UMT PRESS

Journal of Sustainability Science and Management
Journal Homepage: https://jssm.umt.edu.my
eISSN: 2672-7226
DOI: http://doi.org/10.46754/jssm.2026.04.010

AGRONOMIC PERFORMANCE AND GENETIC ANALYSES OF RESISTANCE TO *Xanthomonas oryzae* pv. *oryzae* IN RICE MUTANT LINES (*Oryza sativa* L.)

ALIIF IHSAAN AKMAL SHUKRI[1], NOR AISHAH HASAN[1]*, KOGEETHAVANI RAMACHANDRAN[2], FAIZ AHMAD[3], SOBRI HUSSEIN[3], AFFRIDA ABU HASAN[3], ABDUL RAHIM HARUN[4] AND SHAMSIAH ABDULLAH [5]

[1]Faculty of Applied Sciences, Universiti Teknologi MARA, Negeri Sembilan Branch, Kuala Pilah Campus, 72000 Kuala Pilah, Negeri Sembilan, Malaysia. [2]Laboratory of Plant Pathology, Rice and Industrial Crops Research Centre, MARDI Seberang Perai, P.O. Box 203, Kepala Batas, 13200 Penang, Malaysia. [3]Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, 43400 Kajang, Selangor, Malaysia. [4]Pertama Padi (Malaysia) Sdn. Bhd., Kampung Padang, Mukim Tajar, 06500 Langgar, Kedah, Malaysia. [5]Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Melaka Branch, Jasin Campus, 77300 Merlimau, Melaka, Malaysia.

**Corresponding author: aishahnh@uitm.edu.my*

ARTICLE INFO

Article History:
Received: 9 September 2024
Revised: 21 August 2025
Accepted: 24 August 2025
Published: 15 April 2026

Keywords:
Bacterial leaf blight, gamma irradiation, MR297, sustainability, Xanthomonas oryzae pv oryzae.

ABSTRACT

Rice plays a crucial role in global food security, yet it is highly vulnerable to diseases, particularly bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Sustainable rice production, such as mutagenesis, can help in the development of new rice varieties with imrpoved agronomic performances. Consequently, this investigation was designed to develop potential rice mutant lines resistant to *Xoo* from the MR297 rice variety using gamma radiation. In the preliminary stage (radiosensitivity test), MR297 seeds achieved a 50% lethality rate (LD50) or the highest mutation frequency at 382.245Gy. The M_1 generation was established using 10,000 irradiated seeds at a dose of 384.245 Gy. In the subsequent M_2 and M_3 generations, the derived lines demonstrated superior yield and agronomic traits compared with the parental cultivar MR297. Disease screening against the *Xoo* pathotype indicated that M_2 and M_3 plants exhibited resistance scores ranging from 1 to 3. Genotypic evaluation of 30 selected M_3 individuals showed that all carried the recessive *Xa*5 allele, while seven plants possessed a gene combination of the dominant *Xa*21 and recessive *Xa*5. These seven lines were classified within Cluster II of the UPGMA dendrogram at a similarity coefficient of 0.78. Overall, the results highlight these lines as promising breeding resources for developing high-yielding rice varieties with durable resistance to BLB.

Introduction

Rice (*Oryza sativa* L.) serves as the primary staple crop globally, contributing over 20% of the daily caloric intake for the human population. In 2018, worldwide rice production was reported at approximately 728 million tonnes (IRRI, 2019). Demand for rice continues to rise steadily, driven largely by population expansion and economic growth, particularly in Asian and African countries (The Food and Agriculture Organisation of the United Nations [FAO], 2019).

In Malaysia, annual rice production has ranged between 1.6 and 1.8 million tonnes over the past 10 years (MAFI, 2021). As of 2020, Malaysia achieved only 73.4% of its self-sufficiency level (SSL), necessitating the import of around 26.6% of rice to meet local demand (MAFI, 2021). Since the early 2000s, multiple high-yielding varieties have been developed by the Malaysian Agricultural Development & Research Institute (MARDI), including MR84,

MR219, MR284, MR297, MR303, and MR307 (Ramli, 2019; Shukri *et al.*, 2021). Unfortunately, the production of all Malaysian rice varieties has been declining due to a common limitation: susceptibility to the alarming bacterial leaf blight (BLB) disease (Zakaria & Misman, 2018).

Rice productivity is severely challenged by BLB, a destructive disease caused by *Xanthomonas oryzae*pv. *oryzae* (*Xoo*). Infection by this pathogen leads to considerable yield reduction and stunted growth as a result of severe biochemical, molecular, and physiological alterations in the host plant (Doucoure *et al*., 2018). In Malaysia, BLB outbreaks have affected approximately 12,080 hectares of rice fields across Peninsular Malaysia. The most severe cases were documented in Selangor (5,945 ha), followed by Kedah (4,415 ha), Pulau Pinang (620 ha), Terengganu (440 ha), Negeri Sembilan (291 ha), Perak (174 ha), Perlis (141 ha), Pahang (46 ha), Johor (5 ha), Kelantan (1 ha), and Melaka (<1 ha) (DOA, 2019). Such yield losses have significantly impacted the livelihoods of farming communities, particularly those who rely heavily on rice cultivation as their primary source of income.

Therefore, various techniques were implemented to minimise the risk of BLB disease, including the application of chemical and biological controls. Unfortunately, these strategies proved to be less effective in terms of time and cost, as well as low practicality for application. Utilising resistant varieties remains the most sustainable and economical measure to suppress the bacterial pathogen *Xoo* and mitigate BLB (Viana *et al*., 2019). With the rapid pace of technological advancement, conventional breeding is no longer a viable option. After numerous developments using the same breeding technique, the yield rate continued to decline, along with the resistance rate over the years, necessitating the release of a new variety (Niones *et al*., 2022). Several technology-friendly breeding techniques, such as mutagenesis, have emerged as the new methods applied by most researchers and breeders (Spencer-Lopes *et al*., 2018).

Through mutagenesis, random alterations occur in the genetic makeup of an organism, leading to the emergence of new characteristics that are transmitted to progeny, thereby promoting evolutionary processes (Kumar *et al*., 2020). As stated by Spencer-Lopes *et al*. (2018), mutagenesis has a significant impact on plant growth and development by inducing various changes in cells and tissues, including biochemical, cytological, genetic, and physiological alterations. Despite the extensive alterations in DNA, mutagenesis or mutation breeding in rice is exempt from genetically modified organism (GMO) techniques, as it has a long safety record and beneficial alterations to agronomic quality for human benefits (Sprink *et al*., 2016). In addition, gamma irradiation mimics natural mutation processes, albeit at an accelerated rate (Niones *et al*., 2022). Oladosu *et al.* (2016) also noted that mutagenesis induces useful mutations by enhancing the probability of natural variations, often avoiding the controversies associated with GMOs.

Since 2004, gamma cells have ranked first as the most used emitters for mutation breeding globally. Cobalt-60, the source of gamma irradiation, has been widely used for its higher penetration power, greater dose of irradiation, and faster time to irradiate samples (Lee & Kim, 2018). Many past and current studies have documented the development of new rice varieties with agronomic improvements, including Aljumaili *et al*. (2021) and Acharya *et al*. (2018). Despite this, few investigations have reported the application of gamma irradiation in rice breeding projects to either increase agronomic performance or combat disease. Ocloo *et al*. (2017) applied gamma irradiation to enhance the agronomic traits of rice cultivars in Ghana by observing the physicochemical, functional, and pasting properties. Meanwhile, in Southeast Asia, Alamsyah *et al*. (2017) was part of many studies that focused on the use of gamma irradiation concerning seed viability and rice plant growth against various diseases. In terms of developing BLB-resistant varieties through gamma irradiation, several mutants

have been released or commercialised, including Pandan Putri (Indonesia), BT62.1 (Vietnam), and Pusa-NR-456 (India) (FAO/IAEA Mutant Variety Database, 2023). These varieties share common characteristics, including moderate to high resistance to BLB disease, high yield rates, and semi-dwarf stature (FAO/IAEA Mutant Variety Database, 2023).

Artificial inoculation is considered an effective and preferred approach for screening resistant rice varieties, as it allows disease symptoms to appear more rapidly (Ramalingam *et al*., 2020). Nevertheless, the durability of resistance is often short-lived, with some cultivars losing resistance within only a few years. This makes continuous resistance breeding essential, particularly for exploring genetic diversity in germplasm collections to identify useful resistance genes. Advances in DNA marker technologies have greatly enhanced the detection of BLB resistance genes (*Xa* genes) in rice (Viana *et al*., 2019). By 2021, a total of 47 *Xa* genes had been identified, comprising 29 dominant, 14 recessive, and 4 novel genes conferring resistance to BLB (Chukwu *et al*., 2019; Chen *et al*., 2020; Xing *et al*., 2021). With the aid of molecular markers, many commercial cultivars have been improved by incorporating race-specific resistance genes such as *Xa*4, *Xa*5, *Xa*7, *Xa*13, and *Xa*21, providing them with enhanced defence against *Xoo* (Jiang *et al*., 2020; Pradhan *et al*., 2020). Among the available markers, Simple Sequence Repeats (SSRs) are frequently used because of their high polymorphism, reproducibility, and cost-effectiveness (Nadeem *et al*., 2017). Furthermore, modern molecular breeding tools—including marker-assisted selection, QTL mapping, and genetic transformation—are offering new opportunities for the enhancement of rice breeding programmes aimed at producing BLB-resistant cultivars.

Malaysia was on the right track with the release of some high-yield varieties developed through gamma irradiation, including the Tongkat Ali rice variety and the IS21 rice variety (Sobri *et al*., 2020). However, the country was still lacking in the exploration of the gamma irradiation technique to develop a mutant rice variety with strong resistance against the threatening BLB disease. As mentioned earlier, local production was severely impacted as all Malaysian rice varieties were susceptible to BLB disease caused by *Xanthomonas oryzae*pv. *oryzae*. Despite this limitation, MR297 was widely adopted by farmers due to its high yield potential (8.6-9.5 t/ha) and strong resistance against other diseases and pests, including blast, sheath blight, and brown planthopper (MARDI, 2016; Zakaria & Misman, 2018). Furthermore, a preliminary screening of all the aforementioned varieties revealed that MR297 was identified as a superior cultivar, excelling in eight of the twelve evaluated traits, including yield potential, flowering time, maturity period, plant stature, tiller number, panicle number, grain weight (1000-seed basis), and percentage of unfilled spikelets per panicle (Shukri *et al*., 2021; Shukri, 2022). Therefore, the present research utilised MR297 as the parental line to generate an improved, high-yielding variety with broad-spectrum resistance to *Xanthomonas oryzae*pv. *oryzae* through induced mutagenesis via gamma irradiation.

Materials and Methods

Plant Material and Irradiation Treatment

Seeds of MR297, along with IRBB60 (used as the positive control) and MR284 (used as the negative control), were sourced from the GeneBank of the Malaysian Agricultural Research and Development Institute (MARDI). At the acute gamma facility of the Malaysian Nuclear Agency, the seeds of MR297 were irradiated using a Biobeam GM8000 (Germany) machine, which contains Cobalt-60 as a source. Treatments consisted of seed irradiation with gamma rays at nine different doses: 50, 100, 150, 200, 250, 300, 400, 600, and 800 Gy, with a dose rate of 4.64 Gy/min. The non-treated (0 Gy) seeds of rice were considered as the control. Each group consisted of six replicates, with ten seeds in each replicate.

Radiosensitivity Test (Determining LD_{50})

Following irradiation, ten seeds were placed on germination blotter paper (5 cm × 11 cm) with the embryos oriented downwards, using the sandwich blotter method as outlined by Myhill and Konzak (1967) and later adopted by Asrapil *et al*. (2021). Measurements of shoot and root length were taken 14 days after sowing. A Completely randomised block design (CRBD) with three replicates was employed for this experiment to minimise environmental variation and ensure that differences in seedling growth responses were attributable to the irradiation treatments rather than uncontrolled experimental factors, thereby increasing the accuracy and reliability of LD_{50} estimation. A graph of treatment against shoot length was constructed using Curve Expert software version 1.4 to measure the 50% lethality dose (LD_{50}).

Breeding Scheme and Experimental Design

The breeding scheme for the development of mutant lines, including methodologies and replication design, was accomplished in accordance with the guidelines of the IAEA *Mutation Breeding Manual* (Spencer-Lopes *et al*., 2018), with slight modifications. A total of 10,000 healthy and undamaged seeds of the MR297 variety (1,000 seeds x 10 replicates) were irradiated and cultivated as the M_1 generation. Agronomic selection was conducted to identify plants with desirable traits, particularly early maturity. The seeds were then grown and harvested for M_2 seeds. A total of 5,000 M_1 seeds (500 seeds x 10 replicates) were planted to develop the M_2 population. Disease screening was carried out in this mutant generation, coupled with agronomic selection. Only the resistant rice lines demonstrating good agronomic characteristics (early maturity) were selected for development as the M_3 population. In total, 50 lines were identified based on the aforementioned criteria. A total of 500 seeds (50 lines x 10 seeds x 1 replicate) from the M_2 generation were selected for the development of the M_3 generation. According to Spencer-Lopes *et al*. (2018), only 1 replicate is applied to narrow the selection of the best M_3 lines. Stringent disease screening and agronomic selection were then conducted to choose the best lines. Based on the results, only 30 plants exhibiting the best resistance against *Xanthomonas oryzae*pv *oryzae* (Score: 1) and possessing good agronomic traits were selected for further genotyping analysis. All plants were grown in the greenhouse of the Malaysia Nuclear Agency, Selangor, in a large

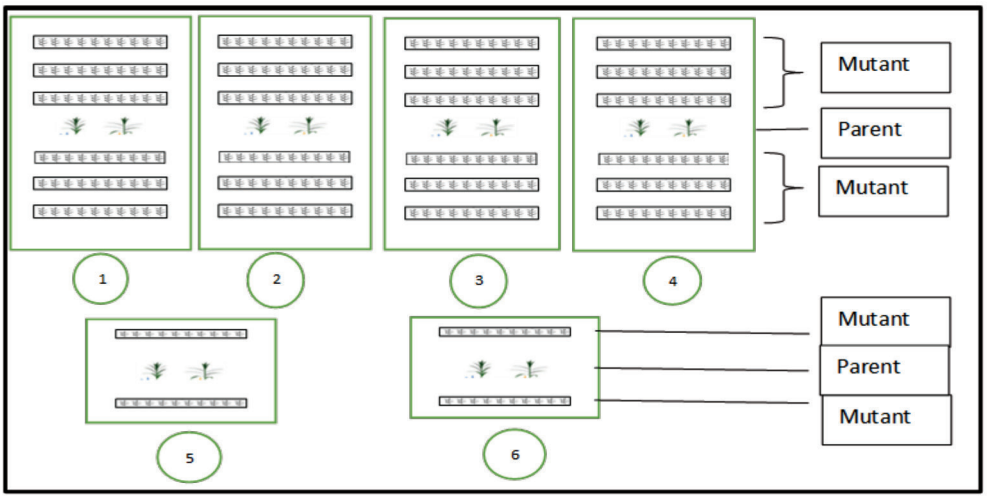

Figure 1: The arrangement of parent and mutant lines in the ARBD across six troughs (M_1 Generation)

trough (size 8 x 4 m²) and a small trough (4 x 2 m²), maintained under narrow plant spacing to limit tiller or branch numbers and safeguard against outcrossing. The parent line, MR297, was planted alongside each mutant line for phenotypic comparative observation. The lines were arranged in an augmented randomised block design (ARBD), as presented in Figure 1. Fertiliser and water were supplemented according to standard practices for rice cultivation (MARDI, 2020).

Screening of Antagonistic Activity Against Bacterial Leaf Blight (BLB) Disease

The *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolate MXO1573 was sourced from the Plant Pathology Laboratory at the Rice and Industrial Crops Research Centre, MARDI Seberang Perai. The culture was grown in peptone sucrose broth and maintained at 30°C for 48 hours. A bacterial suspension was adjusted to approximately 10^8 colony-forming units (cfu) per mL, calibrated with a spectrophotometer at 600 nm to an OD of 0.6 (Azman *et al*., 2017). Rice seedlings aged 45 days, with fully expanded leaves, were inoculated with the MXO1573 strain using the leaf-clipping method. In this procedure, sterilised scissors immersed in the bacterial suspension (10^8 cfu/mL) were used to trim 2–3 cm from the leaf tips (Kauffman, 1973; Buddachat *et al*., 2021). Disease progression was evaluated 21 days post-inoculation, following the Standard Evaluation System (SES) guidelines established by the International Rice Research Institute (IRRI, 2013).

Molecular Characterisation for Identification of Bacterial Leaf Blight (BLB) Resistance Gene(s)

Genomic DNA was extracted from young leaves harvested from three-week-old rice seedlings. The isolation was performed using the DNeasy® Plant Mini Kit (Qiagen Group, Germany), with slight adjustments to the manufacturer's protocol. Polymerase chain reaction (PCR) assays followed the procedures outlined by Lau *et al*. (2016) and Aljumaili *et al*. (2018). Each reaction mixture had a total volume of 25 µL, consisting of 5 µL of 5× PCR buffer, 0.5 µL of 10 mmol L^{-1} dNTPs (Promega, Madison, WI, USA), 0.2 µL of Taq DNA polymerase (Promega, Madison, WI, USA), 13.3 µL of nuclease-free ultrapure water, 1 µL of template DNA (50 ng), and 1 µL each of forward and reverse primers.

Nine SSR markers were employed, targeting four major BLB resistance genes: *Xa*4, *Xa*5, *Xa*13, and *Xa*21 (Table 1). IRBB60 was used as the resistant control, whereas MR284 was included as the susceptible check (Chukwu *et al*., 2019; Jiang *et al*., 2020). PCR amplification was conducted using an Eppendorf thermal cycler (Eppendorf, Germany) under the following programme: initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. A final extension was carried out at 72°C for 7 min, after which samples were cooled to 4°C.

PCR products were separated on 2.5% agarose gels prepared with 1× TBE buffer (0.05 M Tris, 0.05 M boric acid, 1 mM EDTA, pH 8.0). Electrophoresis was conducted at 75 V for 80 min, and DNA bands were visualised using the ChemiImager™ Gel Documentation System (Alpha Innotech Corporation, USA).

Cluster Analysis Based on UPGMA

The binary data obtained from polymorphic SSR markers were analysed using Darwin software version 6.0. A dissimilarity matrix was generated and used as the basis for cluster formation. The tree was constructed following the unweighted pair group method with arithmetic mean (UPGMA), which organises clusters according to average genetic dissimilarities.

Statistical Analysis

Agronomic traits were analysed using analysis of variance (ANOVA) in SPSS software version 25. Treatment means were compared using Tukey's honestly significant difference (HSD) test, applying a significance level of $p \leq 0.05$ (Taheri *et al*., 2016).

Table 1: Simple sequence repeat (SSR) markers used in the experiment

Gene	SSR Markers	Primer Sequence	PCR Product	References
*Xa*4	MP	F : 5'-ATCGATCGATCTTCACGAGG-3' R : 5' -TGCTATAAAAGGCATTCGGG -3'	150bp	Arif *et al*. (2008)
	RM224	F : 5' - ATCGATCGATCTTCACGAGG – 3' R : 3' -TGCTATAAAAGGCATTCGGG – 5'	160bp	Singh *et al*. (2015)
*Xa*5	RM13	F : 5' - TCCAACATGGCAAGAGAGAG – 3' R : 5' - GGTGGCATTCGATTCCAG - 3'	140bp	McCouch *et al*. (1996); Singh *et al*. (2015).
	RM122	F 5'-GAGTCGATGTAATGTCATCAGTGC -3' R : 5'- GAAGGAGGTATCGCTTTGTTGGAC -3'	240bp	Blair *et al*. (2003); Ji *et al*. (2016).
	RM21	F : 5' -ACAGTATTCCGTAGGCACGG – 3' R : 3' -GCTCCATGAGGGTGGTAGAG – 5'	163bp	El-Malky *et al*. (2007)
*Xa*13	*Xa*-13prom	F : 5' -GGCCATGGCTCAGTGTTTAT – 3' R : 3' -GAGCTCCAGCTCTCCAAATG – 5'	498bp	Zhang *et al*. (1996); Singh *et al*. (2011); Dokku *et al*. (2013).
	RG136	F : 5' -TCCCAGAAAGCTACTACAGC – 3' R : 3' - GCAGACTCCAGTTTGACTTC – 5'	530-490bp	Zhang *et al*. (1996)
*Xa*21	pTA248	F : 5'-AGACGCGGAAGGGTGGTTCCCGGA-3' R : 3'- AGACGCGGTAATCGAAGATGAAA –5'	1100/925bp	Ronald *et al*. (1992); Dokku *et al*. (2013).
	*Xa*21FR	F : 5' –TCCAACATGGCAAGAGAGAG – 3' R : 3' – GGTGGCATTCGATTCCAG – 5'	132bp	Ronald *et al*. (1992); Chukwu *et al*. (2019).

Results and Discussions

Development of Mutant Population

Agronomic traits are among the most important characteristics used for early screening in mutation breeding. In the M_1 generation, eight agronomic parameters were evaluated: days to maturity (DM), plant height (PH), number of tillers (NT), number of panicles (NP), panicle length (PL), filled grains per panicle (FGPP), unfilled grains per panicle (UFGPP), and yield per plant (YPP). These traits were selected because they are desirable characteristics targeted in major rice breeding projects, either through conventional methods or mutation approaches (Huang *et al*., 2020). Meanwhile, FGPP and UFGPP were calculated to define the yield rate (YPP) of lines in the M_1 generation.

Agronomic traits for rice lines in the M_1 generation were measured and compared with the parental cultivar, MR297 (Table 2). All measured traits showed significant differences from MR297 according to the paired-samples t-test ($p < 0.05$). Seven out of eight traits, including yield rate, showed a reduction in values when compared to the parental MR297 lines. As stated by Spencer-Lopes *et al*. (2018), the M_1 generation typically suffers from physiological disorders, thereby limiting the selection of subsequent generations based on phenotypic traits. This was confirmed by Viana *et al*. (2019), where most induced mutants were recessive and took an extended period—up to three or four generations—for the mutation level to become dominant. Additionally, the M_1 population was still adapting to unnatural changes at the cellular level, hence beginning to express random morphological characteristics at undefined quality levels. The decrease in trait

Table 2: Agronomic data for the M_1 generation

Traits	MR297 (n = 50)	M_1 Generation (n =10,000)
DM (days)	$107.60^a \pm 0.55$	$106.02^b \pm 0.82$
PH (cm)	$98.30^a \pm 1.80$	$98.79^b \pm 0.39$
NT (n)	$25.60^a \pm 0.89$	$15.81^b \pm 0.38$
NP (n)	$19.80^a \pm 0.84$	$9.76^b \pm 0.27$
LP (cm)	$26.92^a \pm 0.04$	$23.51^b \pm 0.34$
FGPP (n)	$127.84^a \pm 2.32$	$50.84^b \pm 5.05$
UFGPP (n)	$30.64^a \pm 1.40$	$47.18^b \pm 4.91$
YPP (%)	$80.69^a \pm 0.94$	$51.77^b \pm 4.40$

Values are presented as mean ± standard deviation (SD). Different letters denote significant differences based on the paired-samples t-test at $p < 0.05$. Trait abbreviations: DF = days to flowering, DM = days to maturity, PH = plant height, NT = number of tillers, NP = number of panicles, PL = panicle length, LS = seed length, 1000GW = weight of 1000 grains, FGPP = filled grains per panicle, UFGPP = unfilled grains per panicle, TGPP = total grains per panicle, YPP = yield per plant

quality or quantity was caused by numerous mutation by-products, including the generation of toxic reactive oxygen species (ROS), abortion of embryos before maturity, and disruption of seed structure, including the tunica and operculum (Mhamdi & Van Breusegem, 2018). Besides the aforementioned effects, mutation also caused specific mutational changes in every morphological trait measured. Days to maturity (DM) was the only trait with a positive outcome for M_1 lines. The potential mutant lines reached maturity earlier at 106 days compared to MR297 lines (107 days). Deepak *et al*. (2017) also recorded early maturity in the M_1 generation of gamma-induced rice varieties, Dubraj and Sakha 104, respectively.

A total of 5,000 M_1 seeds were selected and progressed to the M_2 generation. The plants were cultivated under a Randomised Block Design (RBD) to evaluate the impact of gamma irradiation on yield and yield-related traits of the rice variety under study.

All traits measured in the M_2 generation were significantly different from MR297, except for the number of tillers (NT) and the length of panicle (LP). In terms of days to flowering (DF), the M_2 generation reached flowering four days earlier than MR297. Most findings also illustrated earlier flowering in the M_2 lines compared to the parental lines, including those reported by Yunus *et al*. (2018) and Cabusora *et al*. (2021). Early flowering allowed plants to initiate the grain-filling phase (part of the maturity phase) at the most desirable time and under optimal conditions (Zhao *et al*., 2018). Additionally, rice crops that reached the flowering stage earlier will eventually mature sooner than expected. Regarding days to maturity (DM), the M_2 lines reached maturity earlier, at approximately 104 days, compared to 108 days for the MR297 lines. Fallah *et al*. (2019) and Mien *et al*. (2022) also observed early maturity in the M_2 generation of gamma-induced rice lines in Vietnam and Iran, respectively. Early maturity is important as it minimises the risks of plants encountering biotic or abiotic stresses throughout their development. Several costs, including fertilisation, irrigation, and maintenance, could also be reduced due to the shortened rice development period leading to harvest.

As observed in Table 3, FGPP and TGPP of the M_2 lines were greater than those of the MR297 lines, resulting in a higher yield rate per plant (YPP). At 81.97%, the average yield

Table 3: Agronomic data for the M_2 generation

Traits	MR297 (n = 50)	M_2 Generation (n = 5,000)
DF (days)	$79.40^a \pm 0.548$	$75.80^b \pm 2.25$
DM (days)	$107.60^a \pm 0.55$	$104.23^b \pm 0.52$
PH (cm)	$98.30^a \pm 1.80$	$95.23^b \pm 0.52$
NT (n)	$25.60^a \pm 0.89$	$25.74^a \pm 0.53$
NP (n)	$19.80^a \pm 0.84$	$20.41^b \pm 0.48$
LP (cm)	$26.92^a \pm 0.04$	$26.80^a \pm 0.57$
100GW (g)	$29.00^a \pm 0.00$	$30.47^b \pm 0.00$
LS (cm)	$0.989^a \pm 0.00$	$0.994^b \pm 0.00$
FGPP (n)	$127.84^a \pm 2.32$	$133.36^b \pm 6.73$
UFGPP (n)	$30.64^a \pm 1.40$	$29.36^b \pm 4.52$
TGPP (n)	$158.48^a \pm 1.56$	$162.72^b \pm 7.10$
YPP (%)	$80.69^a \pm 0.94$	$81.97^b \pm 2.61$

Values are expressed as mean ± standard deviation (SD). Different letters indicate significant differences according to the paired-samples t-test at $p < 0.05$. Trait abbreviations: DF = days to flowering, DM = days to maturity, PH = plant height, NT = number of tillers, NP = number of panicles, PL = panicle length, LS = seed length, 1000GW = thousand-grain weight, FGPP = filled grains per panicle, UFGPP = unfilled grains per panicle, TGPP = total grains per panicle, YPP = yield per plant

rate increased by more than 1% compared to the parental MR297 lines. Kato *et al*. (2020) also recorded an increase in yield rate in M_2 generations compared to their recurrent parents. According to Saeed and Hassan (2009), favourable yield-related mutations appear infrequently in M_2, with rates estimated to be between 1/1000 and 1/500 plants. However, the findings in this project reveal a high rate of beneficial mutagenic effects on yield rate, as the average value recorded is higher than that of the recurrent MR297 lines. There is potential for the yield rate to increase again in the M_3 generation, resulting in a more stable line in terms of inheritance, as recorded in a previous study by Putranto *et al*. (2021).

The M_3 generation is important for further selection and verification of heritable traits in mutant lines obtained from the M_2 generation. The quality of traits in M_3 generation is normally better than in M_2 generation, as the seeds were selected directly from panicles with the best agronomic traits. As observed in Table 4, all traits showed better values in the M_3 generation than in the parental MR297 lines, except for the length of panicle (LP). Hafsyari *et al.* (2021) also observed an increase in quality for all traits in the M_3 generation compared to the parental population. In contrast, Kasim *et al.* (2018) discovered a decrease in some quality traits in the M_3 generation, including plant height, number of tillers, and grain weight. According to Spencer-Lopes *et al.* (2018), there are common instances where M_2 mutant phenotypes (with good agronomic traits) do not reappear in the M_3 generation, even though the seeds are directly selected from good M_2 lines. In this experiment, the length of panicle is the only trait that showed less to no change in quality from the M_1 to M_3 generation. This situation is accepted as the main traits targeted for mutation breeding are achieved in the M_3 generation, including days to maturity, plant height, and yield rate (Khanam *et al.*, 2021).

Results showed that the average M_3 lines reached the flowering state 7 days earlier than the MR297 lines. Early flowering is important for plants to have a sufficient period to initiate the grain filling phase (part of the maturity phase) under the most desirable biotic and abiotic

Table 4: Agronomic data for the M_3 generation

Traits	MR297 (n = 50)	M_3 Generation (n = 500)
DF (days)	79.40 ± 0.548	72.66^b ± 1.19
DM (days)	107.60^a ± 0.55	103.54^b ± 0.68
PH (cm)	98.30^a ± 1.80	91.49^b ± 1.18
NT (n)	25.60^a ± 0.89	26.22^b ± 0.81
NP (n)	19.80^a ± 0.84	20.90^b ± 1.04
LP (cm)	26.92^a ± 0.04	26.83^b ± 0.60
1000GW (g)	29.00^a ± 0.00	30.49^b ± 0.00
LS (cm)	0.989^a ± 0.00	0.998^b ± 0.00
FGPP (n)	127.84^a ± 2.32	133.10^b ± 4.82
UFGPP (n)	30.64^a ± 1.40	29.55^b ± 7.77
TGPP (n)	158.48^a ± 1.56	162.65^b ± 6.71
YPP (%)	80.69^a ± 0.94	81.95^b ± 4.00

Values are shown as mean ± standard deviation (SD). Different superscript letters represent significant differences based on the paired-samples t-test at $p < 0.05$. Abbreviations: DF = days to flowering, DM = days to maturity, PH = plant height, NT = number of tillers, NP = number of panicles, PL = panicle length, LS = seed length, 1000GW = thousand-grain weight, FGPP = filled grains per panicle, UFGPP = unfilled grains per panicle, TGPP = total grains per panicle, YPP = yield per plant

conditions (Zhao *et al.*, 2018). As for days to maturity (DM), M_3 lines reached maturity earlier at approximately 103 days compared to 108 days in MR297 lines. Harahap *et al.* (2019) and Kurniawati *et al.* (2019) also observed early maturity in the M_3 generation of gamma-induced *Sanbei*and *Sigah*rice lines. In the M_3 generation, higher values were observed for filled grains per panicle (FGPP) and total grains per panicle (TGPP), whereas the number of unfilled grains per panicle (UFGPP) was reduced. At 81.95%, the average yield rate increased by more than 1% compared to the parental MR297 lines. Several findings by Kato *et al.* (2020) and Khanam *et al.* (2021) also recorded an increase in the yield rate of M_3 generations compared to their recurrent parents. According to Tuyen *et al.* (2015) and Spencer-Lopes *et al.* (2018), the M_3 generation is the best period to identify the best mutant progeny based on agronomic traits and resistance rates, which are normally not discernible in the M_2 generation. Heritability and environmental factors could decrease the frequency of obtaining large numbers of mutants possessing the best values for most agronomic traits and resistance rates (Vikal *et al.*, 2017). Hence, Spencer-Lopes *et al.* (2018) suggest obtaining a large number of good seeds from the M_2 generation to be planted in a panicle-by-row design for ease of mutant identification and quantitative analysis in the M_3 generation. As a result, 30 resistant lines against BLB with good agronomic traits were identified and further analysed through molecular characterisation to screen for the resistance gene.

Bacterial Leaf Blight (BLB) Phenotypic Screening

Phenotypic scoring is conducted in the M_3 generation to identify the inheritance pattern of resistance from the previous generation, as well as to create a larger number of formidable resistant lines for future cultivation (Chukwu *et al*., 2019). According to Figure 2, most M_3 lines were classified as moderately resistant to BLB (n = 347). A total of 45 lines exhibited the highest resistance rate (Score = 1), resulting in a total of 391 resistant lines in the M_3 generation out of

500 total lines. As stated by Ramli (2019), MR 297 was classified as moderately susceptible to BLB disease. Through gamma irradiation and precise selection in the M_2 generation, a group of formidable resistant lines was successfully developed. However, there were a few M_3 lines classified as susceptible (Scores 5, 7, and 9 = 108 lines).

Molecular Analysis using SSR Markers

All 30 mutant lines were previously selected for molecular identification of *Xa* genes, as they displayed a high resistance rate against BLB based on measurements of *Xoo* lesions. Table 5 summarises the response of all 30 mutant lines to *Xanthomonas oryzae*, as well as the *Xa* genes carried by each line. The selected mutant lines included, ML-1, ML-3, ML-9, ML-16, ML-17, ML-28, and ML-29, consist of 2 *Xa* genes (*Xa*21 and *Xa*5), while 23 mutant lines recorded only 1 *Xa* gene (*Xa*5).

Based on the SSR marker analysis, a dendrogram was constructed using the UPGMA and SAHN clustering methods. As illustrated in Figure 3, all 33 rice lines (30 mutant lines + IRBB60 + MR284 + MR297) are broadly clustered into four groups at a similarity coefficient of 0.78. According to Shukri *et al*. (2021), a higher Euclidean distance between clusters indicates a wider spectrum of performance between them. In this experiment, the performances of each cluster were differentiated based on the number of *Xa* genes carried by the rice lines and the number of SSR markers that amplified desirable (resistant) bands.

Based on Figure 3, Cluster I and Cluster IV consist of one rice line each: IRBB60 (positive control) and MR284 (negative control), respectively. IRBB60 is the sole rice line that carries four *Xa* genes, as identified through successful amplification of all eight SSR markers. This variety was originally developed at the International Rice Research Institute (IRRI), Los Baños, Philippines, and is widely adopted as a reference cultivar in studies of BLB resistance genes (Chukwu *et al*., 2019). Meanwhile, Cluster IV consists of MR284, which recorded no successful amplification for any *Xa*markers. As mentioned by Zakaria & Misman (2018) and Chukwu *et al.* (2019), MR284 and other Malaysian varieties were widely used as negative controls for *Xa*gene amplification tests, as they are phenotypically susceptible to BLB disease and have been proven to possess minor to no *Xa* genes.

In contrast, Cluster II recorded the highest number of rice lines, with 23 mutant lines and one parental line (MR297). These rice lines are clustered together as they carry one *Xa* gene (_Xa_5) and were successfully amplified by two SSR markers (RM122 and RM21). MR297 is grouped in Cluster II as it possesses the _Xa_5 gene too, which was then inherited by all mutant lines, as identified by RM122 and RM21 markers. Previously, Wongkhamchan *et*

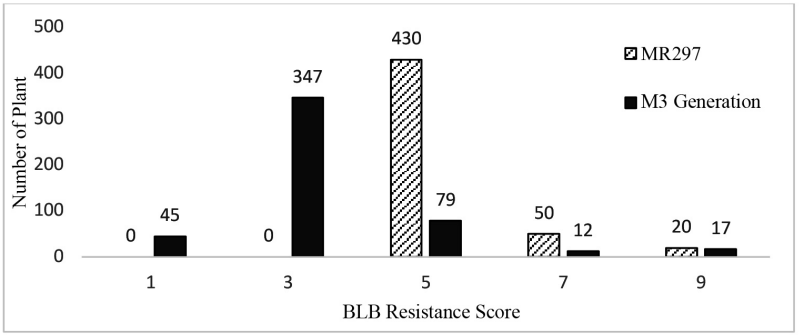

Figure 2: Segregation analysis of BLB score in the M_3 generation (IRRI, 2013): Resistance scale: 1 = resistant, 3 = moderately resistant, 5 = moderately susceptible, 7 = susceptible, 9 = highly susceptible

Table 5: Integrated phenotypic and genotypic analysis of BLB resistance in 30 elite M_3 rice lines

Rice Lines	Host Response	*Xa* Gene				No. of Carried *Xa* Gene
		*Xa*21 (Dominant)	*Xa*4 (Dominant)	*Xa*13 (Recessive)	*Xa*5 (Recessive)	
Resistant control (IRBB60)	R	+	+	+	+	4
Susceptible control (MR284)	MS	-	-	-	-	0
Parent (MR297)	MS	-	-	-	+	1
ML-1	R	+	-	-	+	2
ML-2	R	-	-	-	+	1
ML-3	R	+	-	-	+	2
ML-4	R	-	-	-	+	1
ML-5	R	-	-	-	+	1
ML-6	R	-	-	-	+	1
ML-7	R	-	-	-	+	1
ML-8	R	-	-	-	+	1
ML-9	R	+	-	-	+	2
ML-10	R	-	-	-	+	1
ML-11	R	-	-	-	+	1
ML-12	R	-	-	-	+	1
ML-13	R	-	-	-	+	1
ML-14	R	-	-	-	+	1
ML-15	R	-	-	-	+	1
ML-16	R	+	-	-	+	2
ML-17	R	+	-	-	+	2
ML-18	R	-	-	-	+	1
ML-19	R	-	-	-	+	1

ML-20	R	-	-	+		1
ML-21	R	-	-	+		1
ML-22	R	-	-	+		1
ML-23	R	-	-	+		1
ML-24	R	-	-	+		1
ML-25	R	-	-	+		1
ML-26	R	-	-	+		1
ML-27	R	-	-	+		1
ML-28	R	+	-	+		2
ML-29	R	+	-	+		2
ML-30	R	-	-	+		1

Note : R = resistant, MS = Moderate susceptible, + = present, - = absent.

al. (2021) revealed the susceptibility to BLB in rice lines introgressed with _Xa_5 alone. The lines exhibited a moderately susceptible rate, similar to the parental line. _Xa_5 is responsible as a supportive *Xa*gene to another dominant or recessive gene in rice lines with great resistance against BLB (Jiang *et al*., 2020). In addition, Jeung *et al*. (2006) observed the breakage of _Xa_5 genes in rice lines introgressed with the _Xa_5 gene alone. After a short while, the progenies were transformed into susceptible rice lines, similar to their parents.

In Cluster III, the rice lines ML-1, ML-3, ML-9, ML-16, ML-17, ML-28, and ML-29 are grouped together. This cluster is top tier, as the aforementioned rice lines carry two *Xa* genes (_Xa_21 and _Xa_5) and were successfully amplified by three SSR markers. According to Niones *et al.* (2022), the combination of at least two *Xa*genes will increase the resistance spectrum of rice in the long term. As mentioned by Chukwu *et al*. (2019), the _Xa_5 gene has a smaller resistance spectrum than _Xa_21 or _ Xa_4. The presence of both *Xa*genes in the lines of Cluster III provides larger coverage at the allelic region, hence simultaneously expressing wider resistance in rice defensive mechanisms (Niones *et al*., 2022). In the future,these lines in Cluster III could be cultivated in the M4 – M8 generation for local verification tests (LVT) and Multi Locations Trials (MLT) before commercialisation.

Conclusions

Thirty mutant plants (n = 30) were identified with the recessive *Xa_5* gene. Among these, seven lines (ML-1, ML-3, ML-9, ML-16, ML-17, ML-28, ML-29) carried both the dominant *Xa*21 and recessive *Xa*5 alleles. These seven lines also exhibited high yield performance, ranging from 76.22% to 87.01%, along with favourable expression of other agronomic traits. Consequently, these lines can be considered promising candidates for breeding programmes targeting improved resistance to *Xanthomonas oryzae*pv. *oryzae*.

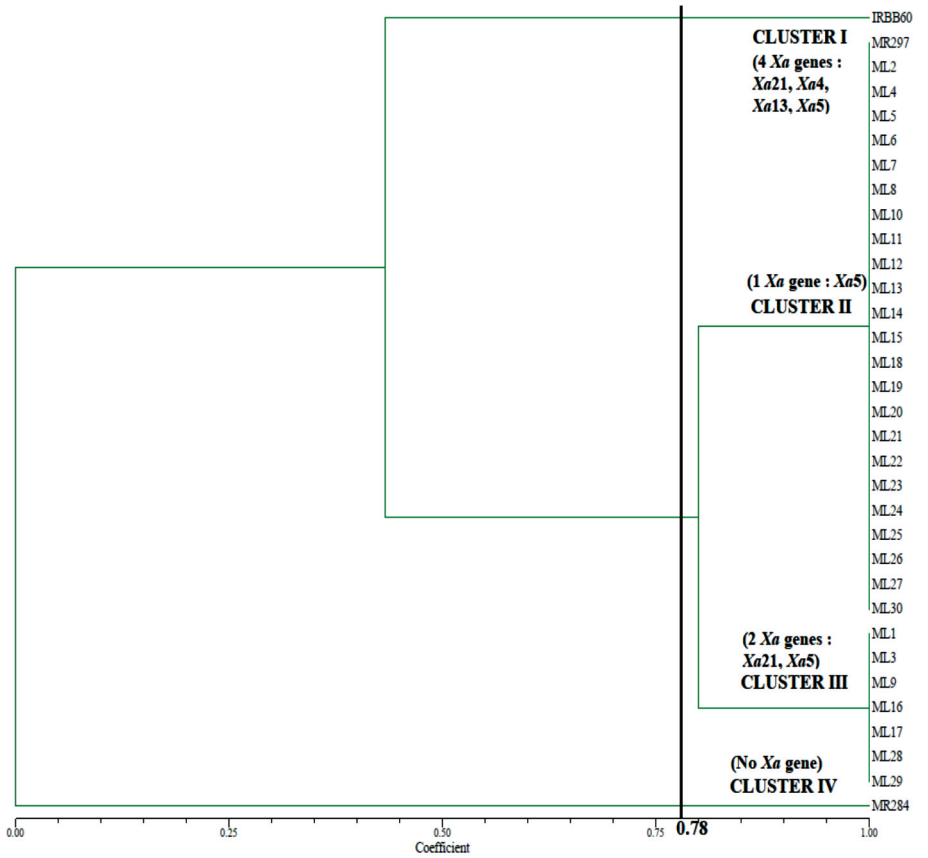

Figure 3: Clustering of the 30 best M_3 lines, parental MR297, and control varieties based on SSR marker analysis

Acknowledgements

This research was supported by the International Atomic Energy Agency (IAEA) through the Technical Cooperation Project: Enhancing Crop Productivity and Quality through Mutation by Speed Breeding (Grant No. RAS5088). The authors would like to thank the Malaysian Nuclear Agency, Universiti Teknologi MARA (UiTM), Universiti Putra Malaysia, and MARDI for providing radiation facilities and plant materials.

Conflict of Interest Statement

The authors declare that there are no competing interests, whether financial, commercial, or personal, associated with this study or its funding sources.

References

Acharya, A., Adhikari, N., Amgain, R., Poudel, A., Yadav, R., & Poudyal, K. (2018). Identification of rice genotypes resistant to bacterial leaf blight disease using SSR markers. *Journal of the Institute of Agriculture and Animal Science*, *35*(1), 113–120.

Alamsyah, W., Zakaria, S., & Supriatna, N. (2017). The effect of gamma ray irradiation on seed viability and plant growth of Aceh's local rice (*Oryza sativa* L.). *Advances in Natural and Applied Sciences*, *11*(3), 91–97.

Aljumaili, S. J., Rafii, M. Y., Zaharah, S. S., Khairulmazmi, A., & Yusuff, O. (2021). Genetic diversity of aromatic rice

germplasm revealed by SSR markers. *Biomed Research International*, *2021*.

Arif, M., Jaffar, M., Babar, M., Sheikh, M. A., Kousar, S., Arif, A., & Zafar, Y. (2008). Identification of bacterial blight resistance genes Xa4 in Pakistani rice germplasm using PCR. *African Journal of Biotechnology*, *7*(5).

Azman, N. A., Sijam, K., Hata, E. M., Othman, R., & Saud, H. M. (2017). Screening of bacteria as antagonist against *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight of paddy and as plant growth promoter. *Journal of Experimental Agriculture International*, *16*, 1–15.

Blair, M. W., Garris, A. J., Iyer, A. S., Chapman, B., Kresovich, S., & McCouch, S. R. (2003). High resolution genetic mapping and candidate gene identification at the Xa5 locus for bacterial blight resistance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, *107*, 62–73.

Buddhachat, K., Ritbamrung, O., Sripairoj, N., Inthima, P., Ratanasut, K., Boonsrangsom, T., & Sujipuli, K. (2021). One-step colorimetric LAMP (cLAMP) assay for visual detection of *Xanthomonas oryzae* pv. *oryzae* in rice. *Crop Protection*, *150*, 105809.

Cabusora, C. C., Desamero, N. V., Borromeo, T. H., Buluran, R. D., Hernandez, J. E., & Cruz, P. C. S. (2021). Characterisation of a novel floral mutation induced by gamma irradiation of Philippine rice variety NSIC Rc9 (Apo). *International Journal of Sustainable Agricultural Research*, *8*(1), 43–55.

Chen, S., Wang, C., Yang, J., Chen, B., Wang, W., Su, J., & Zhu, X. (2020). Identification of the novel bacterial blight resistance gene Xa46(t) by mapping and expression analysis of the rice mutant H120. *Scientific Reports*, *10*(1), 12642.

Chukwu, S., Rafii, M., Ramlee, S., Ismail, S., Hasan, M., Oladosu, Y. A., Magaji, U. G., Akos, I., & Olalekan, K. K. (2019). Bacterial leaf blight resistance in rice: A review of conventional breeding to molecular approach. *Molecular Biology Reports*, *46*(1), 1519–1532.

Deepak, S. B. K., Vikash, D. K., Ashish, T. K. S., Satyapal, P. S., & Samrath, B. (2017). Identification of semi-dwarf and high yielding mutants in Dubraj rice variety of Chhattisgarh through gamma ray based induced mutagenesis. *International Journal of Genetics*, *9*(9), 298–303.

DOA. (2019). Rice production in Malaysia. Retrieved from https://www.data.gov.my/data/ms_MY/organisation/ministry-of-agriculture-and-agrobased-industry

Dokku, P., Das, K., & Rao, G. (2013). Genetic enhancement of host plant resistance of the Lalat cultivar of rice against bacterial blight employing marker-assisted selection. *Biotechnology Letters*, *35*(8), 1339–1348.

Doucoure, H., Perez-Quintero, A. L., Reshetnyak, G., Tekete, C., Auguy, F., Thomas, E., & Cunnac, S. (2018). Functional and genome sequence-driven characterisation of tal effector gene repertoires reveals novel variants with altered specificities in closely related Malian *Xanthomonas oryzae* pv. *oryzae* strains. *Frontiers in Microbiology*, *9*, 1657.

El-Malky, M. M., Fahmi, A. I., & Kotb, A. A. (2007). Detection of genetic diversity using microsatellites in rice (*Oryza sativa* L.). In *African Crop Science Conference Proceedings* (Vol. 8, pp. 597–603).

Fahad, S., Adnan, M., Noor, M., Arif, M., Alam, M., Khan, I. A., Ullah, H., Wahid, F., Mian, I. A., Jamal, Y., Basir, A., Hassan, S., Saud, S., Amanullah, M., Riaz, C., Wu, K. M. A., & Wang, D. (2019). Major constraints for global rice production. In M. Hasanuzzaman, M. Fujita, K. Nahar, & J. K. Biswas (Eds.), *Advances in rice research for abiotic stress tolerance* (pp. 1–22). Woodhead Publishing.

Fallah, A., Bagheri, L., & Nabipour, A. (2019). Agronomical characteristics of M2–M5 mutants of rice in saline paddy fields of Mazandaran. *Iranian Journal of Field Crop Science*, *51*(1), 127–135.

FAO. (2019). *Global food demand by 2050*. Retrieved from http://www.fao.org/statistics/en/

FAO. (2022). *Crop prospects and food situation* (#1, March 2022).

FAO/IAEA. (2022). *IAEA Mutant Variety Database*. Retrieved on December 2021 from https://nucleus.iaea.org/sites/mvd

Hafsyari, S. K., Purwanto, E., & Yunus, A. (2021). Rice strain selection for Mentik Wangi variety M3 generation resulting from 200 gray gamma irradiation. In *IOP Conference Series: Earth and Environmental Science* (Vol. 637).

Harahap, I., Efendi, A., & Syafruddin, I. (2019). Analisis variabilitas dan heritabilitas mutan padi (*Oryza sativa* L.) M3 hasil iradiasi sinar gamma pada sistem pertanian organik. *Jurnal Ilmiah Mahasiswa Pertanian Unsyiah*, *4*(1).

Hosnan, M. A. (2016). *Padi Siraj MR297 - varieti baru*. Retrieved from http://animhosnan.blogspot.com/2016/02/padi-siraj-mr297-varieti-baru.html

Huang, M., Shan, S., Cao, J., Fang, S., Tian, A., Liu, Y., Cao, F., Yin, X., & Zou, Y. (2020). Primary-tiller panicle number is critical to achieving high grain yields in machine-transplanted hybrid rice. *Scientific Reports*, *10*(1), 2811.

International Rice Research Institute. (2013). *Standard evaluation system for rice* (5th ed.). IRRI.

International Rice Research Institute. (2019). *Global rice statistics*. Retrieved from http://ricestat.irri.org:8080/wrsv3/entrypoint.htm

Jeung, J., Heu, S., Shin, M., Vera Cruz, C., & Jena, K. (2006). Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathology*, *96*(8), 867–875.

Ji, Z., Yang, S., Zeng, Y., Liang, Y., Yang, C., & Q, Q. (2016). Pyramiding blast, bacterial blight and brown planthopper resistance genes in rice restorer lines. *Journal of Integrative Agriculture*, *15*(7), 1432–1440.

Jiang, N., Yan, J., Liang, Y., Shi, Y., He, Z., Wu, Y., & Peng, J. (2020). Resistance genes and their interactions with bacterial blight/leaf streak pathogens (*Xanthomonas oryzae*) in rice (*Oryza sativa* L.). *Rice*, *13*(1), 3.

Kasim, N., Sjahril, R., Riadi, M., & Arbie, F. (2018). The phenotype performance of M3 red rice mutant (*Oryza sativa* L.). Paper presented at the IC-FSSAT.

Kato, H., Li, F., & Shimizu, A. (2020). The selection of gamma-ray irradiated higher yield rice mutants by directed evolution method. *Plants (Basel)*, *9*(8).

Kauffman, H. (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Disease Reporter*, *57*, 537–541.

Khanam, S., Noor, M. M. A., Haque, M. S., & Azad, M. A. K. (2021). Developing high yielding, lodging tolerant Biroi type rice lines through mutation breeding technique. *Bangladesh Journal of Nuclear Agriculture*, *35*, 53–61.

Kumar, A., Kumar, R., Sengupta, D., Das, S. N., Pandey, M. K., Bohra, A., & Sundaram, R. M. (2020). Deployment of genetic and genomic tools toward gaining a better understanding of rice–*Xanthomonas oryzae* pv. *oryzae* interactions for development of durable bacterial blight resistant rice. *Frontiers in Plant Science*, *11*, 1152.

Kurniawati, S., Chaniago, I., & Suliansyah, I. (2019). Variability and heritability of Sigah brown rice mutants (M3) in vegetative phase. *International Journal of Environment, Agriculture and Biotechnology*, *4*(6), 1745–1749.

MAFI. (2021). *Dasar agromakanan negara 2021–2030*.

MARDI. (2016). *MARDI Siraj 297: Varieti padi berhasil tinggi*. Penerbitan MARDI (Malaysian Agricultural Research and Development Institute).

MARDI. (2020). *Manual penanaman padi lestari*. Penerbitan MARDI (Malaysian Agricultural Research and Development Institute).

McCouch, S. R., Sebastian, L. S., Ikeda, R., Huang, N., Imbe, T., & WR, C. (1996). Molecular mapping of resistance to rice tungro spherical virus and green leafhopper. *Phytopathology*, *86*, 25–30.

Mhamdi, A., & Van Breusegem, F. (2018). Reactive oxygen species in plant development. *Development*, *145*(15).

Miền, N. T., Khanh, N. T., & Quang, T. V. (2022). Breeding new promising mutant rice lines by gamma Co60. *Vietnam Journal of Agricultural Science*, *20*(1), 1–10.

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., & Baloch, F. S. (2017). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, *32*(2), 261–285.

Niones, J. T., Sharp, R. T., Donayre, D. K. M., Oreiro, E. G. M., Milne, A. E., & Oliva, R. (2022). Dynamics of bacterial blight disease in resistant and susceptible rice varieties. *European Journal of Plant Pathology*, *163*(1), 1–17.

Ocloo, F. C., Owureku-Asare, M., Agyei-Amponsah, J., Agbemavor, W. S., Egblewogbe, M. N., Apea-Bah, F. B., & Ofori-Appiah, D. (2017). Effect of gamma irradiation on physicochemical, functional and pasting properties of some locally-produced rice (*Oryza* spp.) cultivars in Ghana. *Radiation Physics and Chemistry*, *130*, 196–201.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., Miah, G., & Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: A review. *Biotechnology & Biotechnological Equipment*, *30*(1), 1–16.

Pradhan, S. K., Barik, S. R., Nayak, D. K., Pradhan, A., Pandit, E., Nayak, P., & Pathak, H. (2020). Genetics, molecular mechanisms and deployment of bacterial blight resistance genes in rice. *Critical Reviews in Plant Sciences*, *39*(4), 360–385.

Putranto, B. S., Parjanto, & Yunus, A. (2021). Selection of M3 genotype short stem results of ray gamma 250 gray in Mentik Wangi rice. In *IOP Conference Series: Earth and Environmental Science* (Vol. 637).

Ramalingam, J., Raveendra, C., Savitha, P., Vidya, V., Chaithra, T. L., Velprabakaran, S., & Vanniarajan, C. (2020). Gene pyramiding for achieving enhanced resistance to bacterial blight, blast, and sheath blight diseases in rice. *Frontiers in Plant Science*, *11*, 591457.

Ramli, A. (2019). Rice R&D at MARDI [Press release].

Ronald, P. C., Albano, B., Tabien, R., Abenes, L., Wu, K.-S., McCouch, S., & Tanksley, S. D. (1992). Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21. *Molecular and General Genetics*, *236*(1), 113–120.

Saeed, I., & Hassan, M. F. (2009). High yielding groundnut (*Arachis hypogea* L.) variety "Golden". *Pakistan Journal of Botany*, *41*(5), 2217–2222.

Shukri, A. I. A. (2022). *Agronomic performance and genetic analyses of resistance against Xanthomonas oryzae pv. oryzae in rice mutant lines (Oryza sativa L.)* [MSc (Applied Biology) thesis, Universiti Teknologi MARA (UiTM)].

Shukri, A. I. A., Hasan, N. A., Ahmad, F., Ramachandran, K., Rafii, M. Y., Harun, A. R., & Manaf, M. N. A. (2021). Genetic

diversity of selected Malaysian mega rice varieties based on agro-morphological traits. *Malaysian Journal of Biochemistry & Molecular Biology*, *2*, 1–9.

Singh, A. K., Dharmraj, E., Nayak, R., Singh, P. K., & Singh, N. K. (2015). Identification of bacterial leaf blight resistance genes in wild rice of eastern India. *Turkish Journal of Botany*, *39*, 1060–1066.

Singh, A., Gopalakrishnan, S., Singh, V., Prabhu, K., Mohapatra, T., Singh, N. K., Sharma, T. R., Nagarajan, M., Vinod, K. K., & Singh, D. (2011). Marker assisted selection: A paradigm shift in Basmati breeding. *Indian Journal of Genetics and Plant Breeding*, *71*(2), 120.

Sobri, H., Harun, A. R., Simoli, J. M. A., Wahab, M. R. A., Salleh, S., Ahmad, F., & Hashim, N. M. (2020). *Mutation breeding of rice for sustainable agriculture in Malaysia*. Paper presented at the Forum for Nuclear Cooperation in Asia (FNCA), Japan.

Spencer-Lopes, M. M., Forster, B. P., & Jankuloski, L. (2018). *IAEA manual on mutation breeding* (3rd ed.). Plant Breeding and Genetics Subprogramme, Joint FAO/ IAEA Division of Nuclear Techniques in Food and Agriculture.

Sprink, T., Eriksson, D., Schiemann, J., & Hartung, F. (2016). Regulatory hurdles for genome editing: Process-vs. product-based approaches in different regulatory contexts. *Plant Cell Reports*, *35*, 1493–1506.

Taheri, S., Abdullah, T., Ahmad, Z., Sahebi, M., & Azizi, P. (2016). Phenotypic and molecular effects of chronic gamma irradiation on *Curcuma alismatifolia*. *European Journal of Horticultural Science*, *81*, 137–147.

Tuyen, V. M., Hong, N. T., My, P. Q., Hue, N. T., & Ham, L. H. (2015). Applications of gamma rays irradiation and marker assisted selection for improving of bacterial leaf blight resistant rice variety, BT62.1. *Journal of Agricultural Technology*, *11*(8), 2441–2449.

Viana, V. E., Pegoraro, C., Busanello, C., & Costa de Oliveira, A. (2019). Mutagenesis in rice: The basis for breeding a new super plant. *Frontiers in Plant Science*, *10*, 1326.

Vikal, Y., & Bhatia, D. (2017). Genetics and genomics of bacterial blight resistance in rice. In J. Li (Ed.), *Advances in international rice research* (pp. 59–67). IntechOpen.

Wongkhamchan, A., Chankaew, S., Monkham, T., Saksirirat, W., & Sanitchon, J. (2018). Broad resistance of RD6 introgression lines with Xa5 gene from IR62266 rice variety to bacterial leaf blight disease for rice production in Northeastern Thailand. *Agriculture and Natural Resources*, *52*(3), 241–245.

Xing, J., Zhang, D., Yin, F., Zhong, Q., Wang, B., Xiao, S., & Chen, L. (2021). Identification and fine-mapping of a new bacterial blight resistance gene, Xa47(t), in G252, an introgression line of Yuanjiang common wild rice (*Oryza rufipogon*). *Plant Disease*, *105*(12), 4106–4112.

Yunus, A., Parjanto, & Pratama, I. Y. (2018). The performance of M2 generation of Mentik Wangi Susu rice resulted from gamma ray irradiation. In *IOP Conference Series: Earth and Environmental Science*, *142*(1).

Zakaria, L., & Misman, N. (2018). The pathogen and control management of rice blast disease. *Malaysian Journal of Microbiology*, *14*, 705–714.

Zhang, G., Angeles, E., Abenes, M., Khush, G., & Huang, N. (1996). RAPD and RFLP mapping of the bacterial blight resistance gene xa-13 in rice. *Theoretical and Applied Genetics*, *93*(1), 65–70.

Zhao, C., Xu, W., Song, X., Dai, W., Dai, L., Zhang, Z., & Qiang, S. (2018). Early flowering and rapid grain filling determine early maturity and escape from harvesting in weedy rice. *Pest Management Science*, *74*(2), 465–476.