Mutation Breeding of Rice for Sustainable Agriculture in Malaysia

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Abstract

National Agriculture Policy (DPN 3) 1998-2010 and the National Agrofood Policy (NAP) 2011-2020 are the evidence of Malaysian government's efforts in transforming as well as improving the country's food quality. The National Agrofood Policy 2011-2020 was formulated with a special focus on improving the food production sector including rice. Even though Malaysia is still depending on imported rice to fulfil consumer's demand, rice industry has always been a national priority based on its strategic importance as a staple food commodity. Nevertheless, the rice industry in Malaysia is hampered by several challenges such as global climate change, lacking of new variety, insufficient certified seed, emerging of major diseases such as Bacterial Panicle Blight

(BPB), Leaf Blast, drought season, flash flooding, decreasing of planting area, lost interest of the farmers due to high cost of rice production and many others. Thus, the NAP had highlighted that local rice production should be increased to ensure the country's demand in the future. FNCA project is one of the initiatives that supported the national policy. In 2019, Malaysian Nuclear Agency has moved one step forward in the area of mutation breeding by signing the Memorandum of Understanding (MoU) with Certified Seed Company HMN (M) Sdn. Bhd and Bayer Co. (MALAYSIA) Sdn. Bhd. With this agreement, HMN (M) Sdn. Bhd. will collaborate with Malaysia Nuclear Agency to produce certified seeds, multiply and commercialize the rice mutants. Meanwhile, Bayer Co. (MALAYSIA) Sdn. Bhd. will be responsible in coating the mutant seeds with plant growth promoters. This collaboration has added value to NMR 151 (PBR 0159) and NMR 152 (PBR 0156) mutant seeds and resulting in more competitive seeds as compared to other varieties in the market. In addition, from 2017 until 2020, several local verification trails (LVT) were also conducted from the Northern part until the Southern part of Peninsular Malaysia. The data obtained from Sekinchan, Selangor revealed that NMR 152 consistently produced between 7-10 t/ha in granary area as compared to 6 t/ha produced by other varieties within the same planting areas. Field trials also showed that the production cost was reduced by 10%, mainly due to the reduction in fertilizer and pesticide usage. At the same time, the yield could be increased between 40 - 60% depending on the planting areas. The preliminary studies on estimation of glycemic index (GI) revealed that two mutant lines could be consumed by diabetic patients. The two mutant lines (ML3 and ML30) were recorded to have normal glucose reading which was identified to have a moderate GI of 65 and 66, respectively. As low and moderate GI foods are recommended for diabetic patients, these two mutants (ML3 and ML30) have a high potential for their consumption. In addition, two mutant lines were successfully granted with Certificate of Registration of New Plant Variety and Grant of Breeder's Right by Department of Agriculture Malaysia in Feb 2020 with registration number; PBR0156 (for NMR152) and PBR 0159 (for NMR151). Apart from increasing the yield and income of the framers, the mutant lines derived from FNCA project had also greatly impacted the socio-economic status of the farmers as the mutant rice are adaptable to current global climate change conditions. Furthermore, through the MoU with the companies, around 4000 metric tons of seeds will be produced in 2020 for the supply of 28,571 ha planting areas in Peninsular Malaysia. Basically, the project has addressed the national agenda and policy in generating new rice varieties and thus, increase the well-being and livelihood of the farmers.

Keywords: Gamma Irradiation, Ion Beam, Mutation Breeding, MINT, NMR152, NMR151, Rice.

Introduction

In Malaysia, rice industry has always been a priority based on strategic importance of rice as a staple food commodity. Although the production of rice is increasing towards population increase, Malaysia still depends on imported rice to meet the consumer's demand. Malaysia managed to achieve 72% self-sufficiency level in rice with the current average rice yield of 3.7t/ha/season. In this situation, about 28% of the local demand will have to depend on imported rice. In Peninsula Malaysia, rice production depends largely on the irrigated lowland production system. Currently, there are 241,741ha of irrigated rice in Peninsula Malaysia, which contributes more than 85% of the national rice production. However, growing irrigated rice requires large amount of water. It was estimated that about 3,000 liters of water are required to produce 1kg of rice. Unfortunately, there are signs of declining water supply that threatens the sustainability of irrigated rice production. The largest water withdrawal (more than 75%) in Malaysia is for irrigation in the agriculture sector and is mainly confined to irrigated rice production. However, the future agriculture's share of water will decline because of increasing competition for available water from urban and industrial sectors. Water is becoming a scarce entity in the future and in some areas, it has become a limiting factor in rice production (Bhuiyan, 1992). Water supply is affected by the loss of watersheds due to deforestation and soil erosion. There is severe depletion of valuable groundwater resources as water is taken up for agricultural and industrial purposes. Rice will be the crop most affected by water scarcity as it depends most heavily on irrigation. Some varieties of rice can be grown without irrigation. However, their growth totally depends on the rain fall. About 15% of the rice varieties in Sabah and Sarawak are rainfed. Rainfed rice varieties are lower in yield due to uncertainty of water supply. In view of this water shortage, there is an urgent need to develop new varieties of rice with high yield potential and stability under water stress conditions. Therefore, in an attempt to develop water stress tolerance lines, morphological and agronomic traits that are related to water stress condition, can be useful in establishing a successful breeding program for rice improvement.

Induced mutation is an important supplementary approach to plant breeding, particularly when it is desired to improve one or two easily identifiable traits in an otherwise good lines or varieties. The successful use of plant breeding for improving crops requires the existence of genetic variation of useful traits. Unfortunately, the desired variation is often lacking. However, radiation can be used to induce mutations and thereby generate genetic variation from which desired mutants may be selected. Mutation induction has become a proven way of creating variation within a crop variety. Heavy ion beams, such as carbon ions, are more effective in plant for inducing mutations compared with electron beam (Hidema et al., 2003). Novel mutants have been obtained by the carbon ion irradiation in several plant species (Hase et al., 2000). To date, a number of rice mutants with various significant improvements in morphological traits have been recommended for planting (Mohamad et al., 2006). In recent years, the field drought-resistance screening facility was established through mutation breeding program and the evaluation standard was developed. Some advanced lines of drought tolerance rice varieties were identified and will be used in both molecular mapping and breeding programme. Therefore, the objectives of FNCA rice mutation breeding project were to screen for mutant lines derived from gamma rays and ion beam irradiation for high yield potential and stability under water stress conditions with improved quality trait.

Materials and Methods

Part 1: Evaluation of mutant lines derived from gamma irradiation

The seeds of popular local variety, MR219 were irradiated with 300 Gy of gamma radiation from the Cobalt-60 (60Co source) at Malaysian Nuclear Agency. Irradiated seeds were sown at Mardi Research Station in Tanjung Karang, Selangor. The M1 seedlings were transplanted into the field with 25cm x 25cm planting distance. A total of 10,000 M1 seedlings were planted to produce M2 seeds and a total of 5,250 plants were selected from which 2 panicles per hill were randomly harvested from each hill. After M6 generation, the best selected mutant lines were further evaluated for MLT (Multi Location Trial), LVT (Local Verification Trial), disease screening, morphology and agronomy characteristics. The best selected lines were planted for 4 seasons and the data on culm height, panicle length, number of panicle per plant, flowering time, day of maturity, weight for 1000 grain seed, seed length, width length and yield in t/ha were collected. This study was conducted in collaboration with MARDI (Malaysian Agriculture Research and Development Institute), MADA (Muda Agricultural Development Authority), KADA (Kemubu Agricultural Development Authority), IADA (Integrated Agricultural Development Area), Department of Agriculture Malaysia (DOA), HMN (M) Sdn Bhd), BAYER Co (M) Sdn. Bhd. and farmers. The locations for field trial were inclusive of Northern part until Southern part of Peninsular Malaysia with the planting area of approximately one hectare per variety.

Part 2: Evaluation of mutant lines derived from ion beam irradiation

As for ion beam irradiation, several cultivars such as MR219, Pongsu Seribu 2, Tongkat Ali, MARDI(B001), MARDI(C002), UKM-1, UKM-2, UKM-3, UiTM-1, UiTM-2 and UiTM-3 were irradiated in the ion beam facility in Takasaki, Japan. The Irradiation Apparatus for seed, connected to a vertical beam line of the AVF-cyclotron (TARRI, Takasaki), were used for the 320 MeV carbon-ion irradiation. The carbon ion irradiation with the doses of 0, 10, 20, 40, 60, 80, 100 and 120 Gy were performed under atmospheric pressure within 3 min (Hidema et al., 2003). The seeds were evaluated at Malaysian Nuclear Agency, UKM, UiTM and MARDI. The optimum doses of these cultivars were identified. The 31 potential mutant lines derived from MR219 rice that produced through Carbon ion radiation (60 Gy) were screened and selected at Malaysian Nuclear Agency up to six generation (M6) based on the targeted traits. These mutant lines (ML1 to ML31) were evaluated on morphological characters, yield and yield components, and compared to the parental variety, MR219. Meanwhile, another 10 potential mutant lines derived from Pongsu Seribu2 were further evaluated at farmer's field in Sekinchan, Selangor. Data on culm height, panicle length, number of panicles per plant, flowering time, day of maturity, weight for 1000 grain seed, seed length, width length and yield in t/ha were collected. This study was conducted in collaboration with MARDI, IADA, UKM (National University of Malaysia), UPM (Universiti Putra Malaysia), DOA, HMN (M) Sdn Bhd, BAYER Co (M) Sdn. Bhd. and the farmers.

Part 3: Evaluation and characterization of advanced mutant lines of MR219-4 (NMR151 / PBR 0159) and MR219-9 (NMR 152/ PBR 0156) under drought conditions

The seeds of popular local variety, MR219 were irradiated with 300 Gy of gamma radiation from the Cobalt-60 (60Co source) at Malaysian Nuclear Agency. Two advanced rice mutant lines MR219-4/NMR151 and MR219-9/NMR152 and cultivars MR211, MR219 and ARN1 were grown in a greenhouse at the Malaysian Nuclear Agency (Nuclear Malaysia), Bangi. The MR219-4 and MR219-9 and check varieties MR211, MR219 and ARN1 were assigned in a randomized complete block design (RCBD) with three replications. The pre-germinated seeds were sown in trays containing wet soil. Healthy seedlings were transplanting 26 days after germination. The seedlings were planted in a row consisting of nine plants each genotype were replication three represented 15 rows, all together. The planting distance was 23 cm within and 23 cm between rows. The space between two adjacent troughs was 1 m. The water was drained at 30 days after transplanting (DAT) and was re-irrigated periodically when soil water tension fell below -50kPa. The control was continued with standing water until maturity.

Morphological traits evaluated on single plant basis were plant height, days to flowering, number of tillers, flag leaf area, panicle length and days to maturity. The plants were scored for leaf rolling and leaf drying by observing visually using 0-9 scale (Table 1 & Table 2) based on Standard Evaluation System adopted for rice (IRRI 1996). The process of harvesting the grains was done manually when the plants reached maturity. Grains from each plant were packed in an envelope. The culms and leaves were cut at ground level and wrapped with newspaper. The grains and plant parts were dried in an oven at 37°C for 48 hours.

The measured agronomic traits for each plant were grain weight, grain yield, 100-grain weight, dried plant weight, biomass and harvest index. Data were analysed using the statistical analysis system (SAS 9.1.3) for windows software. All the data obtained were subjected to a two-way analysis of variance (ANOVA) and the mean differences were compared by least significant differences (LSD).

Part 4: Evaluation of Nitrogen uptake in advanced mutant lines of MR219-4 (NMR151 / PBR 0159) and MR219-9 (NMR 152/ PBR 0156)

The pot trial was conducted at Nuclear Malaysia in April 2013. Mutant rice varieties MR219-9 and MR219 (check variety) with two water regimes, non-flooded at soil water potential of 0-30 kPa and flooded at 5cm water level were tested in a completely randomized design. Nitrogen was applied at 120 kg N ha in three splits at 5, 30 and 60 days after transplanting using ^{15}N-labelled urea with 5.14 % atom excess. Total N content and ^{15}N abundance in the plant samples was determined by Kjeldahl method and emission spectrometry, NOI7. A field trial was conducted at MARDI and crop establishment was conducted in August 2013. Four rice varieties, MRIA 1, mutant MR219-4 (NMR151 / PBR 0159), MR219-9 (NMR 152/ PBR 0156) and MR 219 were grown under aerobic condition. Each treatment is tested in a 5 x 5 m plot arranged in a randomized complete block design with five replications. ^{15}N-labelled urea at 10% atom excess was applied in microplots of 0.5 x 0.5 m and 1 x 1 m.

Part 5: Screening of Foliar blast disease in selected potential mutant lines

The evaluation of 5 test lines from MINT (NM 1= ML 30, NM 2 = ML3, NM 3 = ML 10, NM 4 = NMR 152 and NM 5= NMR 152) against foliar blast disease resistance were conducted in blast nursery during off season 2016. The screening was done under natural infection from the inoculum bed according to method established by IRRI. Inoculum bed for maintenance of natural airborne spores was maintained continuously by planting mixture of susceptible varieties at 3 stages with interval of one to two weeks. Dry test lines seeds were sown in rows in between two inoculum beds. Bombardment rows which consist of mixture of susceptible varieties planted along both side of the test lines. This is to ensure sufficient inoculum for spreading disease spore to the test lines. Seedlings were watered frequently by sprinkler at every three hours throughout the day. Fertilizer was applied at 2 weeks after sowing using N: P: K at the rate of 140:70:80 kg/ha. Disease reaction was assessed at 21 and 28 days after sowing (DAS) using a 0-9 scale as described by IRRI 3rd edition of the 'Standard Evaluation System for rice (SES)' (1988) (Table 1) and the ability of disease resistance was interpretation based on the General Disease Scale as in Table 2.

Table 1: Standard Evaluation System for rice

0	No lesion
1	Small brown specks of pinpoint size or larger brown specks without sporulating center
2	Small roundish to slight elongated, necrotic grey spots, about 1-2 mm in diameter, with a distinct brown margin. Lesion are mostly found on the lower leaves
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions, 3 mm or longer, infecting less than 2% of the leaf area
5	Typical blast lesion infecting 2-10% of the leaf area
6	Typical blast lesion infecting 11-25% of the leaf area
7	Typical blast lesion infecting 26-50% of the leaf area
8	Typical blast lesion infecting 51-75% of the leaf area and many leaves dead
9	More than 75% leaf area affected

Table 2: General disease scale interpretation

Disease scale	Disease Reaction
0	Highly resistant (HR)
1-2	Resistant (R)
3-4	Moderately resistant (MR)
5-6	Moderately susceptible (MS)
7-8	Susceptible (S)
9	Highly susceptible (HS)

Part 6: Evaluation on effects of MR219 mutant rice on glycaemic responses in BALB/c mice

Plant Materials

The MR219 seeds were irradiated with a carbon-ion beam (60 Gy) using AVF-Cyclotron in a collaborative effort by the National Institute of Quantum and Radiological Science and Technology, Japan and Malaysian Nuclear Agency, Bangi, Selangor. After several series of selection and fixation, 31 potential lines with the required adaptive traits were recovered at M4 generation during the 2009 - 2012 seasons (M0 - M4). The M4 seeds of these 31 mutant lines were evaluated including the parental variety MR219. Samples of rough rice were dehulled using a dehusking machine (Motion Smith Co., Singapore). The dehulled rice was passed through a 500 µm sieve screen on a Sample Mill (Cyclotec 1093, Foss analytical, Sweden) to obtain rice powder.

Evaluation of mutant lines for glycaemic responses through in vivo study

Female BALB/c mice of 8 w old were used in the present work and carried out in a Completely Randomised Design with the guidelines of Research and Ethics Committee of International Medical University (IMU). A total of 48 mice were divided into eight groups having six mice each. The study started after one week of mice arrival to allow them to adapt to the new environment. Normal diet was given to them throughout the period of adaptation.

Oral feeding and glucose monitoring of blood in mice

After the adaptation period, the mice were fasted (overnight fasting) for 12 h. However, distilled water was offered ad libitum. The blood samples were taken at time zero before given the test food to measure the glucose level. Blood collection was done following the standard procedure described by IACUC (2011). Blood sample was obtained by snipping not more than 1 mm of mice tail and gently milking the blood from the snipped tail. One droplet of blood was placed on a glucose test strip and was read using a glucometer (Accu-Check glucometer). Then the mice were given the test food samples by force feeding using the feeding tube. The size of the feeding tube used for feeding was 18 gauge. The mice were restrained by grabbing them by the scruff of their neck and grasping the skin over the shoulders with the thumb and middle fingers during force feeding. This extended the fore-legs to the side, thus keeping the front feet from pushing the gavage tube away. The blood glucose levels were determined again at 30, 60, 90 and 120 min after feeding. Data were subjected to statistical analysis such as analysis of variance (ANOVA) and mean comparisons using SAS 9.4 Software.

Estimation of Glycaemic Index

The percentage of GI was calculated using the formula prescribed by FAO-WHO (1998):

$$GI = [\text{area under curve (test food)} / \text{area under curve (glucose)}] \times 100$$

where the area under curve was calculated based on Trapezoid technique in MS Excel to reflect the total rise of blood glucose levels after eating test foods.

Results and Discussion

Part 1: Evaluation of mutant lines derived from gamma irradiation

Malaysian Nuclear Agency has moved one step forward in the area of mutation breeding by signing the Memorandum of Understanding (MoU) with Certified Seed Company HMN (M) Sdn. Bhd and Bayer Co. (MALAYSIA) Sdn. Bhd. on 29 April 2019. With this agreement, HMN (M) Sdn. Bhd. will collaborate with Malaysia Nuclear Agency to produce certified seeds, multiply and commercialize the rice mutants. Meanwhile, Bayer Co. (MALAYSIA) Sdn. Bhd. will be responsible in coating the mutant seeds with plant growth promoters. This collaboration will add value to NMR 151 and NMR 152 mutant seeds and result in more competitive seeds as compared to other varieties in the market. In addition, from 2016 until 2020, several local verification trails (LVT) and multi-location trial (MLT) were also conducted from the Northern part until the Southern part of Peninsular Malaysia (Figure 1). The data obtained from the field trial at Sekinchan, Selangor revealed that NMR 152 consistently produced between 9-10 t/ha in granary area as compared to 6 t/ha produced by other varieties within the same planting areas. Field trials also showed that the production cost was reduced by 10%, mainly due to the reduction in fertilizer and pesticide usage. At the same time, the yield could be increased between 40 - 60%, depending on the planting areas (Table 3). Table 4 shows the yield of NMR 152 using different evaluation methods in the farmers' field. The data revealed that Wheel method that introduced by the Agriculture Department is the most accurate methods in estimating the yield of mutant rice in an open field.

In 2015, Malaysian Nuclear Agency introduced new innovation in the area of rice breeding technology. The innovation was called as "Nuclear Malaysia Rice Agronomic Package", in which the package was aimed to further stimulate the early maturation and yield of the advanced mutant line (Figure 2). The data revealed that the cost of additional fertilizer, pesticide and field management could be reduced up to 5-10% of total expenditure. Results obtained from northern part of Malaysia indicate that mutant variety (MR 219-9/NMR152) treated with the combination of Biofertilizer, Oligochitosan and liquid smoke produce higher yield (13 t/ ha-using CCT Method) as compared to the control (Figure 3a). The data obtained also revealed that similar pattern was observed with mutant line MR 219-4/NMR151 where by 15 t/hectare (using CCT method) was produced. In another farmer's plot treatment with liquid biofertilizer was significantly increased the grain yield of mutant MR219-4 up to 25% as compared with other treatments over the control. A biofertilizer is a substance composed of living microorganisms that colonise the rhizosphere or the interior parts of plants and promote growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003). According to Phua et al. (2019), those microorganisms can improve the uptake of N_2 fixation and P solubilisation. Therefore, the yield for both NMR151 and 152 increase up to 15 t/ha and 13 t/ha respectively (Figure 3b). The yield obtained was higher than the national average yield (4.207t/ha-actual yield from factory). Meanwhile, MR 219-9 (NMR152) showed more tolerant to drought as compared to MR 219-4 (NMR151) and MR 219-control.

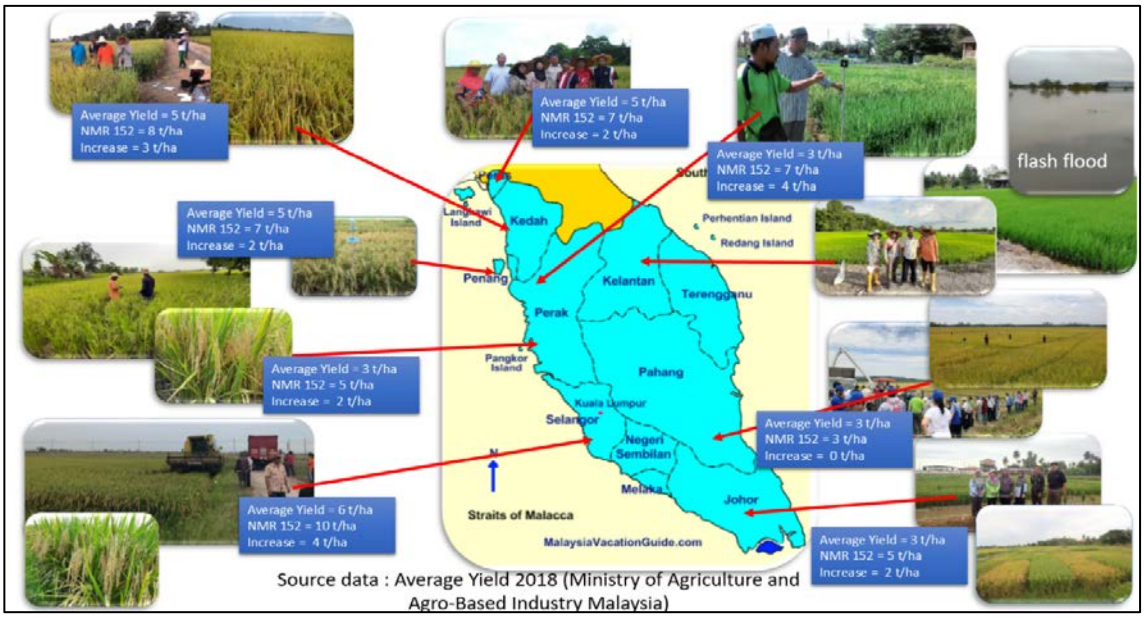


Figure 1: NMR 152 yield increment at different location in Peninsular Malaysia.

Table 3: Yield increment in different state of Malaysia as compared with data from MOA

Location (State)	Average Yield from MOA *(Agrofood Statistics 2018)*	Average Yield NMR152 (PBR 0156)	Yield Increment (t/ha) / (%)
Perlis	5 t/ha	7 t/ha	2 (40%)
Kedah	5 t/ha	8 t/ha	3 (60%)
Penang	5 t/ha	7 t/ha	3 (60%)
Perak	3 t/ha	5 t/ha	2 (67%)
Selangor	6 t/ha	10 t/ha	4 (67%)
Johor	3 t/ha	5 t/ha	2 (67%)

(Note: PBR = Certificate of registration of new plant variety and grant of breeder's right, MOA =Ministry of Agriculture and Food Industries, Malaysia)

Table 4: NMR 152 Local Verification Trial (LVT)

Location	Planting Season	Yield (t/ha)		
		Actual Yield (From factory)	CCT-Method (Yield estimation)	Wheel -Method (Yield estimation)
Sekinchan (IADA)	Main Season	10	15	8.2
	Off Season	9	13	7.5
Jenun (MADA)	Main Season	10	-	-
	Off Season	9	10	7.1
Pendang (MADA	Main Season	8	13	-
	Off Season	7	9	-
Changlun Non-granary area	Main Season	8	-	-
	Off Season	7	7.3	6.1
Utan Aji (MADA)	Main Season	7	10.0	-
	Off Season	6	9.0	-
Seberang Perak (IADA)	Main Season	5	7.0	6.5
	Off Season	4	-	-

(Note: IADA- *Integrated Agricultural Development Area*, MADA - *Muda Agricultural Development Authority* & MADA – *Muda Agricultural Development Authority*)

Figure 2: NMR152/ PBR 0156 field trial in 2015/16 under Malaysia Social Innovation Fund project at Northern Part of Malaysia (Code Project MSI 16010).

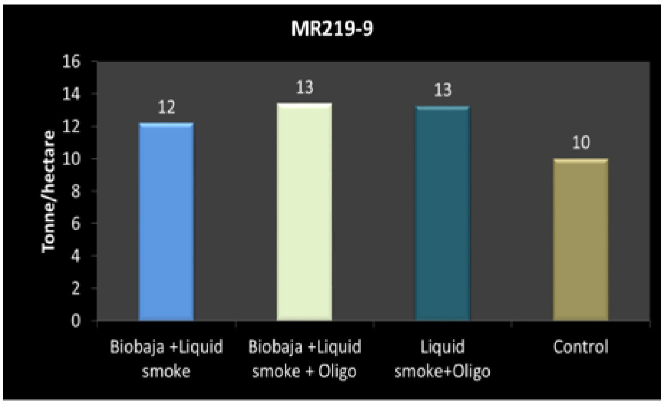


Figure 3a: Effect in yield production on MR219-9/NMR152 using different fertilizer treatment.

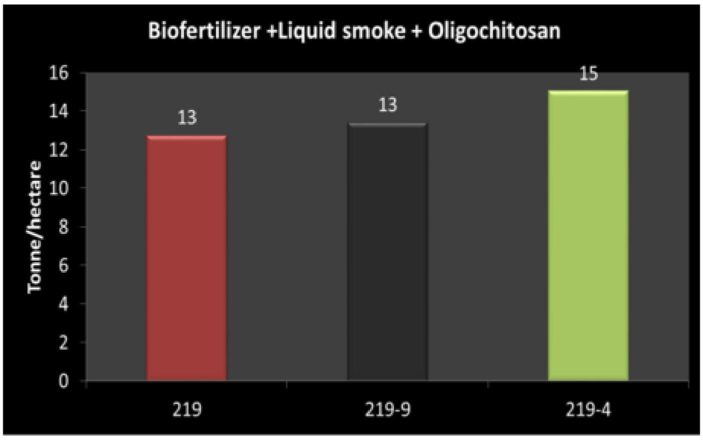


Figure 3b: Effect in yield production using different fertilizer treatment.

Upon completing the evaluation in the field including yield trial, disease screening and DUS trial (Distinctness, Uniformity & Stability), two mutant lines were successfully granted with Certificate of Registration of New Plant Variety and Grant of Breeder's Right by Department of Agriculture Malaysia in Feb 2020 with registration number; PBR0156 (referring to NMR152) and PBR 0159 (referring to NMR151). As for morphology and agronomy characteristics, there was no significant difference between NMR 151 and NMR 152 as both varieties performed similarly in terms of flowering time (82 days), day of maturity between 100 – 110 days and weight for 1000 grain seed of around 31 g (Table 5). The data obtained also showed that NMR 151 produced a high number of tillers per plant and high number of panicles per plant as compared to NMR 152.

Table 5: Morphology and Agronomy Characteristic NMR 152 and NMR 151

	NMR 152 (*PBR0156*)	NMR 151 (*PBR0159*)	Farmer Variety MR219
Characteristics	Remarks	Remarks	Remarks
Flowering time (50% flowering)	82 days	82 days	94 days
Culm height	82 cm	73 cm	76-78 cm
Panicle length	30 cm	29 cm	23 cm
No of panicle per plant	17	21	13
No of tiller per plant	20	22	15
Day of Maturity	100-110 days	105 – 110 days	105 - 111
Weight for 1000 grain seed	31.1 g	31.6 g	27.1 g
Seed length	11.1 mm	9.85 mm	6.58 mm
Width length	2.75 mm	2.67 mm	2.35 mm
Yield	7- 11 t/ha	7 – 9 t/ha	6.5-10.7 t/ha

A total of 4 seasons yield trial at Sekinchan, Selangor recorded that NMR152 showed a high yield (between 9 to 10 t/ha) as compared to other mutant lines (Figure 4). Farmers observation also showed that NMR 152 is resistance to bacteria leaf blight disease (*Xanthomonas oryzae pv oryzae*) as indicated in Table 6. Meanwhile, the inspection conducted by Agriculture Department of Malaysia indicated that NMR 152 could survive under several rice disease conditions such as Leaf Blast (*Pyricularia oryzae* Cavara), Panicle Blast, Brown Leaf Spot (*Helminthosporium oryzae* Breda de Haan), Sheath Blight (*Rhizoctonia solani* Kuhn), Brown Planthopper (Nilaparvata lugens Stal), False smut (Ustiloginoidea virens Cooke), Stem Borers (SB), Rice Ear Bug (*Leptocorisa oratorius* Fabricius)), Green leafhoppers (*Nephotettix virescens* Distant) and Rice leaffolder (*Cnaphalocrocis medinalis* sp). In addition, farmers in Sekinchan also indicated that NMR 152 is resistant to the lodging problem. Nevertheless, the area of infected is very much depending on the farmers' practice.

Figure 4: NMR 151 and NMR 152 rice mutant in Sekinchan, Selangor.

Table 6: Screening of NMR 151 and NMR 152 Varieties for Bacteria Leaf Blight (*Xanthomonas oryzae pv oryzae*)

S.N.	Accession number/variety	Disease severity		Host response	BLB resistance genes				
		14 DAI ± SD	21 DAI ± SD		*Xa21*	*Xa13*	*Xa5*	*Xa4*	*Xa2*
Resistant control	Tetep	3.08 ± 0.405	4.3 ± 0.340	MR	-	-	-	-	+
	Towuti	1.28 ± 0.263	2.03 ± 0.287	R	+	-	-	-	+
Susceptible control	MR 284	8.91 ± 0.557	12.41 ± 1.038	S	-	-	-	-	+
1	NMR 151	3.26 ± 0.332	4.96 ± 0.411	MR	-	-	-	-	+
2	NMR 152	1.44 ± 0.325	2.26 ± 0.272	R	+	-	-	-	+

(Source :Dr Nor' Aishah-UiTM)

Part 2: Evaluation of mutant lines derived from ion beam irradiation

In 2015, a total of 31 potential mutant lines were produced through mutagenesis of MR219 seeds with Carbon ion radiation (60 Gy). They were then planted at the Malaysian Nuclear Agency up to five generation (M5). These mutant lines (ML1 to ML31) were evaluated on morphological, yield and yield components, and compared to the parental variety, MR219. About 5% of M2 populations were selected for further screening in M3. After several series of selection and fixation, 31 potential lines with the required adaptive traits were recovered at M4 generation. The data obtained revealed that the best dose (Shoulder dose) was observed at 60 Gy for Pongsu Seribu2. In terms of growth rate, the data showed that the pattern started to decline from 60 to 120 Gy (Figure 5) and germination rates were more than 90% in the range of 0 – 120 Gy (Figure 6). This observation was similar to those reported by Hidema et al. (2003). However, variety PS2 could still be able to survive at 120 Gy as shown in Figure 7. As for MARDI (B001) the optimum dose was observed between 40 and 80 Gy while the shoulder dose response curve for MARDI C002 was observed at 40 Gy (Figure 8). In terms of spikelet fertility, declining pattern was significantly observed from 60 to 120 Gy for all samples tested (Figure 8), similar pattern was also observed in variety Pongsu Seribu2 (Figure 6). Studies for other varieties are currently in progress.

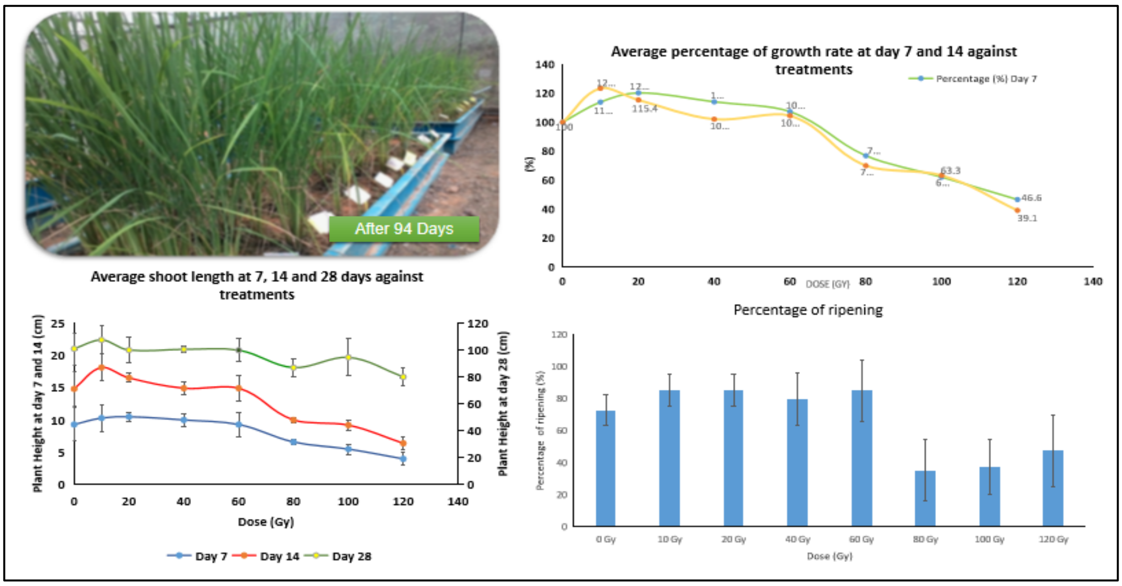
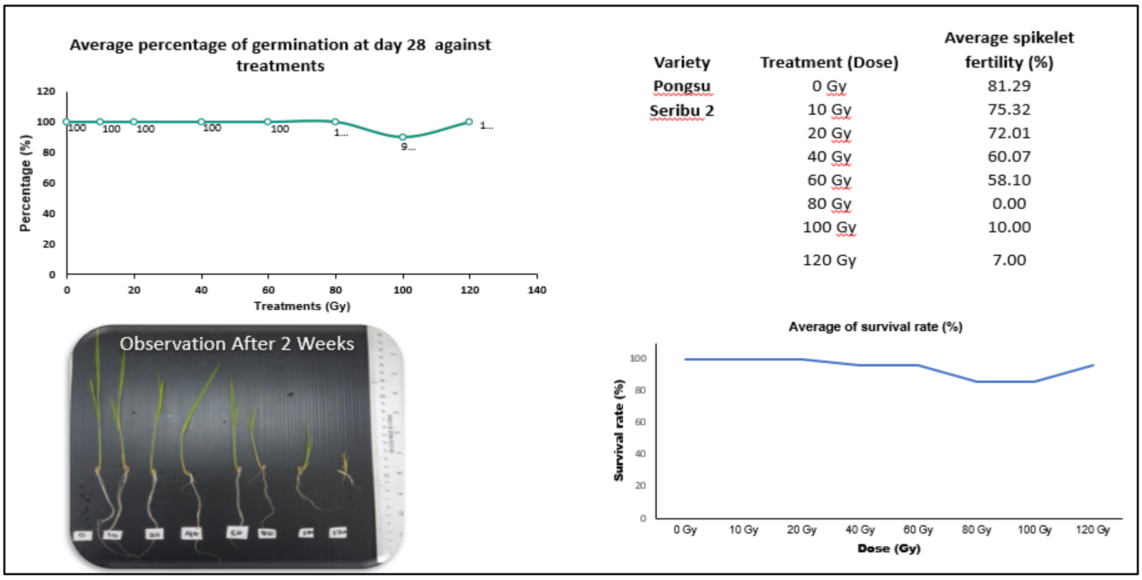


Figure 5: Dose response curves for percentage growth rate, plant height and ripening (Pongsu Seribu2).



Variety	Treatment (Dose)	Average spikelet fertility (%)
Pongsu Seribu 2	0 Gy	81.29
	10 Gy	75.32
	20 Gy	72.01
	40 Gy	60.07
	60 Gy	58.10
	80 Gy	0.00
	100 Gy	10.00
	120 Gy	7.00

Figure 6: Dose response curves for germination rate, survival rate and spikelet fertility (Pongsu Seribu2).

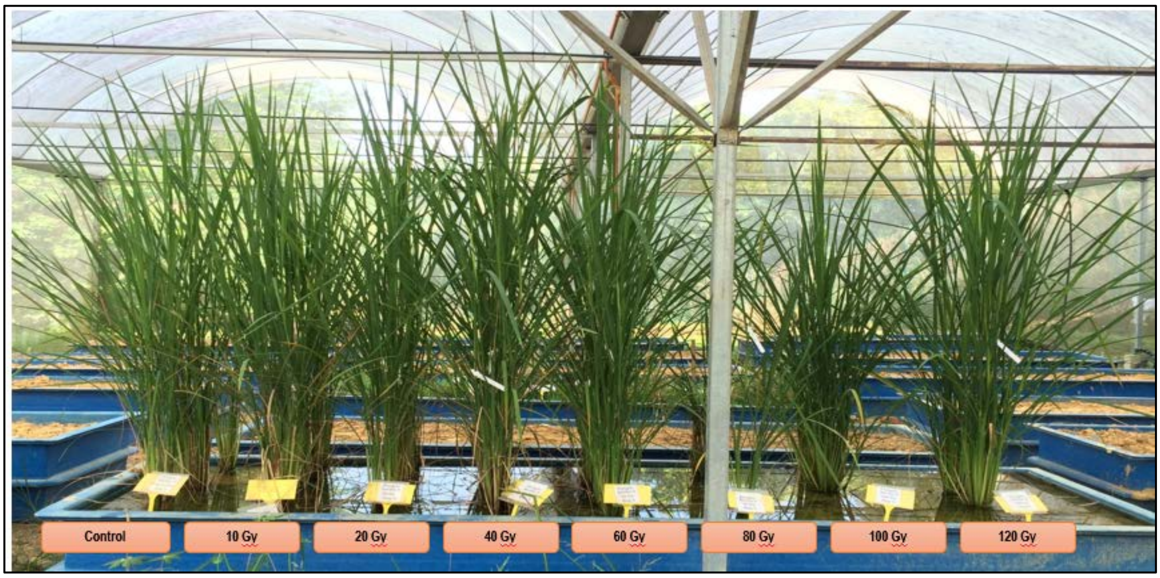


Figure 7: Biological effect of carbon ion on rice (Pongsu Seribu2).

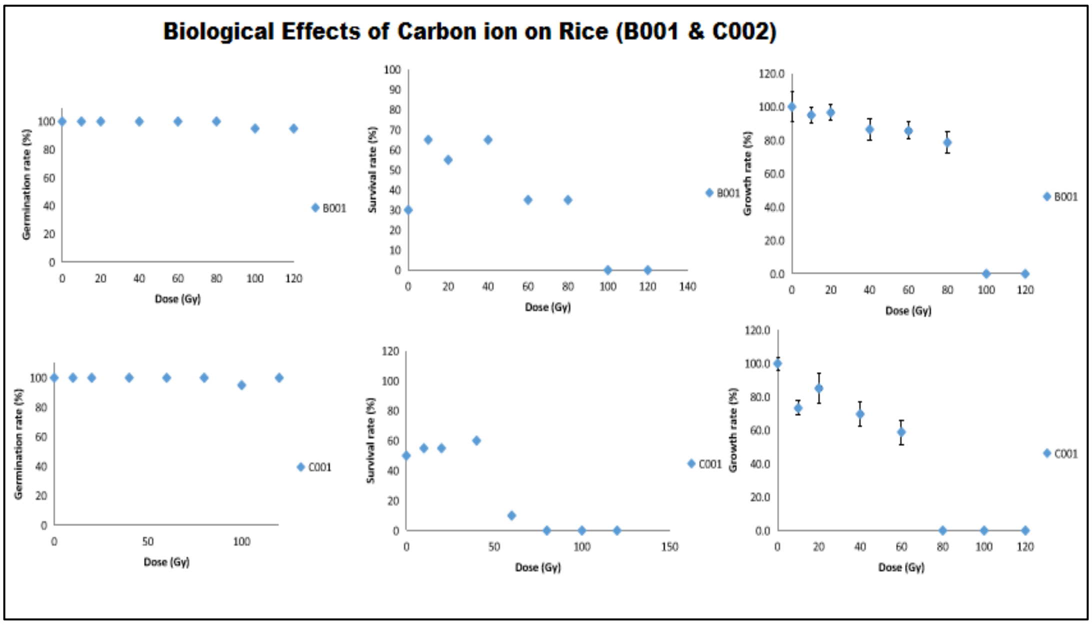


Figure 8: Biological effect of carbon ion on rice (B001 & C002).

The data obtained after M6 generation showed that the highest yield was significantly observed in MINT 4 (10.04 ± 3.38 t/ha) and followed by MINT 3 (9.52 ± 2.80 t/ha), MINT 10 (9.42 ± 3.24 t/ha), MINT 5 (8.68 ± 2.99), MINT 9 (8.46 ± 0.43). In the meantime, similar pattern was observed in MINT 2, MINT 7 and MINT 8 which only produced 7 t/ha (Table 7). In terms of weight for 1000 seeds, most of the potential mutant lines recorded a value between 26g to 32g. The data also showed some correlation between weight for 1000 seeds and actual yield as indicated in MINT 4 with 32.20 ± 0.07 g of seeds were produced around 10.04 ± 3.38 t/ha. This result showed that the highest weight of 1000 seeds will contribute to the highest yield. Contrarily, the number of tillers was not correlated to a high yield in mutant line. Figure 9 shows the potential mutant line derived from ion beam at Sekinchan Selangor.

Figure 9: Mutant derived from ion beam at M6 generation (Sekinchan, Selangor).

Table 7: Morphology and Agronomy Characteristic MINT 1 -MINT 10 (Ion Beam)

Mutant Line	Actual Yield (t/ha) (From factory)	Weight 1000 Seeds (g)	No of Tiller	Average Height (cm)
MINT 1	8.13 ± 0.03	30.02 ± 0.09	27 ± 3	100.40 ± 3.40
MINT 2	7.86 ± 1.66	29.40 ± 0.11	28 ± 2	115.00 ± 0.90
MINT 3	9.52 ± 2.80	29.30 ± 0.16	23 ± 2	124.40 ± 3.30
MINT 4	10.04 ± 3.38	32.20 ± 0.07	21 ± 2	115.80 ± 4.50
MINT 5	8.68 ± 2.99	29.40 ± 0.07	25 ± 3	116.40 ± 1.30
MINT 6	6.87 ± 1.32	29.40 ± 0.08	16 ± 3	123.00 ± 2.00
MINT 7	7.00 ± 0.42	30.70 ± 0.11	19 ± 3	126.40 ± 6.40
MINT 8	7.22 ± 0.30	31.40 ± 0.17	14 ± 3	118.00 ± 2.80
MINT 9	8.46 ± 0.43	29.10 ± 0.12	20 ± 3	102.00 ± 1.90
MINT 10	9.42 ± 3.24	26.0 ± 0.56	27 ± 6	111.63 ± 2.20

(Note : Data from Sekinchan mutant plot – Using farmers practice)

Part 3: Evaluation and characterization of advanced rice mutant lines of rice MR219-4 (NMR151 / PBR 0159) and MR219-9 (NMR 152/ PBR 0156) under drought conditions

Leaf rolling and leaf drying are used as an indication of tolerance in drought studies. In this study, leaf rolling and leaf drying were recorded under water stress condition. Table 8 shows the results of ANOVA for leaf rolling and leaf drying for the evaluated lines. The results showed that there were significant differences among the evaluated lines for leaf rolling and leaf drying. The mean values for leaf rolling and leaf drying of each line are presented in Table 8.

Table 8: Mean value of leaf rolling and leaf drying traits of evaluated lines

Line	Leaf rolling mean score	Leaf drying mean score
MR219-4	3	1
MR219-9	1	1
MR211	7	7
MR219	5	3
ARN 1	0	1
$LSD_{(0.05)}$	0.29	1.70

An early sign of soil water declining is leaf rolling which is a simple expression of leaf wilting. Fischer et al. (2003) have suggested leaf rolling as a criterion for scoring drought tolerance in rice cultivars. Therefore, leaf rolling is useful for quick screening hundreds of lines (Shyful A.A.R et al., 2017). The method of screening is by scoring the plants on a scale 0 to 9 (Table 8) according to Standard Evaluation System adopted for rice (IRRI 1996). A drought resistant check variety, ARN 1 had the best score of 0 for leaf rolling. Meanwhile, MR211, the susceptible check variety had a score of 7 indicating susceptibility to drought. MR219 had a score of 5 which was considered as moderately susceptible. Thus, in this study, MR219-4 can be considered as moderately resistant and MR219-9 as resistant to drought.

According to Fischer et al., (2003), typically leaf drying begins at the tip of the leaf, which is usually under greater water deficit than the basal part that closer to the stem. Leaf drying was observed visually by scoring the plants on a scale 0 to 9 (Table 8) based on Standard Evaluation System adopted for rice (IRRI 1996). Leaf water deficiency can be further reduced beyond the point of turgor loss in which reaching the point of tissue death (Fischer et al., 2003). According to Kadioglu and Terzi (2007), low score of leaf drying can be advantageous in terms of less damage under water stress. In this study, lower score in MR219-9 and MR219-4 which had scored 1 indicating that they were less damage from water stress.

There were significant differences between treatments for number of tillers, days to flowering, plant height, flag leaf area and days to maturity (Table 9). Lines showed significant differences ($p<0.05$) for number of tillers, days to flowering, flag leaf area, panicle length and days to maturity (Table 9). There was significant interaction between treatments and lines for number of tillers, days to flowering, flag leaf area, panicle length and days to maturity.

Table 10 shows that there were significant differences ($p<0.05$) between treatments for grain weight per plant, grain yield per plant, 100-grain weight, dried plant weight and harvest index. The lines were significantly different ($p<0.05$) for grain weight per plant, grain yield per plant, 100-grain weight, dried plant weight, biomass and harvest index (Table 10). There was significant interaction between treatments and lines for grain weight per plant, grain yield per plant, 100-grain weight, dried plant weight, biomass and harvest index.

Table 9: Analysis of variance for morphological traits

Source	Df	Mean squares					
		Number of tillers	Days to flowering	Plant height (cm)	Flag leaf area (cm^2)	Panicle length (cm)	Days to maturity
Treatments (T)	1	0.07	0.0034*	17391	532.12*	30.63	0.0111*
Replications/T (R/T)	4	0.06	0.0001	9296	93.56	11.69	0.0001
Lines (L)	4	0.18*	0.1440*	6385	168.40*	29.25*	0.0820*
T × L	4	0.13*	0.0016*	1113	169.13*	7.96	0.0004*
(R/T) × L	16	0.03	0.0003	9496	45.01	19.85	0.0002
Plants/Plot	60	0.02	0.0001	9061	24.81	7.38	0.0001

*significant at level p<0.05

Table 10: Analysis of variance for agronomic traits

Source	Df	Mean squares					
		Grain weight per plant (g)	Grain yield per plant (g)	100-grain weight (g)	Dried plant weight (g)	Biomass (g)	Harvest index
Treatments (T)	1	267.67*	79.88*	0.41*	596.86*	65.13	0.040*
Replications/T (R/T)	4	20.42	9.80	0.10	173.80	186.63	0.010
Lines (L)	4	104.95*	58.33*	2.16*	636.93*	1214.72*	0.009*
T × L	4	124.87*	55.91*	0.21	350.76*	864.66*	0.005
(R/T) × L	16	21.29	7.65	0.71	170.03	173.51	0.006
Plants/Plot	60	20.59	11.94	2.25	110.17	167.49	0.003

*significant at level p<0.05

Part 4: Evaluation of Nitrogen uptake in advanced mutant lines of MR219-4 (NMR151 / PBR 0159) and MR219-9 (NMR 152/ PBR 0156)

The advanced mutant line MR219-9 (NMR152/PBR0156) showed comparable growth, yield and N uptake under both flooded and non-flooded conditions (Table 11). The yield and yield components are not significantly different from the parent (MR219) but total N uptake was lower than MR219 regardless of water regime. The field trial showed that MR219-9 (NMR152/PBR0156) has a better total N content which is comparable to the aerobic rice check variety (MRIA 1) and this indicates that this advance mutant line MR219-9 is a potential aerobic rice variety. However, further research is needed for the development of a comprehensive agronomic management procedure of MR219-9 (NMR152/PBR0156) rice mutant line.

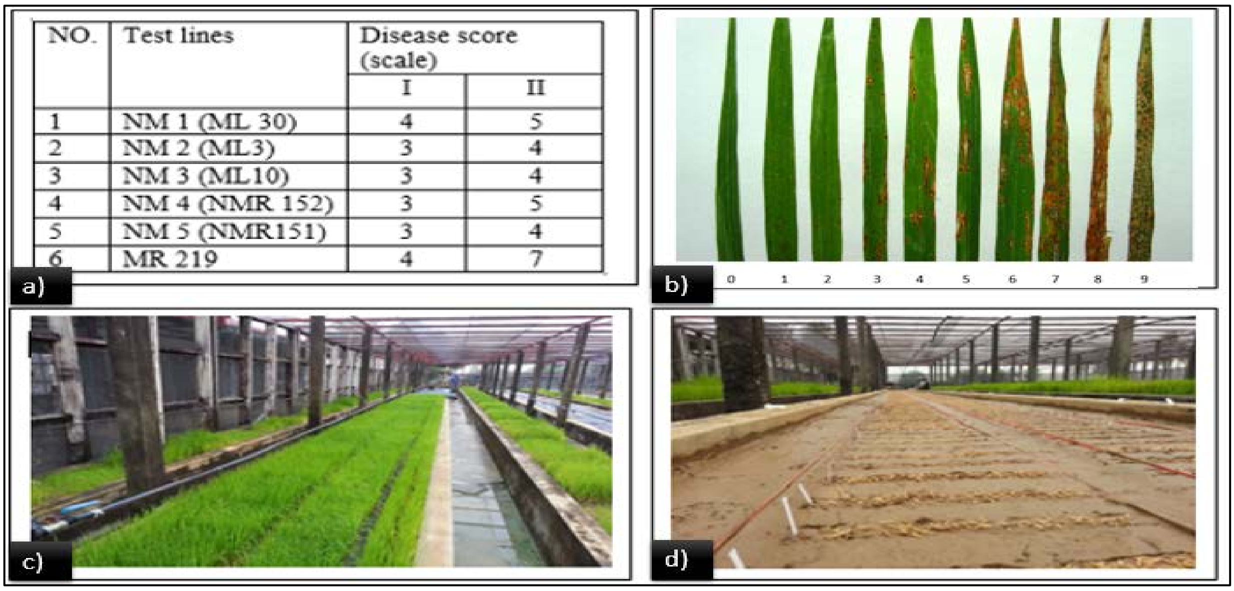
Table 11: Total N uptake and 15N of MR219-9/NMR152/PBR0156 under flooded and non-flooded condition

Treatment	Ndff (mg/pot)	%	Ndfs (mg/pot)	%	Total N (mg/pot)
MR219 (Non flooded)*	133.0 a	15.50	725.0 a	84.50	858.0 a
MR219-9 (Non flooded)	104.7 b	13.95	645.7 c	86.05	750.4 c
MR219 (Flooded)**	128.5 a	15.72	688.9 b	84.28	817.4 b
MR219-9 (Flooded)	96.9 b	12.85	657.0 c	87.15	753.9 c

*Non flooded – 0-30 kPa
** Flooded – 5 cm water depth

Part 5: Screening of Foliar blast disease in selected potential mutant lines

As for disease screening the data revealed that, there were only slight disease progress from 21 DAS to 28 DAS except for control (MR219) (Figure 10). At 21 DAS, all the test lines were scored within the range 3-4, which showed moderately resistant (MR) while at 28 DAS, the test lines scored 4-5, which signified moderately resistant (MR) to moderately susceptible (MS) but the control check MR219 scored 7, which was susceptible. Thus, this study indicated that NM2 (ML3), NM3 (ML10) and NM5 (NMR 151) could be slightly better candidate for foliar blast resistant as compared to NM1 (ML30) and NM4 (NMR 152).



NO.	Test lines	Disease score (scale) I	Disease score (scale) II
1	NM 1 (ML 30)	4	5
2	NM 2 (ML3)	3	4
3	NM 3 (ML10)	3	4
4	NM 4 (NMR 152)	3	5
5	NM 5 (NMR151)	3	4
6	MR 219	4	7

(Source : MARDI)

Figure 10: a) Observation disease reaction after 28 days (DAS),
b) Folia blast scale index,
c) Test lines rows along with both side bombardment rows,
d) Test lines seeds sown in rows between the two inoculum beds.

Part 6: Evaluation on effects of MR219 mutant rice on glycaemic responses in BALB/c mice

Mean comparisons and blood glucose response among the treatments

The ANOVA revealed highly significant differences among the treatments, sampling time and also in the interaction of treatments with sampling time. Most of blood sugar level of food samples reached its maximum reading at 60 min after feeding and significantly dropped at 120 min (Table 12). The mice were recorded to have the highest blood glucose level at this particular time where the carbohydrates of the food were fully digested and reacted in blood glucose change. The extent and duration of the blood glucose response depend on the absorption rate which in turn depends on factors such as gastric emptying as well as the rate of hydrolysis and diffusion of nutrients in the gut (FAO-WHO, 1998).

Table 13 shows the mean values for glucose reading among the treatments. Based on the results, the highest blood glucose reading was recorded in mice fed with standard glucose (8.11 mmol/L). Three food samples namely; ML18, ML21, and the parental line MR219 caused a pre-diagnosis of diabetes on the BALB/c mice. The glucose reading of the blood collected from mice fed with those three food samples were 5.96, 6.34 and 6.21 mmol/L, respectively. Meanwhile, two food samples namely; ML3 and ML30 showed normal glucose level with the values of 5.47 and 5.49 mmol/L, respectively. The low glucose reading was observed in blood collected from mice that were fed with the check variety MRQ74 and saline water (4.87 and 4.78 mmol/L, respectively).

Table 12: Glucose readings after given test samples

Sampling time (minutes)	Mean (glucose reading, mmol/L)
0	4.97
30	5.99
60	6.41
90	6.37
120	5.78

Least Significance Difference (α = 0.05) = 0.21.

Table 13: Mean glucose reading and glycemic index of treatments

Rice line / control	Mean (glucose reading, mmol/L)
Glucose	8.11(D)
Saline water	4.87 (N)
MRQ74	4.78 (N)
MR219	6.21 (PD)
ML3	5.47 (N)
ML18	5.96 (PD)
ML21	6.34 (PD)
ML30	5.49 (N)

Least Significance Difference (α = 0.05) = 0.27.

Glucose reading: (D) Diagnosis of diabetes = ≥ 7.0 mmol/L, (PD) Pre-diabetic condition = 6.0 – 6.9 mmol/L, (N) Normal glucose level = ≤ 5.9 mmol/L (ADA, 2012). Glycemic Index: 0 - 55 = low (L), 56 - 69 = moderate (M), 70 or more = high (H) (Brand-Miller *et al*., 2002).

The glycaemic index (GI) is the incremental area under the blood glucose response curve, and is shown in Figure 11. The higher the amylose content of each food samples, the incremental of the area under curve became smaller and resulted in a low GI. Although the relationship between rice starch and GI is complex, amylose content is generally accepted as being the principal determinant of GI in rice (Larsen et al., 2000).

Here, ML3 and ML30 both had moderate GI rating with a value of 65.00% and 66.00%, respectively. Amylose content of ML3 was the highest among other mutant lines and parental variety, resulted in low GI as compared to others. Meanwhile, ML21 had the highest GI value (75.00%) because of its low amylose content (20.70%). Amylose is a tightly packed structure. Thus, it is more resistant to digestion. It breaks down more slowly, releasing glucose more gradually into the bloodstream and lowers the insulin demand. High amylose rice is less sticky, and have a much lower glycaemic load, which could be beneficial for diabetic patients. As a conclusion, high amylose content food should have a low GI and vice versa. Some researchers reported that low amylose content rice has high GI than intermediate and high amylose content rice (Panlasigui, 1989; Juliano et al., 1989a; 1989b). However, in the present work, the parental variety MR219 amylose content was higher than ML30 but it had high GI value as compared to ML30. This scenario might be due to different response of some variety or rice mutant lines crossed the sampling time or due to a delayed enzymatic hydrolysis as mentioned by O'dea et al. (1980).

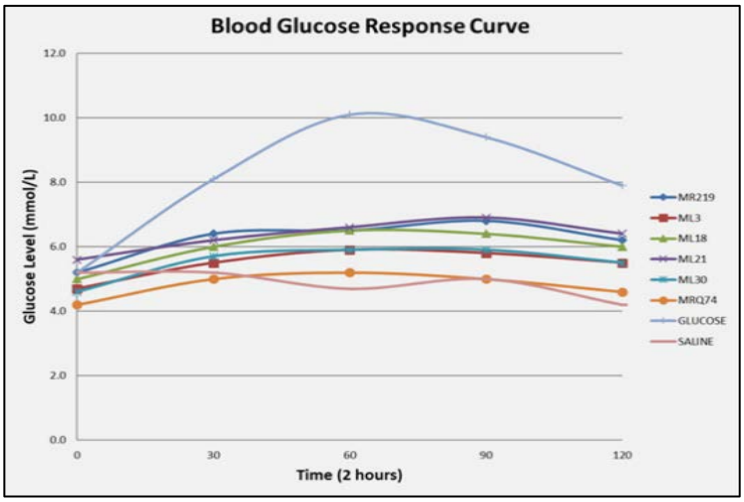


Figure 11: Blood glucose response curves of tested foods at 30minutes intervals

Conclusion

In summary, the data obtained from the farmers revealed that NMR 152 had consistently produced between 9-10 t/ha in granary area as compared to 6 t/ha produced by other varieties within the same planting areas. Field trials also showed that the production cost was reduced by 10%, mainly due to the reduction in fertilizer and pesticide usage. At the same time, the yield could be increased between 40 - 60%, depending on the planting areas. Ion beam irradiation at 60 Gy significantly induced the genetic variability in physicochemical characteristics and nutritional compositions. The estimation of glycemic index revealed that two mutant lines could be consumed by diabetic patients. The two mutant lines (ML3 and ML30) were recorded to have normal glucose reading which was identified to have a moderate GI of 65 and 66, respectively. As low and moderate GI foods are recommended for diabetic patients, these two mutants (ML3 and ML30) have a high potential for their consumption. The findings also raise the value of knowing the GI in our food for awareness. Further study should be carried out on ML3 and ML30 by testing these mutant lines on diabetic patients. As for mutation induction from variety Pongsu Seribu2 using ion beam, data from the M6 generation showed that the highest yield was significantly observed in MINT 4 (10.04 ± 3.38 t/ha) and followed by MINT 3 (9.52 ± 2.80 t/ha), MINT 10 (9.42 ± 3.24 t/ha), MINT 5 (8.68 ± 2.99), MINT 9 (8.46 ± 0.43).

In conclusion, mutation breeding is still one of the promising techniques for the development of new and novel varieties in combination with advanced molecular genetics that can bring plant mutation breeding into new era. Apart from that, the current FNCA project fits well in addressing the climate change issue. The mutant and organic input will help in mitigating production issues (low yield, less water resources, soil fertility) in rice cultivation affected by environment impact as a result of climate change.

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References

Abdul R,H. Strategi Peningkatan Pengeluaran Padi dan Beras Berkualiti ke Arah Pasaran Eksport. Persidangan Padi Kebangsaan 2017. m/s 78-81, 26-28 Sep 2017. Dorsett Grand Subang Selangor.

Abdullah, M.Z., Mohamad,O., Azlan, S., Habibuddin,H., Saad, A., Kahzim,K. and Senawi,M.T. (2003). Rice breeding and germplasm exchange: The Malaysian perspective. Proceedings of an international rice conference 2003, 13-16 October 2003, Alor Setar, Kedah, Malaysia.

Ahmad Nazrul Abd Wahid (2016). Nitrogen Dynamic and Carbon Isotope Discrimination in Aerobic Rice Agroecosystem Under Environmentally Controlled Condition. Thesis MSc.

Asma I., Rafii M.Y., Sobri H., Ling A.P.K., Koh R.Y., Mahmud M.T.M., Siti Zaharah S., Khairuddin A.R., Asfaliza R. Rahim H.A., Yield, Physico-chemical and Nutritional Characteristics of MR 219 M_3 Generation and their Effects on Glycemic Index and Responses in BALB/c Mice. The First International Conference on Genomics, Traits & Business. 21-24 Sep 2014. Hilton Charlotte University, Charlotte, North Carolina. USA.

Asma I.K., Rafii, M.Y., Sobri H., Anna L.P.K., Rahim A.H., Mahmud T.M.M., Siti Z.S., Asfaliza R. and Oladosu Y. 2019. Physicochemical characteristics and nutritional composition of MR219 mutant rice and their effects on glycaemic responses in BALB/c mice. International Food Research Journal 26(5): 1477-1484.

Asma, I, Rafii, Y, Sobri, H, Rahim, A.H, Mahmud. T.M.M, Siti Zaharah, S, Rusli, I, and Asfaliza, R. Penilaian Ciri Morfologi, Hasil Padi Mutan-MR 219. Persidangan Padi Kebangsaan 10-12 Dec 2013.

Azhar M., Sobri H., Faiz A. & Rahim H. Penggunaan Penanda Molekul di Dalam Penyaringan Baka Padi Terpilih Berhasilan Tinggi. Persidangan Pesawah Padi Kebangsaan 2019. 6-7 August 2019. UPM Serdang.

Bhuiyan, S.I. (1992). Water management in relation to crop production: case study on rice: Outlook Agric. 21(4): 293-299.

Fischer, K.S., Lafitte, R., Fukai, S., Atlin, G., Hardy, B., Breeding rice for drought–prone environments, (2003) IRRI, Los Banos.

H. Nor' Aishah, H. Abdul Rahim, A.R. Khairuddin, H. Sobri, M.N. Norain, M.Z. Nursamahah. Assessment of purity of F1 plants derived from the cross of MR264 and Pongsu Seribu 2 using Microsatellite Markers. Proceedings of International Conference on Sustainable on Environment and Agriculture (ICSEA 2013) 17- 18 Nov 2013.

Hase, Y., Tanaka., Baba, T. and H. Watanabe (2000). FRL1 is required for petal and sepal development in Arabidopsis. Plant Journal 24, 21-32.

Hidema, J., Yamamoto, M., Kumagai, T., Hase., Sakamoto A., and Tanaka, A. (2003). Biological Effects of Carbon Ion on Rice (*Oryza sativa* L.) JAERI-review.:85-87.

Juliano, B.O., Perez, C.M., Komindr, S. and Banphotkasem, S. 1989a. Properties of Thai cooked rice and noodles differing in glycemic index in noninsulin-dependent diabetics. Plant Foods for Human Nutrition 39(4): 369-374.

Juliano, B.O., Perez, C.M., Maranan, C.L., Abansi, C.L. and Duff, B. 1989b. Grain quality characteristics of rice in Philippines retail markets. The Philippine Agriculturist 72(2): 113-122.

Juliano, B.O. (1971). A simplified assay for milled rice amylose. Cereal Science Today.16:334-338.

Kadioglu, A. & Terzi, R. 2007. A dehydration avoidance mechanism: Leaf rolling. *Botanical Review* 73(4): 290-302.

Khairuddin Abdul Rahim, Abdul Rahim Harun, Shyful Azizi Abdul Rahman, Ahmad Nazrul Abd Wahid, Faiz Ahmad, Sobri Hussein, Shakinah Salleh, Rusli Ibrahim (2015). Nitrogen Uptake Response By New Rice Varieties, 219-4 And 219-9, Adaptable To Low Water Input In Location Trials, progress report IAEA Research Contract No: 16861.

Larsen, H. N., Rasmussen, O. W., Rasmussen, P. H., Alstrup, K. K., Biswas, S. K., Tetens, I. and Hermansen, K. 2000. Glycaemic index of parboiled rice depends on the severity of processing: study in type 2 diabetic subjects. European Journal of Clinical Nutrition 54: 380-385.

Ling P.K.A., Ung Y.C., Hussein S., Harun A.R., Tanaka A. & Yoshihiro H. Morphological and biochemical responses of Oryza sativa L. (cultivar MR219) to ion beam irradiation. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) ISSN 1673-1581.2013.

Mohamad, O., Mohd Nazir, B., Alias, I., Azlan, S., Abdul Rahim, H.,Abdullah, M.Z.,Othman, O., Hadzim, K., Saad, A., Habibuddin, H. and Golam F. (2006). Development of Improved Rice - 59 - Varieties through the Use of Induced Mutations in Malaysia.Plant Mutation Reports. 1: 27-34.

Morita, R., Kusaba, M., Iida, S., Nishio, T. and Nishimura, M. (2007). Knockout of glutelin genes which form a tandem array with a high level of homology in Rice by Gamma Irradiation.Genes Genet.Syst.82, p.321-327.

Nishimura, M., Morita, R. and Kusaba, M. (2009). Utilization and Molecular Characterization of Seed Protein Composition Mutants in Rice Plants. JARQ 43(1), 1-5.

Norain M. N., Shamsiah A., Abdul Rahim H., Nor'Aishah H., Haslinda Ab. M. & Wan Aminuddin W.A. Correlation Analysis on Agronomic Characters in F2 Population Derived from MR64 and Pongsu Seribu 2. Journal of Applied Science and Agriculture, 9(18) Special 2014, Pages: 143-147.

O'Dea, K., Nestal, P. J. and Antonoff, L. 1980. Physical factors influencing postprandial glucose and insulin responses to starch. The American Journal of Clinical Nutrition 33(4): 760-765.

Panlasigui, L. N. 1989. Glycemic response to rice. Canada: University of Toronto, PhD Thesis.

Phua C, K, H., Halimi M.S., Khairuddin A, R., Che Fauziah I. & Puteri E.M.W. (2019). Effect of gamma-irradiated *Acinetobacter calcoaceticus* on nitrogen and phosphorus uptake of green mustard (*Brassica chinensis*). Malaysian Journal of Soil Science Vol. 23: 149-166. ISSN:1394-7990.

Rahim, A.H, Rafii, M.Y, Sobri, H, Rusli, I, Tanaka, A, Mohd Nasir, A, Nor'Aishah, H, Norain M.N. Kesan Sinaran Ion Terhadap Ciri Agronomi Padi (*Oryza Sativa*) Cv MR21. Persidangan Padi Kebangsaan.

Revandy. I.D., Maziah.M., Ismail.M.R., Syahida.A. Abdullah.M.Z(2010). Responses of the antioxidative enzymes in Malaysian rice (*Oryza sativa* L.) cultivars under submergence conditioin. Acta Physiology Plant.

Rusli Ibrahim, Abdul Rahim Harun, Khairuddin Abdul Rahim, Shyful Azizi Abdul Rahman, Ahmad Nazrul Abd Wahid, Faiz Ahmad, Sobri Hussein, Shakinah Salleh, (2015). Mutation Breeding for the Evaluation of Advanced Mutant Lines of Aerobic Rice for Yield Potential and Stability under Water Stress Condition, progress report IAEA Research Contract No: 16860.

S.A.A. Rahman, P.C.K Hoe, A.N.A. Wahid, S. Hussein, F. Ahmad, S. Salleh, R. Ibrahim, A.R. Harun, K.A. Rahim, M.Mahmud, Z.S. Kamarudin, M.R. Yusop. 2017. Improvement of Advanced Rice Mutant Lines Through Enhancement of Nutrient and Water Use Efficiency in IAEA TECDOC Series-1828, Approaches to Improvement of Crop Genotypes with High Water and Nutrient Use Efficiency for Water Scarce Environments.

Shakinah Salleh, Faiz Ahmad, Sobri Hussein, Abdul Rahim Harun and Khairuddin Abdul Rahim (2016). Chareacterization of Rice Mutants for Registration as New Plant Variety, 3rdInternational Plant Breeding Seminar, 15-16 November, Bangi, Malaysia.

Shyful A. A.R., Zarifth S. K., Phua C. K. H., Maznah M., Ahmad Nazrul A. W., Sobri H., Faiz A., Shakinah S., Mohd R. Y., Rusli I., Abdul Rahim H. & Khairuddin A.R. 2017. Improvement of Advanced Rice Mutant Lines through Enhancement of Nutrient and Water Use Efficiency. IAEA –TECDOC 1828.

Sobri Bin Hussein, Abdul Rahim Bin Harun, Azhar M,Shakinah Binti Salleh, Zaiton A, Sakinah A, Faiz Bin Ahmad, Phua Choo Kwai Hoe, Nur Humaira L.A, Shyful Azizi Bin Abdul Rahman, Ahmad Nazrul A.W, Latiffah Binti Nordin, Atsushi Tanaka, Anna Ling Pick Kiong, Mohd Rafii Bin Yusop4,Kogeethavani. (2018). Mutation Breeding in Malaysia. 3rd World Biotechnology Congress 2018, São Paulo, Brazil.

Sobri H., Faiz A., Azhar M. & Abdul R,H. Pembangunan Varieti Padi Baharu Menggunakan Teknik Mutasi Aruhan. Persidangan Pesawah Padi Kebangsaan 2019. 6-7 August 2019. UPM Serdang.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255: 571 – 586.

Yu, Z.L., Yu, L.D., Vailathong, T. and Brown, I.G. (2006). Introduction of Ion BeamBiotechnology (Transls.), Springer-Verlag, New York.

Yusuff Oladosu, M. Y. Rafii, Norhani Abdullah, Mohammad Abdul Malek, Abdul Rahim Harun, Ghazali Hussin, Mohammad Abdul Latif & Isiaka Kareem. Genetic Variability and Selection Criteria in Rice Mutant Lines as Revealed by Quantitative Traits. The Scientific World Journal. Volume 2014, Article ID 190531, 12 pages,http://dx.doi.org/10.1155/2014/190531.

Zarifth S, K., Mohd Rafii Y., Abdul Rahim H. & Mohd R, I. Prestasi Hasil dan Komponen Hasil Untuk Toleransi Kemarau Ke Atas Titisan-titisan Maju Padi Mutan. Persidangan Pesawah Padi Kebangsaan 2019. m/s 62-65. 6-7 August 2019. UPM Serdang.

Zarifth S,K., Mohd Rafii Y., Mahmud T, M, M.,Mohd R, I. & Abdul Rahim H. Growth Performance and Antioxidant Enzyme Activities of Advanced Mutant Rice Genotypes Under Drought Stress Condition. Agronomy 2018, 8, 279; doi:10.3390/agronomy8120279.

Zarith Shafika Kamarudin (2018). Morpho-Physiology and Yield Performance, and LEA Genes Expression for Drought Tolerance of Advanced Mutant Rice Lines. Thesis PhD.