E-ISSN: 2618-0618
P-ISSN: 2618-060X
© Agronomy
NAAS Rating (2025): 5.20
www.agronomyjournals.com
2025; 8(8): 756-764
Received: 03-06-2025
Accepted: 07-07-2025

Payal Phore
Student; PDM University, Bahadurgarh, Kherka Musalman, Haryana, India

Corresponding Author:
Payal Phore
Student; PDM University; Bahadurgarh, Kherka Musalman, Haryana, India

A comprehensive review of genetic and biochemical approaches for trait improvement in Indian mustard (*Brassica juncea*)

Payal Phore

DOI: https://www.doi.org/10.33545/2618060X.2025.v8.i8k.3633

Abstract
Indian mustard (*Brassica juncea*) is considered an important oilseed crop in India as it possesses a high oil percentage with multiple uses in food (cooking oil and condiment), feed (animal feed), and industrial use, as well as being adaptable to a wide variety of agro-climatic circumstances. In spite of its agronomic and nutritional importance, Indian mustard is subject to limitations from biotic (diseases and pests) and abiotic stresses (drought, salinity, terminal heat) as well as inevitable anti-nutritive factors (high levels of erucic acid and glucosinolates) that limit its usefulness and acceptance within the market. This review discusses recent developments in trait improvement strategies to overcome these limits by using both "traditional" and contemporary methods, such as marker-assisted selection, genomic selection, doubled haploids, and interspecific hybridization. It also discusses widely emerging biotechnological methods (such as CRISPR/Cas9 genome editing and RNA interference (RNAi)) for modifying undesirable traits. The review also highlights areas that can be complemented with biochemical characterization techniques (such as Near-Infrared Spectroscopy (NIR), Gas Chromatography (GC), and ELISA), utilized with genetic tools for determining traits. Finally, the review also discusses the significant prospects of multi-omics and high-throughput phenotyping platforms as integrated strategies to help reduce breeding cycle times. Collectively, these integrated strategies offer a roadmap for developing climate-resilient, high-yielding, and nutritionally improved Indian mustard cultivars suited for future agricultural sustainability.

Keywords: *Brassica juncea*, trait improvement, marker-assisted selection, CRISPR, glucosinolate, erucic acid, biochemical analysis

1. Introduction
Indian mustard (*Brassica juncea*) holds immense importance in Indian agriculture, both as a vital oilseed crop and a contributor to rural livelihoods and national economic stability. It is the second most significant oilseed crop after groundnut and is extensively cultivated across northern, western, and central states such as Rajasthan, Haryana, Uttar Pradesh, Madhya Pradesh, and Gujarat [1]. The crop is valued primarily for its high oil content, ranging from 35% to 45%, with mustard oil being a staple in Indian cuisine and widely used in traditional medicine and the cosmetic industry [2]. Mustard oil is particularly popular in eastern and northern India due to its pungency, long shelf life, and health benefits, including its content of omega-3 and omega-6 fatty acids. Besides its direct consumption, mustard contributes to the country's economy through its by-products; for instance, mustard cake (residue after oil extraction) serves as a protein-rich animal feed and a natural organic fertilizer, supporting integrated and sustainable farming systems [3].
Agronomically, Indian mustard is highly adaptable and resilient, capable of growing under a variety of agro-climatic conditions, especially in rainfed and drought-prone areas where other high-input crops may fail. It has a short growing season (90–130 days), making it a suitable component of double cropping systems, especially when rotated with cereals such as wheat and rice, thereby improving soil fertility, controlling pests and diseases, and enhancing overall farm productivity [4]. Furthermore, Indian mustard plays a key role in the employment of rural populations, offering labor opportunities during sowing, harvesting, and processing stages.

Fig 1: Indian mustard (*Brassica juncea*)

In the past several years, Indian mustard has gained prominence for its role in lowering India's reliance on imported edible oils, which comprise a substantial portion of the national import budget [5]. The Indian government, through programs like the National Mission on Oilseeds and Oil Palm (NMOOP), has championed mustard farming by improving agronomic practices, providing quality seeds, and investing in research for high-yielding and disease-resistant varieties. Mustard is a strategic crop for combating malnutrition and income disparities in rural India because of its nutritional and economic importance. Moreover, mustard's greens (leaves) also provide crucial vitamins and minerals, are regularly consumed in the winter as a vegetable, and improve dietary diversity and micronutrient consumption for rural consumer groups [6]. Overall, Indian mustard has many functional benefits available through oil production, soil health, climate resilient management, rural employment, and nutritional value of mustard greens and oil. Promoting genetic improvement, pest and disease resistance, and biochemical value is imperative to yield and quality, thereby supporting sustainably agriculture and long-term self-sufficiency for food in the country.

2. Need for Trait Improvement in *Brassica juncea*

Brassica juncea (Indian mustard) is a major oilseed crop in India; however, its realized yield consistently falls short of its genetic potential, primarily due to the combined effects of abiotic and biotic stresses, restricted genetic variability, and limitations in oil quality attributes. Mustard is grown mainly under rainfed conditions, leading to susceptibility to terminal heat, drought, and salinity, especially at critical stages of development such as flowering and grain filling, resulting in reduced seed-set and oil content [7]. Seasonal pest and disease outbreaks, particularly Alternaria blight, white rust, Sclerotinia stem rot, and aphid infestations, continue to be a major source of losses. More recently, breeding for resistance to these stresses using more traditional and molecular methods should continue to be a top priority. In addition, the high levels of erucic acid in mustard oil and glucosinolates in seed meal affect the nutritional quality; erucic acid is now associated with specific health claims, and glucosinolates put limits on export quality. It is thus important to work toward the development of low-erucic and low-glucosinolates varieties that will increase nutritional quality and make the crop more competitive in the international marketplace [8]. Moreover, improving plant architecture, harvest index, lodging resistance, early maturity, and nitrogen-use efficiency are critical for enhancing adaptability and productivity across agro-ecological zones.

Another major breeding challenge lies in the narrow genetic base of cultivated *B. juncea*, which reduces the potential genetic gain and limits resistance breeding. Recently, there has been much effort dedicated to enlarging this gene pool through both interspecific hybridization and the use of wild relatives, as well as heterosis using the CMS systems and doubled haploid methods [9]. Although hybrid breeding may seem promising, challenges remain with the stabilization of fertility restoration and the generation of economically relevant hybrid seeds. Breeding for climate change and adapting to current environmental pressures is an increasing challenge, particularly regarding heat, drought and waterlogging tolerance. It is timely due to climate change and because the environmental stresses are becoming more severe to breed for climate resilience, while modern genomic research tools such as MAS, GS and genome editing will be significant and provide opportunities to speed up advances in support of breeding for numerous characteristics. Climate resilience will aid in more accurately integrating the breeding processes for complex traits, stacking resistance genes, and developing climate-resilient cultivars. In summary, sustainable improvement of *B. juncea* will require an integrated breeding program developing cultivars with an adequate yield and maintaining rates of abiotic and biotic tolerance and oil quality [10]. These improvements will aid not only food and nutritional security but will also allow for a decrease in dependence on imported edible oil and improve the economic viability of various mustard cropping areas in India.

3. Genetic Basis of Trait Variation in Indian Mustard

3.1 Taxonomy and Genome Structure

Indian mustard (*Brassica juncea* L. Czern. & Coss.) belongs to the family *Brassicaceae* and is an amphidiploid species (AABB, 2n = 36) derived from the natural hybridization between *Brassica rapa* (AA, 2n = 20) and *Brassica nigra* (BB, 2n = 16). It is a member of U's Triangle, which describes the genetic relationships among the six cultivated *Brassica* species. The genome of *B. juncea* has been sequenced, revealing extensive gene duplication and polyploidy, which have contributed to its adaptability and diversity [11]. The presence of two sub-genomes (A and B) allows for complex interactions in gene expression and inheritance, providing opportunities and challenges in trait selection and improvement. However, the functional redundancy of gene copies in polyploids can also obscure the expression of target traits and complicate marker-trait associations, especially for quantitative traits such as yield and stress tolerance.

3.2 Genetic Diversity and Limitations

B. juncea has a wide geographic distribution and morphological variability, the cultivated Indian gene pool exhibits relatively narrow genetic diversity due to repeated use of a limited number of elite lines in breeding programs [12]. This restricted diversity limits the potential for improving complex traits such as disease resistance, abiotic stress tolerance, and oil quality. While exotic and wild germplasm, including resynthesized *B. juncea* and introgression lines from related species (*B. rapa*, *B. nigra*, and *Sinapis alba*), have been introduced to broaden the gene pool, their integration into mainstream breeding has been limited due to issues like linkage drag and poor agronomic performance. Molecular marker studies using SSRs, SNPs, and AFLPs have confirmed moderate polymorphism among Indian varieties, highlighting the need to explore underutilized genetic resources and wild relatives to widen the breeding base [13].

3.3 Key Agronomic Traits: Glucosinolate and Erucic Acid

Among the critical biochemical traits that affect the utility of

Indian mustard are glucosinolate and erucic acid contents. Glucosinolates are sulfur-containing compounds that are part of the Brassica species. In native Brassica plants, glucosinolates are important for plant defense, but glucosinolates in high levels contribute to the anti-nutritional effects observed in the seed meal used to feed livestock. Similarly, erucic acid is a long-chain monounsaturated fatty acid that is a primary component of mustard oil and constitutes associated with health risks high amounts of myocardial lipidosis [14]. Pungent (or conventional) Indian cultivars exhibit both glucosinolate and erucic acid contents that are considered to be too high for maximal nutritional and commercial appeal. The inheritance of both traits is quantitative; with reports of major and minor QTLs in different linkage groups. Marker-assisted selection (MAS) approaches along with biotechnology have been used to develop "double-low" (low-glucosinolate, low-erucic acid) mustard varieties for edible use and export [15]. However, the uptake of double-low varieties in India has been slow because of lower yield trade-offs and farmer preference for pungent mustard oil. New advances using transcriptomics, QTL mapping and CRISPR-based genome editing point to an opportunity for specific improvement of these traits, without yield detriment.

4. Genotypic Variations in Indian Mustard Cultivars

4.1 Overview of PM (Pusa Mustard) and MST Genotypes

Indian mustard (*Brassica juncea*) is a vital oilseed crop in India, characterized by considerable genotypic diversity, which has been effectively harnessed for the development of cultivars exhibiting improved agronomic performance, enhanced nutritional quality, and greater tolerance to biotic and abiotic stresses [16]. The Pusa Mustard (PM) series by the Indian Council of Agricultural Research - Indian Agricultural Research Institute (ICAR-IARI) is heralded for demonstrating a paradigm shift in mustard cultivation [17] and genotypes PM-21, PM-25, PM-26 and PM-30 have been bred for different agro-climatic zones, with improvements in yield potential, time to maturity, disease resistance (to white rust (*Albugo candida*) and powdery mildew (*Erysiphe cruciferarum*)) and oil content. At the same time, the Mustard Super Trait (MST) genotypes have been developed as elite lines for focused trait stacking [18]. The MST lines that have been bred into value-added traits (improved abiotic stress tolerance and nutrient use efficiency; multiple disease resistance) have combined superior lines with trait improvements [19]. The MST genotypes are bred using traditional methods assisted with molecular markers and genomic selection to precisely accommodate trait additions. The introduction of these diversified genotypes has allowed mustard crop production to transition into non-traditional areas while providing the ability to stabilize productivity in established mustard growing areas [20].

4.2 Double Zero (00) Varieties: Genetic and Nutritional Relevance

The emergence of double zero (00) mustard varieties with low erucic acid content (<2%) and low glucosinolate content (<30 µmol/g) in oil and meal, respectively, represents a breakthrough in Indian mustard improvement [21]. Indian mustard had long been characterized by high erucic acid and glucosinolate content, which can be potentially harmful to human health and restrict the use of mustard meal in animal feeding [22]. To address this issue, breeders successfully bred in double zero traits by using genes from exotic canola-quality lines and crossed these genes into Indian mustard throughout backcrossing and molecular-assisted selection.

Successful double zero cultivars include Pusa Double Zero Mustard-31 (PDZM-31), LES-43 and RLC-3 balance quality traits with yield and agronomic suitability [23]. The double zero cultivars yield healthier edible oils with improved fatty acid profiles, thus improving suitability for human consumption and unlocking opportunities in the international food market. The lower glucosinolate levels in mustard cake are also more desirable as a protein source for livestock and poultry feeding [24]. Genetically, double zero traits are conferred by two major loci with recessive alleles; the first is FAE1 for erucic acid, and the second is the GSL genes for biosynthesis of glucosinolates [25]. Incorporation of these alleles without compromising yield and disease resistance remains a key challenge that has been progressively addressed by breeders using advanced molecular tools.

4.3 Varietal Performance Evaluated Through Genetic Markers (e.g., MYB, GER, FAE)

Molecular breeding has helped significantly characterize and develop improved Indian mustard cultivars. The use of gene-specific markers has improved the ability to dissect complex traits at the molecular level so that varietal development can be more efficient [26]. MYB transcription factors are among the most relevant markers and play a role in the regulation of secondary metabolites and plant responses to environmental stresses such as drought, salinity, and pathogen attacks [27]. MYB transcription factors regulate the expression of genes related to flavonoids, lignin and cuticle production, important traits related to plant defense and stress adaptation in the plant.

Another important molecular marker family is the GER (Germ-like protein) gene group, known for tolerance to oxidative stress and defenses against pathogens [28]. The expression of GER genes is associated with better tolerance to fungal pathogens and oxidative stress to promote better plant growth and survival during the growing season. Conversely, FAE1 (Fatty Acid Elongase 1) genes control the biosynthesis of erucic acid. Functional mutations or gene silencing of FAE1 genes are directly correlated with the development of low-erucic acid genotypes, meaning FAE1 gene markers are very helpful with quality trait improvement [29]. Utilizing SSR (Simple Sequence Repeat) and SNP (Single Nucleotide Polymorphism) markers, breeders have successfully mapped Quantitative Trait Loci (QTLs) associated with key agronomic traits such as seed yield, flowering time, plant height, and stress tolerance [30]. Marker-assisted selection and genomic selection strategies have enabled precise pyramiding of these traits into new cultivars. Furthermore, advancements in high-throughput genotyping platforms have improved the efficiency of screening large germplasm collections and identifying marker-trait associations for use in breeding programs [31].

5. Molecular Approaches for Trait Analysis

5.1 Use of SSR and PCR-Based Markers

Simple Sequence Repeats (SSRs) and Polymerase Chain Reaction (PCR)-based markers are the most widely used markers in Indian mustard researchers because they are highly polymorphic, co-dominant, reproducible, and genome-wide [32]. SSR markers, which can be developed from Genomic and Expressed Sequence Tag (EST) libraries have enabled the identification of polymorphic loci associated with yield, oil content, flowering time and disease resistance [33]. PCR techniques such as RAPD, AFLP and ISSR allowed for the first genetic diversity assessments among Indian mustard genotypes. SSRs have become strong markers for cultivar identification,

analyzing population structure and QTL mapping. Work is also being done on developing gene-based markers and using high-throughput PCR systems for rapid screening of these breeding populations for marker-assisted selection (MAS) [34].

5.2 Primer Design and Genetic Mapping

The successful development of a marker and its ability to amplify a gene is founded on accurate primer design. The application of bioinformatics and genome sequencing on Brassica species has made primer design for species-specific candidate genes associated with important metabolic and regulatory pathways [35] appropriate and feasible. These primers will have been used predominantly in gene expression studies, genotyping applications, and allele mining. Genetic linkage maps have been created using a variety of markers in Indian mustard (*Brassica juncea*) including SSRs and SNPs. This evidence density framework has provided avenues for QTL mapping study traits such as oil quality (erucic acid content), stress tolerance, seed glucosinolate content, and plant architecture. There is also a dense linkage map using next-generation sequencing data and generated using genotyping-by-sequencing analysis, which is useful for identifying tightly linked markers associated with the trait of interest [36].

5.3 Marker-Trait Association Studies

Marker-trait association studies (MTAs) or association mapping is the statistical association of molecular marker variation with phenotypic trait variation in a diverse panel of genotypes [37]. Association mapping exploits the natural variation in alleles in a genetically diverse population rather than analyzing biparental quantitative trait loci (QTL) mapping, and allows much higher mapping resolution. Marker-trait association studies (MTAs) have been successfully used in Indian mustard to understand complex traits such as seed yield, oil composition, drought resistance, flowering time, and disease resistance [38]. With the advent of high-density single nucleotide polymorphism (SNP) array technology and whole genome resequencing, genome wide association studies (GWAS) have been conducted and highlighted a number of novel candidate genes and genomic regions associated with traits such as fatty acid biosynthesis (FAE1, FAD2 genes), glucosinolate metabolism (GSL genes), and abiotic stress response (DREB, HSP, NAC transcription factors) [39]. The combined use of association mapping together with transcriptomics and proteomics has great potential for functional validation of association-mapped trait-linked genes.

6. Biochemical Characterization of Traits

Biochemical profiling serves as a vital component in understanding the phenotypic expression of genetic traits in Indian mustard (*Brassica juncea*). These biochemical parameters, especially oil content, glucosinolate concentration, and fatty acid composition, directly influence the nutritional, industrial, and economic value of mustard cultivars. The integration of biochemical analyses with breeding programs enhances the selection of genotypes with desirable nutritional and quality traits.

6.1 Estimation of Oil Content (NIR and Soxhlet Methods)

Oil content continues to be a major breeding objective in Indian mustard. The two best-known methods for oil estimation are Near Infrared Reflectance (NIR) spectroscopy and the Soxhlet extraction method. The NIR method is a quick, non-destructive and high-throughput technique that uses reflectance spectra of the intact seeds to predict oil contents [40]. The NIR method is especially useful for screening large germplasm collections and segregating populations. The Soxhlet extraction method is a classical gravimetric method and uses organic solvents (e.g., hexane or petroleum ether) to extract oil from seed powder [41]. Although the Soxhlet extraction method is time-consuming, it is also the most accurate method and provides reference values for NIR calibrations.

6.2 Glucosinolate Estimation (ELISA Method)

Glucosinolates are sulfur-rich secondary metabolites that affect both plant defense and meal quality. High concentrations of glucosinolates can adversely affect the palatability and nutritional value of mustard seed cake in livestock feeds. By using the Enzyme-Linked Immunosorbent Assay (ELISA) procedure, glucosinolates can be determined in a sensitive, specific, and quantitative method [42]. ELISA Kits are developed that use antibodies against specific glucosinolate compounds; hence, it is possible to estimate the concentrations of glucosinolates in seed meal extracts. Many researchers have indicated that ELISA is a better option for estimating glucosinolates than using traditional colorimetric methods because ELISA is readily adaptable to high-throughput studies with outstanding reproducibility and is also a more effective structured approach to differentiating between different glucosinolate profiles.

6.3 Erucic Acid Estimation (Gas Chromatography)

Erucic acid content is an important biochemical trait that establishes the edibility and export potential of mustard oil. Potential cardiotoxic effects renders its production at levels above 2% undesirable [43]. Gas chromatography (GC) is the standard for profiling the fatty acid composition of mustard oil. GC follows the derivatization of oil samples to fatty acid methyl esters (FAMEs), separating and quantifying the resulting FAMEs with a gas chromatograph and flame ionization detector (FID). GC is capable of accurately quantifying erucic acid and other important fatty acids (such as oleic, linoleic, linolenic acids) and could be used to detect double zero (00) genotypes [44].

Table 1: Biochemical Trait Estimation Techniques in *Brassica juncea*

Trait	Method Used	Principle	Key Instrument/Tool	Advantages	Limitations
Oil Content	Near Infrared Spectroscopy (NIR)	Measures light absorption by organic bonds (CH, OH, NH)	NIR Spectrophotometer [45]	Rapid, non-destructive, suitable for large samples	Requires calibration, less accurate than Soxhlet
	Soxhlet Extraction	Solvent extraction and gravimetric analysis	Soxhlet Apparatus [46]	Highly accurate, reference method	Time-consuming, uses hazardous solvents
Glucosinolate Content	ELISA	Antigen–antibody reaction; colorimetric detection	ELISA Reader [47]	Sensitive, specific, high-throughput	Costly kits, limited compound specificity
Erucic Acid Composition	Gas Chromatography (GC)	Separation of fatty acid methyl esters (FAMEs) by volatility	Gas Chromatograph + FID [45]	High precision and resolution	Requires derivatization, high cost

6.4 Integrating Genetic and Biochemical Approaches for Trait Enhancement in Indian Mustard (*Brassica juncea*)

The integration of genetic and biochemical information is a robust and strategic direction towards trait improvement in Indian mustard (*Brassica juncea*). High utility genetic markers, including Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNP) and gene-specific loci, such as FAE1, MYB28, and GER provide useful information in understanding the genetic basis of important agronomic and quality attributes. As a component of this integrated breakdown, biochemical data provide functional evidence of these attributes and can appropriately allow researchers to link genotype and observable phenotype, which is essential. Ultimately, this also leads to a more exact and defined genotype selection for breeding purposes, since it not only emphasizes favourable genotypes, but also phenotypes with favourable biochemical properties which include increased oil content, reduced glucosinolate content, and low erucic acid content - which are essentially breeding goals for nutritionally sustainable and oil seed focused types of mustard. Increasingly, whereas genetic improvement of these biochemical traits has depended mainly on gene-linked molecular markers and multiplexed, commercially accessible high-throughput biochemical screening technologies. For instance:

- **Oil Content:** SNP (Single Nucleotide Polymorphism) markers developed in association with FAE1, WRINKLED1 and BjAGPase genes has enabled increased oil yield in *B. juncea*. Gas Chromatography-Flame Ionization Detection (GC-FID) and Near-Infrared (NIR) Spectroscopy are both effective and consistent methods for quantification.
- **Glucosinolate Content:** Genetic markers e.g., GER1, GER5, and MYB28 have enabled lower anti-nutritional glucosinolates and biochemical methods for estimating it includes High-Performance Liquid Chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA), enabling correct profiling of glucosinolate concentration.
- **Erucic Acid Levels:** Erucic acid, the most critical quality characteristic, is assessed using an FAE1-based Cleaved Amplified Polymorphic Sequence (CAPS) marker that enables the differentiation between low-erucic acid genotypes (<2%) and high-erucic acid types (>30%). The primary method for fatty acid content determination has been GC-FID for fatty acid composition profiling.

Overall, the synergy between molecular and biochemical tools accelerates the breeding process by allowing for genotype selection based on DNA that subsequently reliably allows for selection or identification of the biochemical and phenotypic traits of interest. This synergy of tools concomitantly enables the selection for growth of higher quality Indian mustard varieties that have higher yield, enhanced nutritional quality and enhanced industrial end-use value.

Table 2: Integration of Genetic and Biochemical Data

Trait	Genetic Marker/Associated Gene	Biochemical Assay Method	Example Genotypes & Findings	Reference
Oil Content	SNPs from GBS, FAE1, BjAGPase, WRINKLED1	NIR spectroscopy; Soxhlet extraction; GC-FID	High-oil lines DJ-1-2 DT5, PLM-4 (>43 %) via multi-env trials; metabolic engineering (BjAGPase down-regulation + WRI1 expression) increased oil	[48,49]
Glucosinolate Content	GER1, GER5, At5gAJ67, Myb28 (QTL-linked markers)	HPLC; Vis-NIRS; ELISA	Low-GSL developable via RIL population using GER1/GER5 markers; Vis-NIRS models accurately predicted total and aliphatic GSLs across 641 samples; ELISA offers high throughput.	[50,51]
Erucic Acid	FAE1 gene (CAPS, functional markers)	GC-FID analysis of fatty acid methyl esters	Eight “single-zero” genotypes (PM24, PM30, PDZ-1, etc.) <2 % erucic; high-erucic lines 30–45 %	[50]

7. Analytical Tools and Methodologies

Precise and comprehensive trait analysis is a cornerstone of effective crop improvement programs. In Indian mustard (*Brassica juncea*), where oil quality, fatty acid composition and anti-nutritional factors have a major impact, modern analytical instrumentation plays a crucial role. Current breeding implementations are heavily reliant on effective, precise and high-throughput options for biochemical and molecular trait assessment. The capacity to conduct high-throughput phenotyping is paramount, but these devices also offer an element of validation for marker-assisted selection, functional genomics and trait mapping. The following set of instruments and methods are considered the most widely used technologies for trait characterisation in mustard research and breeding programs.

7.1 Near Infrared Spectroscopy (NIR)

Near Infrared Spectroscopy (NIR) has emerged as one of the most efficient and non-destructive tools for rapid biochemical screening in oilseed crops. NIR works by measuring the absorbance of near-infrared light (700–2500 nm) by specific molecular bonds including C–H, O–H, and N–H, which are plentiful in lipids, moisture, and proteins [52]. In the case of mustard breeding, NIR is used to primarily determine oil in seeds and moisture in seeds without chemical removal or grinding.

The primary benefit of NIR is the rapidity, simplicity and minimal amount of sample preparation needed, making it highly suitable for high-throughput phenotyping with large breeding populations. NIR allows breeders to rapidly screen large numbers of genotypes for oil, and facilitate early-stage selection decisions. Although the data from NIR is slightly less precise than procedures using chemical extraction, NIR is a cost-effective, reproducible method that can be adapted for observations made at the field level and is an important first step in quality analysis pipelines.

7.2 Soxhlet Apparatus for Oil Extraction

Despite the growing popularity of rapid techniques like NIR, the Soxhlet extraction apparatus continues to serve as the reference standard for quantitative oil analysis in mustard seeds. The classical Soxhlet extraction method involves extracting oil for a period of several hours using a suitable organic solvent (typically hexane or petroleum ether) in a cyclical manner where the solvent passes through a thimble of finely ground seed powder and continues on to extract oil. Despite the growing

popularity of rapid techniques like NIR, the Soxhlet extraction apparatus continues to serve as the reference standard for quantitative oil analysis in mustard seeds [53]. This method is a valuable tool for instrument calibration of NIR, validating claims of oil content documented in varietal release trials, and documenting accurate information for nutritional labeling or industry standards. While the process is relatively time-consuming and requires solvent handling precautions, its ability to produce precise and repeatable results makes it indispensable for analytical validation.

7.3 ELISA Reader for Secondary Metabolite Quantification

The enzyme linked immunosorbent assay (ELISA) reader is a common tool for biochemical analysis of secondary metabolites, especially glucosinolates, which have a profound impact on the nutritional and anti-nutritional characteristics of Indian mustard [54]. ELISA is a very sensitive immunological method that identifies the presence of a specific molecule through antigen–antibody binding reactions and subsequent colorimetric or fluorescence readings.

ELISA has provided mustard breeders with a method of high-quality and quantitative assessment of seed meal extracts for target glucosinolates or stress-inducible proteins. In comparison to standard methods of identification and quantitation (i.e., colorimetric methods, thin layer chromatography), ELISA provides better specificity, better sensitivity and less human error in the measurement process. It also allows the simultaneous processing of multiple samples, making it well-suited for large breeding experiments and genetic studies focusing on anti-nutritional factor reduction. Furthermore, modern ELISA kits have been adapted for the detection of environmental stress markers, enabling researchers to assess the biochemical responses of different genotypes under abiotic stress conditions, such as drought or salinity [55].

7.4 Gas Chromatograph for Fatty Acid Profiling

The Gas Chromatograph (GC) is a highly sophisticated analytical tool that plays a pivotal role in the quantitative and qualitative analysis of fatty acids in mustard oil [56]. Fatty acids must be converted to the volatile methyl esters (Fatty Acid Methyl Esters; or, FAME) before analysis, via derivitization. These FAMEs are then separated in the GC based on their volatility and interaction with the column's stationary phase. Standard GC systems utilize Flame Ionization Detector (FID), or some may use Mass Spectrometry (MS) detector, which allows for quantification of individual fatty acids (e.g., erucic acid, oleic acid, linoleic acid, and linolenic acid) [56]. The adoption of GC technology is beneficial to characterize oil quality traits, especially the development of double zero (00) mustard with low erucic acid and glucosinolates.

In the development of breeding programs, GC is often used in conjunction with molecular markers associated with fatty acid biosynthesis genes (e.g., FAE1, FAD2, and LPAAT) in breeding programs. Correlating phenotypic and genotypic GC data provide genetic marker-assisted selection (MAS) for advantageous oil profiles and increase crop genetic improvement efficiency towards a canola-quality mustard.

Table 3: Analytical Tools and Their Applications in Mustard Breeding Programs

Analytical Tool	Purpose	Type of Data Generated	Application in Breeding
NIR Spectrophotometer	Rapid oil content screening	Quantitative (oil%)	Selection of high-oil genotypes
Soxhlet Extraction Unit	Accurate oil content estimation	Gravimetric (oil%)	Validation of oil content in elite lines
ELISA Reader	Secondary metabolite estimation	Colorimetric/Fluorescence (µmol/g)	Identification of low-glucosinolate cultivars
Gas Chromatograph (GC-FID)	Fatty acid profiling (esp. erucic acid)	FAME profiles (%)	Development of double zero (00) and canola-quality varieties

8. Advances in Trait Improvement Technologies

The landscape of crop improvement in *Brassica juncea* has undergone a paradigm shift with the incorporation of cutting-edge technologies that enable precision breeding, enhanced trait selection, and accelerated varietal development. These technologies not only augment the conventional methods but also allow for the modification of specific genetic components with unparalleled accuracy, paving the way for the next generation of high-performance mustard cultivars.

8.1 Use of CRISPR/Cas and RNAi for Targeted Gene Editing

The CRISPR/Cas9 genome editing has revolutionized plant genetic modification by providing a reliable and efficient method to modify some DNA sequences in a precise, efficient, and programmable manner [57]. In Indian mustard, CRISPR/Cas systems are being used to edit erucic acid biosynthetic genes (e.g., FAE1), glucosinolate metabolism genes (e.g., MYB28 with GSL genes), and genes related to abiotic stress (e.g., DREB and HSPs). This allowed for the knockout or knock-in of specific target genes without inserting foreign DNA, which ultimately suggests these varieties could be acceptable under some regulatory systems for non-transgenic crops. Additionally, RNA interference (RNAi) has been used to silence undesired gene expression for specific traits, especially to reduce antinutritional factors, including glucosinolates and to alter seed coat color to meet processing industry needs [58]. RNAi constructs were designed to target specific genes in glucosinolate biosynthetic pathways that were able to successfully lower seed meal glucosinolate level while establishment defense remained intact in vegetative plant tissues. Collectively, these gene-editing platforms display a paradigm shift in breeding mustard in ways that traits could be manipulated while having little consequence on the overall plant's physiology and agronomics of the variety.

8.2 High-Throughput Genotyping and Phenotyping Platforms

The integration of high-throughput genotyping (HTG) and phenotyping (HTP) platforms has streamlined the identification of trait-associated genetic variants and their expression across environments [59]. Techniques such as Genotyping-by-Sequencing (GBS), SNP chips, and whole-genome resequencing allow rapid and cost-effective scanning of the mustard genome, enabling the discovery of novel alleles and marker-trait associations [60]. On the phenotyping front, automated imaging systems, drone-based remote sensing, and multispectral cameras are increasingly being used to assess plant traits such as canopy temperature, chlorophyll fluorescence, biomass accumulation, flowering time, and disease severity. These tools reduce human

error, increase precision, and allow for temporal tracking of phenotypic responses under various stress conditions. Such platforms provide a multi-dimensional dataset that, when integrated with genotypic information, offers insights into complex trait architectures and supports genomic prediction models.

8.3 Integration of Genomic Selection in Breeding Programs

Genomic Selection (GS) is a forward-looking breeding strategy that utilizes genome-wide marker data to predict the performance of breeding lines without the need for extensive phenotyping in every generation [61]. In Indian mustard, GS is gaining traction for traits governed by polygenic inheritance, such as oil yield, drought tolerance, nutrient-use efficiency, and disease resistance. By training statistical models on genotype-phenotype datasets, GS enables the estimation of Genomic Estimated Breeding Values (GEBVs) for selection candidates. This significantly reduces breeding cycles and enhances selection accuracy for complex traits. The incorporation of GS into mustard breeding pipelines represents a shift towards data-driven, predictive breeding, offering solutions for accelerating genetic gain and improving resource efficiency.

9. Challenges and Future Directions

While technological advancements have expanded the toolkit available for Indian mustard improvement, several critical challenges remain that must be addressed to realize the full potential of these innovations. Addressing these challenges will require a concerted effort involving multi-disciplinary research, policy support, and farmer-centric approaches**.**

9.1 Environmental Influence on Biochemical Traits

One of the most significant concerns in terms of trait stabilization is the environmental sensitivity of biochemical traits like oil content, fatty acid profile, and glucosinolate levels [62]. Specifically, biochemical traits frequently show genotype × environment (G×E) interactions and often do not conform to predicting the expression of these traits over a range of agro-climatic environments. Additionally, the biochemical makeup of mustard genotypes may be impacted by soil type, temperature, rainfall, and nutrient availability, which ultimately can impact selection and breeding decisions. Because of this, genotypes should ideally be identified among multi-environment trials (METs) along with the model for stability to demonstrate consistent performances. Clearly, genomic prediction models with environmental covariates (i.e., envirotyping) represent new and exciting ways to improve predictability for traits that are variable due environmental conditions.

9.2 Enhancing Nutritional Quality Without Yield Penalty

Improving nutritional quality traits (such as reducing the levels of erucic acid and glucosinolates), has been traditionally seen as a trade-off with lower yield or vigor in plants. This is an important breeding dilemma, as high yielding cultivars are required to be profited, but cultivars must be bred that meet nutritional and safety standards as well. Breeding strategies therefore need to, at least, include marker-assisted backcrossing, QTL pyramiding, multi-trait selection indices that can balance nutritional qualities and agronomical traits. The opportunity for genomic selection facilitates the selection of yield and quality simultaneously by capturing the small-effect loci present across the genome [63]. In addition, using pathway modeling and flux balance analysis can help to identify regulatory points within pathways that can be altered to improve nutritional traits and benefit biomass or yield.

9.3 Role of Multi-Omics in Next-Generation Breeding

The convergence of multi-omics technologies including genomics, transcriptomics, proteomics, metabolomics, and epigenomics has opened new frontiers in mustard research. These approaches provide a holistic understanding of the molecular networks regulating key traits and their interactions under stress or developmental stages. For instance, transcriptome profiling under heat or drought stress has identified differentially expressed genes (DEGs) involved in ABA signaling and antioxidant defense [64]. Similarly, metabolomic analysis can unravel the biochemical basis of glucosinolate biosynthesis and oil metabolism. Integration of these omics layers through systems biology and machine learning models can lead to the identification of novel biomarkers and candidate genes for use in breeding. In the future, the incorporation of pan-genomics and haplotype-based breeding, along with CRISPR-driven gene stacking, could further refine the development of climate-resilient, nutrient-dense, and high-yielding mustard varieties**.**

10. Conclusion

Trait improvement in Indian mustard (*Brassica juncea*) is essential for boosting yield, nutritional value, and stress resilience. This review underscores the integration of conventional breeding with molecular tools, such as SSRs, SNPs, QTL mapping, and gene-specific markers (e.g., FAE1, MYB28, GER) to dissect complex traits. Biochemical assays like NIR, Soxhlet extraction, ELISA, and GC-FID offer essential phenotypic validation. Innovations in genome editing (CRISPR/Cas9), RNAi, and high-throughput genotyping/phenotyping have accelerated the development of double-zero and multi-trait genotypes, including Pusa Mustard and MST lines. Despite progress, environment interactions and quality-yield trade-offs remain challenges. Future breeding must emphasize multi-omics integration, envirotyping, and machine learning to improve trait predictability. Collectively, molecular and biochemical strategies offer a promising pathway for developing climate-resilient, high-yielding, and quality-enhanced cultivars aligned with national goals for edible oil self-sufficiency and sustainable agriculture.

References

1. Kumar A, Premi OP, Thomas L. Rapeseed-Mustard cultivation in India-an overview. National Research Centre on Rapeseed-Mustard; 2008. p. 1-2.
2. Kaur R, Sharma AK, Rani R, Mawlong I, Rai PK. Medicinal qualities of mustard oil and its role in human health against chronic diseases: A review. Asian J Dairy Food Res. 2019 Apr 1;38(2).
3. Sarkar N, Chakraborty D, Dutta R, Agrahari P, Bharathi SD, Singh AA, *et al*. A comprehensive review on oilseed cakes and their potential as a feedstock for integrated biorefinery. J Adv Biotechnol Exp Ther. 2021 Sep;4(3):376-87.
4. Tyagi S, Maman S, Ajesh BR, Shashidhar BR, Tyagi A. Genetics and Plant Breeding to Improve the Yield of Oilseed Crops. In: Oilseed Crops. 2025 Mar 31. p. 265-97.
5. Jat RS, Singh VV, Sharma P, Rai PK. Oilseed brassica in India: Demand, supply, policy perspective and future potential. OCL. 2019;26:8.
6. Tiwari A. Formulation of lito with mustard green leaves (*Brassica juncea* L.) [Doctoral dissertation]. Department of

Nutrition & Dietetics Central Campus of Technology Institute of Science and Technology Tribhuvan University, Nepal; 2021.
7. Rathore SS, Shekhawat K, Meena PD, Singh VK. Climate smart strategies for sustainable production of rapeseed-mustard in India. J Oilseed Brassica. 2018 Jan;9(1):1-9.
8. McVetty PB, Fernando WD, Li G, Tahir M, Zelmer C. 4 High-Erucic Acid, Low-Glucosinolate. In: Biocatalysis and agricultural biotechnology. 2009 Apr 27. p. 43.
9. Bohra A, Tiwari A, Pareek S, Joshi R, Satheesh Naik SJ, Kumari K, *et al*. Past and future of cytoplasmic male sterility and heterosis breeding in crop plants. Plant Cell Rep. 2025 Feb;44(2):1-20.
10. Meena HP, Kiran BU, Bindu H. Genetic Enhancement of Abiotic Stress Tolerance in Oilseeds Through Contemporary Breeding Approaches. In: Breeding Climate Resilient and Future Ready Oilseed Crops. Singapore: Springer Nature Singapore; 2025 Feb 1. p. 43-99.
11. Yang J, Liu D, Wang X, Ji C, Cheng F, Liu B, *et al*. The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. Nat Genet. 2016 Oct;48(10):1225-32.
12. Panjabi P, Yadava SK, Kumar N, Bangkim R, Ramchiary N. Breeding *Brassica juncea* and B. rapa for sustainable oilseed production in the changing climate: progress and prospects. In: Genomic designing of climate-smart oilseed crops. 2019. p. 275-369.
13. Biswas P, Kumar N. Application of molecular markers for the assessment of genetic fidelity of *in vitro* raised plants: current status and future prospects. In: Molecular Marker Techniques: A Potential Approach of Crop Improvement. 2023 Jun 30. p. 233-56.
14. Poddar KH, Sikand G, Kalra D, Wong N, Duell PB. Mustard oil and cardiovascular health: Why the controversy?. J Clin Lipidol. 2022 Jan 1;16(1):13-22.
15. Sachan DS, Naimuddin SK, Patra D, Subha L, Senthilkumar T, Chittibomma K, *et al*. Advancements in enhancing oil quality in rapeseed and mustard: A Comprehensive Review. J Exp Agric Int. 2024;46(5):181-93.
16. Meena BL, Meena HS, Singh VV, Meena MD, Sharma HK, Rai PK, *et al*. Breeding Climate Resilient Rapeseed–Mustard in Climate Change Era: Current Breeding Strategies and Prospects. In: Breeding Climate Resilient and Future Ready Oilseed Crops. Singapore: Springer Nature Singapore; 2025 Feb 1. p. 303-32.
17. Yadava DK, Yashpal NS, Nanjundan J, Vasudev S. Brassica Breeding 15. In: Fundamentals of Field Crop Breeding. p. 779.
18. Meena BL, Meena HS, Singh VV, Meena MD, Sharma HK, Rai PK, *et al*. Breeding Strategies and Prospects. In: Breeding Climate Resilient and Future Ready Oilseed Crops. p. 303.
19. Singh N, Watts A, Rao M, Nanjundan J, Singh R. Achieving genetic gain for yield, quality and stress resistance in oilseed Brassicas through accelerated breeding. In: Accelerated Plant Breeding, Volume 4: Oil Crops. 2022. p. 165-79.
20. Sinha S, Kumar A, Sohane RK, Kumar R. Breeding of oilseeds: a challenge for selfsufficiency. In: Proceedings-cum-Abstract Book. 2020 Jul 29.
21. Banga S, Kumar PR, Bhajan R, Singh D, Banga SS. Genetics and breeding. In: Brassica oilseeds: breeding and management. Wallingford UK: CABI; 2015. p. 11-41.
22. Sharma S, Bala M, Kaur G, Tayyab S, Feroz SR. Chemical composition of oil and cake of *Brassica juncea*: implications on human and animal health. In: The *Brassica juncea* Genome. Cham: Springer International Publishing; 2022 Mar 9. p. 29-55.
23. Yadava DK, Yashpal, Saini N, Nanjundan J, Vasudev S. Brassica breeding. In: Fundamentals of Field Crop Breeding. Singapore: Springer Nature Singapore; 2022 May 6. p. 779-835.
24. Sadeghi Mahoonak A. Studies on Quality Improvement of Mustard Proteins [Doctoral dissertation]. University of Mysore.
25. Wang P, Xiong X, Zhang X, Wu G, Liu F. A review of erucic acid production in Brassicaceae oilseeds: Progress and prospects for the genetic engineering of high and low-erucic acid rapeseeds (*Brassica napus*). Front Plant Sci. 2022 May 11;13:899076.
26. Kage U, Kumar A, Dhokane D, Karre S, Kushalappa AC. Functional molecular markers for crop improvement. Crit Rev Biotechnol. 2016 Sep 2;36(5):917-30.
27. Cao Y, Li K, Li Y, Zhao X, Wang L. MYB transcription factors as regulators of secondary metabolism in plants. Biology. 2020 Mar 24;9(3):61.
28. Govindan G, KR S, Alphonse V, Somasundram S. Role of germin-like proteins (GLPs) in biotic and abiotic stress responses in major crops: A review on plant defense mechanisms and stress tolerance. Plant Mol Biol Rep. 2024 Sep;42(3):450-68.
29. Liu Y, Du Z, Lin S, Li H, Lu S, Guo L, *et al*. CRISPR/Cas9-targeted mutagenesis of BnaFAE1 genes confers low-erucic acid in *Brassica napus*. Front Plant Sci. 2022 Feb 10;13:848723.
30. Ahmad A, Wang JD, Pan YB, Sharif R, Gao SJ. Development and use of simple sequence repeats (SSRs) markers for sugarcane breeding and genetic studies. Agronomy. 2018 Nov 12;8(11):260.
31. Bhat JA, Yu D. High-throughput NGS-based genotyping and phenotyping: Role in genomics-assisted breeding for soybean improvement. Legume Sci. 2021 Sep;3(3):e81.
32. Singh L, Sharma D, Parmar N, Singh KH, Jain R, Rai PK, *et al*. Genetic diversity studies in Indian mustard (*Brassica juncea* L. Czern & Coss) using molecular markers. In: Brassica Improvement: Molecular, Genetics and Genomic Perspectives. 2020. p. 215-44.
33. Aneja B, Yadav NR, Chawla V, Yadav RC. Sequence-related amplified polymorphism (SRAP) molecular marker system and its applications in crop improvement. Mol Breed. 2012 Dec;30:1635-48.
34. Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, *et al*. DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip. 2018 Mar 4;32(2):261-85.
35. Das Laha S, Kundu A, Podder S. Impact of biotic stresses on the Brassicaceae family and opportunities for crop improvement by exploiting genotyping traits. Planta. 2024 May;259(5):97.
36. Su C, Wang W, Gong S, Zuo J, Li S, Xu S. High density linkage map construction and mapping of yield trait QTLs in maize (Zea mays) using the genotyping-by-sequencing (GBS) technology. Front Plant Sci. 2017 May 8;8:706.
37. Kadirvel P, Senthilvel S, Geethanjali S, Sujatha M, Varaprasad KS. Genetic markers, trait mapping and marker-assisted selection in plant breeding. In: Plant Biology and Biotechnology: Volume II: Plant Genomics and

Biotechnology. 2015. p. 65-88.
38. Chand S, Patidar OP, Chaudhary R, Saroj R, Chandra K, Meena VK. Rapeseed-Mustard Breeding in India: Scenario, Achievements and. In: Brassica breeding and biotechnology. 2021 Jul 7. p. 19.
39. Xiao R, Zou Y, Guo X, Li H, Lu H. Fatty acid desaturases (FADs) modulate multiple lipid metabolism pathways to improve plant resistance. Mol Biol Rep. 2022 Oct;49(10):9997-10011.
40. Vaknin Y, Ghanim M, Samra S, Dvash L, Hendelsman E, Eisikowitch D, *et al*. Predicting Jatropha curcas seed-oil content, oil composition and protein content using near-infrared spectroscopy—A quick and non-destructive method. Ind Crops Prod. 2011 Jul 1;34(1):1029-34.
41. Dutta R, Sarkar U, Mukherjee A. Extraction of oil from Crotalaria Juncea seeds in a modified Soxhlet apparatus: Physical and chemical characterization of a prospective bio-fuel. Fuel. 2014 Jan 15;116:794-802.
42. van Doorn HE, van Holst GJ, van der Kruk GC, Raaijmakers-Ruijs NC, Postma E. Quantitative determination of the glucosinolates sinigrin and progoitrin by specific antibody ELISA assays in Brussels sprouts. J Agric Food Chem. 1998 Feb 16;46(2):793-800.
43. Galanty A, Grudzińska M, Paździora W, Paśko P. Erucic acid—Both sides of the story: A concise review on its beneficial and toxic properties. Molecules. 2023 Feb 17;28(4):1924.
44. Ullah S, Rehman ZU, Assogba CM, Haq RU. Molecular profiling and biochemical characterization of *Brassica napus* advanced lines for enhanced polyunsaturated fatty acid production. Discov Agric. 2024 Nov 26;2(1):108.
45. Oblath EA, Isbell TA, Berhow MA, Allen B, Archer D, Brown J, *et al*. Development of near-infrared spectroscopy calibrations to measure quality characteristics in intact Brassicaceae germplasm. Ind Crops Prod. 2016 Oct 30;89:52-8.
46. Saikia SL, Rai GK, Salgotra RK, Rai SK, Singh M, Rai PK. Erucic acid and glucosinolate variability in *Brassica juncea* L. Int J Chem Stud. 2018;6(6):1223-6.
47. Akhatar J, Singh MP, Sharma A, Kaur H, Kaur N, Sharma S, *et al*. Association mapping of seed quality traits under varying conditions of nitrogen application in *Brassica juncea* L. Czern & Coss. Front Genet. 2020 Sep 1;11:744.
48. Saini N, Singh N, Kumar A, Vihan N, Yadav S, Vasudev S. Development and validation of functional CAPS markers for the FAE genes in *Brassica juncea* and their use in marker-assisted selection. Breed Sci. 2016;66(5):831-7.
49. Gohain B, Kumar P, Malhotra B, Augustine R, Pradhan AK, Bisht NC. A comprehensive Vis-NIRS equation for rapid quantification of seed glucosinolate content and composition across diverse Brassica oilseed chemotypes. Food Chem. 2021 Aug 30;354:129527.
50. Li X, Wen D, He Y, Liu Y, Han F, Su J, *et al*. Progresses and Prospects on Glucosinolate Detection in Cruciferous Plants. Foods. 2024 Dec 20;13(24):4141.
51. Weyer LG. Near-infrared spectroscopy of organic substances. Appl Spectrosc Rev. 1985 Mar 1;21(1-2):1-43.
52. López-Bascón MA, De Castro ML. Soxhlet extraction. In: Liquid-phase extraction. Elsevier; 2020 Jan 1. p. 327-54.
53. Ali Redha A, Torquati L, Langston F, Nash GR, Gidley MJ, Cozzolino D. Determination of glucosinolates and isothiocyanates in glucosinolate-rich vegetables and oilseeds using infrared spectroscopy: A systematic review. Crit Rev Food Sci Nutr. 2024 Sep 9;64(23):8248-64.
54. Gowayed SM, Abd El-Moneim D. Detection of genetic divergence among some wheat (*Triticum aestivum* L.) genotypes using molecular and biochemical indicators under salinity stress. PloS one. 2021 Mar 29;16(3):e0248890.
55. Seppänen-Laakso T, Laakso I, Hiltunen R. Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition. Anal Chim Acta. 2002 Aug 16;465(1-2):39-62.
56. Ansori AN, Antonius Y, Susilo RJ, Hayaza S, Kharisma VD, Parikesit AA, *et al*. Application of CRISPR-Cas9 genome editing technology in various fields: A review. Narra J. 2023 Aug 27;3(2):e184.
57. Duraiswamy A, Sneha A NM, Jebakani KS, Selvaraj S, Pramitha JL, Selvaraj R, *et al*. Genetic manipulation of anti-nutritional factors in major crops for a sustainable diet in future. Front Plant Sci. 2023 Feb 15;13:1070398.
58. Singh AK, Singh A, Sarkar S, Ganapathysubramanian B, Schapaugh W, Miguez FE, *et al*. High-throughput phenotyping in soybean. In: High-throughput crop phenotyping. 2021. p. 129-63.
59. Singh VK, Bhoyar PI, Anu, Sharma V. Application of genomics and breeding technologies to increase yield and nutritional qualities of rapeseed-mustard and sunflower. In: Technologies in Plant Biotechnology and Breeding of Field Crops. 2022 Oct 6. p. 103-31.
60. Rajendra V, Charitha KS. 21. Marker-Assisted Selection for Crop Improvement.
61. Kumar S, Singh D, Dutta M. Quality characteristics in rapeseed-mustard and role of some anti-nutritional factors in plant defense: future strategies. J Oilseed Brassica. 2014 Jul;5(2):87-95.
62. Voss-Fels KP, Cooper M, Hayes BJ. Accelerating crop genetic gains with genomic selection. Theor Appl Genet. 2019 Mar 1;132:669-86.
63. Jia J, Zhou J, Shi W, Cao X, Luo J, Polle A, *et al*. Comparative transcriptomic analysis reveals the roles of overlapping heat-/drought-responsive genes in poplars exposed to high temperature and drought. Sci Rep. 2017 Feb 24;7(1):43215.