

Genetic Engineering in Indian Mustard (*Brassica juncea* L.): Current Progress and Future Directions for Enhanced Crop Improvement

Abstract

Genetic tools have revolutionized mustard improvement strategies, offering innovative avenues to enhance its agronomic traits and quality parameters. CRISPR technology, exemplified by research at the National Institute of Plant Genome Research in India, has enabled precise manipulation of glucosinolate levels in mustard plants, improving mustard oil quality. The Barnase-barstar gene system has facilitated the development of mustard hybrids, increasing yield and resilience. Additionally, Microsatellites have emerged as indispensable tools for understanding genetic relationships within mustard populations. These genetic tools hold promise for addressing agronomic challenges and meeting market demands in mustard cultivation. However, their deployment raises ethical, regulatory, and socio-economic considerations that require careful consideration. Responsible stewardship and transparent deployment of these technologies are essential to realize their full potential in enhancing mustard crops and ensuring a sustainable future for food production. In conclusion, genetic engineering offers exciting avenues for mustard improvement, with CRISPR, the Barnase-barstar gene system, and Microsatellites playing pivotal roles in enhancing crop quality, yield potential, and resilience. As mustard continues to play a crucial role in global agriculture and food security, the responsible utilization of these genetic tools holds promise for meeting the evolving needs of farmers and consumers worldwide. Furthermore, the paper briefly discusses the application of these genetic tools in enhancing Dhara Mustard, a popular variety in Indian agriculture, emphasizing its potential impact on addressing agricultural challenges and meeting consumer demands.

Keywords: CRISPR, Barnase-Barstar, Microsatellites, Dhara Mustard, Food Security

1. Introduction

Genetic engineering has emerged as a pivotal tool in agricultural biotechnology, offering innovative solutions to enhance crop productivity, resilience, and nutritional quality. Within the diverse landscape of crop species, Indian mustard (*Brassica juncea* L.) occupies a prominent position as one of India's principal oilseed crops, vital for both domestic consumption and economic sustenance [1]. The application of genetic engineering techniques in Indian mustard holds immense promise for addressing the multifaceted challenges faced by the agricultural sector, ranging from climate change adaptation to food security [2-3]. This review paper provides an in-depth analysis of the current progress and future directions of genetic engineering in Indian mustard, elucidating the key advancements, challenges, and opportunities shaping its trajectory. By exploring recent developments in transgenic technology, gene editing, and molecular breeding approaches, this paper aims to illuminate the potential avenues for enhancing crop improvement and driving sustainable agricultural development in the context of Indian mustard. Through a comprehensive examination of the scientific literature and empirical evidence, this review offers valuable insights into the transformative role of genetic engineering in shaping the future of Indian mustard cultivation, while underscoring the need for concerted efforts to realize its full potential in ensuring global food security and agricultural sustainability.

The *Brassica juncea* hybrid designated as Dhara Mustard Hybrid-11 (DMH-11), conceptualized and developed under the auspices of Professor Deepak Pental at the University of Delhi, represents a significant milestone in the realm of agricultural biotechnology within India [4]. Initiated with the primary objective of mitigating the nation's dependency on edible oil imports, DMH-11 stands as the second genetically modified crop to receive governmental approval in India subsequent to BT cotton. Its inception in 2002 marked a pivotal moment in the utilization of transgenic technology within the Indian agricultural landscape [5]. DMH-11, a hybrid crop, is the progeny resulting from the amalgamation of two distinct parental lines, and its nomenclature derives from the fruition of 11 successive generations [6]. It manifests as a transgenic entity,

wherein its genetic constitution has undergone modification through engineered techniques. Specifically, DMH-11 stems from the crossbreeding of the indigenous Brassica juncea variety "Varuna" with an East European mutant denoted as Early Heera-2 [6, 7-8].

The advent of DMH-11 heralds notable advancements in agricultural productivity, with empirical evidence displaying an average yield escalation of up to 28% vis-à-vis traditional counterparts like Varuna [9]. However, the developmental trajectory of DMH-11 was not devoid of challenges. One such hurdle pertained to the intrinsic reproductive biology of mustard plants, characterized by the capacity for self-pollination owing to their hermaphroditic floral structure. The resultant genetic isolation rendered conventional pollination mechanisms ineffective, necessitating innovative solutions to enable efficient hybridization [10]. Subsequent endeavours led to the successful resolution of these impediments, signifying a commendable achievement for the University of Delhi. Beyond its yield-enhancing attributes, DMH-11 encompasses broader implications within the agricultural domain. Notably, it encapsulates the integration of the barnase gene, sourced from the soil bacterium *Bacillus amyloliquefaciens*. This genetic amalgam endows DMH-11 with heightened fertility and seed production capabilities, underpinned by the orchestrated interplay of three key genes: Bargene, Barnase and Barstar. In the broader context of Indian agriculture, mustard occupies a prominent status among the nation's principal oilseed crops. The advent of DMH-11 not only underscores a paradigmatic shift towards genetically engineered solutions but also underscores the imperative of sustainable agricultural practices to address contemporary challenges. As a pioneering exemplar of biotechnological innovation, DMH-11 epitomizes the transformative potential of interdisciplinary collaboration and technological ingenuity in fostering agricultural resilience and food security within India's agrarian landscape [11].

2. Genetic tools used to improve mustard

Genetic tools have revolutionized mustard improvement strategies, offering innovative avenues to enhance its agronomic traits and quality parameters [6, 12]. Among these tools, CRISPR stands out as a powerful gene editing technology, exemplified by the work of researchers at the National Institute of Plant Genome Research in New Delhi, India, who successfully engineered mustard plants with altered glucosinolate levels, yielding palatable mustard oil [13]. Complementing this, the Barnase-barstar gene system has facilitated the development of mustard hybrids, contributing to increased yield and resilience. Additionally, microsatellites have emerged as indispensable tools, providing molecular insights and enabling the assessment of genetic relationships within mustard populations [14]. Together, these genetic tools represent a dynamic toolkit for mustard improvement, offering promising prospects for addressing agronomic challenges and meeting diverse market demands.

2.1 CRISPR: A gene editing tool

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has emerged as a transformative gene-editing tool, revolutionizing the field of molecular biology and offering unprecedented precision in genetic manipulation [15-16]. In the context of mustard improvement, researchers at the National Institute of Plant Genome Research in New Delhi, India, have harnessed the power of CRISPR to engineer mustard plants with distinct glucosinolate profiles [17]. Glucosinolates, sulfur-containing compounds abundant in cruciferous vegetables like mustard, play crucial roles in plant defense mechanisms and impart characteristic flavors and health benefits to mustard oil [18]. However, the bitter taste associated with high glucosinolate levels in mustard seeds has been a longstanding challenge, limiting the palatability and marketability of mustard oil. The breakthrough achieved by researchers at the National Institute of Plant Genome Research represents a significant milestone in mustard breeding and oil production. By employing CRISPR-mediated gene editing techniques, they successfully modulated the expression of genes involved in glucosinolate biosynthesis, resulting in mustard plants with low glucosinolate levels in seeds while maintaining high levels in leaves [51-53]. This precise manipulation of glucosinolate content has unlocked new possibilities for the production of palatable mustard oil, offering consumers a flavorful and nutritious culinary experience while retaining the health-promoting properties associated with glucosinolates [19].

The implications of this achievement extend far beyond the realm of mustard cultivation, signaling a paradigm shift in crop improvement strategies. CRISPR-mediated gene editing offers

unparalleled precision and efficiency in targeted genome modifications, facilitating the rapid development of novel crop varieties with tailored traits to meet evolving consumer preferences and agricultural challenges [15]. In the case of mustard, the ability to fine-tune glucosinolate levels opens doors to enhanced oil quality, increased market competitiveness, and improved consumer acceptance, driving innovation and growth in the mustard industry. Furthermore, the success of CRISPR-mediated gene editing in mustard underscores the pivotal role of biotechnological advancements in addressing complex agricultural issues and promoting sustainable food production [17-19]. By harnessing the power of molecular tools like CRISPR, researchers can accelerate the breeding process, bypassing traditional breeding barriers and overcoming genetic constraints to develop crops with desired traits, such as disease resistance, drought tolerance, and nutritional enhancement [20]. This transformative approach to crop improvement holds promise for enhancing global food security, mitigating the impacts of climate change, and fostering agricultural sustainability.

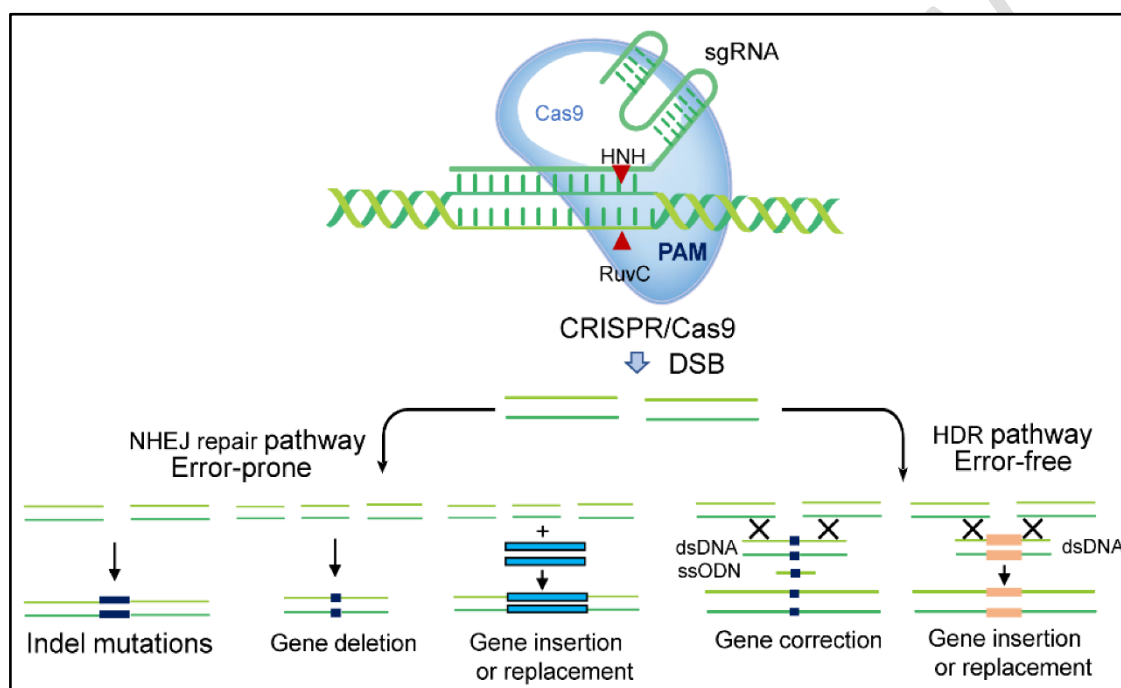


Figure 1: CRISPR-Cas9 Gene editing tool-working principal. (Source: Wan et al. (2020) [21])

However, alongside its immense potential, CRISPR technology also raises ethical, regulatory, and socio-economic considerations that warrant careful scrutiny and responsible stewardship. The precise nature of CRISPR-mediated genome editing raises questions regarding the unintended off-target effects and long-term ecological impacts of genetically modified crops [17-19]. Additionally, regulatory frameworks governing the use of gene editing technologies vary widely across jurisdictions, posing challenges to their widespread adoption and commercialization. CRISPR represents a game-changing tool in the genetic toolkit of mustard improvement, offering unparalleled precision and efficiency in genome editing. The successful application of CRISPR to modulate glucosinolate levels in mustard plants exemplifies its transformative potential in enhancing crop quality, nutritional value, and market competitiveness [15-16, 18]. Moving forward, responsible and transparent deployment of CRISPR technology is essential to realize its full potential in addressing global agricultural challenges and ensuring a sustainable future for food production.

2.2 *Barnase-barstar gene system*

The Barnase-barstar gene system stands as a pivotal genetic engineering tool in the realm of mustard breeding, offering a novel approach to hybridization and crop improvement [22]. Originating from molecular biology principles, this system leverages the precise manipulation of gene expression to facilitate the creation of mustard hybrids with enhanced agronomic traits and improved yield potential. At the heart of the Barnase-barstar system lies a strategic interplay between two key genes: Barnase and Barstar. Barnase, a ribonuclease enzyme, plays a central

role in inducing male sterility within the mustard plant by disrupting the normal development of pollen grains [23]. When expressed in the reproductive organs of the plant, Barnase selectively targets and degrades RNA molecules essential for pollen viability, effectively rendering the plant incapable of producing functional pollen [24]. This engineered male sterility prevents self-pollination and promotes outcrossing, facilitating the controlled hybridization of genetically distinct mustard lines.

Complementing the action of Barnase, the Barstar gene serves as its counterpart, acting as an inhibitor to neutralize the effects of Barnase and restore fertility in the hybrid plants. Barstar functions by binding to Barnase with high affinity, forming a stable complex that prevents Barnase from exerting its ribonucleolytic activity [25]. Through the precise regulation of Barstar expression, breeders can effectively modulate the fertility of the mustard hybrids, ensuring optimal pollination and seed set. The application of the Barnase-barstar gene system in mustard breeding has revolutionized traditional hybridization methods, offering unparalleled control over the reproductive process and accelerating the development of superior mustard varieties [26]. By introducing the Barnase and Barstar genes into parental lines with desirable agronomic traits, breeders can produce hybrid progeny that inherit the favorable characteristics of both parent plants. This hybrid vigor, resulting from the combination of diverse genetic backgrounds, often translates into increased yield, improved disease resistance, and enhanced adaptability to environmental stressors.

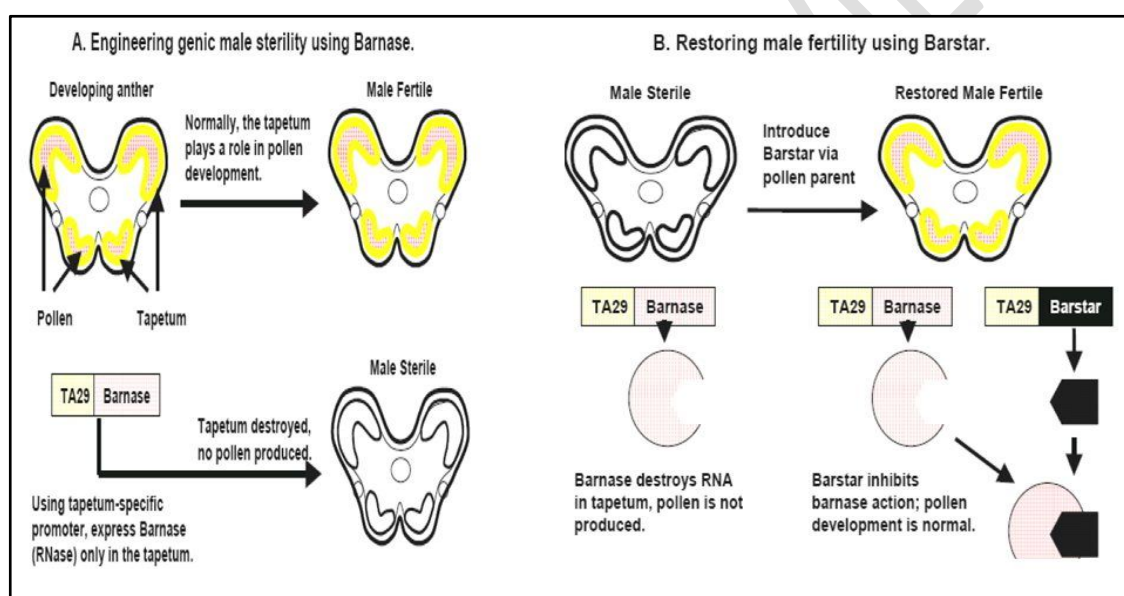


Figure 2: Engineering GMS using Barnase B. Restoring male fertility (MF) using Barstar

Moreover, the Barnase-barstar system facilitates the production of hybrid seeds on a commercial scale, overcoming logistical challenges associated with manual emasculation and pollination. With male sterility conferred by the Barnase gene, hybrid seed production becomes more efficient and cost-effective, enabling breeders to meet the growing demand for high-quality mustard seeds in agricultural markets [27]. The adoption of the Barnase-barstar gene system underscores the pivotal role of biotechnology in addressing the evolving needs of modern agriculture and ensuring food security. By harnessing the power of genetic engineering, researchers and breeders can unlock the full potential of mustard crops, optimizing their performance and resilience in diverse agroecosystems. Furthermore, the development of hybrid mustard varieties through the Barnase-barstar system offers a sustainable solution to increasing global food production while minimizing environmental impact and resource usage. However, alongside its immense potential, the utilization of genetic engineering tools like the Barnase-barstar system also raises ethical, regulatory, and socio-economic considerations that warrant careful deliberation. As mustard hybrids developed through this technology are introduced into agricultural landscapes, it is essential to ensure transparency, safety, and equitable access to these innovations, fostering a balanced approach to crop improvement and sustainable development. Through responsible stewardship and collaborative efforts, the Barnase-barstar gene system holds promise for shaping the future of mustard agriculture and contributing to a more resilient and prosperous food system [24-26].

2.3 Microsatellites as a genetic tool

In mustard breeding, genetic tools like Microsatellites play a pivotal role in facilitating molecular analysis and evaluating genetic relationships among different mustard varieties. Microsatellites, also known as simple sequence repeats (SSRs), are short, tandemly repeated DNA sequences dispersed throughout the genome [28]. These genetic markers exhibit high levels of polymorphism, making them ideal for assessing genetic diversity and population structure in mustard germplasm.

Microsatellites are valuable tools for mustard breeders seeking to understand the genetic basis of agronomic traits, identify superior parental lines for hybridization, and develop targeted breeding strategies [29]. By analyzing the distribution and allelic variation of microsatellite markers across mustard genomes, researchers can discern patterns of genetic relatedness and infer evolutionary relationships among different mustard varieties. This information enables breeders to make informed decisions regarding the selection and utilization of parental lines in breeding programs, optimizing the efficiency and success of hybridization efforts. Furthermore, Microsatellites provide valuable insights into the genetic diversity and population structure of mustard germplasm collections, aiding in the conservation and utilization of genetic resources [29-30]. By characterizing the genetic profiles of diverse mustard accessions using microsatellite markers, researchers can identify unique genotypes, detect genetic bottlenecks, and prioritize germplasm for breeding purposes. This genetic information serves as a foundation for the development of tailored breeding programs aimed at improving mustard varieties for specific traits and environments.

In addition to their utility in genetic diversity analysis, Microsatellites are indispensable tools for marker-assisted selection (MAS) and trait mapping in mustard breeding. By associating microsatellite markers with desirable agronomic traits, such as disease resistance, oil content, and seed quality, breeders can expedite the development of elite mustard varieties with improved performance and marketability [31]. Moreover, Microsatellites facilitate the tracking of target genomic regions during breeding cycles, enabling the efficient introgression of favorable alleles into breeding populations through marker-assisted backcrossing and pyramiding strategies. Overall, Microsatellites represent a practical and versatile tool for mustard breeders, providing essential molecular data for the assessment of genetic diversity, population structure, and trait inheritance in mustard germplasm. By harnessing the power of microsatellite markers, researchers can enhance the efficiency and precision of mustard breeding programs, ultimately contributing to the development of improved varieties with enhanced agronomic traits, yield potential, and resilience to biotic and abiotic stresses [32]. As mustard continues to play a vital role in global agriculture and food security, the utilization of Microsatellites and other genetic tools holds promise for advancing mustard breeding efforts and meeting the evolving needs of farmers and consumers worldwide.

3. Science and Technology as a Public Good

Science and technology have long been recognized as vital components of societal progress, driving innovation, economic growth, and social development. The development of DMH-11 by a team of scientists from Delhi University's Centre for Genetic Manipulation of Crop Plants (CGMCP), led by Dr. Deepak Pental, in collaboration with the National Dairy Development Board (NDDB) and the Department of Biotechnology, exemplifies the potential of publicly-funded research to yield transformative outcomes in agriculture. In an era where scientific research in agriculture and genetic engineering is increasingly dominated by private interests, DMH-11 stands as a notable exception—a product of science driven by the public system of research [33]. This achievement underscores the importance of preserving and nurturing public research institutions as engines of innovation and drivers of societal progress. Historically, national agricultural research systems and international public-access institutions have played pivotal roles in driving agricultural revolutions, such as the green revolution of the 1960s through the 1980s [34]. However, the landscape of agricultural biotechnology has witnessed a shift, with multinational corporations exerting growing control over genetic technologies and food production systems. The consolidation of agri-business monopolies raises significant political concerns, particularly regarding food security, farmer livelihoods, and rural workers' rights. The appropriation of genetic

technologies by corporate interests underscores the need for robust governance mechanisms to safeguard public interests and ensure equitable access to technological advancements.

In the context of DMH-11, its development offers a glimmer of hope for the re-appropriation of scientific research as a public good. However, this optimism is tempered by broader socio-political dynamics, including the recent push for agricultural liberalization and privatization, as evidenced by the now-repealed farm laws. The potential implications of these policy shifts raise valid concerns about the future trajectory of agricultural research and the role of private agri-corporates. While genuine apprehensions exist regarding the co-optation of genetic engineering research by corporate interests, it is imperative to recognize the transformative potential of this technology in advancing agricultural production systems. Balancing the imperative of technological progress with the need to safeguard public interests and ensure equitable access to innovations remains a central challenge for policymakers, researchers, and civil society stakeholders alike.

4. Boost to Production

The prospect of increased mustard yield and production, potentially realized through the adoption of DMH-11 by Indian farmers, offers a ray of hope amidst agricultural challenges. Despite a substantial area under mustard cultivation in India, averaging between 6 to 7 million hectares, and a yearly production of approximately 8 million tonnes over the past five years, the nation faces a yield disparity compared to developed countries [35]. With an average yield of less than 1500 kg per hectare, significantly lower than the 2200 to 2500 kg per hectare seen in developed nations, India grapples with a deficit in domestic edible oil production, necessitating substantial imports. Importantly, India imports about 50 to 60 percent of its edible oil requirements, amounting to 140 lakh tonnes in 2019-20, with notable volumes sourced from palm, soybean, rapeseed (canola), and mustard oils [36]. Interestingly, a significant portion of canola and soybean oil used for production is genetically modified (GM), prompting reflections on the irony of potentially importing GM Indian mustard oil from Australia. While efforts to curb imports have resulted in a decline in imports of rapeseed and mustard oil from 4 lakh tonnes in 2016-17 to about 55,000 tonnes in 2020-21, the market demand for mustard and canola oil remains substantial, underscoring the urgent need for increased domestic yields [30].

Proponents of DMH-11 suggest a potential yield increase of 25 to 30 percent without significant increases in water usage or chemical inputs, offering promise for addressing India's agricultural productivity challenges. However, contrasting viewpoints exist, with some disputing claims of its yield advantage over existing non-GM mustard varieties. These assertions necessitate rigorous verification through impact studies. Nevertheless, the imperative for augmenting mustard yields and production is undeniable, with transgenic technology offering a pathway to achieving this goal. Notably, while DMH-11 utilizes established technology, developed as early as 2002, its significance extends beyond immediate yield expansions [6, 37]. It serves as a foundational platform for the development of future GM hybrid mustard varieties with even greater yield potential. Therefore, the decision surrounding DMH-11 holds implications beyond mere yield increases, offering a springboard for the exploration and realization of enhanced agricultural productivity through genetic engineering in mustard cultivation [38].

5. DMH-1: Hybrid Mustard with Innovative CMS-Restorer System

Hybridization, leveraging the phenomenon of heterosis, has emerged as a powerful strategy to enhance productivity in numerous crop species. *Brassica juncea*, commonly known as oilseed mustard and a staple oilseed crop in the Indian subcontinent, exhibits distinct gene pools, particularly Indian and east European varieties [39]. Addressing this challenge, we present the development of DMH-1, a hybrid mustard variety, facilitated by a novel Cytoplasmic Male Sterility (CMS)-restorer system conducive to large-scale hybrid seed production [40]. The CMS, designated as '126-1' initially identified in a doubled haploid population of a resynthesized *B. napus* line, exhibited stable expression through successive generations of backcrossing in *B. napus*. Subsequently, the CMS was successfully transferred to *B. juncea* via conventional backcross breeding methods, requiring 4 to 7 generations for stable expression in recipient varieties [41]. Notably, '126-1' CMS demonstrated a unique trait, wherein any variety other than the male sterile cytoplasm could act as a restorer, rendering it exceptionally flexible and adaptable for hybrid seed production in *B. juncea*.

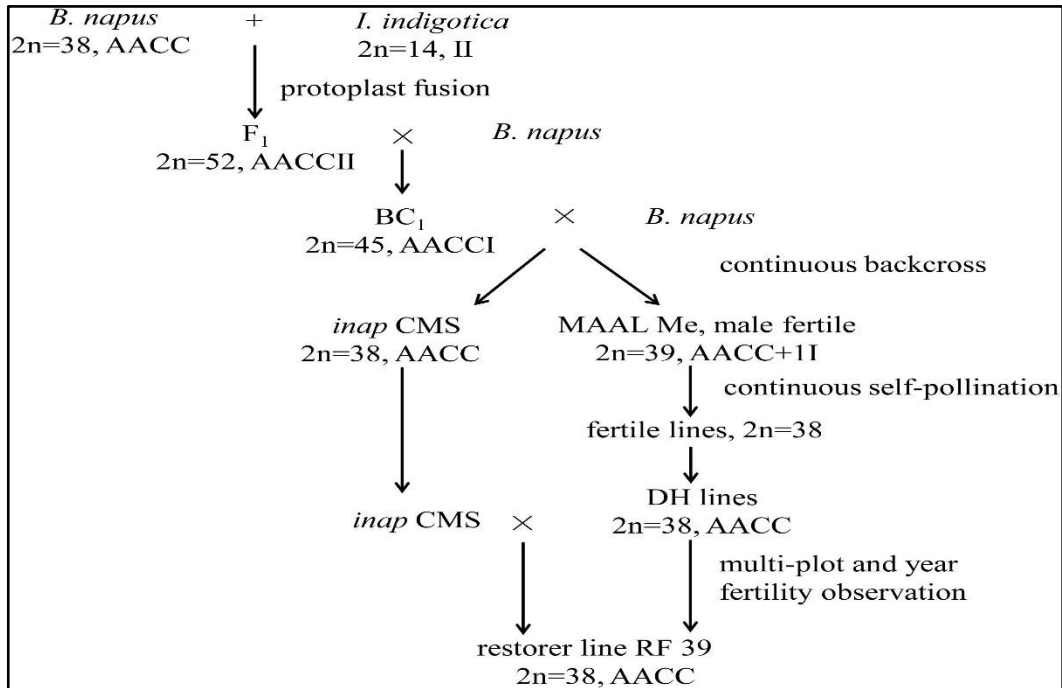


Figure 3:The breeding procedure of restorer line for inap CMS. (Source: Li et al. 2019 [42])

Further characterization revealed that '126-1' CMS did not induce aberrations in floral or vegetative features, maintaining normal flower development, nectar production, and female fertility [39]. The development of DMH-1, utilizing the '126-1' CMS, involved crossing the Indian cultivar Pusa bold with the east European line EH-2. DMH-1 exhibits desirable agronomic traits inherited from the east European parent, including taller stature (~200cm), abundant branching, thin-walled pods containing approximately 15 seeds each, and early maturity. Extensive field demonstration trials conducted across mustard-growing regions of north and north-western India revealed promising results for DMH-1. Average heterosis of 35.6% and 28.5% was recorded in 2004-05 and 2005-06, respectively, with maximum yield potential reaching 3.29 tonnes/hectare in field trials and 3.78 tonnes/hectare in minikit trials. Moreover, DMH-1 hybrids exhibited high resistance to white rust and significant tolerance to *Alternaria* blight, outperforming widely cultivated mustard cultivars in India [43].

6. Environmental Release of GM-Mustard in India: Cause for Hope

The recommendation by the Genetic Engineering Appraisal Committee (GEAC) for the environmental release of genetically modified (GM) hybrid mustard DMH-11 in India signals a significant advancement in agricultural biotechnology [44]. This decision, made on October 26th, 2022, after years of evaluation, allows for seed production and field trials to assess its impact on yield and the environment, with potential commercial release pending favourable results. With the potential for seed production and field trials to evaluate its impact on yield and environmental sustainability, this development offers a glimmer of hope for achieving a more resilient and productive agricultural system in India. Amidst uncertainties, the approval of GM mustard symbolizes a convergence between scientific advancement, sustainable agriculture, and enhanced food security, laying the groundwork for a brighter and more sustainable future for Indian agriculture.

7. Current Progress and Future Directions for Enhanced Crop Improvement

7.1. Opens the Pathway for More Genetic Research

Significantly, this decision also opens up the possibility for further genetic engineering research towards developing mustard hybrids with other selective traits such as better input efficiency, better product quality, higher pest resistance, to name a few. The GEAC has also recommended the field trials for GM hybrids of Banana, Cotton, Potato, and Rubber [45]. Public

sector research institutions, such as ICAR-Potato Research Centre, National Agri-Food Biotechnology Institute, and Rubber Research Institute of India, are expected to lead these research trials. These steps will hopefully expand the scope of genetic engineering research in agricultural production in India after a long hiatus, a development that will go long way in helping expand agricultural productivity in a sustainable fashion. When developed and disseminated by the State such research can also lead to a positive impact on farm profitability. These developments are indicative of a possible convergence of the goals of higher agricultural productivity and production, better incomes for farmers, and environmental sustainability, that is made possible by appropriate development and deployment of agricultural science and technology under proper State Control.

7.2. Concerns regarding Environmental Sustainability

A major criticism of GM crops pertains to the harm that it might cause to ecology, and to human and animal health. The criticism remains strong despite the fact that GM crops have been used, and consumed, for more than two decades now without causing specific harm to any species, or to the ecology in general. In the specific case of GM-Mustard, it has been argued that it can have adverse impact on the population of pollinators, particularly honey bees. While this is a hypothesis that requires systematic scientific inquiry to arrive at any conclusion, there is not much evidence to support the claim. As a matter of fact, GM-Canola (Canola/Rapeseed and Mustard are different species under the same genus), developed using the same Barnase-Barstar technology that has been used to develop DMH-11, has been cultivated for more than a decade in countries such as Canada, without any remarkable harm to bee colonies. Having said that, it is undoubtedly critical that systematic impact studies of any new technology are thoroughly and scientifically conducted under specific agro-climatic conditions, before it is accepted for production. And this is already part of GEAC's recommendations. GEAC has instructed the conduct of field studies under the supervision of Indian Council of Agricultural Research (ICAR) to generate evidence of effects of DMH-11 on honeybees and other pollinators in Indian agro-climatic zones within next two years [47]. Uncertainty is part of any scientific and technological development, and any policy framework dealing with technological developments has to work with some level of uncertainty. A blanket rejection of the technology itself cannot be an answer to all uncertainties, particularly when the technology – genetic engineering, in this case – is critical to a sustainable, and food and nutrition secure future. The way to technological progress requires manoeuvring through uncertainty in a manner that is sustainable from the point of view of human development, including environmental sustainability. In the case of genetic engineering, this implies the presence of a robust and independent regulatory system, under the aegis of the state. Such a system can help define well-established standards of human safety and environmental sustainability, and ensure that the development of high yielding hybrids through genetic engineering technologies adhere to such standards. Constant monitoring and evaluation by robust regulatory agencies must be an essential aspect of ecosystem of genetic engineering research.

7.3. Effects on Employment

Another concern with regard to development and absorption of technology, including transgenic technology, in agricultural production has been regarding its tendency of causing unemployment, and displacing manual work. The concern is one-sided at best. It fails to appreciate that technological development also leads to new kinds of employment, and significant reduction in the drudgery of agricultural tasks. This is not to imply that there would not be any loss of employment by the coming of new technology in agriculture, but rejection of a technology on this premise is naive, at best. The point rather is to account for the loss of employment as the cost of technology, and ensuring those losing out are compensated in a just and fair manner, and have access to better jobs and economic mobility. Research indicates that in any case agriculture in India is not in a situation to absorb the rural work force, and the non-farm sector, particularly manufacturing, has to be strengthened in order to provide meaningful employment. This is of course a macro-economic policy problem, with no easy Luddite-like solution. In this specific case of GM-Mustard, it has been argued by some that the herbicide tolerance in DMH-11 will lead to expansion in the use of herbicides that will, in turn, take away the manual work of weeding [48]. As a temporary solution, the GEAC recommendation restricts the use of Glyphosate (herbicide) to registered "Pest Control Operators." The attempt is to prohibit the use of herbicide, except in the

production of hybrid seeds of DMH-11 [49]. This step may prevent the application of herbicides in the actual production of GM-Mustard by farmers, as and when it begins. This, of course, is a temporary solution. And one has to think of more permanent ways to deal with the larger issue of technology induced unemployment in agriculture, without taking recourse to a reactionary position of rejecting the technology itself. As a matter of fact, it would not be wrong to argue that with developments in genetic engineering technology the need for herbicide tolerance in developing GM-Hybrids will be reduced, if not eliminated. Such possibilities in genetic engineering, however, can only be explored after the technology is allowed to function, and more public investments are directed towards research in biotechnology.

7.4. Concluding Concerns

The approval from GEAC for “environmental release” for DMH-11 implies a horizon, according to experts, of, at least, two years before it becomes available for widespread use and adoption, provided the field trials yield favourable results in terms of yields and safety concerns among other parameters [50]. However, given the history of slow promotion of genetic engineering research in India, this horizon is marked by uncertainties

8. Conclusion

Genetic tools have significantly advanced mustard improvement strategies, offering precision, efficiency, and novel approaches to address agronomic challenges and meet market demands. CRISPR technology has enabled precise manipulation of glucosinolate levels, enhancing the palatability and marketability of mustard oil. The Barnase-barstar gene system has revolutionized mustard hybridization, increasing yield and resilience while facilitating commercial-scale seed production. Microsatellites have provided invaluable insights into genetic relationships within mustard populations, aiding in trait mapping and marker-assisted selection. While these genetic tools offer promising prospects for mustard crop improvement, their deployment necessitates careful consideration of ethical, regulatory, and socio-economic implications. Responsible stewardship and transparent deployment of these technologies are essential to harness their full potential while ensuring safety, sustainability, and equitable access. As mustard continues to play a crucial role in global agriculture and food security, the adoption of genetic engineering holds promise for meeting the evolving needs of farmers and consumers worldwide. Furthermore, the application of these genetic tools in enhancing varieties like Dhara Mustard underscores their potential impact on addressing agricultural challenges and meeting consumer demands. In the journey towards sustainable agriculture and food security, the ongoing advancements in genetic engineering offer exciting opportunities to enhance mustard crops, contributing to a more resilient, productive, and equitable food system. Through collaborative efforts and responsible innovation, the future of mustard cultivation holds promise for meeting the challenges of the 21st century while ensuring a sustainable and prosperous agricultural landscape.

9. REFERENCES

1. Lal S, Kumar V, Gupta U, Sushma, Shirke PA, Sanyal I. Overexpression of the chickpea Metallothionein 1 (MT1) gene enhances drought tolerance in mustard (*Brassica juncea* L.). *Plant Cell, Tissue and Organ Culture (PCTOC)*. 2024;157(1):6.
2. Saini P, Saini P, Kaur JJ, Francies RM, Gani M, Rajendra AA, Negi N, Jagtap A, Kadam A, Singh C, Chauhan SS. Molecular approaches for harvesting natural diversity for crop improvement. *Rediscovery of genetic and genomic resources for future food security*. 2020:67-169.
3. Sachan DS, Naimuddin SK, Patra D, Subha L, Senthilkumar T, Chittibomma K, Khan N, Prasad SV. Advancements in Enhancing Oil Quality in Rapeseed and Mustard: A Comprehensive Review. *Journal of Experimental Agriculture International*. 2024;46(5):181-93.
4. Akhatar J, Kumar H, Kaur H. Recent Progress in Brassica Hybrid Breeding. *Plant Male Sterility Systems for Accelerating Crop Improvement*. 2022:195-219.
5. Priyanka N, Rajashekhar M, Bharghavi K, Akhil G, Asritha CH, Keerthana B, Sruthi K. Insect Resistance Transgenic Crops and their Current Status in India. *Chronicle of Bioresource Management*. 2020;4(1):1-5.

6. 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. Genomic Designing of Climate-Smart Oilseed Crops. 2019:275-369.
7. Singh RK, Arunachalam A, Mohapatra T. Present status of GM crops in India. Indian Farming. 2020 May;70(5).
8. Yaduraju NT. The saga of genetically-modified (GM) and herbicide-tolerant (HT) crops in India. Weeds-Journal of the Asian-Pacific Weed Science Society. 2021;3(1):38-55.
9. Agnihotri A, Gupta P, Dwivedi A, Seth CS. Counteractive mechanism (s) of salicylic acid in response to lead toxicity in *Brassica juncea* (L.) Czern. cv. Varuna. Planta. 2018;248:49-68.
10. Rogo U, Fambrini M, Pugliesi C. Embryo rescue in plant breeding. Plants. 2023;12(17):3106.
11. Barrett CB. Overcoming global food security challenges through science and solidarity. American Journal of Agricultural Economics. 2021;103(2):422-47.
12. Mohd Saad NS, Severn-Ellis AA, Pradhan A, Edwards D, Batley J. Genomics armed with diversity leads the way in Brassica improvement in a changing global environment. Frontiers in Genetics. 2021;12:600789.
13. Yashpal, Saini N, Singh N, Chaudhary R, Yadav S, Singh R, Vasudev S, Yadava DK. Genetic improvement of oil quality using molecular techniques in Brassica juncea. Brassica Improvement: Molecular, Genetics and Genomic Perspectives. 2020:109-25.
14. Singh L, Nanjundan J, Singh KH, Sharma D, Parmar N, Watts A, Jain R, Thakur AK. Development of a set of SSR markers for characterization of Indian mustard germplasm and varieties. Journal of Plant Biochemistry and Biotechnology. 2021:1-1.
15. Wang JY, Doudna JA. CRISPR technology: A decade of genome editing is only the beginning. Science. 2023;379(6629):eadd8643.
16. Zhu H, Li C, Gao C. Applications of CRISPR–Cas in agriculture and plant biotechnology. Nature Reviews Molecular Cell Biology. 2020;21(11):661-77.
17. Kaul T, Verma R, Sony SK, Bharti J, Motelb KF, Thangaraj AP, Kaul R, Nehra M, Eswaran M. CRISPR/Cas Enables the Remodeling of Crops for Sustainable Climate-Smart Agriculture and Nutritional Security. Global Climate Change and Plant Stress Management. 2023:71-111.
18. Liu Z, Wang H, Xie J, Lv J, Zhang G, Hu L, Luo S, Li L, Yu J. The roles of cruciferaeglucosinolates in disease and pest resistance. Plants. 2021;10(6):1097.
19. Chhajed S, Mostafa I, He Y, Abou-Hashem M, El-Domiaty M, Chen S. Glucosinolate biosynthesis and the glucosinolate–myrosinase system in plant defense. Agronomy. 2020;10(11):1786.
20. Rauf S, Al-Khayri JM, Zaharieva M, Monneveux P, Khalil F. Breeding strategies to enhance drought tolerance in crops. Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. 2016:397-445.
21. Wan L, Wang Z, Tang M, Hong D, Sun Y, Ren J, Zhang N, Zeng H. CRISPR-Cas9 gene editing for fruit and vegetable crops: strategies and prospects. Horticulturae. 2021;7(7):193.
22. Singh P. Genetically modified crops in India: Politics, policies, and political economy. In Policy Issues in Genetically Modified Crops 2021 Jan 1 (pp. 75-96). Academic Press.
23. Jain HK, Kharkwal MC, editors. Plant breeding: Mendelian to molecular approaches. Springer Science & Business Media; 2012 Dec 6.
24. Sood R, Chauhan A. Applications of Genetic Engineering and DNA Markers in Plant Breeding. Research and Review in Genetics and Plant Breeding. 2023;1:45.
25. Gotte G, Menegazzi M. Biological activities of secretory RNases: Focus on their oligomerization to design antitumor drugs. Frontiers in Immunology. 2019;10:488880.
26. Tuteja N, Gill SS, Tuteja R, editors. Improving crop productivity in sustainable agriculture. John Wiley & Sons; 2012 Oct 11.
27. Cabrera-Ponce JL, Valencia-Lozano E, Trejo-Saavedra DL. Genetic modifications of Corn. In Corn 2019 Jan 1 (pp. 43-85). AACC International Press.
28. Abdurakhmonov IY. Introduction to microsatellites: basics, trends and highlights. Microsatellite markers. 2016;1:13.
29. Nanjundan J, Radhamani J, Thakur AK, Berliner J, Manjunatha C, Sindhu A, Aravind J, Singh KH. Utilization of rapeseed-mustard genetic resources for Brassica improvement: A retrospective approach. Brassica Improvement: Molecular, Genetics and Genomic Perspectives. 2020:1-30.
30. Thakur AK, Singh KH, Sharma D, Parmar N, Nanjundan J. Breeding and genomics interventions in Ethiopian mustard (*Brassica carinata* A. Braun) improvement—A mini review. South African Journal of Botany. 2019;125:457-65.

31. Akhatar J, Kumar H, Kaur H. Recent Progress in Brassica Hybrid Breeding. *Plant Male Sterility Systems for Accelerating Crop Improvement*. 2022:195-219.
32. Chand S, Patidar OP, Chaudhary R, Saroj R, Chandra K, Meena VK, Limbalkar OM, Patel MK, Pardeshi PP, Vasisth P. Rapeseed-mustard breeding in India: Scenario, achievements and research needs. *Brassica breeding and biotechnology*. 2021:174.
33. Vinod KK, Gopala Krishnan S, Senapati M, Singh AK. Breeding Field Crops: History, Current Status and Introspections. In *Fundamentals of Field Crop Breeding 2022* May 6 (pp. 1-38). Singapore: Springer Nature Singapore.
34. Kumar R, Pandey MK, Chand P. Correlation and Path-coefficient Analysis for Oil and Its Fatty Acids Traits in Indian Mustard [*Brassica Juncea* (L.) Czern & Coss.].
35. Kumar R, Pandey MK, Priyanka N, Kumar V. Character Association and Path Analysis Studies in Indian Mustard [*Brassica juncea* L.].
36. Johnson H. Path-Breaking or History-Repeating? Analysing the Paris Agreement's Research and Development Paradigm for Climate-Smart Agriculture. *Intellectual property and clean energy: The Paris Agreement and climate justice*. 2018:555-84.
37. Das A, Saha S, Layek J, Babu S, Saxena R, Ramkrushna GI. Agricultural Technologies. In *Trajectory of 75 years of Indian Agriculture after Independence 2023* Aug 29 (pp. 57-78). Singapore: Springer Nature Singapore.
38. Ghosh N, Halder G. Current progress and perspective of heterogeneous nanocatalytic transesterification towards biodiesel production from edible and inedible feedstock: A review. *Energy Conversion and Management*. 2022;270:116292.
39. Adak T, Kumar K, Singh VK. An Appraisal of seasonal variations in thermal indices, heat and water use efficiency in mango. *Climate change and its implications on Crop production and food security*. Mahima Research Foundation and Social Welfare, Banaras Hindu University, Varanasi. 2016:183-8.
40. 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*). *Frontiers in Plant Science*. 2022;13:899076.
41. Meena BL, Sharma P, Rai PK. Genetic Resources of Brassicas. *Cash Crops: Genetic Diversity, Erosion, Conservation and Utilization*. 2021 Oct 18:285.
42. Singh VK, Bhoyar PI, Anu, Sharma V. Application of Genomics and Breeding Technologies to Increase Yield and Nutritional Qualities of Rapeseed-Mustard and Sunflower. *Technologies in Plant Biotechnology and Breeding of Field Crops*. 2022:103-31.
43. Kaur G, Singh VV, Singh KH, Priyamedha, Rialch I, Gupta M, Banga SS. Classical genetics and traditional breeding in *Brassica juncea*. In *The Brassica juncea Genome 2022* Mar 9 (pp. 85-113). Cham: Springer International Publishing.
44. Li P, Kang L, Wang A, Cui C, Jiang L, Guo S, Ge X, Li Z. Development of a fertility restorer for inap CMS (*Isatisindigotica*) Brassica napus through genetic introgression of one alien addition. *Frontiers in Plant Science*. 2019;10:442143.
45. Shrivastava A, Tripathi MK, Tiwari S, Singh P, Tripathi N, Tiwari PN, Parihar P, Singh J, Chauhan S. Screening of Indian mustard genotypes against white rust disease based on disease indexing. In *Biological Forum—An International Journal*. 2023c 2023 (Vol. 15, No. 4, pp. 268-72).
46. Nuthalapati CS, Zilberman D, Qaim M, Pingali P. Hybrid Mustard and Biotechnology. *Economic & Political Weekly*. 2023;58(43):47.
47. Ahuja V. Genetically modified organisms and biosafety regulations in India: Status and capacity building initiatives. *Special issue on environment*. 2019;65:64.
48. Tripathi KK, Rao SR. Regulatory Systems and Requirements for Genetically Engineered Cotton from Lab to Land. *Cotton: Biotechnological Advances*. 2010:197-220.
49. Singh P. Genetically modified crops in India: Politics, policies, and political economy. In *Policy Issues in Genetically Modified Crops 2021* Jan 1 (pp. 75-96). Academic Press.
50. Mathew D. Biosafety and Bioregulatory Mechanisms for Transgenic Crops. In *Genetic Engineering of Horticultural Crops 2018* Jan 1 (pp. 273-314). Academic Press.
51. Yaduraju NT. The saga of genetically-modified (GM) and herbicide-tolerant (HT) crops in India. *Weeds-Journal of the Asian-Pacific Weed Science Society*. 2021;3(1):38-55.
52. Verma V, Negi S, Kumar P, Srivastava DK. Global status of genetically modified crops. In *Agricultural biotechnology: latest research and trends 2022* Jan 8 (pp. 305-322). Singapore: Springer Nature Singapore.

53. Mohammadhassan R, Tutunchi S, Nasehi N, Goudarziasl F, Mahya L. The prominent characteristics of the effective sgRNA for a precise CRISPR genome editing. InCRISPR Technology-Recent Advances 2022 Aug 19. IntechOpen.
54. Hadipour K, Asadishad T, Mahya L, Mohammadhassan R, Rahbar A, Goudarziasl F. A comparative review on genome editing approaches. Biointerface Res Appl Chem. 2023;13(6):567.
55. Mohammadhassan R, Ebli PE, Behrouz MA. CRISPR Technology for Diagnosis: A Brief Review: CRISPR for Diagnosis. Everyman's Science. 2022;57(1).
56. Singh S, Ram M, Gupta D, Meena MK, Nayak PK, Choudhary K, Kumar R, Chouhan S. Assessing genetic variability in taramira (*Erucasativa* Mill.) germplasm for enhanced breeding strategies. International Journal of Economic Plants. 2024;11(Feb, 1):018-25.

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