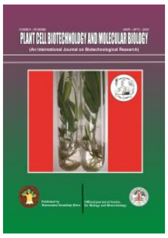

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Advances in Breeding for Oil Quality Enhancement in Indian Mustard (*Brassica spp.* L.): Achievements, Challenges, and Research Opportunities

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Major objectives in oil crop improvement are enhancement of seed and oil yield, quality of oil according to its use, *i.e.* edible or industrial uses, breeding of varieties that fit in different cropping systems and breeding biotic and abiotic stress resistant/tolerant varieties. Despite traditional breeding approaches, including pure line breeding, yielding only modest gains in productivity, recent advancements in mustard breeding have led to significant breakthroughs in both productivity and oil quality. This review discusses the innovative breeding strategies that have contributed to these advancements, with a focus on hybrid development, oil quality enhancement, and

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biotechnological approaches. To enhance productivity, researchers at the University of Delhi have developed hybrid seed production techniques using transgenic Barnase-barstar systems and cytoplasmic male sterility (CMS) systems. These systems enable large-scale hybrid seed production, with field trials demonstrating significant yield heterosis ranging from 31% to 55% compared to national check varieties. In addition to productivity, improving oil and meal quality has been a key objective. By integrating genes from canola-quality mustard lines, breeders have achieved reductions in erucic acid and glucosinolates, enhancing the health profile and industrial applicability of mustard oil. A high-density linkage map developed using an F1 double haploid mapping population has facilitated the marker-assisted backcross breeding of desirable traits, enabling precise transfer of key quality traits. Transgenic approaches, such as antisense RNA technology, have led to the development of high-oleic, low-linoleic mustard lines with improved fatty acid profiles. These advancements reflect a strategic combination of conventional and biotechnological methods, demonstrating a clear pathway for boosting mustard yields while enhancing oil quality. Molecular markers reported for genetic diversity assessment, mapping and tagging genes/QTLs for different qualitative and quantitative traits and their use in marker-assisted selection have been presented. This progress not only addresses current challenges but also sets the stage for future research aimed at further optimizing productivity, oil quality, and resistance to pests and diseases in mustard cultivation.

Keywords: Breeding; hybrid; CMS; antisense RNA technology; high-oleic; low-linoleic; molecular markers and QTLs.

1. INTRODUCTION

Indian mustard (*Brassica juncea* L.), is a major oilseed crop in India, cultivated across six million hectares, primarily in rainfed regions of northern India during the winter season [1]. *Brassica juncea*, commonly known as Indian mustard, holds particular significance due to its amphidiploid nature, with a chromosome count of 2n = 36 (AABB) [62]. This amphidiploidy arises from the combination of genomes from two diploid species: *Brassica rapa* (AA) with 2n = 20 chromosomes and *Brassica nigra* (BB) with 2n = 16 chromosomes [63-64]. Traditional breeding approaches, particularly pure line breeding, have yielded only marginal improvements in productivity, largely due to the limited genetic variability among the existing elite germplasm [2]. To significantly enhance productivity and oil quality, Indian breeding programs are now focusing on exploiting new sources of genetic variation and utilizing innovative biotechnological tools. To overcome the limited genetic base in traditional mustard breeding, breeders are exploring heterosis, or hybrid vigour, by crossing lines from two distinct gene pools—the east European and Indian gene pools. Hybrids from these diverse backgrounds have shown increased yields due to heterosis, with research indicating that these crosses are particularly effective for improving productivity [3]. However, the exotic east European germplasm, known for its beneficial traits like low erucic acid and glucosinolates, has proven challenging to integrate into Indian mustard varieties due to issues like linkage drag and adaptation to Indian agro-climatic conditions.

Addressing these challenges requires a combination of conventional and biotechnological approaches. The University of Delhi's laboratory is at the forefront of such efforts, focusing on two primary objectives: increasing productivity through hybrid breeding and improving oil and meal quality by developing canola-quality mustard with low erucic acid and glucosinolates. The lab has successfully developed cytoplasmic male sterility (CMS)-based restorer systems and transgenic barnase-barstar systems to enable large-scale hybrid seed production [4]. Additionally, high-density linkage maps using AFLP, RFLP, and SSR markers have been constructed to tag genes related to crucial traits, like erucic acid, linoleic acid, and seed coat color. These maps also facilitate the dissection of quantitative trait loci (QTLs) involved in yield components and glucosinolates. Biotechnological tools like antisense RNA and microspore-derived doubled haploids offer precise methods for transferring desirable traits from exotic germplasm into elite Indian varieties [5]. Marker-assisted backcross breeding provides a way to integrate specific traits from east European lines into Indian cultivars without compromising the heterotic gene pool. These innovative strategies are essential for developing high-yielding, high-quality mustard varieties that meet both productivity and nutritional goals, ensuring a brighter future for *Brassica juncea* cultivation in India [6].

Mustard, a staple crop in many regions, has gained significant attention due to its high oil content and adaptability. Breeding efforts in mustard have made remarkable strides, leading to enhanced oil quality and improved yields [7]. This review explores the latest advances in mustard breeding techniques, focusing on innovations that have enhanced oil content, yield, and resistance to pests like aphids. Key advancements in mustard breeding include the use of modern molecular techniques, such as marker-assisted selection (MAS), genomic selection, and CRISPR/Cas9-based gene editing. These methods allow breeders to target specific traits, speeding up the process of developing high-yielding and high-oil-content mustard varieties [8]. Moreover, breeders are leveraging traditional breeding techniques, like hybridization and backcrossing, to create varieties that are more resilient to environmental stressors and diseases.

A significant area of focus has been on improving oil quality in mustard [9]. This involves not only increasing oil content but also enhancing the fatty acid composition to meet health and industry standards. Breeders are working on reducing erucic acid levels and increasing oleic acid content to produce oil that is healthier for consumption and has a broader industrial application [10]. Additionally, efforts to increase the levels of essential fatty acids, such as omega-3 and omega-6, are ongoing, with promising results. In addition to oil quality, mustard breeding programs aim to increase overall yield and resistance to common pests and diseases. Breeding for aphid resistance is crucial, as aphids can significantly impact crop health and yield [11]. By incorporating genes from wild relatives and using advanced genomic tools, breeders are developing mustard varieties that require fewer pesticides and are more environmentally sustainable. This comprehensive review provides a detailed analysis of the latest advancements in mustard breeding, emphasizing oil quality enhancement and yield improvement. It also highlights the challenges that remain and suggests future research directions to further enhance the crop's potential. Through continued innovation and collaboration, mustard breeding can contribute significantly to food security and sustainable agriculture [12].

2. ENHANCEMENT OF PRODUCTIVITY THROUGH DEVELOPMENT OF HYBRIDS

To boost mustard productivity through hybrid development, innovative approaches in creating male sterility and restorer systems have been employed. The lab has pioneered the use of transgenic barnase-barstar systems to induce male sterility and create restorer lines in *Brassica juncea* [13]. This technique involves transferring barnase and barstar transgenes to appropriate heterotic combiners via recurrent backcrossing, providing a reliable method for large-scale hybrid seed production. The resulting hybrids based on this male sterility system have demonstrated significant yield advantages. In field trials conducted in northern India over two consecutive mustard-growing seasons, yield heterosis of 50–55% was recorded in transgenic hybrids compared to national check varieties [14]. The hybrid will undergo multi-location trials during the 2005-06 growing season to further validate its performance [15].

Additionally, the lab has developed a novel cytoplasmic male sterility (CMS) and restorer system for large-scale hybrid seed production [16]. This CMS system is unique in that it allows any mustard line to maintain sterility after a series of backcrosses or restore fertility in the F1 generation, providing a broad range of combiners and restorers for hybrid seed production (Fig. 1). The first hybrid derived from this CMS system was tested in demonstration trials on farmers' fields in northern India during the 2004-05 growing season, yielding an average heterosis of 31% (with a range from 16-58%) compared to local check varieties [17]. Plans are underway to test this hybrid in larger trials across 500 farmers' fields during the 2005-06 season, with each trial covering one acre [18]. These advancements in hybrid breeding techniques offer promising avenues for enhancing mustard productivity and can potentially revolutionize mustard cultivation in India by providing high-yielding, resilient hybrids for large-scale commercial production.

3. IMPROVEMENT OF OIL AND MEAL QUALITY

Enhancing oil and meal quality in Indian B. juncea (mustard) has been a key focus of breeding programs, with a particular emphasis on reducing erucic acid and glucosinolates—two traits associated with canola-quality mustard [19]. Low erucic acid in B. juncea is controlled by two recessive genes, while low glucosinolates are governed by 6-7 recessive genes [20]. These traits are being transferred from the canola-quality line "Heera" to Indian cultivars through marker-assisted backcross breeding using double haploids. A significant step in this process involved the development of a high-density linkage map using an F_1 double haploid (DH) mapping population derived from a cross between "Varuna" (an Indian cultivar) and "Heera'' [21]. The initial map consisted of 1,029 Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLP) markers, later augmented with additional AFLP, RFLP, and Simple Sequence Repeat (SSR) markers [22]. This map facilitated the identification and tagging of critical loci. Through a candidate gene approach, two loci associated with erucic acid were identified, while microsatellite markers helped tag two loci for seed coat color [23]. The markers from this map are instrumental in the marker-assisted backcross transfer of low erucic acid and yellow seed coat color traits from East European lines to Indian varieties [24].

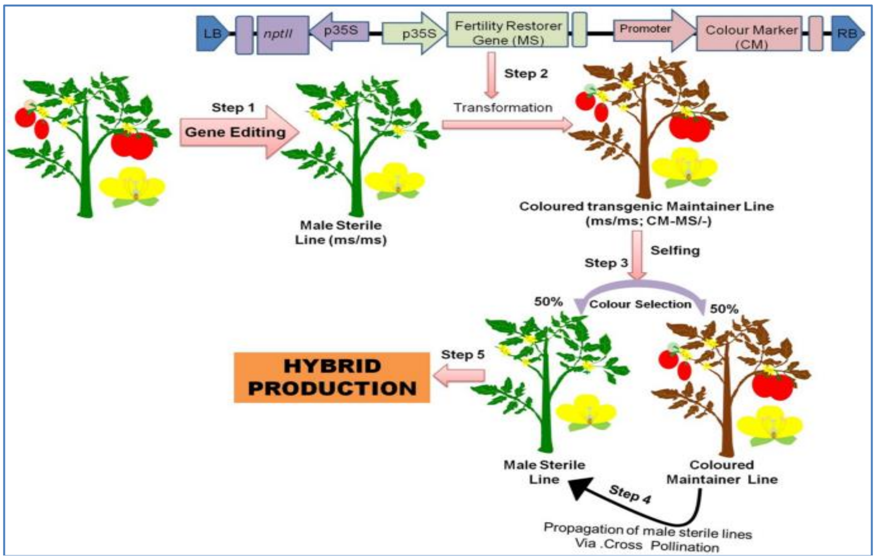

Fig. 1. Advancements from natural CMS to genetically manipulated systems for hybrid seed production [61]

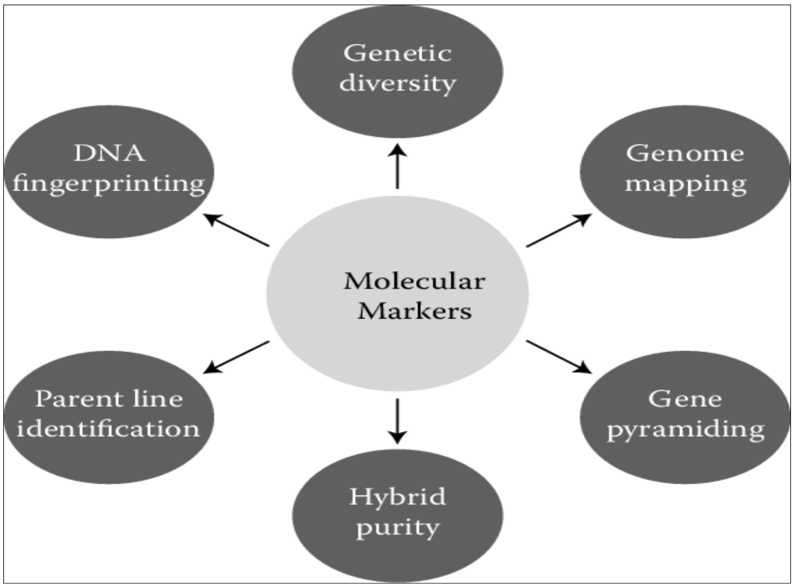

Fig. 2. Molecular markers and their applications in plant breeding

Transgenic approaches have also played a vital role in improving oil quality. A high-oleic, low-linoleic mustard line was developed using antisense RNA technology [25]. This involved modifying the fatty acid composition of a zero-erucic acid mustard line by introducing antisense constructs targeting the fad2 gene from B. rapa. The resulting homozygous lines achieved 74% oleic acid and 8-9% each of linoleic and linolenic acids [26]. Further work aims to make these transgenic lines marker-free through the Cre-Lox technique, with plans to transfer them to zero-erucic acid Indian mustard varieties. This progress underscores the innovative breeding strategies employed to enhance oil quality, ultimately contributing to higher-value mustard products with improved nutritional profiles and broader market appeal.

4. USE OF MOLECULAR TOOLS FOR OIL CROP IMPROVEMENT

In the quest for improving oil crops, particularly with respect to biotic and abiotic stress resistance, traditional breeding approaches often face limitations [27]. These challenges stem from complex genetic control, lack of resistant germplasm, inadequate screening techniques, and the influence of environmental factors on traits. However, molecular tools, particularly Marker-Assisted Selection (MAS), offer promising solutions by expediting the process of trait improvement and enhancing precision in selection. Conventional crop improvement typically involves the exploitation of genetic variation within crossable boundaries, focusing on recombination breeding and heterotic hybrid development [28]. This process heavily relies on the selection of appropriate parental lines, often derived from available germplasm and passport data. While these resources provide a foundation for breeding, many potentially valuable genotypes remain underutilized within the primary gene pool. Furthermore, conventional selection methods predominantly depend on phenotype, which, due to the interaction between genotype and environment, may not always accurately reflect the underlying genetic potential.

MAS overcomes these limitations by allowing breeders to focus directly on the genotype, reducing the dependency on phenotypic expressions that can be misleading, especially for complex traits like oil yield, disease resistance, and drought tolerance [29,65,66]. The integration of molecular markers into breeding programs provides a more efficient and accurate approach to selecting desirable recombinants [30]. MAS enables the identification of key genetic markers associated with specific traits, allowing for more targeted breeding and reducing the time required for trait introgression [31].

Additionally, when no resistance source exists within the germplasm, transgenic approaches can be explored to incorporate resistance traits from other species, broadening the genetic base for improvement [32]. These molecular tools not only streamline the breeding process but also enhance the overall effectiveness of oil crop improvement by providing a more reliable and rapid path to achieving desired traits. This review underscores the significance of molecular tools in oil crop improvement, with a focus on the successful implementation of MAS and its potential to revolutionize breeding practices. By utilizing molecular markers and transgenic techniques, breeders can address the complex challenges associated with traditional methods, leading to enhanced oil yields, improved disease resistance, and greater sustainability in oil crop production.

5. MARKER-ASSISTED SELECTION IN IMPROVEMENT OF OILSEED CROPS

Over the past quarter-century, since the landmark paper in 1986 on Restriction Fragment Length Polymorphism (RFLP) markers for constructing linkage maps in tomato and maize, significant advancements have been made in applying molecular techniques to oilseed crops [33]. This has catalyzed a paradigm shift in plant breeding, with a focus on Sequence Tagged Site (STS) and Simple Sequence Repeat (SSR) markers to generate high-density genome maps and tag genes/quantitative trait loci (QTLs) in Brassica, soybean, sunflower, and groundnut. Single Nucleotide Polymorphisms (SNPs) have also emerged, providing insights into genetic diversity and enhancing the precision of marker-assisted selection (MAS) [34]. The early limitations of MAS, such as recombination between markers and target genes, low polymorphism levels, and reduced resolution of QTLs due to environmental interactions, are gradually being addressed [35]. Recent developments in genome-wide sequence-based SSR and SNP markers hold the key to overcoming these challenges, especially in key oilseed crops [36]. High-density genetic and physical maps are aiding in finding markers that are physically close to target genes, minimizing the risk of marker-trait linkage breakdown due to recombination [37]. The construction of these high-density genome maps using SSR markers is critical for rapid tagging of useful genes and map-based genome characterization [38].

For qualitative traits like nematode and virus resistance in soybean, MAS is immediately applicable, while quantitative traits require further validation and fine mapping to identify tightly linked markers [39]. Pyramiding multiple genes for resistance against various pathogens, nematodes, and insects could significantly boost productivity and ensure more robust oilseed crops [40]. Strategic use of markers in conjunction with conventional breeding is crucial for stabilizing production, particularly under abiotic stresses [41]. Many QTLs for seed and oil yield, as well as for salt and drought tolerance, have been mapped, yet their validation in varied genetic backgrounds and across different locations is necessary for broader application (Fig. 3). Achieving the full potential of MAS in oilseed crop improvement demands considerable research efforts, substantial funding, enhanced infrastructure for phenotyping and genotyping, and innovative experimental strategies [42]. The ongoing reduction in genotyping costs, coupled with rapid technological advancements, makes MAS more accessible for large-scale breeding experiments [43]. However, successful MAS implementation requires strategic integration with traditional breeding, emphasizing that these molecular tools are not replacements but rather enhancements to conventional approaches. The potential benefits in terms of time, effort, and cost savings in the long run justify continued investment in robust sequence-based validated markers for key traits in oilseed crops [44, 64].

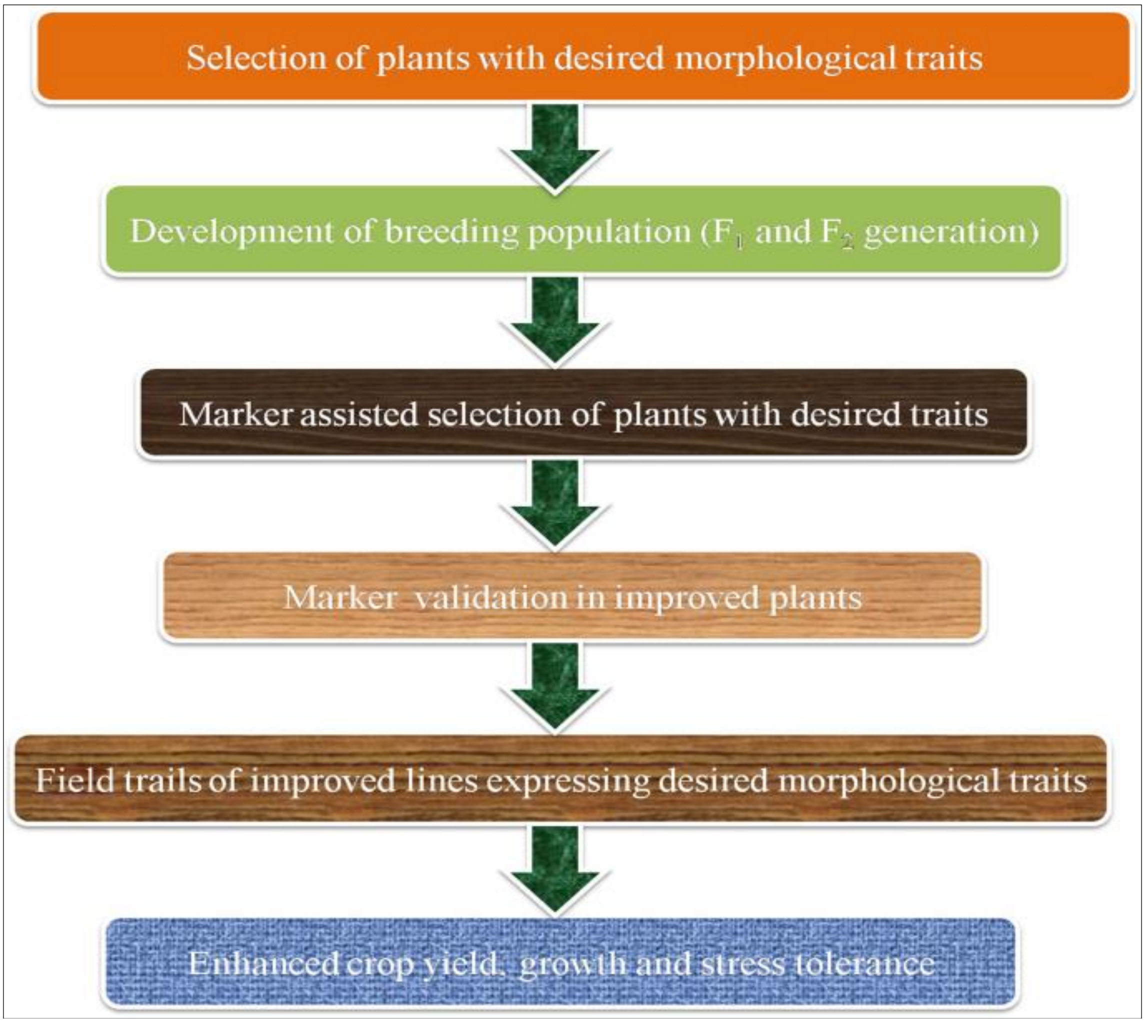

Fig. 3. Marker-assisted breeding for abiotic stress tolerance in crop plants

6. ACHIEVEMENTS IN MUSTARD BREEDING AND OIL QUALITY

Advances in mustard breeding have played a pivotal role in transforming the oilseed landscape in India [45]. A significant achievement in this field is the development of high-yielding hybrid varieties, enabled by innovative breeding techniques such as cytoplasmic male sterility (CMS) and the transgenic barnase-barstar system [46]. These approaches have revolutionized hybrid seed production, allowing for large-scale commercial cultivation with substantial yield gains. In field trials, these hybrid mustard varieties have shown yield heterosis of up to 50-55% compared to traditional national check varieties, indicating a tremendous potential for boosting productivity [47]. The success of these hybrids has provided farmers with new opportunities to increase their output, contributing to food security and rural income [48].

Another notable achievement in mustard breeding is the significant improvement in oil quality. The focus has been on reducing erucic acid and glucosinolates, compounds that are generally considered undesirable in edible oils due to health and flavor concerns [49]. Through marker-assisted backcross breeding and transgenic techniques, researchers have successfully introduced canola-quality traits into Indian mustard cultivars. These traits include lower erucic acid content and reduced glucosinolates, making the oil healthier for consumption and expanding its market potential. Transgenic approaches, such as the use of antisense RNA, have further enhanced the oil's composition by increasing oleic acid content and reducing linoleic and linolenic acids [50]. These advancements have improved the nutritional profile of mustard oil, making it more appealing to health-conscious consumers.

Furthermore, the integration of molecular tools has played a crucial role in enhancing the efficiency of breeding programs. High-density linkage maps and marker-assisted selection (MAS) have allowed breeders to identify and transfer specific traits more accurately, reducing the time required for breeding new varieties [51]. These molecular techniques have enabled precise selection of traits related to yield, oil quality, and even resistance to biotic and abiotic stresses. This precision has resulted in a more consistent and reliable breeding process, leading to mustard varieties that are not only more productive but also more resilient to environmental challenges. Overall, the achievements in mustard breeding and oil quality enhancement are a testament to the successful marriage of traditional breeding with modern biotechnological tools, paving the way for a brighter future for mustard cultivation in India.

7. CHALLENGES IN MUSTARD BREEDING AND OIL QUALITY

Despite the remarkable achievements in mustard breeding and oil quality enhancement, several challenges persist that require ongoing attention. A key challenge is the complex genetic control of many traits, particularly those related to yield, disease resistance, and stress tolerance. Mustard breeding involves managing a variety of genetic factors, which can complicate the selection process [52]. This complexity is exacerbated by the interaction of multiple genes and environmental influences, making it difficult to isolate and stabilize desired traits. The high degree of genetic variation required for effective breeding also poses a challenge, as it necessitates extensive resources for genotyping and phenotyping to maintain the diversity needed for robust breeding programs [53].

Another significant challenge is the potential environmental impact of new breeding techniques, particularly transgenic approaches. Public perception and regulatory hurdles often complicate the introduction of genetically modified organisms (GMOs), leading to slower adoption rates and increased costs associated with regulatory compliance [54]. Moreover, the use of transgenic methods in mustard breeding may face opposition from certain consumer groups and environmental organizations, who express concerns about biodiversity and ecosystem health. Addressing these concerns requires careful risk assessments and transparent communication to ensure that breeding innovations are both safe and publicly accepted.

Additionally, ensuring equitable access to advanced mustard varieties is a challenge that impacts the broader agricultural community. Although high-yielding hybrids and improved oil quality can benefit farmers and consumers alike, disparities in resource availability can create barriers to adoption. Smallholder farmers may lack the financial resources, knowledge, or infrastructure needed to effectively implement these advanced breeding techniques. This disparity can limit the widespread adoption of improved mustard varieties and exacerbate existing inequalities in the agricultural sector. To overcome this challenge, it's crucial to focus on capacity building, knowledge transfer, and inclusive policies that ensure all stakeholders can benefit from advances in mustard breeding and oil quality enhancement.

8. FUTURE PERSPECTIVES FOR MUSTARD BREEDING AND OIL QUALITY ENHANCEMENT

The field of mustard breeding and oil quality enhancement in India stands at the cusp of a transformative era, with significant achievements paving the way for new research opportunities and innovative approaches [55]. The integration of advanced biotechnological tools, such as marker-assisted selection (MAS), genomic selection, and CRISPR/Cas9 gene editing, has revolutionized the ability to identify and transfer desirable traits into mustard crops with precision and efficiency [56]. This capability opens doors to overcoming traditional breeding limitations, accelerating the development of high-yielding, disease-resistant, and nutritionally enhanced mustard varieties. In the coming years, researchers are expected to focus on optimizing the balance between productivity and sustainability [57]. This will involve not only improving yields but also enhancing oil quality, such as increasing oleic acid content while reducing erucic acid and glucosinolates. The adoption of integrated nutrient management (INM) practices, combining organic and inorganic nutrient sources, is poised to play a pivotal role in promoting sustainable agricultural practices and soil health [58]. This holistic approach to mustard cultivation aligns with the broader sustainability goals outlined by global agricultural frameworks.

Challenges remain, including the need to address climate change's impact on mustard production, ensuring resistance to emerging diseases and pests, and overcoming socio-economic barriers to technology adoption [59]. Future research must focus on developing climate-resilient mustard varieties, understanding the complex genetic mechanisms underpinning yield and quality traits, and promoting wider adoption of innovative breeding techniques among Indian farmers. Collaborative efforts among academic institutions, government agencies, and private sector stakeholders will be essential to drive these advancements forward [60]. Furthermore, there is an opportunity to explore alternative uses for mustard crops, such as biofuel production and industrial applications, which could diversify market opportunities for Indian farmers. By addressing these challenges and leveraging emerging technologies, India can lead the way in mustard breeding and oil quality enhancement, ensuring food security, promoting sustainable agriculture, and contributing to the economic well-being of rural communities. The journey ahead is both exciting and promising, with immense potential to redefine mustard cultivation in India.

9. CONCLUSION

Mustard breeding and oil quality enhancement in India has witnessed significant advancements, driven by both conventional and biotechnological approaches. Through the implementation of innovative breeding techniques like cytoplasmic male sterility (CMS), transgenic barnase-barstar systems, and marker-assisted selection (MAS), Indian mustard breeding programs have made notable strides in improving yield, oil quality, and resistance to biotic and abiotic stresses. These achievements have translated into tangible benefits for farmers, with higher-yielding and more resilient mustard varieties contributing to increased agricultural productivity and sustainability. However, these advancements come with a set of challenges that require strategic solutions. The complex genetic structure of mustard, along with environmental influences, makes it challenging to achieve stable and predictable outcomes in breeding programs. By leveraging molecular tools and integrating conventional breeding with modern biotechnological methods, researchers can continue to improve oil quality, yield, and environmental resilience. The continued exploration of genetic diversity and the development of high-density genetic maps will be critical in identifying and harnessing desirable traits. Ultimately, the success of future mustard breeding initiatives will depend on collaborative efforts among researchers, policymakers, and the farming community to ensure that the benefits of these advancements are maximized and widely accessible. Through this approach, India can continue to lead the way in mustard breeding and oil quality enhancement, contributing to a more sustainable and productive agricultural sector.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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