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# MUTAGENESIS IN AROMATIC RICE: CURRENT PROGRESS, CHALLENGES AND FUTURE PROSPECTS

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## Abstract

Aromatic rice, renowned for its unique aroma and higher market value, is of considerable significance in global rice production. Mutation has become a potent method for strengthening agronomic features, increasing stress tolerance, and expanding the genetic diversity of crop plants. This review highlights the advancements in mutagenesis research focused on aromatic rice, emphasizing successful implementations in enhancing yield, fragrance, grain quality, and resilience to problematic conditions. Advanced mutation techniques, including gamma irradiation, EMS treatment, and the incorporation of genomic tools, have significantly expedited the induction and characterization of mutants. Notwithstanding these gains, obstacles, including restricted genetic diversity, difficulties in trait stabilization, and inadequate high-throughput screening methodologies. Furthermore, the integration of mutagenesis with contemporary genomics, transcriptomics, and genome editing techniques is yet inadequately investigated in the enhancement of aromatic rice. This review also observes advanced opportunities, such as TILLING platforms and CRISPR-based reverse genetics, to utilize mutagenesis more efficiently and precisely. A thorough comprehension of induced genetic diversity and the functional validation of responsible genes will be vital for future breeding programs. Mutagenesis continues to be a potential approach for creating enhanced fragrant rice varieties to satisfy the requirements of quality-focused markets and sustainable agriculture.

**Keywords:** Mutation breeding, Random mutation, Targeted mutation, Grain aroma contents, Genetic variation

## Introduction

Rice is an indispensable dietary component for almost fifty percent of the global population (Fukagawa and Ziska, 2019). In addition to yield, grain quality attributes such as milling percentage, appearance, grain size, cooking quality, and fragrance influence consumer acceptance of a variety, thus affecting its adoption by farmers and marketability (Custodio *et al.*, 2019). Aroma is regarded as one of the most favored quality factors, following cooking quality, flavor, and elongation post-cooking (Verma and Srivastav, 2020). Over 300 volatile compounds regulate rice grain aroma contents, and identifying additional ones is crucial for improving aromatic rice (Wakte *et al.*, 2017).

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Esters, aldehydes, alcohols, ethylene, ketones, and furans are the major groups of volatile compounds significantly responsible for rice aroma, with 2-Acetyl-1-pyrroline (2-AP) being the key compound (Yi *et al.*, 2024).

Aromatic rice represents a limited yet distinctive category of rice cultivars and landraces regarded as superior quality (Mondal *et al.*, 2024). Aromatic rice is becoming more popular worldwide, including in Bangladesh, due to its unique fragrance, taste, and nutritional value. The demand is also increasing as more people choose healthier foods with lower glycemic indexes (Cabral *et al.*, 2024). Aromatic rice is recognized for its nutritional advantages, especially when eaten whole grain, providing essential nutrients like fiber, vitamins, and minerals (Carcea, 2021). Additionally, it contains unique biomolecules like 2-AP, which are associated with its antioxidant characteristics (Verma and Srivastav, 2020).

The rice growers in Bangladesh cultivate various aromatic rice landraces and varieties, including Chinigura, Kalijira, Kataribhog, and Badshabhog, which are integral to local cuisine and are experiencing increasing export demand. In October 2023, Bangladesh restricted rice exports, encompassing aromatic varieties, to safeguard domestic food security (The Daily Star, 2023). Prior to the prohibition, the nation exported more than 10,000 tonnes of aromatic rice each year to 136 nations, including the UAE, USA, and UK (Anonymous, 2023). Through governmental assistance and research aimed at enhancing yield, disease resistance, and aroma, Bangladesh may solidify its standing in the global aromatic rice market. Aromatic rice provides farmers with a more lucrative option compared to non-aromatic high yielding rice, enhancing agricultural development and the national economy (Ariff *et al.*, 2019).

Maximum aromatic rice exhibits inferior ideotype, lodging incidence, long duration, and increased susceptibility to biotic and abiotic stresses (Roy *et al.*, 2020). In the case of hybridization genetic incompatibility, complex genetic pathways of the major aroma producing gene *Badh2* make it difficult to transfer in a high-yielding elite cultivars (Singh *et al.*, 2000). Aroma is a complex quantitative trait that exhibits loss or dilution of the traits in hybrids and segregating generations (Lorieux *et al.*, 1996). Linkage drag, or the co-inheritance of undesirable agronomic traits, is a common challenge in aromatic rice breeding programs when crossed with high-yielding parents (Yi *et al.*, 2009). Pollen sterility is also a well-known issue in inter-subspecies crosses (e.g., indica × japonica hybrids), often resulting in a higher number of unfilled grains (Lorieux *et al.*, 1996). Thus, improving aromatic rice through conventional hybridization frequently proves to be a non-viable option.

Mutation breeding is a superior method for enhancing aromatic rice, since it facilitates both targeted and non-targeted mutagenesis, providing a versatile and effective strategy to increase specific traits while preserving the distinctive aromatic and grain quality attributes of elite varieties (Bado *et al.*, 2015). Non-targeted mutagenesis, using physical (e.g., gamma rays, ion beams) or chemical mutagens (e.g., EMS), induces random genetic variation across the genome, creating novel alleles for important traits such as early maturity, semi-dwarfism, stress tolerance, and disease resistance (Abdallah *et al.*, 2002).

Targeted mutagenesis, utilizing modern tools such as CRISPR/Cas9, base editors, or TALENs, facilitates precise editing of specific genes, including *Badh2* for aroma enhancement or restoration, as well as genes that are responsible for yield and stress responses (Ashokkumar *et al.*, 2020). This approach enables accelerated and more predictable trait development while minimizing linkage drag. In contrast to conventional hybridization, which frequently compromises the intricate inheritance of aroma and grain quality due to segregation and linkage drag (Lorieux *et al.*, 1996). Mutation breeding facilitates trait enhancement within the same genetic background, maintaining the identity and market value of fragrant rice. Combining mutation breeding with Marker-Assisted Selection (MAS) and high-throughput screening technologies speeds up the breeding cycle and improves selection accuracy (Mehta *et al.*, 2019). Moreover, mutation breeding is a non-transgenic approach, making it more socially acceptable and easier to deploy in regulatory environments with restrictions on genetically modified organisms. Therefore, this review comprehensively outlines mutagenesis in aromatic rice and provides guidelines to address relevant challenges that will facilitate future aromatic rice breeding programs.

## Historical milestones in rice mutagenesis

### Chemical mutagen

The successful journey of chemical mutagenesis in rice breeding began in India in 1972 with the development of IIT 48 through 0.3% Ethylene Oxide (EtO) and IIT 60 using 0.5% Ethyl Methanesulfonate (EMS) (Figure 1). The mutagenic line IIT 60 matured 30 days earlier than its parent variety, IR8, while maintaining the same yield potential. India later introduced Ethyl Iodide (EI) for developing the photosensitive variety Intan Mutant through 0.2% EI treatment for 4 hours. In 1999, Sodium Azide ($NaN_3$) was introduced, followed by a combination of 0.2% Diethyl Sulfate (DES) and Pyrazofurin (PYM) for 2 hours, leading to the development of Huayou 446.

Japan emerged as a leading country in employing chemical mutagens for rice breeding. They first used N-Nitroso-N-methylurea (NMU) to develop Moretsu in 1985 and applied N-Methyl-N-nitrosourea (MNU) treatment to fertilized egg cells to produce the low-amylose rice variety Chuukan-bohon Nou-13 in 1991. Additionally, Japan integrated somaclonal variation techniques with Anther culture to develop Sumi-takara (1991) and utilized protoplast culture techniques to create Yume-kaori in 1993. Vietnam introduced 0.02% N-nitroso-N-ethyl urea (NEU) for 18 hours to develop the deep-water rice variety NN 22-98 in 1983. Later, in 1987, Ethyl Nitrosourea (ENH) was used to develop the low-temperature-tolerant rice variety DB-2, and 0.02% Dimethyl Sulfate (DMS) was applied to hybrid seeds from the cross IR8 x X6.

In 1987, Russia introduced N-Methyl-N-nitrosourea (MNU) to develop a lodging-tolerant rice variety, Madjan, using a 0.01% MNU treatment for 18 hours (IAEA, 2025). The biological mutagen Datura (*Datura stramonium*) extract was initially utilized by Bangladesh in 2013 to create a lodging-tolerant aromatic rice variety, Binadhan-13. The seeds were immersed in Datura extract for 18 hours following exposure to 150 Gy of gamma radiation (IAEA, 2025).

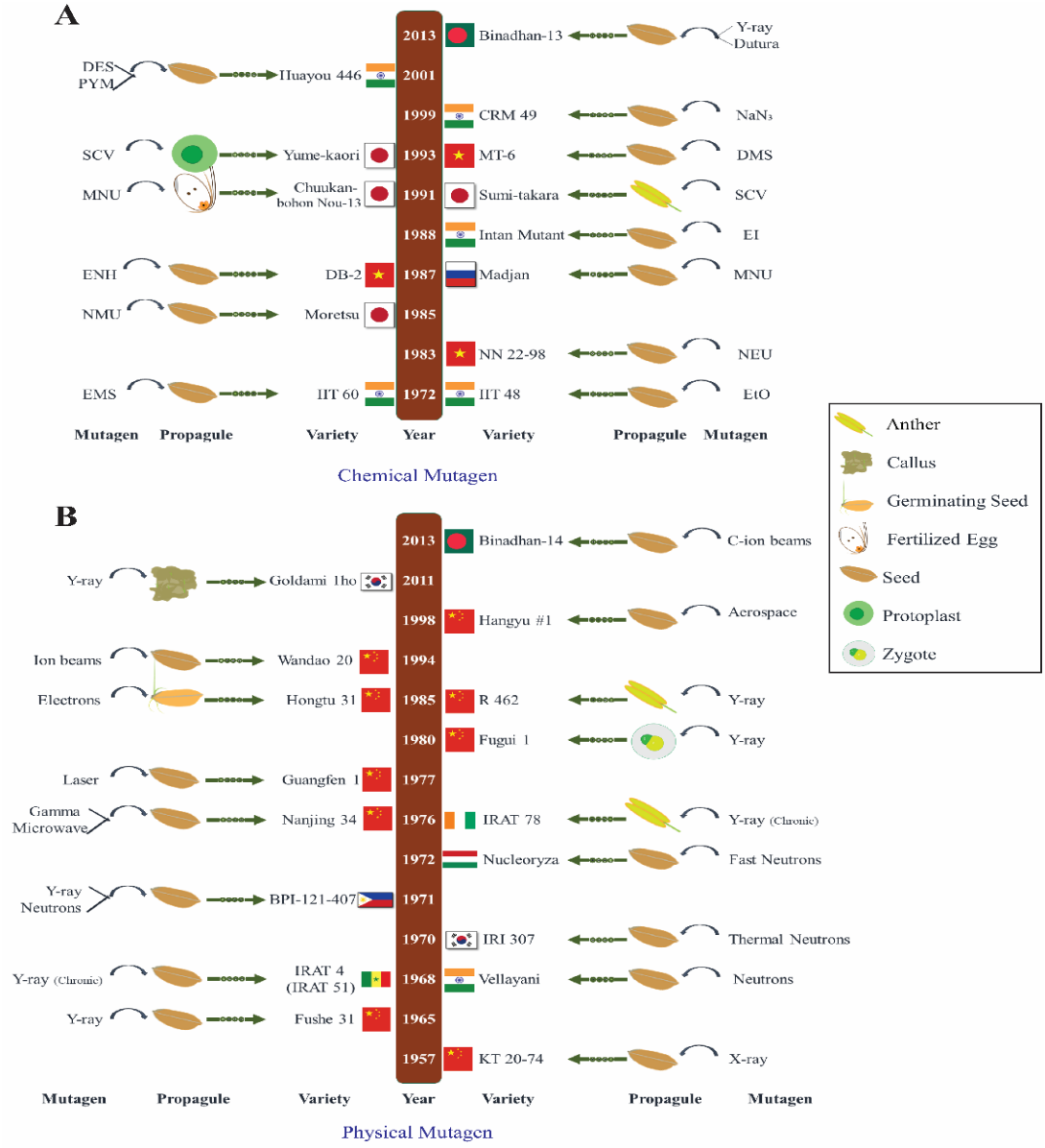


**Fig. 1. First reports of various chemical (A) and physical (B) mutagens used in rice breeding, including the country, target propagule, and registration year according to Mutant Variety Database 2025**.

(EtO: Ethylene Oxide; EMS: Ethyl Methanesulfonate; NEU: N-nitroso-N-ethyl urea; EI: Ethyl Iodide; NMU: N-Nitroso-N-methylurea; MNU: N-Methyl-N-nitrosourea; ENH: Ethyl Nitrosourea; EI: Ethyl Iodide; SCV: Somaclonal variation; DMS: Dimethyl Sulfate; $NaN_3$: Sodium Azide; DES: Diethyl Sulfate; PYM: Pyrazofurin).

**Physical mutagen**

L. Stadler began mutant breeding in 1928 by X-raying wheat, maize, and barley. In 1957, China sanctioned their initial mutant rice variety, KT 20-74, produced using X-ray irradiation, therefore asserting its dominance in mutant rice breeding and mutagenesis.

The most commonly used mutagen in rice mutation breeding program is gamma rays (γ-rays). Their use began in 1965 when China developed Fushe 31, an early-maturing dwarf rice variety for mountains regions. Senegal approved the first mutant rice variety in 1968 by utilizing chronic gamma irradiation, while India developed the Vellayani variety using neutron irradiation on rice seeds. South Korea utilized thermal neutrons in 1970, while the Philippines employed gamma rays and neutrons. In 1971, Hungary approved fast neutron-based blast-tolerant rice.

Beyond seed irradiation, Ivory Coast was the first to develop a mutant rice variety by irradiating anthers with chronic gamma rays in 1976.

China later used gamma rays to irradiate protoplasts in 1980 germinate seeds with electrons in 1985, and irradiate anther callus in South Korea in 2011. China developed Nanjing 34, a high-yielding semi-dwarf rice, in 1976 using microwaves and gamma rays. Laser treatment introduced lodging-tolerant Guanfen 1 the following year. China developed Wandao 20, a fungal disease-resistant rice, in 1994 using ion beams. Bangladesh developed Binadhan-14 in 2013 using C-ion beams. China pioneered aerospace radiation in 1998 by exposing rice seedlings to space conditions at 30-38 km altitude for eight hours to improve plant structure.

**Recent progress in rice mutagenesis**

About 876 mutant rice varieties have been developed worldwide through diverse mutation breeding approaches. According to the Mutant Variety Database, 2025, 34 countries have successfully released mutant rice varieties using various mutation breeding techniques. China leads global efforts with 296 registered mutant rice varieties, followed by Japan with 234. Meanwhile, Bangladesh ranks 11th, having released a total of 13 mutant varieties so far (Figure 2). The majority of mutant rice varieties have been developed in Asian countries, which dominate this field due to their ideal climate, fertile lands, and advanced cultivation practices, while several African and American nations have also made noteworthy contributions. The widespread adoption of mutation breeding highlights its significance in global rice improvement efforts.

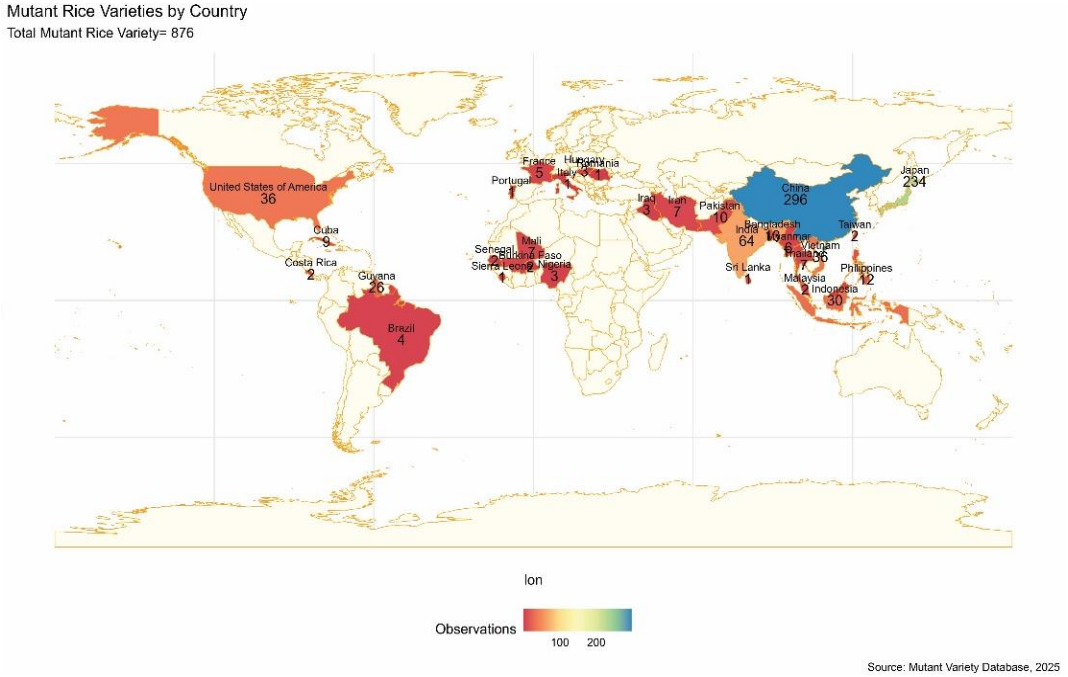


**Fig. 2. Distribution of rice mutant varieties by country.**

## Mutagenesis in aromatic rice

### Random physical mutation in aromatic rice

### Gamma rays

More than 92% of mutant rice varieties were developed through the gamma rays (Viana *et al.*, 2019) by the small (1-16 bp) to large (9.4-129.7 kb) deletion of DNA fragments or frameshift mutation (Morita *et al.*, 2009). Gamma-ray is electromagnetic energy emitted by the radioactive decay of U, Th, and Ac and from naturally occurring K-40 and C-14 and different artificial gamma emitted from Co-60, Cs-137, and Am241 were used in plant breeding (Pathirana, 2021). Aromatic rice varieties developed through gamma-ray treatment exhibit higher levels of 2-acetyl-1-pyrroline (2AP) (Sansenya *et al.*, 2017). One such variety, 'Rojolele,' is an Indonesian aromatic rice developed through mutation breeding. The initial seeds were first subjected to gamma irradiation and subsequently soaked in sodium azide (Dwiningsih and Alkahtani, 2022).

Roy *et al.*, 2018 reported the development of lodging tolerant, photo-insensitive, semi-dwarf with high yield potential and retained the aroma by gamma irradiation from the local landrace Tulpanji. Sarshar and Khushboo-95 are two popular aromatic rice varieties of Pakistan developed through 150 and 200 Gy gamma irradiation (Bughio *et al.*, 2007). Bordoloi *et al.*, 2023 reported an improvement of morpho-agronomic traits of the popular Joha rice cultivar Kon Joha by 100-400 Gy of gamma rays from a Co-60 source.

**Fast neutrons**

Fast neutrons, which emit ionizing radiation in the range of 1-20 MeV, can break DNA strands, leading to genetic variability and alterations in various traits (Ali *et al.*, 2024). Neutron irradiation led to small changes in DNA, changes in chromosomes, and mixing of genes, resulting in beneficial features like better crop yield, resistance to diseases, and improved aroma. Shua-92 is an aromatic rice variety developed through 15 Gy gamma irradiation of IR8 seeds. This variety was created in Pakistan in 1993 (Bughio *et al.*, 2007). Similarly, the Thai aromatic rice variety 'Khao Dawk Mali 105' was developed by irradiating its parent varieties with fast neutrons (Wangsomnuk *et al.*, 2009).

**Ion beam**

Different types of ion beams were used in plant mutation breeding such as Ionised nuclei, e.g. $^{12}C$ 6+, $^{14}N$ 7+, $^{56}Fe24+$, $^{34}Ar18+$, etc (Pathirana, 2021). Have very high energy to break the DNA molecule and can generate energy up to 100 – 320 MeV (Pathirana *et al.*, 2021). Using Argon ion beam irradiation on the Japanese aromatic mutant variety Pare Bau, researchers developed mutants that exhibited a higher number of filled grains and shorter plant height (Okasa *et al.*, 2020).

**Random chemical mutation in aromatic rice**

**Ethylmethanesulfonate (EMS)**

Alkylation of G-bases leading to pairs with thymine (T) rather than cytosine (C) results from G/C to A/T transitions (Talebi *et al.*, 2012). EMS is an alkylating agent that causes point mutations with concentrations of 0.2% to 2.0% and soaks the plant materials for 10 to 20 h (Viana *et al.*, 2019). EMS mutation is more effective than the physical mutagens (Khan *et al.*, 2007). It can easily modify nucleotides, which results in various missense, nonsense, and silent point mutations (McCallum *et al.*, 2000). Pakistan developed the aromatic rice variety Shadab by applying 0.5% ethyl methane sulphonate (EMS 0.5%) from IR6 line (Bughio *et al.*, 2007). EMS treatment was also reported effective for creating variability in Basmati (Super basmati and Basmati 370) rice (Wattoo, 2012).

**N-methyl-N-nitrosourea (MNU)**

MNU is the most used mutagen in rice, inducing mutations by alkylating guanine and cytosine. O6-alkylguanine formation promotes G/C to A/T transitions, influenced by DNA sequence and structure (Satoh *et al.*, 2010). Kakar *et al.*, (2019) reported a change in antioxidant and phenolic compounds in rice by MNU treatment.

**Sodium azide ($NaN_3$)**

Sodium azide produces a metabolite L-azido alanine that may penetrate to nucleus and create point mutations in the genome, causing G/C to A/T transitions with a mutation rate of 1.4 to 2.9 mutations/kb (Gruszka *et al.*, 2012). ) Herwibawa and Kusmiyati., 2017 have reported genetic variations caused by sodium azide in the Indonesian popular aromatic rice variety Inpago Unsoed1.

**Targeted mutagenesis in aromatic rice**

Targeted mutation is a technique that precisely induces specific genetic changes at a defined location in the genome without introducing foreign DNA. Its origins in plants trace back to the 1970s with genetic engineering, which involved randomly introducing gene sequences via homologous recombination (HR) to knock out specific genes. The discovery of mega nucleases in the 1980s improved targeted genome engineering, rapidly establishing genome editing technologies as powerful tools for manipulation (Chakraborty *et al.*, 2017). To create gene knockout mutants through insertion/deletion or gene replacement, sequence-specific nucleases like zinc-finger nucleases (ZFNs), TALENs, and CRISPR-associated proteins (Cas9 and Cas12) can be utilized.

**Zinc finger nucleases (ZFNs)**

The zinc finger-based DNA-binding domain is designed to selectively attach to specific target DNA sequences, while the FokI domain is responsible for cleaving the DNA at the designated site (Osakabe and Osakabe, 2015).

Customizing the zinc finger DNA-binding domain lets ZFNs target any genomic sequence. In rice, engineered ZFNs have successfully targeted the SSIVa locus, inducing mutations that influence plant height, grain filling, and starch content (Jung *et al.*, 2018). This result suggests the potential use of ZFNs in aromatic rice by introducing semi-dwarfism and reducing starch content.

**Transcription activator-like effector nucleases (TALENs)**

Phytopathogenic bacteria *Xanthomonas* naturally produce transcription activator-like effector (TALE) proteins (Boch *et al.*, 2009). TALE's DNA-binding domain is made of monomers of repeating 34 amino acids that bind unique base pairs. TALEs' DNA-binding domain (DBD) is fused with the FokI restriction enzyme to create TALENs, like ZFNs (Joung and Sander, 2013). TALEs' repeat sequences allow precision targeting of single genomic locations, making them easier to design than ZFNs.

Plant genome editing with TALENs is common. TALENs mutated the pathogen TAL effector binding site in the OsSWEET14 promoter, which promotes pathogen survival and virulence in rice, thus impeding gene transcription and reduced pathogen pathogenicity (Li *et al.*, 2012). TALEs also construct artificial transcriptional regulators for gene regulation by binding to gene activators and receptors. These results show that TALENs can improve aromatic rice's disease resistance, addressing significant production constraints.

**CRISPR/Cas system**

In recent years, genome editing has emerged as a powerful tool for rapidly and efficiently generating beneficial genetic variations, offering new opportunities for crop improvement (Eshed and Lippman, 2019). In this context, leveraging CRISPR/Cas9 to

introduce mutations that confer aroma in high-yielding elite genotypes offers a highly efficient strategy. This approach significantly reduces the time required to develop desired genotypes while also mitigating the challenges posed by linkage drag, which often complicates the retention of superior traits in traditional breeding methods (Roldan *et al.*, 2017). Recent advancements in the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats-Associated Protein 9) system now allow precise gene modifications, including gene knockouts, knock in, base editing, and fine-tuning of gene expression (Huang *et al.*, 2022).

In rice, many target traits can be precisely edited using genome editing techniques (Zeng *et al.*, 2019). Researchers have used CRISPR/Cas9 to create new alleles of *BADH2* and *Wx*, leading to the development of aromatic and glutinous rice varieties (Ashokkumar *et al.*, 2020). Additionally, genome editing has been applied to modify multiple susceptibility genes, providing broad-spectrum resistance against blight disease in rice (Hutin *et al.*, 2015). Subsequently, CRISPR/Cas9 has been used to improve key quantitative and qualitative traits in rice. This includes editing *OsSAPK2* for drought tolerance (Lou *et al.*, 2024), *OsGIF1* for stem, leaf, and grain size (He *et al.*, 2017), *OsMGD2* for grain quality (Basnet *et al.*, 2019), and *OsBADH2* to enhance aroma in rice (Shao *et al.*, 2017). Based on these studies, CRISPR/Cas9 can accelerate aromatic rice development. It allows precise *OsBADH2* alterations that increase rice aroma content. The simplicity, precision, and efficiency of CRISPR/Cas9 make it a popular technique for targeted mutagenesis across species.

**Success in aromatic rice mutation breeding**

In aromatic rice breeding, the most successful approach involves improving specific traits through irradiation and hybridizing the resulting mutant with a high-yielding parent. According to the mutant variety database, a total of 26 aromatic mutant rice varieties have been registered across nine different countries (Table 1). Japan initiated this journey in 1992 by developing the first aromatic mutant variety, Hagi-no-kaori, through the hybridization of one mutant previously developed from the Variety Reimei with 200 Gy gamma irradiation. To date, Japan has registered eight aromatic mutant rice varieties, all of which have been developed by crossing two mutants or a mutant with a non-mutant genotype. Bangladesh, India, and the United States have each registered three aromatic mutant rice varieties. The Indian varieties ADT-41, Pusa-NR-546, and TCDM-1 were developed through direct mutation using gamma rays to enhance semi-dwarf traits and increase yield. Notably, Pusa-NR-546 also exhibits resistance to various fungal diseases and insect pests (Table 1). Indonesia, Iran, Pakistan, and Thailand have each registered two aromatic mutant rice varieties, all developed using gamma rays, except for HOM Rangsi from Thailand, which was developed using fast neutrons. Vietnam has registered one variety, VN124, which is of export quality and exhibits tolerance to brown planthopper, blast, and grain smut virus (Table 1).

**Table 1. List of the aromatic mutant rice varieties developed by different countries with their key features**

| Variety | Country | Year | Mutagenesis approach | Characteristics |
|---|---|---|---|---|
| Binadhan-9 | Bangladesh | 2012 | Crossing with one mutant | High-yielding, aromatic rice variety |
| Binadhan-13 | Bangladesh | 2013 | Gamma rays with Dutura Extract | Aromatic, high-yielding, with leaves that remain green until maturity, and moderately resistant to lodging |
| Binadhan-18 | Bangladesh | 2015 | Carbon ion beams | High-yielding (7.25 t/ha) and aromatic rice variety |
| ADT-41 | India | 1994 | Gamma rays | Semi-dwarf variety with long grains, a mild aroma, and a higher yield |
| Pusa-NR-546 | India | 2003 | Gamma rays | High-yielding, semi-dwarf with super fine grain quality, tolerance to brown spot, leaf blast, sheath blight, bacterial leaf blight (BLB), stem borer (SB), white-backed planthopper (WBPH), and gall midge (GM) |
| Trombay Chhattisgarh Dubraj Mutant-1 (TCDM-1) | India | 2019 | Gamma rays | Aromatic, dwarf stature, and early maturity |
| Sinar 1 | Indonesia | 2020 | Gamma rays | High-yielding, aromatic rice variety with a higher aroma level than its parent and moderate resistance to brown planthopper strain |
| Sinar 2 | Indonesia | 2020 | Gamma rays | Resistant to BLB diseases, high yield, aromatic rice (higher aroma level than the parent) |
| Roshan | Iran | 2019 | Gamma rays | High-yielding aromatic variety tolerant to stem borer and blast disease |
| Shahriar | Iran | 2019 | Gamma rays | Short stature, early mature, high yield (8-8.6 $th^{-1}$), aromatic, tolerant to blast disease |
| Akigumo | Japan | 2004 | Crossing of two mutants | Low amylose content and aromatic |
| Benika | Japan | 2004 | Crossing with one mutant | Colored grain rice with aromatic qualities |
| Benisarasa | Japan | 2004 | Crossing with one mutant | Colored grain rice with aromatic qualities |
| Chiho-no-kaori | Japan | 2002 | Crossing of two mutants | High yield, good quality with aroma; strong resistance to leaf blast and panicle blast, and medium maturity |

**Table 1. Continued**

| Variety | Country | Year | Mutagenesis approach | Characteristics |
|---|---|---|---|---|
| Hagi-no-kaori | Japan | 1992 | Crossing of two mutants | Aromatic taste |
| Natsugumo | Japan | 2004 | Crossing of two mutants | Low amylose content and aromatic |
| Shi-hou | Japan | 2004 | Crossing of two mutants | Colored grain rice with aromatic qualities |
| Silky Pearl | Japan | 2004 | Crossing of two mutants | Aromatic, short culm, low amylose content, with superior lodging resistance and high yield, along with medium resistance to leaf and ear blast |
| Khushboo 95 | Pakistan | 1996 | Gamma rays | Reduced plant height, highly productive tillers, early maturity, increased panicle length, more grains per panicle, and good grain quality with a distinct aroma |
| Mehak | Pakistan | 2006 | Gamma rays | High yield, good quality, and a strong aroma |
| Hawm Thammasart | Thailand | 2017 | Gamma rays | Photo-period insensitive with good grain quality and aroma. |
| Hom Rangsi | Thailand | 2019 | Fast neutron | Shorter plant type with photoperiod insensitivity, excellent seed quality, aroma, and high yield |
| A-201 | United States | 1997 | Crossing of two mutants | Aromatic rice with semidwarf characteristics and long grain size |
| Aromatic SE | United States | 2004 | Crossing of two mutants | Aromatic, semidwarf, early maturing, with good quality |
| Dellmont | United States | 1993 | Crossing with one mutant | An early-maturing, aromatic, semidwarf variety with long grain size |
| VN124 | Vietnam | 2008 | Crossing with one mutant | A short-duration, aromatic variety with good quality for export, and tolerant to brown planthopper (BPH), blast (BL), and grain smut virus (GSV) |

Source: Mutant Variety Database, 2025

## Challenges in mutagenesis in aromatic rice

The intricate and delicate characteristics of aroma and grain quality present numerous challenges for mutant breeding in fragrant rice. The primary problem is preserving aroma quality, predominantly regulated by the badh2 gene, as random mutations may disrupt this or other volatile biosynthetic pathways, leading to a loss or reduction of aroma. Pleiotropy

and linkage drag significantly impact customer acceptance by unintentionally altering several agronomic or quality traits, such as grain morphology, amylose concentration, and culinary performance. The infrequent occurrence of desired mutations necessitates the screening of extensive populations, a process that is time-consuming, labor-intensive, and resource-intensive. Although advanced methods like as GC-MS are costly and require specialist apparatus, conventional scent screening procedures like sensory evaluation and KOH assays are low-throughput and often subjective. Moreover, it is challenging to maintain grain quality while including stress tolerance or yield-enhancing traits due to genetic trade-offs. The approach is further complicated by limited availability to contemporary genotyping technologies, a deficiency of high-throughput molecular markers for small effect QTLs, and insufficient marker-assisted selection methodologies. In mutants, background genome recovery may be insufficient, and in the absence of precise backcrossing and marker-assisted selection, deleterious mutations may persist. In the absence of speed breeding, the slow advancement of generations prolongs the breeding cycle to almost ten years. Farmers may hesitate to transition from traditional varieties to promising mutant lines due to perceived alterations in fragrance or grain quality, unless unambiguous advantages are shown.

**Enhancing mutation breeding strategies for aromatic rice improvement**

A proposed future strategy for mutation breeding in aromatic rice should adopt a holistic and integrated approach combining mutagenesis, phenotypic selection, molecular screening, and advanced evaluation protocols. The strategy begins with a radiosensitivity test to determine the optimal dose of physical or chemical mutagens, followed by mutagenesis of $M_0$ seeds to induce broad-spectrum genetic variability (Figure 3). $M_1$ plants are grown under close spacing, and a single panicle per plant is harvested to ensure genetic integrity. In the $M_2$ generation, phenotypic selection should be employed for key traits such as semi-dwarfism, early maturity, grain type, and tolerance to biotic and abiotic stresses, along with initial aroma screening through sensory and biochemical tests. In subsequent generations ($M_3$ to $M_4$), marker-assisted selection (MAS) using PCR-based or high-throughput genotyping platforms should be applied to select stable lines for essential traits-such as the *badh2* gene for aroma, *Pi* genes (*Pi-1a*, *Pi-b*, *Pi-kh*) for blast resistance, *Xa* genes (*Xa4*, *Xa21*) for bacterial blight resistance, and the *Wx* gene for grain quality. The selected lines should then be evaluated in replicated yield trials ($M_5$ to $M_7$) to assess agronomic performance, aroma retention, and quality traits. Promising genotypes should undergo multilocation yield trials from $M_8$ to $M_{10}$ generations to validate performance across diverse environments, followed by official varietal registration based on performance and farmer preferences.

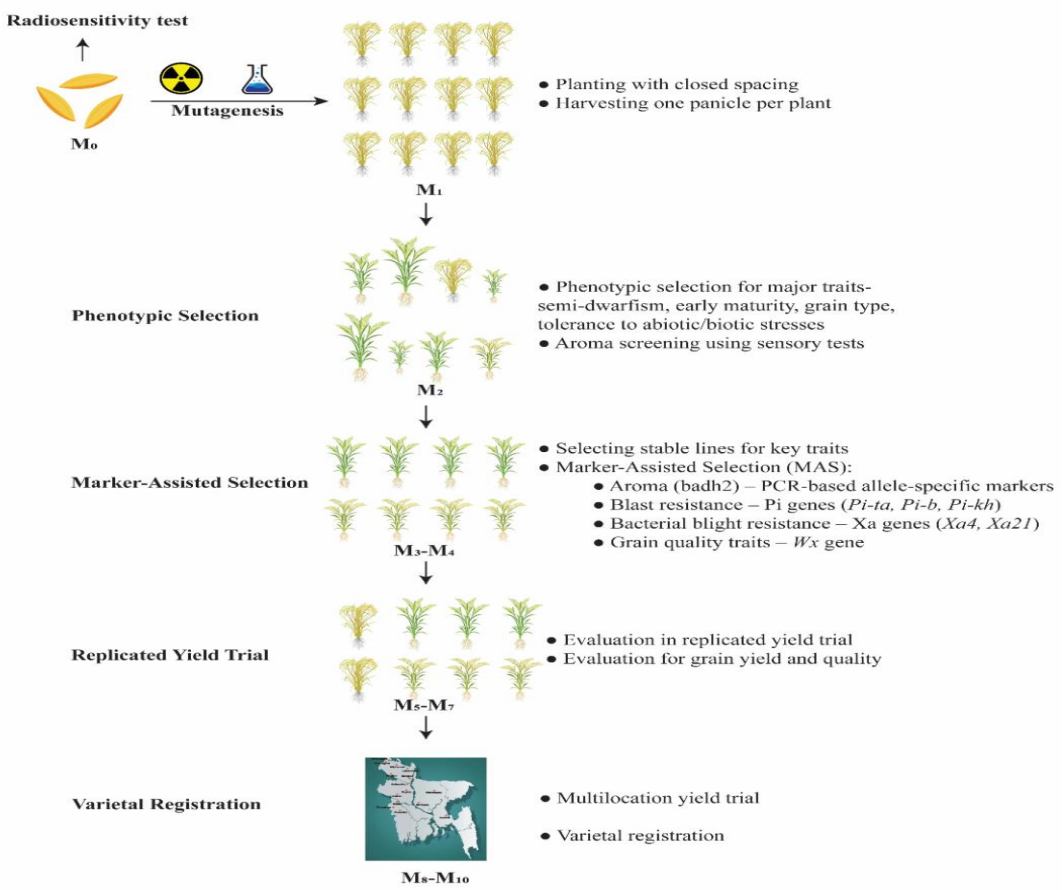


**Fig. 3. Mutation breeding scheme for aromatic rice.**

## Future prospects and conclusions

The future scope of mutation breeding in aromatic rice is highly promising, with cutting- edge technologies offering new opportunities to overcome existing limitations and accelerate varietal development. Traditional non-targeted mutation breeding, using physical (e.g., gamma rays, ion beams) and chemical mutagens (e.g., EMS), will continue to play a significant role in creating novel genetic variability, particularly for traits like early maturity, semi-dwarfism, stress tolerance, and improved grain yield, while efforts will increasingly focus on integrating marker-assisted selection (MAS), high-throughput genotyping, and genomic selection (GS) to streamline the selection of beneficial mutants. Enhanced screening platforms such as metabolomics, aroma phenotyping using gas chromatography-electronic nose systems, and digital phenotyping tools will help in rapid identification of high-aroma content lines with superior grain quality. Development of mutant libraries and TILLING platforms will enable reverse genetic approaches to discover new alleles controlling aroma and other agronomic traits.

In contrast, targeted mutation breeding using gene-editing tools like CRISPR/Cas9, TALENs, and base editors opens a new frontier, allowing precise modifications of specific genes without disturbing the desirable aromatic background. For instance, CRISPR can be employed to knockout repressors or modify regulatory regions influencing aroma intensity, enhance abiotic stress tolerance by editing stress-responsive transcription factors, or fine-tune starch biosynthesis genes to optimize grain quality traits such as amylose content and gelatinization temperature. Gene editing can also be used to convert non-aromatic elite varieties into aromatic types by precisely disrupting the Os*badh2* gene, enabling the rapid development of high-yielding aromatic rice lines with minimal genetic drag. With the advancement of speed breeding, controlled environment agriculture, and artificial intelligence (AI)-driven trait prediction models, the time required for developing improved aromatic rice cultivars can be significantly reduced. Overall, the synergy between conventional mutation breeding and modern genome-editing technologies will redefine the future of aromatic rice improvement. Thus, enabling breeders to develop climate-resilient, high-aroma, high-quality rice varieties that meet both market preferences and food security goals.

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