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Heterosis Breeding for Enhanced Oil Yield and Meal Quality in Indian Mustard

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Abstract: ***Indian mustard (Brassica juncea L.) is a major oilseed crop with significant economic and nutritional importance. Enhancing its seed yield and oil quality is essential to meet the increasing global demand for edible oils. Heterosis breeding, also known as hybrid vigour, has emerged as an effective strategy to improve productivity and quality traits in mustard. This review focuses on the role of heterosis in increasing oil yield and improving meal quality. It highlights key advancements in breeding techniques, including cytoplasmic male sterility (CMS) systems and transgenic barnase–barstar technology for hybrid seed production. Additionally, the review discusses the chemical composition of mustard seeds, emphasizing fatty acids and glucosinolates that influence oil quality. Efforts to reduce erucic acid and glucosinolate content while enhancing desirable traits are also examined. Overall, heterosis breeding offers a promising approach for developing high-yielding, nutritionally superior mustard varieties suitable for future agricultural demands.***

Keywords: Indian mustard, Heterosis breeding, Oil quality, Hybrid seed production, Mustard meal

1. Introduction

Indian mustard (*Brassica juncea L.*) is one of India's leading oilseed crops, cultivated over nearly six million hectares. It is predominantly grown in the rainfed regions of northern India during the winter season [1]. A member of the Brassicaceae family, it is widely grown for its sharp-flavoured seeds, which are notable for their high oil content. Indian mustard is an amphidiploid species with a chromosome number of 2n = 36. It has 38 to 42% oil and 24% protein. Enhancing the seed yield and oil content of Indian mustard requires prioritizing advanced breeding strategies. One promising approach lies in utilizing the concepts of combining ability and heterosis [2]. Enhancing both the productivity and oil quality of *Brassica juncea* is essential to satisfy the growing demands of national and international markets. In plant breeding, heterosis is widely utilized as a mechanism through which hybrids outperform their parental lines. Heterosis breeding is considered one of the most effective strategies to overcome yield limitations [3]. To address the narrow genetic base in conventional mustard breeding, researchers are turning to heterosis, or hybrid vigour, by crossing lines from two distinct gene pools—the Indian and East European. Hybrids derived from these diverse backgrounds have demonstrated higher yields through heterosis, with studies showing that such crosses are particularly effective in boosting productivity [4]. Developing superior parental lines with enhanced oil and seed meal quality can significantly boost both the production and the nutritional value of oil and seed meal cake in the country. A major focus in mustard improvement has been enhancing oil quality [5]. This includes not only raising the overall oil content but also refining the fatty acid profile to meet health and industrial requirements. Breeders are working to lower erucic acid levels while increasing oleic acid content, thereby producing oil that is healthier for human consumption and more versatile for industrial applications [6]. The laboratory has successfully developed **cytoplasmic male sterility (CMS)-based restorer systems** along with **transgenic barnase-barstar systems**, enabling large-scale production of hybrid seeds [7].

2. Materials and Methods

We included scientific publications from July 2020 to December 2025 focusing on heterosis breeding in Indian mustard, particularly studies related to future perspectives in mustard breeding, chemical composition of mustard seeds, superior performing hybrids, heterotic effects, achievements in mustard breeding, and oil quality. The selected literature provided valuable insights into the role of heterosis breeding in enhancing oil yield and improving desirable agronomic traits in Indian mustard.

Heterosis Breeding and Hybrid Development Strategies in Indian Mustard

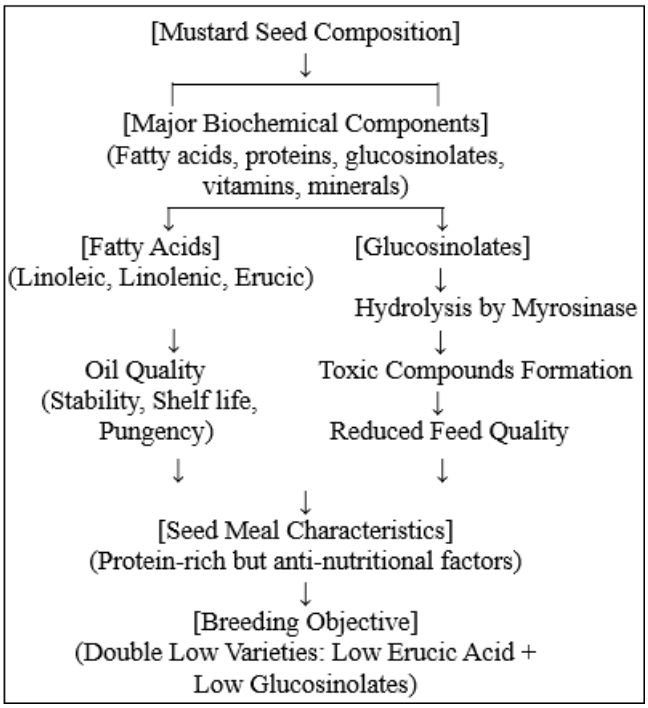
Heterosis breeding is among the most effective technological methods for improving Indian mustard varieties, enhancing both seed yield quality and quantity, along with other yield-contributing traits. Heterosis is widely used in producing vigorous hybrids, as it reflects the heterozygous condition of crosses formed between genetically diverse parents through hybridization. Such hybrids often display stronger heterotic effects than their parents and may show improved yield, enhanced disease resistance, and greater tolerance to both biotic and abiotic stresses [8]. To enhance mustard productivity through hybrid development, advanced methods for creating male sterility and restorer systems have been applied. The laboratory pioneered the use of transgenic barnase-barstar systems to induce male sterility and establish restorer lines in *Brassica juncea [9]*. This approach involves transferring barnase and barstar transgenes into suitable heterotic combiners via recurrent backcrossing, offering a dependable technique for large-scale hybrid seed production. Hybrids developed through this system have shown remarkable yield improvements.

Chemical Composition of Indian Mustard

Component/ Constituent	Presence in Indian Mustard	Role / Significance	Major Details Mentioned
Fatty Acids	Major constituent of mustard oil	Determine oil quality and stability	Includes linoleic acid, linolenic acid, and erucic acid
Linoleic Acid	Important fatty acid	Contributes to oxidative stability of oil	Helps improve shelf life and stability
Linolenic Acid	Polyunsaturated fatty acid	More prone to oxidation	Causes reduced shelf life and off-flavours
Erucic Acid	Major monounsaturated omega-9 fatty acid	Undesirable in high concentration for human consumption	Reduction through breeding is required
Proteins	Present in seed meal	Nutritional importance	Balanced amino acid composition
Glucosinolates	Sulphur- and nitrogen-containing secondary metabolites	Important in plant defence	Hydrolysis produces anti-nutritional compounds
Myrosinase Enzyme	Enzyme associated with glucosinolates	Breaks down glucosinolates	Produces isothiocyanates, nitriles, and oxazolidinethiones
Isothiocyanates	Major bioactive compounds	Responsible for pungency of mustard oil	Major constituents of essential oil
Allyl Isothiocyanate	Major isothiocyanate compound	Contributes to pungency	54.8–68.8% of essential oil
3-Butenyl Isothiocyanate	Minor isothiocyanate	Contributes to oil composition	4.8–5.9% of essential oil
Phenethyl Isothiocyanate	Minor isothiocyanate	Functional bioactive compound	2.4–3.4% of essential oil
Sulphur-containing Compounds (Sulphides)	Present in minor amounts	Influence aroma and composition	Occur along with isothiocyanates
Phytosterols	Present in mustard seeds	Health-promoting and functional properties	Contribute to nutritional value
Antioxidants	Present naturally	Improve stability and health benefits	Protect against oxidation
Vitamins and Minerals	Essential micronutrients	Nutritional and functional importance	Enhance health-promoting properties
Anti-nutritional Compounds	Produced from glucosinolate hydrolysis	Limit use of seed meal in animal feed	Includes nitriles and oxazolidinethiones
Double Low Varieties	Developed through breeding	Improve oil and meal quality	Reduced erucic acid and glucosinolate content

Mustard seeds possess a complex biochemical composition that underpins their nutritional and industrial importance. Although influenced by genetic, environmental, and processing factors, they consistently contain major constituents such as fatty acids, proteins, glucosinolates, phytosterols, antioxidants, vitamins, and minerals. Among these, the fatty acid profile is a key determinant of oil quality. Linoleic acid contributes to oxidative stability, whereas linolenic acid is more prone to oxidation, resulting in reduced shelf life and the development of off-flavours. Erucic acid, a monounsaturated omega-9 fatty acid, is another major component; however, its high concentration in traditional varieties makes it undesirable for human consumption, necessitating its reduction through breeding strategies. Mustard oil also contains bioactive compounds, primarily isothiocyanates, which impart its characteristic pungency. In *Brassica juncea*, essential oil is predominantly composed of isothiocyanates, including allyl isothiocyanate (54.8–68.8%), 3-butenyl isothiocyanate (4.8–5.9%), and phenethyl isothiocyanate (2.4–3.4%), collectively accounting for more than 62.9% of the oil, along with minor amounts of sulphur-containing compounds such as sulphides [10].

Glucosinolates, sulphur- and nitrogen-containing secondary metabolites, play an important role in plant defence; however, their hydrolysis by the enzyme myrosinase produces compounds such as isothiocyanates, nitriles, and oxazolidinethiones, which can adversely affect the nutritional quality of seed meal, particularly for animal feed. Although mustard seed meal is rich in protein with a balanced amino acid composition, its utilization is limited by these anti-nutritional factors. Additionally, phytosterols, antioxidants, vitamins, and minerals contribute to the functional and health-promoting properties of mustard. Consequently, current breeding efforts are focused on developing "double low" varieties with reduced erucic acid and glucosinolate content, thereby improving both oil quality and the nutritional value of seed meal for wider acceptance in food and industrial applications.



Oil and Meal Quality

Mustard seed oil contains long-chain fatty acids like erucic and eicosenoic acids, making it useful for both food and industry. However, erucic acid can reach up to 50%, while international standards limit it to below 2% due to health

risks such as myocardial infarction and lipidosis. Mustard oilseed cakes are protein-rich with balanced amino acids but contain glucosinolates, which are anti-nutritional and hinder animal and poultry growth. Breeding efforts therefore aim to develop varieties with low erucic acid (<2%) and reduced glucosinolate levels (<30 µmol/g defatted seed meal) [11].

India's mustard oil and seed meal quality program focuses on four goals:

- Evaluating low-erucic acid and low-glucosinolate lines of *Brassica juncea* and *B. napus* for yield and quality.
- Developing improved varieties with reduced erucic acid and/or glucosinolate content.
- Studying the genetics and breeding behaviour of these traits.
- Assessing '0/00' types for resistance to local pests and diseases to aid resistant variety development.

Achievements in Mustard Breeding and Oil Quality

Advances in mustard breeding have significantly reshaped India's oilseed sector[12]. A key milestone is the development of high-yielding hybrids, achieved through innovative techniques such as cytoplasmic male sterility (CMS) and the transgenic barnase-barstar system [13].

Cytoplasmic Male Sterility (CMS)

Cytoplasmic male sterility (CMS) is a maternally inherited trait in plants characterized by the inability to produce functional pollen while maintaining normal female fertility. Since the trait is controlled by genes present in the mitochondrial genome, it is transmitted through the cytoplasm of the female parent. CMS has become an essential tool in hybrid seed production because it eliminates the need for manual emasculation and facilitates large-scale hybrid breeding programs.

The phenomenon of CMS arises due to alterations or rearrangements in mitochondrial DNA that interfere with normal pollen development. These mitochondrial abnormalities disrupt energy metabolism during microsporogenesis, leading to sterile pollen formation. However, fertility can often be restored through nuclear-encoded fertility restorer (Rf) genes, which suppress the sterility-inducing mitochondrial effects and enable normal pollen production in hybrids.

Barnase–Barstar Transgenic System

The barnase–barstar system is a genetically engineered male sterility mechanism widely used for hybrid seed production in crop plants. This system was originally developed from the bacterium *Bacillus amyloliquefaciens*, where barnase acts as a ribonuclease enzyme and barstar functions as its natural inhibitor.

In this system, the **barnase gene** is introduced into plants under the control of a tapetum-specific promoter. The tapetum is a nutritive tissue essential for pollen development. Expression of barnase in tapetal cells leads to degradation of cellular RNA, causing premature destruction of the tapetum and resulting in male sterility due to non-functional pollen formation.

To restore fertility in hybrids, another parental line carrying the **barstar gene** is crossed with the barnase-containing sterile line. The barstar protein specifically inhibits barnase activity, thereby protecting tapetal cells and allowing normal pollen development in hybrid plants. Consequently, the F_1 hybrid becomes fully fertile while maintaining the benefits of hybrid vigor.

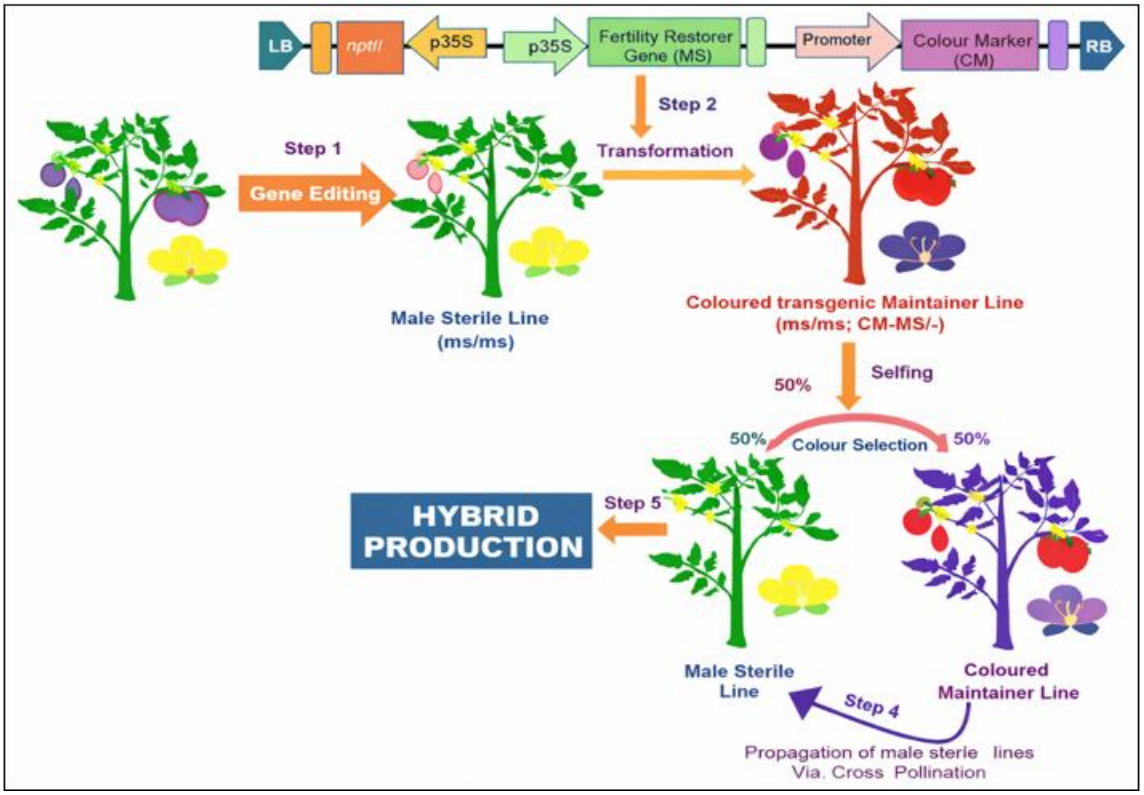


Figure: Advancements from natural CMS to genetically manipulated systems for hybrid seed production [14]

Another major success in mustard breeding is the improvement of oil quality, achieved by reducing erucic acid and glucosinolates- compounds considered undesirable in edible oils due to health risks and flavour issues [15].

Seed Meal Quality Improvement through Heterosis

Seed meal obtained after extraction of oil from mustard seeds is a valuable protein-rich by-product of Indian mustard (*Brassica juncea*). It has considerable economic importance due to its applications in livestock feed, organic fertilizers, and industrial protein sources. Despite its nutritional potential, the wider utilization of mustard seed meal is restricted by the presence of anti-nutritional constituents such as glucosinolates, elevated fibre levels, and traces of erucic acid. Consequently, enhancement of seed meal quality has become a major focus in mustard improvement programs. In recent years, heterosis breeding has gained importance as an efficient strategy for simultaneously improving seed yield and desirable quality traits associated with mustard seed meal.

Heterosis breeding has emerged as an effective approach for improving seed meal quality traits in Indian mustard, particularly those linked to both nutritional value and anti-nutritional components. Research on combining ability indicates that important traits such as glucosinolate concentration, fatty acid profile, and protein composition are influenced by a combination of additive and non-additive genetic effects [16]. Metabolomic studies have further demonstrated that hybrids possess distinct biochemical compositions compared to their parental lines, often exhibiting desirable negative heterosis for glucosinolates, which contributes to improved meal quality *[17]*. Moreover, the predominance of non-additive gene action suggests that heterosis can be effectively utilized to enhance both yield and quality traits simultaneously *[18]*. Genetic investigations focusing on erucic acid and glucosinolate levels also support the potential of heterosis breeding in reducing anti-nutritional factors, thereby enhancing the suitability of mustard seed meal for animal feed and industrial uses.

3. Conclusion

Heterosis breeding has proven to be a highly effective approach for enhancing seed yield, oil content, and overall quality in Indian mustard. The integration of advanced technologies such as cytoplasmic male sterility (CMS) and transgenic barnase–barstar systems has significantly improved hybrid development and seed production efficiency.

In addition to yield improvement, considerable progress has been made in enhancing oil quality by reducing undesirable components such as erucic acid and glucosinolates. These improvements not only make mustard oil safer for consumption but also increase its industrial applicability.

Future research should focus on expanding the genetic base, improving stress tolerance, and developing climate-resilient hybrids. The integration of molecular breeding tools and biotechnology will further accelerate the development of superior mustard varieties. Overall, heterosis breeding holds great promise for ensuring food security, nutritional quality, and sustainable agricultural development.

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