

The Potential of Mutation Breeding for Ensuring Sustainable Food Security

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ABSTRACT

Plants are subject to various environmental stresses, which demand the development of crops that can withstand these challenges, especially with the ongoing impacts of climate change, increased global food demand, and other factors. Abiotic stresses such as drought, salinity, heavy metal toxicity, and extreme temperatures (both high and low) severely limit plant growth and agricultural productivity. The continued loss of fertile land, diminishing water resources, and the accelerating pace of global warming and climate change are expected to result in decreased yields of key food crops across many regions. Genetic diversity is a critical source of phenotypic variation and has historically driven evolutionary processes. Thousands of years ago, humans began harnessing heritable genetic variation in the domestication of plants and animals. Today, inducing mutations offers the possibility of introducing novel traits. These induced mutations have played an essential role in improving global food and nutritional security by enhancing mutant germplasm and developing new crop varieties. Physical and chemical mutagens have generated significant genetic variability. Over the past few decades, induced mutations have greatly contributed to developing better crop varieties. With the help of modern cellular and molecular biology tools, the efficiency of mutation induction, detection, and application has improved. Consequently, more than 3,400 mutant-derived crop varieties have been officially released worldwide, with over 60% of these coming from Asia, especially China, Japan, and India. This paper presents an in-depth review of mutagenesis principles, its applications, and the potential for crop improvement. It also explores molecular and genomic advances in mutation technologies, particularly in building stress-tolerant agriculture.

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Introduction

Plant breeding techniques have played a pivotal role in advancing genetically enhanced crop varieties. These methods continue to expand the crop germplasm base by developing genetically superior varieties for agricultural use. However, the existing genetic resources may not be sufficient to address the growing food demands of a human population projected to reach nine billion by 2050 (Green et al., 2005). Achieving further increases in agricultural productivity, while maintaining equity and environmental sustainability amidst resource limitations, remains a significant challenge. Although both domestication and modern breeding have led to crops with superior agronomic traits compared to their wild relatives, this progress has often come at the cost of reduced genetic diversity (Tanksley & McCouch, 1997; Lee, 1998). For many crops, it is estimated that less than 5% of the available biodiversity

is currently being utilized in agriculture, particularly in self-pollinating species (Tanksley & McCouch, 1997).

Induced mutations have played a critical role in enhancing plant varieties by introducing specific, desirable traits (Ahloowalia & Maluszynski, 2001; Maluszynski et al., 2004; Jain, 2005; Khah et al. 2024). Numerous mutant varieties, developed through mutation breeding, have been released for commercial cultivation across various crop species, highlighting the economic value of this technology (Kharkwal & Shu, 2009; Jain & Suprasanna, 2011). In response to global food security concerns, there is an increasing need to complement current genetic improvement strategies with innovative research aimed at sustainable agriculture (Khah and Verma 2017a, b; Alam et al. 2022). Mutation breeding offers a significant advantage over traditional crossbreeding by allowing for the precise alteration of one or a few traits in a promising variety,

without disrupting the rest of its valuable genetic makeup. Moreover, advances in molecular biology have enhanced the effectiveness of mutagenesis techniques. When combined with molecular marker technologies and high-throughput mutation screening methods, mutation breeding has become even more powerful for crop improvement (Shu, 2009; Khah et al. 2024). This integration not only accelerates the identification of beneficial mutations but also enables breeders to effectively utilize the vast potential of genetic diversity that remains untapped in many crops. Looking forward, these approaches offer promising pathways to address the growing food demands while ensuring resilience against climate change and other environmental challenges.

Methodology

This review on “the potential of mutation breeding for ensuring sustainable food security” employs a systematic approach to gather and synthesize existing literature, focusing on the advancements and applications of mutation breeding for enhancing resilience in crops. First, a comprehensive literature search was conducted using key databases such as PubMed, ScienceDirect, Google Scholar, and Web of Science, chosen for their extensive coverage of peer-reviewed scientific articles. Search terms included combinations of “induced mutation,” “mutation breeding,” “plant stress resilience,” “abiotic stress tolerance,” and “genomic mutagenesis,” with Boolean operators used to refine search results. Inclusion criteria encompassed studies published in reputable journals that specifically address mutation breeding in the context of stress resilience, while studies not directly relevant or lacking in-depth data were excluded. Key information, such as research objectives, mutagen types (e.g., chemical, physical, CRISPR/Cas), crop species, targeted stress types (e.g., drought, salinity), and major findings, was extracted from each study. This data was then categorized according to mutagenesis techniques and stress types, facilitating a thematic analysis that identified trends in breeding practices and technology application. Technological integration with modern tools, such as gene editing, molecular markers, and phenotypic screening, was noted, showcasing how traditional mutation techniques are enhanced by precision technologies. Critical review of these sources also allowed for the identification of gaps in current research, such as the need for improved mutagenesis efficiency for certain crop species and field-based validation of laboratory results. This methodology provides a robust framework for evaluating the role of mutation breeding in agricultural resilience, yielding insights that are invaluable for guiding future research and practice. Through this structured analysis, the review underscores both the achievements and challenges in mutation breeding, ultimately highlighting its importance in supporting sustainable crop development under evolving environmental stresses.

Induced mutagenesis

Mutations in plants can be induced using both physical agents, such as gamma radiation and high or low-energy beams, and chemical mutagens like ethyl methane sulfonate

(EMS). These mutagen treatments are applied to seeds and vegetatively propagated crops. The underlying mechanism involves mutagens causing breaks in nuclear DNA, and as the DNA repair processes occur, new mutations are introduced randomly, which are heritable. These changes can also take place in cytoplasmic organelles, leading to a variety of mutations, including chlorophyll, chromosomal, and genomic mutations. Such genetic alterations enable plant breeders to identify and select mutants with desirable traits, such as resistance to abiotic and biotic stresses. Induced mutations offer the potential to develop crops with multiple beneficial traits. For example, mutant varieties can be better adapted to changing environmental conditions, improving their survival rates under climate change. A notable success of this technique is seen in Vietnam, where eight rice mutant varieties have been developed. These varieties exhibit a range of favorable traits, including high grain quality, tolerance to salinity, and short growth duration, allowing farmers to harvest up to three times per year. As a result, these innovations have generated an additional income of 300 million US dollars for Vietnamese farmers. Furthermore, mutant crop varieties are generally well-accepted by consumers. Induced mutations have been instrumental in advancing global food security, as mutant varieties have contributed significantly to crop productivity. By enabling the development of new food crops with enhanced traits, mutation breeding has allowed farmers to cultivate crops more efficiently in various regions, directly impacting food production and availability (Kharkwal & Shu, 2010). This strategy continues to provide promising solutions for meeting the growing demand for food in the face of environmental challenges.

Gamma radiation has long been the predominant physical mutagen used for inducing mutations in both seed and vegetatively propagated crops (Verma and Khah 2016; Khah and Verma 2020). However, with recent advances in ion energy technologies, heavy ion beam (HIB) and low energy ion beam (LIB) have emerged as promising tools for mutation induction in a broad range of crops. HIB, in particular, has become increasingly popular due to its higher efficiency in mutation induction, largely because of its ability to transfer linear energy transfer (LET), which results in more pronounced biological effects than other mutagens. The use of HIB has successfully generated various *Arabidopsis* mutants with diverse genetic changes, including deletions, insertions, and chromosomal translocations. This technology offers precise control over genetic alterations, making it a highly effective tool for plant mutation breeding. It facilitates the development of desirable traits that can significantly improve crop varieties. These innovations highlight the potential of ion beam technology as a powerful alternative to conventional physical mutagens, providing greater efficiency in mutation induction and enhancing genetic diversity in plant breeding programs.

Significant advancements have been made in plant cell tissue culture, particularly in the regeneration of all major food and horticultural crops. One of the most prominent techniques is micropropagation through organogenesis, which is routinely employed for the clonal propagation of

ornamental plants and various vegetatively propagated species, especially woody plants and fruit trees. In this process, explants, such as shoot meristems, adventitious buds, and microspores, are treated with mutagens, leading to the regeneration of direct shoots, followed by root formation (Suprasanna et al., 2010). The regenerated plants are subsequently maintained in greenhouses and exposed to selective pressure to identify mutants with desirable traits. Somatic embryogenesis, particularly in vegetatively propagated crops like banana, date palm, and cassava, is also readily induced. One of the advantages of applying mutagenic treatments to embryogenic cell suspensions is the significant reduction or complete elimination of chimeras. This enables the development of mutant somatic embryos that can be regenerated into whole plantlets. During this process, embryogenic cells are typically placed on filter paper, transferred onto agar-solidified media, treated with gamma radiation, and then cultured to induce somatic embryo formation. Following this treatment, the cells can also be subjected to selective pressures to isolate mutants with desirable traits, such as tolerance to disease, salt, or drought conditions. Once selected, these mutant plants are transferred to greenhouses and eventually to the field for further evaluation. They may also be crossed with other varieties to enhance genetic diversity and improve crop traits.

One critical aspect of this approach is determining the radiosensitivity of each experimental plant species, which involves calculating the LD50 (Lethal Dose) to avoid excessive or insufficient radiation dosages. This is essential because different plants and even varieties exhibit varying levels of radiosensitivity. For instance, low doses of gamma radiation have been shown to promote growth in citrus, depending on the cultivar. They also help maintain the embryogenic nature of date palm for up to 2-3 years, stimulate growth in orchids, enhance the production of secondary metabolites in medicinal plants, and improve the shelf life of post-harvest products. Overall, the integration of plant tissue culture with mutagenic treatments has opened up new possibilities for crop improvement, allowing researchers to accelerate the development of mutants with desirable characteristics. By harnessing this technology, scientists can effectively enhance the productivity, resilience, and quality of crops, addressing the challenges posed by a growing global population, climate change, and the need for sustainable agriculture. This approach not only facilitates the propagation of plants with superior traits but also offers a powerful tool for breeding programs aimed at developing crops that can withstand various environmental stresses while maintaining high yields.

A diverse array of mutants has been successfully isolated in various crops, including ornamental plants, maize, rice, and wheat, contributing significantly to crop breeding efforts. Likewise, low-energy ion beam (LIB) technology has been effectively utilized for mutation breeding and gene transfer. LIB offers several advantages over traditional mutagenesis methods, such as a lower damage rate, a higher mutation rate, and a broader mutation spectrum. In rice, for instance, 11 new lines of mutants were developed in China, featuring

traits such as higher yields, improved disease resistance, a shorter growing period, and enhanced grain quality. These new rice lines are now being cultivated extensively. In addition, mutation breeding has been applied successfully to jasmine rice in Thailand. A wide variety of mutants have been identified in this crop, exhibiting traits such as short stature and unique coloration in various plant parts. These mutations include red or purple coloring in the leaf sheath, collar, auricles, and ligules, as well as dark brown stripes on the leaf blade. Other notable mutations include changes in the seed coat and pericarp, which also turned dark brown. This diversity of mutants showcases the potential of mutation breeding to produce valuable traits in crops, enhancing both their agricultural and economic viability.

Mutation induction for quality and nutrition improvement

In addition to increasing crop yield, improving the quality and nutritional content of crops is crucial for human diets. There is a growing need to enhance mineral elements (biofortification) and essential amino acids for both human and animal consumption. Modifying protein and fatty acid profiles for better nutrition and health, altering the physicochemical properties of starch for diverse industrial applications, increasing phytonutrients in fruits, and reducing anti-nutritional factors in staple foods are also important goals. Induced mutations offer a valuable tool in achieving these enhancements in crop plants. Out of the 3,000 mutant varieties developed worldwide, over 750 mutants have been specifically induced for improved nutritional quality (www-mvd.iaea.org). These efforts underscore the potential of mutation breeding to contribute to the development of crops that meet both the dietary and health needs of a growing global population.

The collaborative research initiative between the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) has concentrated on utilizing nuclear techniques to advance crop improvement through induced mutations. One of the primary objectives of this program is to generate new cereal strains with significantly higher concentrations of essential micronutrients, such as vitamins and minerals, while simultaneously improving their bioavailability. A key aspect of this effort is reducing the levels of phytic acid, an antinutrient that inhibits the absorption of important minerals like iron and zinc in the human body (Jain, 2000). By targeting the reduction of phytic acid, the program aims to enhance the nutritional value of staple crops, which can be a critical step in addressing malnutrition on a global scale.

In this context, breeding crops with enriched micronutrient content in their edible parts can be a highly effective strategy to reduce the ongoing costs associated with traditional methods of addressing nutrient deficiencies, such as food fortification and supplementation programs. Such biofortification efforts, by increasing the inherent nutrient content of crops, would offer a sustainable, long-term solution (Shetty, 2009). However, the success of these initiatives will depend on several important factors. First and foremost, farmers must be willing to adopt these new

varieties. For this to happen, the improved crops must be economically viable and capable of producing yields comparable to or better than current varieties. Additionally, the edible parts of these crops must be palatable, meaning they should meet consumer preferences in terms of taste, texture, and appearance. This is crucial for ensuring widespread consumer acceptance of the new biofortified varieties (Bouis, 2002). Furthermore, the biofortification strategy will only be effective if the nutrients embedded in the crops can be readily absorbed by the human body. This involves ensuring that the increased micronutrient content is bioavailable, meaning that it can be efficiently absorbed and utilized by the body after consumption. A successful strategy also requires a thorough understanding of how increased nutrient content affects the overall nutritional balance of consumers, particularly when these nutrient-rich varieties become staple foods. For example, altering the micronutrient profile of staple crops could have unintended effects on the diet, which should be carefully monitored and managed (Bouis, 2002).

There are also several broader considerations that need to be addressed before implementing a plant breeding strategy focused on combating micronutrient deficiencies, particularly in developing countries. These considerations include the technical feasibility of breeding staple crops with high micronutrient density, the potential effects of this breeding on overall plant yields, and the willingness of farmers to adopt and cultivate these new varieties. Additionally, it is essential to evaluate alternative strategies for addressing micronutrient malnutrition that might be more easily implemented or sustained in specific regions. This comprehensive approach ensures that any breeding strategy adopted will not only be technically feasible but also economically viable and socially acceptable (Bouis, 2002). The successful introduction of such nutrient-rich crops has the potential to make a significant contribution to global food security and public health by reducing dependence on external fortification methods and ensuring a more sustainable source of essential nutrients directly from the foods people consume regularly. This approach, if widely adopted, could play a key role in alleviating micronutrient deficiencies and improving the overall nutritional status of populations, especially in regions where malnutrition remains a critical public health issue.

Numerous mutant genes have been successfully integrated into commercial crop varieties, leading to significant enhancements in the nutritional profiles of various crops, including maize, barley, soybean, and sunflower. For instance, quality protein maize (QPM) varieties are cultivated across extensive areas, demonstrating nearly double the levels of lysine and tryptophan compared to their parent lines, while exhibiting a 30% reduction in leucine content. This remarkable nutritional improvement has had a profound impact on both human and animal growth and performance. In cassava, researchers have isolated three mutants with varying starch grain sizes, which present considerable economic potential for industrial starch applications and improved cooking qualities. Notably, the smaller starch grains are particularly advantageous for bioethanol production. Similarly, a mutant variety of sweet

sorghum, designated Yuantian No.1, has been developed in China, offering 20% more total carbohydrates than its parental lines, making it ideal for food, feed, and bioenergy applications.

Furthermore, five rice mutants with giant embryos—characterized by their enlarged sizes compared to the wild type—have shown increased levels of protein, vitamins B1 and B2, vitamin E, essential amino acids (including arginine, aspartic acid, glutamic acid, lysine, and methionine), and key mineral elements such as calcium, iron, potassium, phosphorus, and zinc (Zhang et al., 2007). In the case of bananas, several mutants exhibiting diverse traits have been identified, including reduced height, resistance to *Fusarium* wilt, early flowering, increased fruit size, and tolerance to Black Sigatoka disease (Jain, 2010). In date palms, various mutant lines that are tolerant to Bayoud disease, caused by the fungus *Fusarium oxysporum* f. *albedinis*, have been successfully established in the field for over four years in Algeria and are thriving (Jain, 2007). Additionally, new mutant varieties of barley, wheat, rice, and soybean have been released with low phytic acid (LPA) content. This advancement has significantly contributed to reducing phosphorus pollution while enhancing the bioavailability of phosphorus and essential micronutrient minerals in cereals and legumes. Such progress underscores the critical role of induced mutations in improving crop nutritional quality and sustainability.

Mutant crop varieties and their impact on food security

Mutation breeding techniques, particularly those involving gamma radiation and other physical mutagens, have proven invaluable in generating a diverse array of mutants, resulting in significant genetic variability that is crucial for plant breeding and genetics research, as well as modern genomic studies. These mutants can be directly released as new varieties or serve as foundational resources in breeding programs aimed at creating further genetic variation. The mutant varieties that have been developed typically demonstrate enhanced yields, improved disease resistance, better quality traits, and greater resilience to environmental fluctuations. A substantial number of these cultivars have been successfully released in developing regions, significantly contributing to the economic advancement of these nations. Currently, these varieties are cultivated across hundreds of millions of hectares of agricultural land, and their impact on the national economies of these countries is substantial, often measured in the billions of dollars. This highlights the critical role that mutation breeding plays in not only improving agricultural productivity but also in fostering economic growth and sustainability in various regions.

The technique of mutation breeding has proven to be remarkably effective, leading to the successful release of approximately 3,000 mutant varieties derived from over 200 plant species, including staple crops like rice and wheat, as well as various fruits such as grapefruit and vegetables like lettuce. This achievement spans more than 73 countries worldwide, as reported by the Food and Agriculture Organization and the International Atomic Energy Agency (FAO/IAEA, 2018). Notably, more than

1,000 of these mutant varieties pertain to major food crops, covering millions of hectares of agricultural land. These advancements have made significant contributions to improving rural economies, enhancing nutrition, and promoting sustainable food security.

Despite these successes, food insecurity continues to rise globally, with an estimated 2 billion people—particularly in low- and middle-income countries—facing undernourishment. Concurrently, the impacts of climate change pose severe threats to the global food supply chain, leading to rapid losses in biodiversity essential for food and agriculture. The unpredictable climate conditions, characterized by extreme weather patterns and shifting environmental norms, are a primary factor hindering crop yields across the globe. Consequently, the ongoing development of new and improved crop varieties is essential for ensuring sustainable agricultural production. While natural mutations occur infrequently in crop plants, the application of induced mutation techniques is vital for generating the genetic diversity necessary for breeding programs aimed at developing desired traits. The extensive use of mutation breeding techniques has resulted in the official release of over 3,200 mutant varieties from more than 200 different plant species across more than 70 countries. In Pakistan specifically, the impact of mutation breeding is notable, with more than 59 varieties of various crop species—including wheat, rice, cotton, sugarcane, mungbean, lentil, sesame, castor bean, mandarin, rapeseed, mustard, chickpea, and groundnut—successfully released through both chemical and physical mutation breeding techniques. This advancement not only showcases the effectiveness of mutation breeding in addressing agricultural challenges but also highlights its potential to contribute significantly to food security and rural development in regions facing economic and nutritional hardships.

In China, just three mutant varieties have made a significant impact, covering over 30 million hectares and generating approximately \$4.9 billion in economic benefits, thus contributing to the enhancement of socio-economic conditions. India has also seen substantial advancements in the development of mutant varieties, resulting in considerable economic returns for farmers and the agricultural sector. Bangladesh has made notable progress with mutant rice varieties that can be harvested a month earlier than traditional options while yielding similar quantities with superior quality. This specific variety is cultivated in three crop rotations, and around 10,000 farmers grow it, accounting for nearly 80% of the rice-growing area in the country. In Indonesia, the economic gains from a single top rice variety have reached an estimated \$2 billion, benefitting countless farmers and millions of citizens who rely on these mutant varieties for their livelihoods. Meanwhile, in Peru, improved mutant varieties of barley and amaranth have provided farmers with approximately 7 million Andean, significantly enhancing their food security and economic standing. Vietnam has also benefited from the introduction of mutant varieties, particularly in rice and soybean. A leading rice mutant cultivar has generated \$3.3 billion in revenue, with

an impressive increase of \$537.6 million compared to older varieties. Soybean mutant varieties have similarly made a considerable impact, bringing in around \$3 billion and benefiting approximately 3.5 million farmers with a 30% boost to their economic returns. In Pakistan, the National Institute for Agriculture and Biology (NIAB) has developed 43 mutant varieties, demonstrating a notable economic impact with earnings of \$6 billion recorded in 2018. These examples illustrate the transformative potential of mutation breeding in enhancing agricultural productivity, economic stability, and the livelihoods of farmers across various countries.

Economic Impact of mutant varieties developed in India

Mutation breeding offers a promising avenue for inducing novel genetic changes in plants, thereby enhancing traditional plant breeding efforts aimed at developing superior crop varieties with desirable characteristics. A significant advancement in this field has been the introduction of two herbicide-tolerant basmati rice varieties in India, Pusa Basmati 1979 and Pusa Basmati 1985. These varieties are designed to effectively address weed challenges in rice fields, thereby reducing labor costs for farmers. Another remarkable outcome of mutation breeding in India is the linseed variety TL99, which features a low linolenic acid content, making it more suitable for edible oil production. The process of induced mutagenesis has also led to the creation of India's first summer cowpea variety, TC 901. Furthermore, the Trombay groundnut (TG) variety TAG 24 has been recognized as a national check variety within the All India Coordinated Research Project on Groundnut, boasting a genetic yield potential exceeding 7000 kg/ha under optimal agro-ecological conditions, including a summer environment, balanced nutrition, and controlled irrigation. According to breeder seed statistics, the production of superior mutant groundnut varieties in India from 1998 to 2008 generated 1022 metric tons valued at approximately \$1.18 million. Additionally, other TG varieties, such as TG 37A and TPG 41, have demonstrated impressive yield advantages, recording a 48-59% increase in productivity over local varieties in regions like Rajasthan and Maharashtra. These achievements underscore the significant contributions of mutation breeding to agricultural productivity and economic viability in crop production.

The early maturing mutant groundnut variety, TG 51, has shown exceptional performance in India's Kadapa district in Andhra Pradesh, yielding an impressive 7.8 tons per hectare (t/ha) compared to the national average of just 1.8 t/ha. This variety, along with others possessing high genetic yield potential and specific desirable traits, was disseminated to farmers through a robust network of partnerships. These partnerships involved the Indian Council of Agricultural Research (ICAR), State Agricultural Departments, State Agricultural Universities, National and State Seed Corporations, NGOs, National Institutes, Krishi Vigyan Kendras, seed companies, and progressive farmers. Through these collaborative efforts, large-scale breeder seed production programs were successfully initiated in India. Over the past decade (2010–2020), around 1000

metric tons of breeder seeds and approximately 6400 metric tons of certified seeds of these high-performing groundnut varieties were produced and distributed. This seed production program, valued at \$45.2 million, reached National and State Seed Corporations, State Agricultural Universities, seed companies, NGOs, and farmers alike. As a result of this large-scale distribution, these groundnut mutant varieties were introduced in major groundnut-growing states across India, including Gujarat, Andhra Pradesh, Maharashtra, Karnataka, Odisha, and Rajasthan. The adoption of these improved varieties enabled farmers to achieve significantly higher yields, with some harvesting up to seven metric tons per hectare. This success translated into considerable economic benefits, with farmers earning net profits as high as \$1,200 per hectare.

In the field of black gram cultivation, the mutant variety TAU 1, released in 1985, has gained immense popularity among farmers in Maharashtra. This variety has proven so successful that it now covers nearly 50% of the total area under black gram cultivation in the state, significantly improving productivity and farmer income (Souframanien 2018). In addition to TAU 1, another high-yielding black gram mutant variety, TU 40, is rapidly gaining traction, particularly in the southern states of India. This variety is well-regarded for its improved yield potential and adaptability, making it increasingly popular among farmers in these regions (Souframanien and Ganapathi 2021). In pigeon pea cultivation, the early-maturing mutant variety TJT 501 has been a game changer for farmers in Madhya Pradesh. This variety now occupies 60% of the cultivated pigeon pea area in the state, and its introduction has resulted in a remarkable transformation in both area and productivity. Over the last decade, the area under pigeon pea cultivation has nearly doubled, and the seed replacement rate has increased fivefold, from 10.48% to an impressive 48.11% (Souframanien 2020). This has not only boosted the state's overall agricultural productivity but has also had a significant impact on farmer livelihoods. In addition to black gram and pigeon pea, several high-performing mutant varieties of mungbean, including TMB 37, TM 96-2, and TM 2000-2, are being cultivated across large areas under rice fallows in states like Andhra Pradesh, Chhattisgarh, and West Bengal. These varieties have shown great adaptability to the conditions in these regions, contributing to enhanced crop productivity and more efficient land use. These mungbean mutants are highly valued for their early maturity, disease resistance, and higher yields, making them an ideal choice for farmers looking to maximize the utility of their agricultural land (Souframanien and Ganapathi 2021).

From 2014 to 2019, approximately 155 tons of breeder seeds of various mutant pulse varieties, including black gram, pigeon pea, and mungbean, were produced as part of large-scale seed production programs (Badigannavar et al. 2021). This breeder seed production has made a substantial impact on the overall pulse cultivation landscape in India. It is estimated that, based on seed production over the last five years, Trombay mutant pulse varieties now cover approximately 5% of the total area under pulse cultivation in the country, accounting for an area of around 1.5 million

hectares (Souframanien 2020). The widespread adoption of these varieties is a testament to their superior performance and the tangible benefits they bring to farmers in terms of increased yield and improved economic returns. The introduction of these mutant varieties across various regions of India has had a transformative effect on pulse farming, contributing to national food security, enhancing farmer incomes, and promoting sustainable agricultural practices. These achievements highlight the importance of mutation breeding in addressing the challenges of modern agriculture, particularly in the context of food security and the ever-present threat of climate change (Murty et al. 2007, Souframanien and Ganapathi 2021).

New breeding techniques (NBTs)

New Breeding Techniques (NBTs) represent a cutting-edge innovation in the realm of plant breeding, fundamentally transforming how genetic diversity is incorporated into crop plants. These advanced techniques rely on site-specific, targeted mutagenesis to achieve genetic alterations with greater accuracy and reduced off-target mutations compared to traditional breeding methods (Holme et al. 2019). Precision breeding, as it is often called, provides a refined approach to modifying plant genomes, enhancing efficiency, and accelerating the breeding process to achieve desired traits, such as higher yields, improved quality, and better resistance to environmental stresses. The seven core techniques under the NBT umbrella include Zinc Finger Nuclease (ZFN) technology, oligonucleotide-directed mutagenesis (ODM), cisgenesis and intragenesis, grafting on genetically modified (GM) rootstocks, RNA-dependent DNA methylation, agro-infiltration "sensu stricto," and reverse breeding. Of these, ZFN, a powerful tool within the category of site-directed nucleases (SDNs), is particularly effective in enabling precise, site-specific mutations within plant genomes. This opens up new avenues for breeders to introduce beneficial traits at specific locations in the DNA, avoiding the broader, random mutations characteristic of classical mutagenesis.

In addition to ZFN, other SDN tools, such as Transcription Activator-Like Effector Nucleases (TALEN) and CRISPR/Cas systems, have been introduced, with CRISPR/Cas rapidly becoming one of the most widely used genome editing technologies in plant science due to its versatility, efficiency, and precision (Holme et al. 2019). These techniques allow breeders to make precise, predetermined changes to the genetic makeup of plants, leading to more reliable and predictable results, as well as fewer unintended genetic alterations, which has long been a challenge with traditional mutation breeding techniques. The impact of these advancements has been far-reaching, prompting global institutions to harness their potential. In this regard, the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) launched the Plant Mutation Breeding Network (PMBN), aimed at enhancing collaboration and knowledge-sharing in the Asia Pacific region. This initiative builds on the success of previous efforts that have resulted in the development of over 2000 crop varieties in the region through mutation breeding. The PMBN seeks to further expand these efforts

by encouraging the use of mutagenesis to develop new crop varieties with enhanced yield, stability, quality traits, and resistance to diseases and climate change (Gil 2019).

This network provides immense benefits to both farmers and researchers by facilitating the development of improved crop varieties that are not only higher-yielding but also resilient to environmental stresses and better suited to the changing agricultural landscape. Moreover, these improved varieties are essential for enhancing food security, particularly in regions that are most vulnerable to climate change and resource limitations. One of the key advantages of NBTs over classical mutation breeding is their precision. Classical mutation techniques often lead to random genetic changes, requiring extensive screening to identify the desired mutations. In contrast, NBTs, especially when combined with tools like Targeting Induced Local Lesions in Genomes (TILLING), allow for more targeted mutations. This precision makes NBTs incredibly useful for achieving specific genetic alterations without the unintended side effects commonly seen with traditional methods. As a result, NBTs greatly expedite the breeding process, enabling scientists and breeders to develop new, improved crop varieties more efficiently (Jankowicz-Cieslak et al. 2017).

While the classical mutation breeding approach remains highly valuable, especially in terms of its ability to generate a wide range of genetic diversity, the inclusion of NBTs into breeding programs significantly enhances the efficiency and precision of the process. Traditional methods, although slower and more labor-intensive, can still play an important role in creating large populations of mutants for subsequent screening. By combining these approaches, plant breeders can take advantage of both the broad genetic diversity generated by traditional mutation breeding and the targeted precision offered by NBTs. Furthermore, the use of NBTs in plant breeding is increasingly being recognized as a key tool in addressing some of the most pressing challenges facing global agriculture today. As food insecurity continues to rise worldwide—impacting nearly 2 billion people, particularly in low- and middle-income countries—there is an urgent need for more resilient, high-yielding crop varieties that can thrive in changing environmental conditions. The unpredictable nature of climate change poses significant threats to global food production, including reduced crop yields, biodiversity loss, and disruptions to the food supply chain. In this context, the development of improved crop varieties through NBTs offers a critical solution to enhancing agricultural sustainability and food security.

Challenges of mutation breeding

Mutation breeding has emerged as a powerful tool for addressing the global challenge of ensuring sustainable food security, especially in the face of climate change, population growth, and dwindling natural resources. By inducing genetic variation through radiation or chemical treatments, mutation breeding allows scientists to develop new crop varieties with improved traits such as higher yields, disease resistance, and resilience to environmental stresses. However, despite its immense potential, the

technique faces several challenges that must be addressed to maximize its impact. From the complexities of identifying beneficial mutations to the hurdles of farmer adoption and public perception, mutation breeding is not without obstacles. Understanding these challenges is critical to unlocking its full potential in securing the global food supply.

- *Limited Genetic Diversity in Certain Crops:* Mutation breeding relies on inducing genetic changes in existing plant varieties, but if a crop species already has a limited genetic base, creating new, favorable traits can be difficult. This can slow down the development of improved varieties, especially in crops with narrow genetic diversity.
- *Unintended Genetic Mutations:* While mutation breeding is designed to generate beneficial traits, it can also result in unintended or off-target mutations. Some of these unintended changes might negatively affect plant growth, yield, or resistance to diseases, leading to a long and labor-intensive process of identifying and eliminating undesirable traits.
- *Time-Intensive Screening:* Identifying beneficial mutations from thousands or millions of induced genetic changes requires extensive field trials and screening, which can be time-consuming and costly. This challenge makes the process of developing new varieties slower compared to modern gene-editing technologies like CRISPR/Cas.
- *Environmental Variability:* One of the main goals of mutation breeding is to produce crops that are resilient to environmental stresses like drought, salinity, or extreme temperatures. However, the unpredictable nature of climate change adds complexity, as it is difficult to breed varieties that can withstand multiple environmental stresses simultaneously.
- *Adoption by Farmers:* Even when superior mutant varieties are developed, their success depends on the willingness of farmers to adopt them. Factors such as the cost of seeds, the perceived risk of new varieties, access to resources, and the suitability of the crops for local farming practices can all impact farmer adoption rates.
- *Regulatory and Public Perception:* Although mutation breeding does not involve genetic modification (GM) in the conventional sense, public perception of "mutated" crops can still pose a challenge. Some consumers may be wary of consuming crops developed through radiation or chemical mutagenesis, even though these techniques have been proven safe. Additionally, regulatory approval for new varieties can vary between countries, adding to the complexity of widespread adoption.
- *Resource Limitations in Developing Countries:* While mutation breeding has been successful in many regions, particularly in developing countries,

resource limitations such as insufficient funding, lack of access to advanced technologies, or inadequate infrastructure can hinder the full potential of mutation breeding programs. These challenges are particularly pronounced in rural areas where agricultural modernization is slow.

- **Integration with Modern Breeding Techniques:** Mutation breeding, when combined with modern biotechnological methods like molecular marker-assisted selection or genomic selection, can enhance efficiency. However, integrating these technologies requires investment in capacity building, training, and access to cutting-edge tools, which may be a challenge for resource-constrained countries.
- **Sustainability of Seed Systems:** Ensuring that improved mutant varieties are available to all farmers, including smallholders in remote areas, requires a robust seed production and distribution system. In many developing countries, the lack of such systems results in low seed replacement rates, undermining the widespread adoption of improved varieties.
- **Economic and Market Uncertainties:** Even with increased yields and improved traits, market dynamics such as fluctuating commodity prices, trade barriers, and agricultural subsidies can affect the economic viability of crops developed through mutation breeding. Farmers may hesitate to adopt new varieties if they are unsure about future market conditions or profitability.

Conclusions

Mutation breeding has significantly advanced crop improvement worldwide, leading to the release of thousands of mutant varieties that enhance agricultural traits such as yield potential, nutritional quality, and resistance to both biotic and abiotic stresses. These stresses, including pests, diseases, drought, and salinity, are critical challenges in global agriculture. Mutant varieties with desirable traits have been incorporated into breeding programs as key parental lines, playing a vital role in the development of new, high-performing cultivars. In addition to generating improved varieties, mutation breeding has enriched the genetic diversity available for crop improvement, offering valuable traits for breeders to tap into. This diversity is essential for addressing the specific agricultural needs of different regions and enhancing major crops and vegetables worldwide. Recent advancements in molecular marker-assisted selection, genome editing, and genomic selection have further improved the efficiency of mutation breeding. These tools, combined with speed breeding techniques, enable the rapid development of new crop varieties, allowing for quicker responses to emerging challenges. In the context of climate change and a growing global population, mutation breeding plays a crucial role in ensuring sustainable food security. It creates crops that are not only high-yielding but also resilient to environmental

stressors, addressing urgent needs for both productivity and sustainability in agriculture.

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