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Genomic Approaches for Enhancing Yield and Quality Traits in Mustard (*Brassica spp*.): A Review of Breeding Strategies

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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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ABSTRACT

Mustard, a vital oilseed crop, plays a significant role in global agriculture due to its versatile applications in food, feed, and biofuel industries. However, meeting the increasing demands for yield and quality traits poses a substantial challenge to mustard breeders. In response, genomic approaches have emerged as powerful tools to expedite mustard breeding programs by unraveling the genetic basis of key agronomic traits. This review provides a comprehensive overview of genomic strategies aimed at enhancing yield and quality traits in mustard. Beginning with an exploration of traditional breeding methods and their limitations, we delve into the advancements in genomics, including next-generation sequencing technologies, marker-assisted selection (MAS), and genome editing techniques. We discuss how these tools are leveraged to identify yield-related genes, quantitative trait loci (QTLs), and markers for efficient trait selection. Furthermore, we examine genomic approaches for improving oil content, nutritional profiles, and phytochemical

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composition, crucial for enhancing mustard quality. Case studies demonstrating the successful integration of genomics into breeding programs are highlighted, along with discussions on challenges such as regulatory concerns and technical hurdles. Finally, we outline future directions and the potential of genomic approaches to revolutionize mustard breeding, paving the way for sustainable crop improvement. This study offers valuable insights into the application of genomics in mustard breeding and underscores its importance in addressing the evolving needs of agriculture in the 21st century.

Keywords: Mustard; genomic approaches; yield, quality; breeding; Marker-assisted Selection (MAS).

1. INTRODUCTION

Mustard, with its rich historical legacy, stands as an ancient crop deeply intertwined with human civilization. Its cultivation traces back thousands of years, with evidence suggesting its presence in civilizations such as the Egyptians, Greeks, and Romans [1]. Over time, mustard has evolved from a wild plant to a domesticated crop, adapting to various climates and soil conditions. Geographically, mustard finds its place in diverse regions across the globe, from temperate zones to subtropical and tropical climates. Its adaptability to different environments makes it a versatile crop for cultivation. However, regions with well-drained soils and moderate temperatures are most conducive to mustard growth. The crop's distribution spans continents, contributing significantly to agricultural landscapes worldwide [2]. Agronomic practices play a pivotal role in mustard cultivation, ensuring optimal yield and quality. Land preparation, sowing techniques, irrigation, fertilization, and pest and disease management are among the key aspects of mustard farming. Farmers employ various methods to enhance crop productivity while minimizing environmental impact, balancing traditional wisdom with modern agricultural practices [3]. Beyond its agricultural significance, mustard holds economic and cultural importance. As a cash crop, it contributes substantially to the livelihoods of farmers, particularly in regions where it serves as a primary source of income. Moreover, mustard occupies a prominent place in culinary traditions worldwide, adding flavor and nutrition to a myriad of dishes. Its seeds are not only valued for oil extraction but also for their culinary and medicinal properties, reflecting its multifaceted role in human society [4].

2. SIGNIFICANCE OF YIELD AND QUALITY TRAITS IN MUSTARD

The significance of yield and quality traits in mustard is multifaceted, impacting both agricultural productivity and end-use applications. Higher yields directly translate to increased production, which is essential for meeting the growing demand for food, feed, and biofuel. Mustard serves as a vital oilseed crop, contributing to edible oil production, livestock feed, and industrial applications. Enhancing yield traits biomass production is imperative for sustainable agriculture and meeting global dietary needs [5]. Moreover, yield traits play a pivotal role in determining the economic viability of mustard cultivation. Farmers rely on high-yielding varieties to maximize their returns and maintain profitability. Improved yield traits not only increase farm income but also contribute to rural livelihoods, supporting the socio-economic well-being of farming communities. A robust mustard industry stimulates economic growth by generating employment opportunities and driving market demand for related products [6]. Quality traits are equally essential as they determine the market value and consumer acceptance of mustard products. In the case of mustard oil, quality parameters such as oil content, fatty acid composition, and flavor profile influence its utility in cooking, food processing, and pharmaceutical applications (Table 1). Mustard seeds are also valued for their protein content, nutritional composition, and phytochemical properties, which contribute to their dietary and health benefits. Improving quality traits enhances the market competitiveness of mustard products, facilitating trade and market access both domestically and internationally [7].

3. GENOMIC APPROACHES IN MUSTARD BREEDING

The integration of genomic approaches in mustard breeding is driven by the desire to the development of improved varieties with desirable traits. Traditional breeding methods, while effective, are often time-consuming and labor-intensive, relying on phenotypic evaluation and selection based on visible characteristics [8]. Furthermore, genomic approaches enable the identification and utilization of genetic variation present within mustard germplasm and introduce novel traits (Table 2). High-throughput sequencing technologies facilitate the rapid generation of genomic data, including whole-genome sequences [9]. This wealth of genetic information empowers breeders to perform targeted introgression of favorable alleles from wild relatives or exotic germplasm, thereby enhancing the genetic diversity and adaptive potential of mustard cultivars. In addition, genomic tools such as (MAS) offer precision and efficiency in trait improvement by enabling the selection of desired traits at the molecular level. MAS involves the use of DNA markers linked to target traits to screen and select breeding lines possessing the desired genetic makeup. This targeted approach minimizes the need for laborious field evaluations and accelerates the breeding cycle, leading to faster cultivar development and release [10].

Genomics, powered by a diverse genetic pool, is emerging as a key driver in modern crop improvement. This approach involves tapping into a wide range of genetic diversity to create robust genomic resources, which serve as the foundation for trait discovery and improvement in crops like Brassica. Fig 1 illustrates this crop improvement cycle, highlighting the critical role of genetic variation and genomic resources in accelerating the process from the laboratory to the field and back. The cycle encompasses a holistic approach to crop improvement, beginning with the exploration of genetic diversity in existing germplasm resources. This genetic information forms the basis for genomic selection (GS) and genomic engineering (GE), allowing for rapid identification and incorporation of desirable traits. These techniques significantly reduce the time and effort required for conventional breeding methods, offering more precise and efficient ways to enhance crop characteristics. Moreover, genomic engineering enables targeted modifications for specific traits, facilitating the development of climate-resilient and adaptive crops capable of withstanding extreme environmental conditions. By capitalizing on genomic diversity, the crop improvement cycle can deliver more robust and sustainable Brassica varieties, better equipped to meet the demands of modern agriculture.

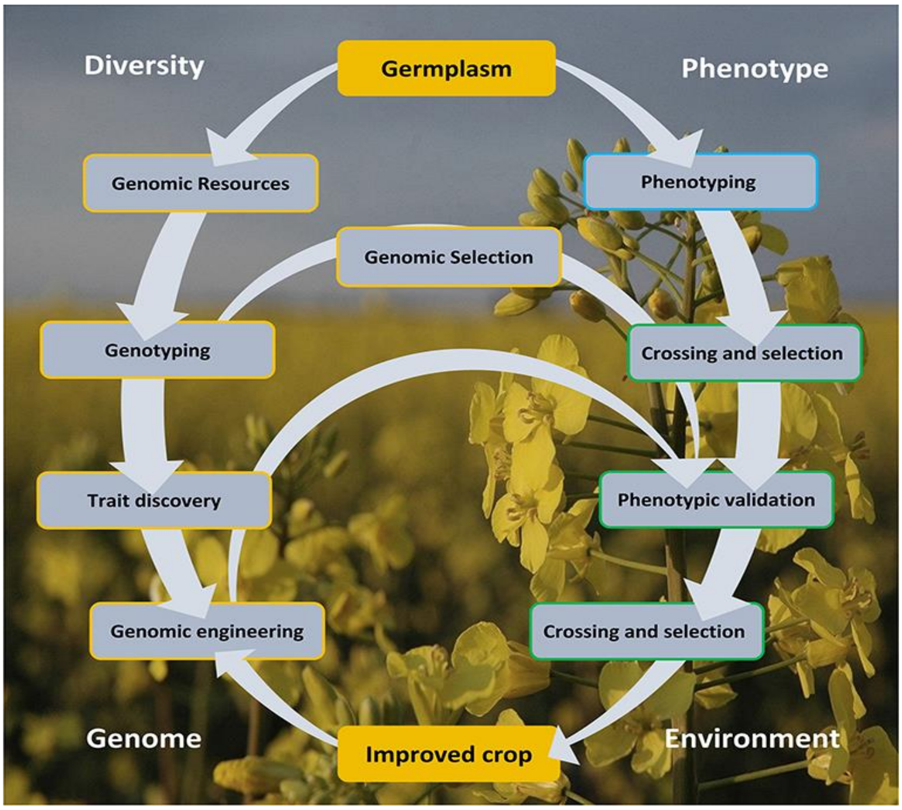

Fig. 1. Genomics armed with diversity leads the way in brassica improvement [21]

Table 1. Yield and quality traits in mustard along with their different properties

Trait	Significance	Properties
Yield Potential	Determines overall productivity	Influenced by genetics, environment, and management
Seed Size	Affects market value and processing	Larger seeds often preferred for oil extraction
Oil Content	Determines oil yield and quality	High oil content desirable for oil production
Protein Content	Affects nutritional value and end-use	Higher protein content often preferred for food products
Disease Resistance	Reduces crop losses and improves stability	Resistance to fungal, bacterial, and viral pathogens
Water Use Efficiency	Maximizes water utilization	Efficient use of water resources for better yields
Harvest Index	Indicates resource allocation efficiency	Higher harvest index indicates more yield per unit of resource invested
Maturity Period	Determines the time to harvest	Early-maturing varieties allow for flexible cropping systems and reduced risks
Lodging Resistance	Prevents plant lodging and yield losses	Strong stems and root systems help plants withstand adverse weather conditions
Pod Shattering	Minimizes seed loss during harvesting	Reduced pod shattering leads to higher yield and easier harvesting
Leaf Area Index	Reflects canopy development and light interception	Higher leaf area index enhances photosynthesis and yield potential
Root Depth	Improves nutrient and water uptake	Deeper roots access moisture and nutrients from deeper soil layers
Flowering Duration	Influences pollination and seed set	Longer flowering duration allows for extended pollination and seed development
Seed Viability	Determines seed germination rates	High seed viability ensures better establishment and uniform crop stand
Nutrient Content	Affects crop quality and nutritional value	Adequate levels of essential nutrients contribute to better crop performance

Table 2. Historical methods for mustard improvement

Methods	Properties	References
Mass Selection	Involves selecting superior individuals based on observable traits	[11]
Pure Line Selection	Selection of individual plants with desirable characteristics for propagation	[12]
Hybridization	Crossing of genetically diverse mustard lines to generate hybrid progeny	[13]
Mutagenesis	Induction of genetic variability through chemical or radiation mutagens	[14]
Polyploidy Induction	Generation of plants with multiple sets of chromosomes to enhance genetic diversity	[15]
Heterosis Breeding	Utilization of hybrid vigor by crossing genetically diverse mustard lines	[16]
Interspecific Hybridization	Crossbreeding between different species of mustard to introduce novel traits	[17]
Grafting	Joining tissues of different mustard varieties to combine desirable traits	[18]
Induced Polyploidy	Artificially induced multiplication of chromosome sets to create polyploid plants	[19]
Selection for Resistance	Selection of mustard lines with resistance to pests, diseases, or abiotic stress	[20]

4. GENOMIC TOOLS AND TECHNIQUES

Genomics, the study of an organism's entire genetic makeup, offers unprecedented opportunities to unravel the complexities of plant genomes and identify key genes underlying agronomical important traits. By deciphering the genetic code of plants, researchers can gain insights into the molecular mechanisms governing traits such as yield. This knowledge enables breeders to make informed decisions in selecting parental lines and designing breeding strategies aimed at enhancing desired traits [22]. Traditional plant breeding methods, while effective, often rely on time-consuming and labor-intensive phenotypic evaluations, which are limited by the availability of genetic variability within breeding populations (Table 3) [23]. Genome editing enables breeders to introduce beneficial alleles or edit specific genes

Table 3. These genomic tools and techniques empower mustard breeder

Genomic Tool/Technique	Significance	Properties
Genotyping by Sequencing	Identifies genetic variations	High-throughput sequencing of DNA fragments allows for SNP discovery and genotyping
Genome-Wide Association Studies (GWAS)	Links genotype to phenotype	Identifies genetic markers associated with traits of interest, aiding in trait improvement and marker-assisted selection
Marker-Assisted Selection (MAS)	Accelerates breeding process	Targets specific genomic regions associated with desired traits for selection, reducing breeding cycles
Quantitative Trait Loci (QTL) Mapping	Identifies regions of interest in the genome	Maps genomic regions linked to quantitative traits, facilitating marker development and breeding
Genome Editing (CRISPR/Cas9)	Precision gene modification	Enables targeted modifications of specific genes for trait improvement, enhancing crop performance
Transcriptomics	Studies gene expression patterns	Analyzes RNA transcripts to understand gene function and regulation, aiding in trait characterization
Genomic Selection	Predicts breeding values based on genomic data	Uses genomic information to estimate breeding values, enhancing selection accuracy and genetic gain
Single Nucleotide Polymorphism (SNP) Genotyping	Identifies genetic variation	Detects single nucleotide variations in the genome, serving as genetic markers for trait mapping and selection
Comparative Genomics	Studies evolutionary relationships	Compares genomes of different species or varieties to understand genetic diversity and trait evolution
Next-Generation Sequencing (NGS)	Rapid genome sequencing	Enables high-throughput sequencing of DNA, providing comprehensive genomic information for analysis
Metagenomics	Studies microbial communities	Analyzes DNA sequences from environmental samples to understand microbial diversity and function
Epigenomic	Studies heritable changes in gene expression	Investigates modifications to DNA and histones, providing insights into gene regulation and phenotype variation
Bioinformatics	Analyzes and interprets genomic data	Utilizes computational tools and algorithms to process, analyze, and interpret large-scale genomic datasets
Genomic Databases	Stores and shares genomic information	Central repositories of genomic data facilitate data sharing, collaboration, and access to valuable resources

responsible for agronomical desirable traits, accelerating the development of tailored crop varieties with enhanced yield, quality, and resilience to biotic and abiotic stresses [24]. Furthermore, genomics fosters international collaborations and data sharing initiatives, enabling breeders to access vast genomic resources and accelerate genetic gain across diverse crop species. The 1001 Genomes Project in Arabidopsis thaliana, researchers collaborate to sequence and annotate entire plant genomes, providing valuable genomic resources for crop improvement efforts worldwide [25, 26].

4.1 Deciphering Plant Genomes

"Genomics allows researchers to decode the complete genetic information of plants, providing insights into the organization, structure, and function of their genomes. By sequencing plant genomes, scientists can identify genes responsible for various traits, including yield, disease resistance, and stress tolerance. This knowledge forms the basis for understanding the genetic basis of plant traits and facilitates targeted breeding efforts to develop improved crop varieties [27].

4.2 Next-Generation Sequencing (NGS)

"Next-generation sequencing technologies enable rapid and cost-effective sequencing of plant genomes. NGS platforms generate massive amounts of DNA sequence data, allowing researchers to comprehensively analyze the genetic variation within plant populations. This information is crucial for identifying genomic regions associated with target traits through methods such as genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping [28].

4.3 Marker-Assisted Selection (MAS)

"MAS involves the use of molecular markers linked to target genes or genomic regions associated with desirable traits. By genotyping breeding populations with molecular markers, breeders can identify individuals carrying favorable alleles and accelerate the selection process. MAS enables more efficient selection of desired traits compared to traditional phenotypic evaluation methods, leading to faster development of improved crop varieties [28].

4.4 Genomic Selection (GS)

"Genomic selection leverages genomic information to predict the breeding value of individuals based on their entire genetic makeup. Through the use of statistical models and machine learning algorithms, GS integrates genome-wide marker data with phenotypic information to estimate the genetic merit of individuals for target traits. This approach enables breeders to make selections at earlier stages of breeding programs, leading to faster genetic gain and increased breeding efficiency [29].

4.5 Genome Editing Technologies

"Genome editing tools, such as CRISPR-Cas9, enable precise modification of plant genomes by targeting specific DNA sequences. CRISPR-Cas9 allows researchers to introduce targeted mutations, insertions, or deletions in plant genomes, facilitating gene knockout, gene replacement, or gene editing. Genome editing offers unprecedented opportunities for crop improvement by enabling precise modification of genes associated with agronomical important traits, leading to the development of tailored crop varieties with enhanced performance and resilience [30].

5. GENOMIC APPROACHES FOR QUALITY IMPROVEMENT

5.1 Understanding the Genetic Basis of Quality Traits

The genetic basis of quality traits is essential for improving the overall quality of crops. Genomic approaches allow researchers to dissect the complex genetic architecture underlying various quality traits such as flavor, aroma, texture, and nutritional composition. Through techniques like genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping, researchers can identify genomic regions associated with specific quality traits. By pinpointing candidate genes and regulatory elements responsible for quality traits, breeders gain insights into the molecular mechanisms governing trait expression. This knowledge serves as a foundation for targeted breeding efforts aimed at enhancing quality attributes in crop plants [31].

5.2 Genomic Approaches for Enhancing Oil Content

Oil content is a critical quality trait in oilseed crops, influencing the economic value and utility of the harvested produce. Genomic approaches offer powerful tools for enhancing oil content in crop plants. Through genomic selection and marker-assisted breeding, breeders can identify and introgression favorable alleles associated with high oil content into elite breeding lines [32]. Additionally, genome-editing technologies such as CRISPR-Cas9 enable precise modifications to genes involved in oil biosynthesis pathways, leading to the development of high-yielding oilseed varieties [33]. By targeting key regulatory genes and metabolic pathways, breeders can manipulate oil accumulation in seeds, ultimately improving the oil content and quality of oilseed crops [34].

5.3 Improving Nutritional and Phytochemical Profiles

Genomic selection strategies facilitate the breeding of nutrient-rich varieties by prioritizing individuals with desirable nutritional traits based on their genomic profiles. [35,36] Moreover, understanding the genetic basis of phytochemical compounds such as antioxidants, vitamins, and secondary metabolites enables breeders to develop crops with enhanced functional properties and therapeutic benefits. Through targeted manipulation of metabolic pathways and gene regulatory networks, genomic approaches offer unprecedented opportunities for improving the nutritional quality and health-promoting attributes of crop plants.

5.4 Integration of Genomic Approaches into Breeding Strategies

- **Combining Genomic Tools with Conventional Breeding Methods**
  For instance, marker-assisted selection (MAS) allows breeders to identify and introgress favorable alleles associated with target traits into elite breeding lines more efficiently than traditional phenotypic selection methods.
- **Accelerating Breeding Cycles Through Genomics:**
  Genomic approaches offer unprecedented opportunities for accelerating breeding cycles and increasing genetic gain in crop improvement programs.
- **Case Studies Demonstrating Successful Implementation**
  Numerous case studies demonstrate the successful implementation of genomic approaches in breeding programs across various crop species.

5.5 Identification of Yield-Related Genes and QTLs

Genomic approaches have revolutionized. “the identification of genes and quantitative trait loci (QTLs) associated with yield in crops. Genome-wide association studies (GWAS) and linkage mapping have been instrumental in pinpointing genomic regions harboring genes influencing yield-related traits. By analyzing genetic variation within breeding populations, researchers can identify candidate genes and QTLs associated with traits such as grain yield, biomass production, and stress tolerance [37]. Furthermore, “advances in high-throughput sequencing technologies have facilitated transcriptomic, proteomic, and metabolomic analyses to uncover the molecular mechanisms underlying yield determination. Transcriptomic studies, in particular, enable the profiling of gene expression patterns associated with yield under different environmental conditions and developmental stages. Integration of multi-omics data allows for a comprehensive understanding of the regulatory networks governing yield-related processes, providing valuable insights for crop improvement [38]. Recent studies have identified key genes and QTLs involved in various aspects of yield, including photosynthesis, nutrient uptake, hormone signaling, and reproductive development. Functional characterization of these genes using transgenic approaches or gene editing technologies offers opportunities for targeted manipulation of yield-related traits in crops. Overall, the identification of yield-related genes and QTLs through genomic approaches provides a foundation for targeted breeding efforts aimed at enhancing crop productivity and resilience [39].

5.6 Genomic Selection Strategies

Genomic selection (GS) has emerged as a powerful approach for accelerating genetic gain in breeding programs by leveraging genomic information to predict the breeding value of individuals. Unlike traditional phenotypic selection, which relies on observed performance, GS integrates genome-wide marker data with phenotypic information to estimate the genetic merit of individuals for target traits [40]. Using statistical models and machine learning algorithms, GS enables breeders to predict the performance of untested individuals based on their genomic profile. By selecting individuals with the highest predicted breeding values as parents for the next breeding cycle, GS enhances the efficiency of selection and accelerates the rate of genetic gain [41]. One of the key advantages of GS is its ability to capture the cumulative effects of numerous small-effect QTLs contributing to complex traits such as yield. By considering the entire genome rather than individual marker-trait associations, GS offers improved prediction accuracy and robustness across diverse genetic backgrounds and environments [42].

6. CHALLENGES AND LIMITATIONS OF TRADITIONAL BREEDING

The challenges and limitations of traditional breeding methods:

1. **Time-consuming Process**: Traditional breeding methods often require multiple generations of crosses and selections to achieve desired traits, leading to lengthy breeding cycles and delayed variety development [43].
2. **Limited Genetic Variation**: Conventional breeding relies on the existing genetic diversity within the crop species or closely related wild relatives. This limited genetic pool may hinder the discovery of novel alleles for trait improvement [44-45].
3. **Dependence on Phenotypic Evaluation**: Traditional breeding relies heavily on phenotypic evaluation, which involves visually assessing plant characteristics. This subjective evaluation may lead to inaccuracies and biases, especially for traits that are difficult to quantify or are influenced by environmental factors [46].
4. **Inefficient Selection for Quantitative Traits**: Traditional breeding methods often lack precision in selecting for such traits, resulting in slow progress and limited genetic gain [47].
5. **Undesirable Linkage Drag**: Introducing a desired trait into a breeding line may inadvertently carry along undesirable genetic traits linked to it, known as linkage drag. Breaking undesirable linkages while retaining the desired trait can be challenging through conventional breeding methods [48].
6. **Limited Capacity for Trait Introgression**: Incorporating novel traits from wild relatives or exotic germplasm into cultivated varieties through traditional breeding approaches can be labor-intensive and time-consuming due to reproductive barriers and genetic incompatibilities [49-50].
7. **High Cost and Resource Intensiveness**: Traditional breeding programs require significant investments in infrastructure, manpower, and field trials. The cost and resource intensiveness associated with maintaining breeding populations and conducting field evaluations can pose financial constraints [51].
8. **Regulatory Hurdles**: The release of new crop varieties developed through traditional breeding methods may be subject to stringent regulatory requirements, including field trials, safety assessments, and compliance with intellectual property rights [52].

6.1 Addressing Regulatory and Ethical Concerns

The adoption of genomic breeding in agriculture raises various regulatory and ethical concerns that need to be addressed. Regulatory frameworks must be developed to ensure the safe and responsible use of genomic technologies, including genome editing tools such as CRISPR-Cas9 [53].

6.2 Overcoming Technical and Bioinformatics Challenges

The widespread adoption of genomic breeding faces technical and bioinformatics challenges that require innovative solutions. Generating and analyzing large-scale genomic data require advanced computational infrastructure and bioinformatics expertise. Standardization of data formats, development of bioinformatics pipelines, and integration of diverse datasets from multiple sources are critical for harnessing the full potential of genomic technologies in breeding programs [54].

6.3 Future Prospects for Genomic Breeding in Mustard

In mustard breeding, genomic approaches hold significant promise for addressing key challenges such as yield enhancement, quality improvement, and abiotic stress tolerance. Furthermore, advancements in genome editing technologies offer opportunities for targeted trait improvement, including the enhancement of oil content, disease resistance, and nutritional quality in mustard crops. Future research efforts should focus on integrating genomic breeding approaches with conventional breeding methods, addressing technical challenges, and exploring novel genetic resources to unlock the full potential of mustard genomics for sustainable crop improvement [55-56].

7. CONCLUSION

In this review, we have explored the application of genomic approaches in mustard breeding, focusing on yield enhancement, quality improvement, and the integration of genomic tools into breeding strategies. Key findings include the identification of yield-related genes and QTLs, the implementation of genomic selection strategies, and the successful integration of genomic data into breeding programs. Additionally, we discussed challenges such as regulatory concerns, technical hurdles, and bioinformatics challenges associated with genomic breeding in mustard. The integration of genomic approaches into mustard breeding programs holds significant promise for accelerating genetic gain, enhancing breeding precision, and developing improved mustard varieties with enhanced yield, quality, and resilience. Moving forward, future mustard breeding programs should prioritize the adoption of genomic tools such as marker-assisted selection, genome-wide association studies, and genomic selection to expedite variety development and address agronomic challenges. Efforts to address regulatory and ethical concerns, overcome technical and bioinformatics challenges, and foster collaboration and data sharing among researchers are essential for realizing the full potential of genomic breeding in mustard. Genomic approaches offer transformative opportunities for mustard breeding, enabling breeders to develop resilient, high-yielding varieties tailored to meet the demands of modern agriculture and contribute to global food security.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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