *Alternaria* blight, crucial for maintaining yield under disease pressure. In *B. napus* (canola), the cultivar Westar has been the focus of QTL mapping to identify genetic loci associated with drought tolerance, facilitating the selection of beneficial traits for breeders. These tools facilitate the precise mapping of genes linked to traits such as salt and drought tolerance, aiding the development of high-yielding, stress-tolerant mustard cultivars (84). Despite challenges in breeding for stress tolerance, the research focus on creating robust mustard genotypes capable of withstanding increasing environmental stresses (85).

Integrating genomics-assisted breeding strategies, biofortification techniques, and resource-efficient cultivation practices is critical for the sustainable intensification of mustard-based cropping systems. Integrating genomics-assisted breeding with biofortification techniques offers a promising strategy to enhance the nutritional value of mustard while ensuring resilience to environmental stressors. By precisely identifying and selecting quantitative trait loci (QTLs) linked to essential nutrients like iron and zinc, breeders can develop mustard varieties that are not only rich in these micronutrients but also exhibit traits such as drought and heat tolerance. This integration accelerates the breeding process through marker-assisted selection, enabling rapid identification of nutrient-rich plants, thus reducing the time and cost associated with traditional breeding methods. Additionally, genomics can improve nutrient stability, ensuring that enhanced nutrient levels are retained during post-harvest processing and cooking. As a result, mustard varieties enriched with multiple nutrients can thrive in diverse agro-climatic conditions, supporting sustainable agriculture and addressing global malnutrition challenges. By improving nitrogen and water use efficiency, and breeding for biotic stress tolerance, reliance on chemical inputs such as pesticides can be reduced, aligning with sustainable agriculture principles (86). Participatory breeding and variety testing under organic conditions are also key components of an integrated approach to mustard breeding that seeks to achieve long-term production gains without compromising environmental health (87).

Future directions in mustard breeding and genetics

The future of mustard breeding holds intriguing possibilities, driven by next-generation genomics techniques that promise to revolutionize crop enhancement. Advances in long-read sequencing are enabling the scaffolding of mustard genomes at the chromosomal level, significantly improving the development of comprehensive marker maps and the identification of potential genes underlying quantitative trait loci (QTLs). These developments will be instrumental in enhancing the precision and efficiency of mustard breeding programs. Additionally, the integration of genotyping assays with high-throughput phenotyping technologies will facilitate precise and early screening of breeding populations, accelerating the selection of desira-

ble traits. Genome editing techniques, such as CRISPR-Cas9, offer tremendous potential for the rapid introduction of beneficial allelic variations, allowing for the targeted improvement of specific traits in mustard. Moreover, synthetic biology approaches are emerging as a powerful tool for the rational design of complex biochemical pathways, which could lead to the production of mustard varieties with enhanced nutritional profiles and stress resistance. Synthetic biology plays a crucial role in the development of mustard varieties with improved nutritional profiles and stress resistance by enabling the rational design and engineering of complex biochemical pathways. This approach involves the application of engineering principles to biological systems, allowing researchers to construct and modify genetic components in a precise manner. By synthesizing genes and regulatory elements, scientists can create new metabolic pathways that enhance the biosynthesis of essential nutrients, such as vitamins and minerals, within mustard plants. For instance, synthetic biology techniques can be used to optimize the expression of specific genes responsible for the accumulation of micronutrients like iron and zinc, thereby increasing their levels in the seeds. Moreover, synthetic biology can facilitate the development of mustard varieties that are more resilient to environmental stresses, such as drought, salinity, and extreme temperatures. By introducing or modifying genes associated with stress tolerance, researchers can enhance the plant's ability to cope with challenging growing conditions, ensuring that the biofortified crops maintain their nutritional benefits. Overall, the integration of synthetic biology into crop improvement programs provides a powerful toolset for enhancing the nutritional qual-

ity and resilience of mustard, contributing to food security and improved human health. The integration of multi-omics data through systems biology models is expected to provide a comprehensive understanding of genotype-phenotype relationships and gene regulatory networks, thereby enabling knowledge-based trait improvement. This holistic approach will be crucial in optimizing breeding strategies for multiple target traits, ensuring sustainable productivity and adaptability of mustard in the face of climate change. By leveraging cooperative networks and harnessing cutting-edge technologies, the future of mustard breeding will see significant genetic gains across multiple traits. This progress will pave the way for the development of superior mustard varieties. These varieties will meet the growing global demand for sustainable and high-quality agricultural products. However, several potential challenges and limitations may arise in applying these advanced techniques. Regulatory hurdles could impede the swift approval and adoption of genetically engineered crops. The costs associated with developing and implementing these technologies may also be a barrier. This barrier could particularly affect smallholder farmers and breeding programs with limited resources. Additionally, technology adoption can vary among regions. This variation can impact the uniformity of advancements across different agricultural contexts. Addressing these challenges will be essential for realizing the full potential of modern mustard breeding strategies.

Conclusion

The immense genetic potential of mustard coupled with diverse challenges necessitates integrated breeding ap-

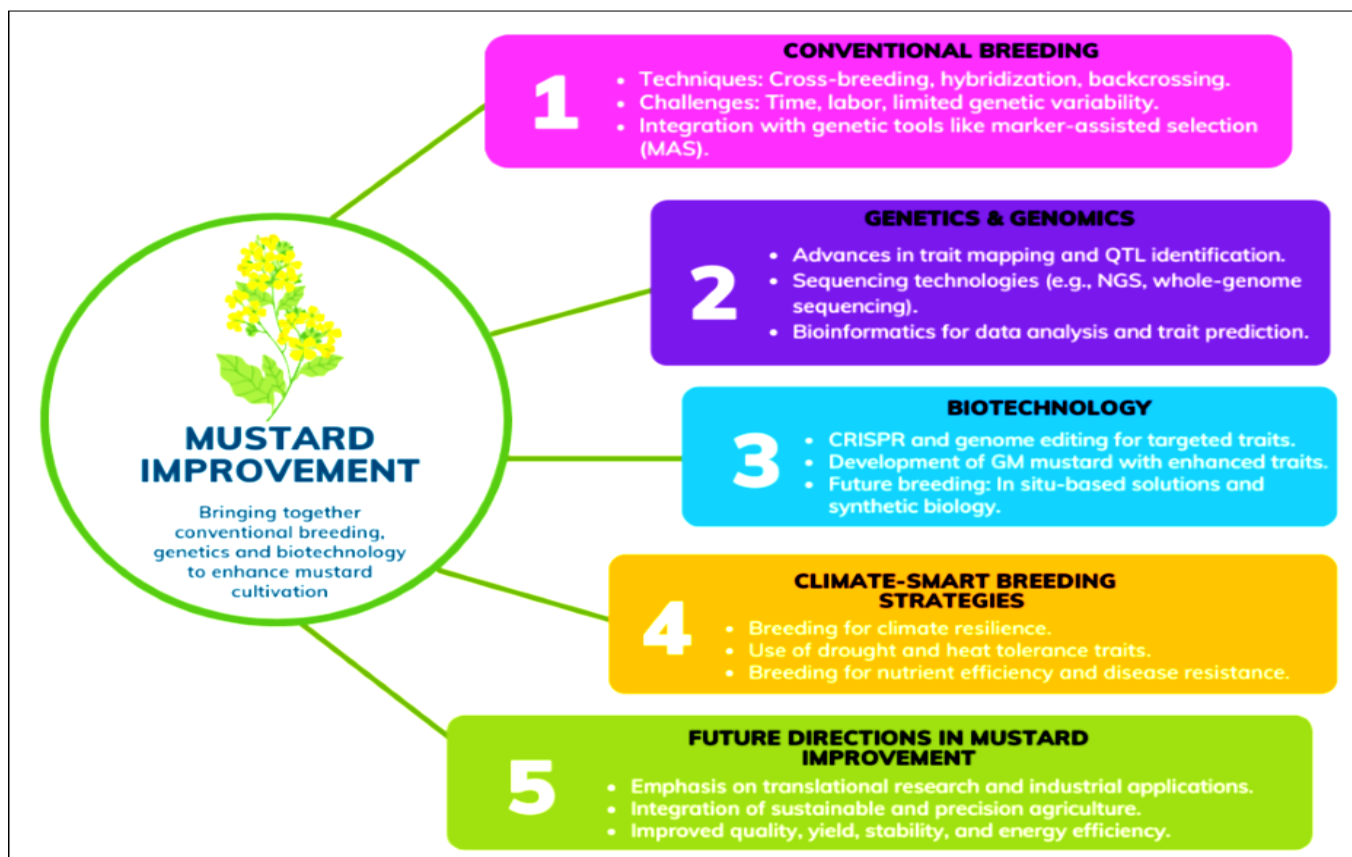


Fig. 1. Advancements in Plant Breeding Techniques. This figure illustrates different plant breeding techniques used to introduce new traits into elite crop varieties. The techniques are categorized based on biotechnological developments and their applications in improving crop yield and resistance, which are crucial for enhancing the productivity of crops such as rapeseed-mustard.

proaches. Advances in deciphering trait genetics and genomics are enabling knowledge-driven breeding and germplasm improvement. While conventional breeding methods retain relevance, emerging technologies like MAS, transgenics and genome editing are accelerating improvement for complex and novel traits. Climate-resilient mustards developed using sustainable breeding pipelines will be crucial for long-term productivity and food security. Sustainable breeding pipelines encompass strategies that prioritize environmental stewardship, resource efficiency, and reduced reliance on chemical inputs. By integrating modern biotechnologies with conventional breeding wisdom through collaborative networks, researchers can develop mustard varieties that are not only high yielding, but also environmentally friendly. This approach emphasizes minimizing environmental impact by selecting traits that enhance nutrient use efficiency and promote soil health. Additionally, sustainable breeding pipelines focus on reducing the need for chemical fertilizers and pesticides, thereby lowering the ecological footprint of mustard production. Improved resource use efficiency can be achieved through the development of varieties that require less water and nutrients while maintaining robust growth and yield. By continuing to integrate these cutting-edge technologies with time-tested breeding practices, mustard breeding programs have the potential to transform global mustard production, particularly in regions most vulnerable to climate change and food insecurity.

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Authors' contributions

GP contributed to writing the article, including searching, collecting, drafting, and editing the manuscript. US conceived the idea of writing the review and designed its content. SP analyzed the manuscript and provided regular assistance in revising and finalizing it. RR, SN, and SM critically reviewed and edited the manuscript. All authors have read and approved the final version for publication.

Compliance with ethical standards

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