FNCA／MEXT
Technical Material

Achievement
Sub-Project on Mutation Breeding of Rice for Sustainable Agriculture

(FY 2013 - 2017)

Mutation Breeding Project

Forum for Nuclear Cooperation in Asia (FNCA)

June, 2020

Forward

Mutation breeding is not a new technology but its continued application throughout the world proves its usefulness. Mutation breeding is a simple and ubiquitously applicable technique, and is advantageous in improving one or a few specific traits of the preferred variety. The rice is the most important staple food crop particularly in Asian countries. The fist subproject on rice under the FNCA Mutation Breeding Project, namely "Composition or Quality Improvement in Rice", was successfully implemented from FY2007 to FY2012. Subsequently, the subproject "Mutation Breeding of Rice for Sustainable Agriculture" was carried out from FY2013 to FY2017 with ten participating countries. This report covers the research activities of the last subproject.

Sustainable agriculture is a very important issue that we have to address under the global climate change. The last subproject was aimed to develop mutant varieties and promising mutant lines that contribute to the sustainable agriculture. Most of the participating countries succeeded to obtain high-yielding mutant lines with enhanced tolerance to biotic and/or abiotic stresses. Some mutant lines have already been released and brought economic benefits. We have to say that sustainable agriculture cannot be achieved in a short time; however, the last subproject was successful in that people involved in this subproject started to consider what we should do and demonstrated that the mutation breeding can contribute to this issue.

I believe mutation breeding technology has been built on years of research experience. I hope this report can be of any help to share the knowledge among the people involved in this research field.

Finally, on behalf of the people involved in this subproject, I would like to extend my gratitude to the Ministry of Education, Culture, Sports, Science and Technology, Japan, and Nuclear Safety Research Association for their continuous support.

April, 2020

Yoshihiro HASE
Project Leader of Japan, Mutation Breeding Project, FNCA
Senior Principal Researcher,
Takasaki Advanced Radiation Research Institute,
National Institutes for Quantum and Radiological Science and Technology (QST)

Contents

Development of Rice Mutant Variety with Higher Yield and Improved Agronomic Traits through Carbon Ion Beam Irradiation

A. N. K. Mamun[1], M. H. Kabir[1], A. K. Azad[2], M. A. Azam[2], M. R. Islam[1], M. T. Jahan[1], Md. Monirul Islam[1], Pronabananda Das[1] and Md. Monirul Islam[1]

[1] Plant Biotechnology and Genetic Engineering Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Bangladesh,

[2] Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Bangladesh.

Summary

BINA dhan14 is becoming more popular to the farmers due to the advantage of higher yield, early maturity, long grain fine rice and less water requirement over parent as it is cultivated in rain-fed condition. Advanced promising mutant line obtained from BRRI dhan 29 was released as a variety called BINA dhan18 and BINA dhan19 was developed from NERICA10. These varieties were developed through carbon ion beam irradiation from the QST, Takasaki, Japan with kind cooperation of FNCA during the period of late 2013 to early 2018. The main criteria of these varieties are lodging resistant with higher yield, long grain fine rice. BINA dhan14 is cultivated during Boro and Aus season, BINA dhan18 is moderate duration with higher yield Boro rice and BINA dhan19 is highly drought tolerant and also cultivated round the year. BINA dhan18 is about 100 cm tall and requires 148-153 days (seed to seed) to mature. Maturity was observed 13-15 days earlier than that of the parent variety BRRI dhan29. Thousand grain weight is about 26.5 g, amylose and protein contents are 23.2% and 7.06%, respectively. The average yield of 7.25 t/ha and the highest yield of 10.5 t/ha was recorded in favorable environmental condition. The average yield of BINA dhan19 is about 3.84 t/ha and the maximum yield is 5.0 t/ha. The plant of BINA dhan19 is about 80-90 cm tall and does not get lodged. During severe drought the plant became almost stop growing but when rain is started then it starts growing vigorously and gave almost equal yield like favorable condition. It matures within 90-105 days and thousand grain weights is about 23 g. The grain contains 7.32% protein and 23.8% amylose. Efforts are continuing towards indigenous rice cultivars of Lombur, B-11 and Hori dhan to develop new mutant variety with higher yield, improved agronomic traits and tolerant to abiotic stress.

Introduction

The continuous increase of population in the country demands more food. In turn, it requires more land for rice cultivation as rice is the staple food for 166 million people in Bangladesh and also the most important food crop in Asia and rest of the world (AIS, 2008). We are facing severe threat of climate change challenges in crop sector. In Bangladesh, rice is grown three seasons in a year i.e., Aman season (July to December), Boro season (December to May) and Aus season (March to June). Rice provides 75% of total calories and 51% of protein in our population. About 60% of the total agricultural labor force is engaged for rice production (AIS, 2008). It covers 77.96% of total cropped area in Bangladesh (AIS, 2007) and contributing 14.6% of our national GDP (BBS, 2004). It is a diploid species, with a small genome in comparison to other cultivated cereals (Moin *et al.*, 2017). The average rice yield in the country is about 2.5 t/ha. It is very low yield comparing to other rice growing countries in the world (Islam, 1989). The low yield performance of rice in the country is the effect of the cultivation of low yield potential genotypes, drought, flash-flood and soil salinity. Salinity is also one of the significant abiotic constraints to rice cultivation in the coastal areas of Bangladesh. Salt stress brings about huge losses in worldwide agricultural productivity (Moradi *et al.*, 2003; Yu *et al.*, 2016). In the world, about 400 million hectares of land are affected by high soil salinity and about 1 million hectares of land are affected by high salinity in the coastal regions in Bangladesh (Karim *et al.*, 1990). Salinity hindrances almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. A number of indigenous cultivars are prone to lodging but had some degree of tolerant to salinity and submergence. Its grains are medium bold and contains 10.3% protein and 28.6% amylose (Kamruzzaman and Asaduzzaman, 2004). Mutation Breeding Project of FNCA aimed for breeding technology with major crops using irradiation either by gamma-ray or by ion-beam. Mutations are the major source of genetic variability and artificial mutations can be induced by mutagens (Wei *et al.*, 2013; Oladosu *et al.*, 2016). Many reports are available in induced mutation of rice for genetic improvements with higher yield and other agronomic traits (Azam and Uddin, 1999; Hidema *et al.*, 2003). Recently, ion beam irradiation has been appeared to utilize an effective means of inducing mutations. The biological effects of ion beams have also been investigated and observed to be shown a high relative biological effectiveness (RBE) in lethality, mutation and also to transfers high energy to the target compared to low linear energy transfer (LET) radiation such as Gamma-rays, X-rays and electrons (Blakely, 1992). It has been also demonstrated that ion beams induce mutations at high frequency and induce novel mutants in *Arabidopsis* (Hase *et al.*, 2000, Shikazono *et al.*, 2003, Tanaka *et al.*, 1997, 2002). The use of ion beams for inducing mutations in rice (*Oryza satva* L.) breeding has also been attempted (Hayashi *et al.* 2007; Hidema *et al.* 2003; Rakwal *et al.* 2008). It is generally and widely accepted that in mutation breeding induced traits become fixed during M_2 to M_4 generations (Azad *et al.*, 2010; Hamid *et al.*, 2006; Shamsuzz*aman et al.*, 1998; Azam and Uddin, 1999). Indigenous genotypes have been indicated as the tremendous sources of genes for new alleles (Evenson and Gollin, 1997; Guevarra *et al.*, 2001; Hoisington *et al.*, 1999; Jackson, 1999; Tanksley and McCouch, 1997). Based on the information mentioned above, the study was undertaken to develop new mutant variety with higher yield and yield contributing traits among the local rice genotypes in Bangladesh using carbon ion beam irradiation. In rice, mutants were developed from immature embryos (Ookawa *et al.*, 2014), calli obtained from seeds (Serrat *et al.*, 2014) and cell suspension cultures (Chen *et al.*, 2013). Seeds are, however, easier to handle and do not require a specialized structure, and is therefore, the most widely used material (Da Luz *et al.*, 2016; Oladosu *et al.*, 2016). Screening of existing landraces as well as creation of new mutant variety with improved salt tolerant traits is also a major goal for future study. In this study, we aimed to describe the possibility to get mutants with higher yield, photoperiod insensitive, and shorter plant height from local T. aman rice genotypes Ashfal and also to get mutant with higher yield and early maturity from BRRI dhan29 and it is extended to obtain mutant from NERICA10 in rice by ion beam irradiation.

Materials and Methods

Hulled rice seeds of Ashfal, BRRI dhan29 and NERICA10 were exposed to 26.7 MeV/n carbon ions with different doses of 0, 10, 20, 40, 60, 80, 100, 120,140, 160, 180 and 200 Gy at Japan Atomic Energy Agency (currently QST, Takasaki, Gunma, Japan). One hundred and fifty seeds were used for each irradiation dose. The irradiated seeds were allowed to germinate in petri-dishes. Germinated seeds were sown in seed bed to grow seedlings. Seedlings were transplanted in the field. Single seedlings were transplanted into each hill. Seedlings were transplanted at the PBGED experimental field, IFRB, AERE, Savar, Dhaka, and also at the experimental field of BINA, Mymensingh. Plot size was 5.0 x 2.0 m^2 and distance between rows and plants were maintained 20 cm and 15 cm respectively. The experiments followed RCB design with three replications. Recommended dose of fertilizers, cultural and intercultural practices were done as and when required. Porous pipe was depth into 15 cm of soil to maintain irrigation in rice field. All fertilizers were applied during final land preparation excluding urea. Urea was applied at three equal installments at 7, 30 and 55 days after transplanting. Three hand weeding were made and the plots were kept saturated with irrigation till maturity during Boro season. Heights were measured randomly from ten mutant plants of each irradiated dose at the time of harvesting. Number of days to heading was recorded as the number of days required from sowing time when 50% of plants of each line headed. Days of maturity was recorded as the number of days required from sowing time when 90% of plants of each line appeared with yellowish grains. These two data were recorded through visual observation by visiting the plots every alternate day. Number of effective tillers per plant and panicle length was measured at the time of maturity with randomly selected five competitive plants. Filled and unfilled grains/panicle was recorded from five hills per plot at harvest. Grain yield was recorded from 1 m^2 area which was later converted to yield at ton/ha at 14% moisture. Moisture data was recorded with a grain moisture meter.

Results

Table 1 showed that BINA dhan18 was cultivated at seven different zones in Bangladesh to observe the growth uniformity and yield performance. Table 2 indicated that data of yield attributing traits of mutant (RM(2)-40(C)-1-1-10) and parent (BRRI dhan29) are non-significant. Maturity period and yield was found significantly different between mutant and parent and also among the location cultivated in Bangladesh (Table 3). It was observed that highest (7.8 t/ha) yield with short (145 days) duration was observed at mutant grown at Pakkhifanda, Rangpur area over the parent. Table 3 also indicated that mutant yielded higher and required short duration to mature over parent at every location grown in Bangladesh. Table 4 proved that mutant produced long grain fine rice as grain length was recorded 9.0 (7.4) mm over parent 8.0 (6.5) mm.

Table 1. Multi location trial of BINA dhan18 at different sub-station of BINA.

Location	Date of seed sowing	Date of transplanting	Age of seedlings (days)
BINA farm, Mymensingh	02 December 2013	09 January 2014	39
Maijbari, Mymensingh	02 December 2013	10 January 2014	40
BINA sub-station farm, Magura	02 December 2013	14 January 2014	44
Farmer's Field, Magura	02 December 2013	14 January 2014	44
BINA sub-station farm, Rangpur	01 January 2014	12 February 2014	43
Pakkhifanda, Rangpur	01 January 2014	13 February 2014	44
BINA sub-station farm, Barisal	02 December 2013	31 January 2014	61

Table 2. Yield attributing traits of mutant (RM(2)-40(C)-1-1-10) over parent (BRRI dhan29).

Mutant/variety	Plant height (cm)	Effective tiller (no.)	Panicle length (cm)	Filled grains/ panicle (no.)	Unfilled grains/ panicle (no.)
RM(2)-40(C)-1-1-10	101	11.42	24.7	113	33
BRRI dhan29	100	11.87	24.5	111	33
LSD(0.05)	NS	NS	NS	NS	NS

NS- not significant

Table 3. Comparison of maturity period and yield of mutant (RM(2)-40(C)-1-1-10) with the parent variety (BRRI dhan29) at different sub-station of BINA.

Location	Maturity period (days)		Yield (t/ha)	
	RM(2)-40(C) -1-1-10	BRRI dhan29	RM(2)-40(C) -1-1-10	BRRI dhan29
BINA farm, Mymensingh	150	165	6.9	6.5
Maijbari, Mymensingh	152	165	5.8	6.3
BINA sub-station farm, Magura	157	168	7.6	5.7
Farmer's Field, Magura	155	168	5.8	5.5
BINA sub-station farm, Rangpur	155	165	7.5	7.1
Pakkhifanda, Rangpur	145	160	7.8	7.2
BINA sub-station farm, Barisal	155	170	5.1	4.9
Farmer's Field, Barisal	151	162	5.2	5.2
LSD (0.05)	-	-	0.36	
Average	153	165	6.46	6.05

Table 4. Comparison of grain characters of the mutant (RM(1)-40- (C)-1-1-10) and the parent variety (BRRI dhan29).

Mutant/variety	1000-grain weight (g)	Grain length (mm)	Grain breadth (mm)	Length: breadth ratio
RM(1)-40(C)-1-1-10	26.0	9.0 (7.4)	3.0 (2.5)	3.0 (2.96)
BRRI dhan29	20.6	8.0 (6.5)	2.5 (2.2)	3.2 (2.95)

Numbers in the parentheses indicate the values of dehusked grain.

Control (NERICA10)

BINAdhan19 (Mutant)

Discussion

On the basis of lethality, ion beams induced higher frequencies of mutation than gamma rays. In view of this, seeds of Ashfal rice, BRRI dhan29 and NERICA10 were irradiated with different doses of carbon ion beams and were grown in Boro and Aman season. These results showed that photoperiod sensitivity was altered by carbon ion irradiation in case of BINA dhan14 whilst it was developed from highly photoperiod sensitive cultivar Ashfal. It was also found that plant heights of the three studied land races at M_2 progenies were significantly shortened than that of their parents. This is also observed consistent with the fact that the fertile M_2 plants had shorter plant height than their parents. This might be due to the fact that the fertile mutant lines shifted from vegetative phase to reproductive phase earlier than parents and it is caused by ion beam irradiation. It has been reported that one dominant and one recessive gene are responsible for photoperiod sensitivity in rice (Yu and Yao, 1968; Yokoo and Fujimaki, 1971; Yokoo *et al.*, 1980). It could be happened that carbon ion beams irradiation inactivated or down regulated the activity of the dominant gene in the irradiated seeds which makes the mutant lines homozygous monogenic recessive for photoperiod insensitivity. BINA dhan18 showed higher yield and early maturity compared to parent of BRRI dhan29 which could be due to the useful effect of carbon ion beam. BINA dhan19 also showed higher yield, early maturity and highly drought tolerant compared to parent of NERICA10 which also thought to the useful effect of carbon ion beam. It was obvious from the study that BINA dhan14 was developed using 200 Gy and BINA dhan18 and also BINA dhan19 was developed using 40 Gy respectively through carbon ion beam irradiation. The rest of the doses of the particular genotypes under investigation were omitted from these evaluations because they were prone to lodging for weak culm although they had shortened plants. Moreover, these progenies had shorter panicle with smaller grains and lower number of seed grains and also their inferior performance. Although the grain characteristics such as size and shape altered in case of most of the mutants but grain weight almost similar as the parent. Hence, it could be concluded that it is possible to induce fixed mutants in *indica* type rice Ashfal, BRRI dhan29 and NERICA10 through 40-200Gy doses of carbon ion beams irradiation. This result is also in agreement with the results of Honda *et al.* (2006) who reported genetically fixed mutants in M_1 generation of sweet pepper for dwarf height and yellow pericarp. Therefore, all of these suggest that heavy ion beams irradiation has unique properties that induce fixed mutation.

Acknowledgements

This work was carried out under the Forum for Nuclear Cooperation in Asia (FNCA) and IAEA/RCA Mutation Breeding Project. Special thanks to Dr. Atsushi Tanaka, Dr. Hase, National institutes for Quantum and Radiological Science and Technology (QST); Ms. Aki Koike, Nuclear Safety Research Association (NSRA); Dr. Liu Luxiang, RCA Coordinator of China for their kind help and cooperation.

References

Azad MAK, Hamid MA, Yasmine F (2010). Binachinabadam-4: a high yielding mutant variety of groundnut with medium pod size. Plant Mutation Reports 2(2):45-47.

Azam MA, Uddin I (1999). Binadhan-4, an improved rice variety bred through induced mutation. Bangladesh J. Nuclear. Agric. 15: 59-65.

AIS (2008). (Agriculture Information Service). Krishi Diary. Agric. Info. Service. Khamarbari, Dhaka. P. 66.

AIS (2007). (Agriculture Information Service). Krishi Diary. Agric. Info. Service. Khamarbari, Dhaka. P. 53.

BBS (2004). Monthly Statistical Bulletin of Bangladesh Bureau of Statistics, Statistics Division, Min. Plan. Govt. Peoples Repb. Bangladesh, Dhaka. Pp. 54-58.

Blakely EA (1992). Cell inactivation by heavy charged particles. Radiat. Environ. Biophys. 31:181-196.

Chen Y-L, Liang H-L, Ma X-L, Lou S-L, Xie Y-Y, Liu Z-L (2013). An efficient rice mutagenesis system based on suspension-cultured cells. J. Integr. Plant Biol. 55: 122-130. doi: 10.1111/jipb.12000.

Da Luz VK, da Silveira, da Fonseca SFS, Groli GM, Figueiredo EL, Baretta RG (2016). Identification of variability for agronomically important traits in rice mutant families. Bragantia 75: 41-50. doi: 10.1590/1678-4499.283.

Evenson RE, Gollin D (1997). Genetic resources, international organizations and improvement in rice varieties. Econ. Dev. Cult. Change 45: 471-500.

Guevarra E, Loresto GC, Jackson MT (2001). Use of conserved rice germplasm. Plant Genet. Resour. Newslett. 124: 51-56.

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

Hayashi Y, Takehisa H, Kazama Y, Ichida H, Ryuto H, Fukunishi N, Abe T (2007). Effects of ion beam irradiation on mutation induction in rice. In: Proc. Cyclotrons and their applications 2007, 18th International Conference, pp. 237-239.

Hamid MA, Azad MAK, Howelider MAR (2006). Development of three groundnut varieties with improved quantitative and qualitative traits through induced mutation. Plant Mutation Reports 1(2): 14-16.

Hidema J, Yamoto M, Kumagai T, Hage Y, Sakamoto A, Tanaka A (2003). Biological effect of carbon ion on rice (*Oryza sativa* L.). JAERI-Review 2003, 033: 85-87.

Hoisington D, Khairallah M, Reeves T, Ribaut J-M, Skovmand B, Taba S, Warburton M (1999). Plant genetic resources: What can they contribute toward increased crop productivity? Proc. Natl. Acad. Sci. USA 96: 5937-5943.

Honda I, Kikuchi K, Matsuo S, Fukuda M, Saito H, Ryuto H, Fukunishi N, Abe T (2006). Heavy ion induced mutants in sweet pepper isolated by M1 plant selection. Euphytica 152: 61-66.

Islam MA (1989). A study on public oriented cropping patterns under rainfed and irrigated conditions. Ph.D. Thesis, Dept. Agron., Bangladesh Agril. Univ., Mymensingh, P. 172.

Jackson MT (1999). Managing the world's largest collection of rice genetic resources. In Proceedings of the Intl. Symposium on Rice Germplasm Evaluation and Enhancement. Eds. J N Rutger, J F Robinson, R H Dilday. pp. 22-28. Arkansas Agr. Expt. Station Special Report, Arkansas, USA.

Karim ZH, Hossain SG, Ahmed M (1990). Salinity problems and crop intensification in the coastal region of Bangladesh. soils publication no. 33. BRAC, Dhaka, Bangladesh.

Kamruzzaman M, Asaduzzaman M (2004). Cultivation of saline tolerant rice varieties in the coastal area to cope with salinity in Protapkhali, paikgacha, Khulna, Bangladesh. enviroscope.iges.or.jp/contents/APEIS/inventory/0145.pdf.

Moin M, Bakshi A, Saha A, Dutta M, Kirti PB (2017). Gain-of-function mutagenesis approaches in rice for functional genomics and improvement of crop productivity. Brief. Funct. Genom. 16, 238-247. doi: 10.1093/bfgp/elw041.

Moradi F, Ismail AM, Gregorio G, Egdane J (2003). Salinity tolerance of rice during reproductive development and association with tolerance at seedling stage. Indian J. Plant. Physiol. 8: 105-116.

Oladosu Y, Rafii MY, Abdullah N, Hussin G, Ramli A, Rahim HA (2016). Principle and application of plant mutagenesis in crop improvement: a review. Biotechnol. Biotechnol. Equip. 30: 1-16. doi: 10.1080/13102818.2015.1087333.

Ookawa T, Inoue K, Matsuoka M, Ebitani T, Takarada T, Yamamoto T, (2014). Increased lodging resistance in long-culm, low-lignin gh2 rice for improved feed and bioenergy production. Sci. Rep. 4, 6567. doi: 10.1038/srep06567.

Rakwal R., Kimura S, Shibato J, Nojima K, Kim YK, Nahm BH, Jwa NS, Endo S, Tanaka K, Iwahashi H (2008). Growth retardation and death of rice plants irradiated with carbon ion beams is preceded by very early dose- and time-dependent gene expression changes. Mol. Cells 25(2): 272-278.

Serrat X, Esteban R, Guibourt N, Moysset L, Nogués S, Lalanne E (2014). EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods 10, 5. doi: 10.1186/1746-4811-10-5Serrat, X., Esteban, R., Guibourt, N., Moysset, L., Nogués, S., Lalanne, E. (2014). EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods 10, 5. doi: 10.1186/1746-4811-10-5.

Shamsuzzaman KM, Hamid MA, Azad MAK, Shaikh MAQ (1998). Development of early maturing and high yielding cotton genotypes through induced mutations. Bangladesh J. Nuclear Agric. 14: 1-8.

Shikazono N, Yokota Y, Kitamura S, Suzuki C, Watanabe H, Tano S, Tanaka A (2003). Mutation rate and novel *tt* mutants of *Arabidopsis thaliana* induced by carbon ions. Genetics 163: 1449-1455.

Tanaka A, Tano S, Chantes T, Yolota Y, Shikazono N, Watanabe H (1997). A new Arabidopsis mutant induced by ion beams affects favonoid synthesis with spotted pigmentation in testa. Genes Genet.Syst. 72:141-148.

Tanaka A, Sakamoto A, Ishigaki Y, Nikaido O, Sun G, Ase Y, Shikazono N, Tano S, Watanabe H (2002). An ultra violet-B- resistant mutant with enhanced DNA repair in Arabidopsis. Plant Physiol. 129: 64-71.

Tanksley SD, McCouch SR (1997). Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277: 1063-1066.

Wei F-J, Droc G, Guiderdoni E, Hsing YC (2013). International consortium of rice mutagenesis: resources and beyond. Rice 6: 39. doi: 10.1186/1939-8433-6-39.

Yokoo M, Fujimaki H (1971). Tight linkage of blast resistance with late maturity observed in different Indica varieties of rice. Japan J. Breed. 21: 35-39.

Yokoo M, Kikuchi F, Nakane A, Fujimaki H (1980). Genetical analysis of heading data by aid of close linkage with blast resistance in rice. Bull. Nat. Inst. Agric. Sci. D31: 95-126.

Yu CJ, Yao TT (1968). Genetische studien beim Reis. II. Die Koppelung des Langhullspelzengens mit dem Photoperiodizitatsgen. Bot. Bull. Acad. Sin. 9: 34-35.

Yu Y, Cui YC, Ren C, Rocha PSCF (2016). Transgenic rice expressing a cassava (*Manihot esculenta* Crantz) plasma membrane gene MePMP3-2 exhibits enhanced tolerance to salt and drought stresses. Genet. Mol. Res. 15.

Breeding of Rice Variety(s) for High Yielding and Early Maturity Through a Wide Cross and Mutation

Sobrizal

National Nuclear Energy Agency, Center for the Application of Isotopes and Radiation, Jl. Cinere Pasar Jumat P. O. Box 7002 JKSKL, Jakarta 12070 Indonesia.

Abstract

To fulfill the Indonesian domestic demand in which the annual population growth is still more than 1.4%, Indonesian rice production should be increased. This can be achieved by increasing rice productivity and harvesting index through growing high yielding and early maturity of rice varieties. In previous research, breeding lines that have wide genetic diversity have been constructed through Indica-Japonica cross of IR36/Koshihikari. To gather the desirable characters in one lines some of breeding lines were crossed each other and some improved lines were obtained. To remove undesirable characters in improved lines some of these limes were irradiated by gamma ray and 12 promising mutant lines with high yield and early maturities were selected. The growth durations of these mutant lines were ranging from 93.7 to 99.3 days from sowing to harvesting, significantly shorter than those of original line SKI 88, national leading variety Ciherang and the early maturity national leading variety INPARI 13. Yield of most mutant lines were not significantly difference with that of original line, SKI 88 and national leading variety, Ciherang, but significantly higher than that of INPARI 13. To fulfill the requirement of variety release in Indonesia, other multi-location yield trials and other examinations such as pests, diseases as well as other grain quality examinations should be conducted.

Key words: rice, high yield, early maturity, wide cross, mutation

Introduction

Rice is a staple food of majority Indonesian population. In the mid-1980s Indonesia firstly achieved self-sufficiency for rice, however the growth of rice production has slowed down since the 1990s (BAPENAS, 2002). The decline in rice production is frequently attributed to loss of rice fields caused by conversion to non-agricultural uses, and to the declining productivity. To fulfill the domestic demand in which the annual population growth is still more than 1.4 %, Indonesian rice production should be increased. This can be achieved by increasing rice productivity and harvesting index through growing high yielding and early maturity of rice varieties.

Since the release of IR8 varieties in 1966 (Cantrell and Hettel, 2004), the potential for paddy production has not been significantly increased (Sobrizal and Ismachin, 2006). The formation of new varieties is only able to maintain the potential of IR8 by improving the growth duration, grain quality, and its adaptability to environmental stress. The narrowness of the genetic diversity of the released rice varieties contributes to the occurrence of slowed down of growth yield potential. Many released rice varieties are related to each other, so the diversity is less and yield potentials are almost same (Susanto *et al.*, 2003). In 1982 the national average production was 4.04 t / ha, while in 2016 it was 5.24 t / ha (BPS, 2017), so the 34-year increase was only 1.20 t / ha. In order to increase the yield potential for future, different genetic resources are needed in the new varieties assembly program.

In previous research, breeding lines that have wide genetic diversity have been constructed through Indica-Japonica cross of IR36/Koshihikari (Sobrizal, 2008). Intra sub-specific crossing with large genetic distances between the two parents allow for the occurrence of transgressive segregation so that it will produce a progeny with a relatively large genetic diversity. Transgressive segregation is the emergence of an individual in a segregated population in which the individual phenotype exceeds that of both parental phenotypes and is commonly found in progeny derived from inter and intra-specific crosses (Vega and Frey, 2004). Great genetic diversity will provide flexibility in selection, including selection towards increased yield potential. The selected plants are purified to become pure lines, and can be used as breeding materials in subsequent breeding programs. By using these unique germplasms as well as mutations 12 rice promising lines with high yield and early maturities have been constructed. The objective of this research is to develop high yielding and early maturities of rice varieties.

Materials and Methods

Construction of Breeding Materials

To increase the genetic variability of breeding materials, IR36 and Koshihikari were crossed, and developing of pure lines from this cross was conducted as described in Sobrizal 2013. To gather desirable characters in a line some of selected pure lines were crossed as shown in Fig. 1. From these crosses selected pure lines (SKI 64, SKI 88, SKI 153, and SKI 276) were used as further breeding materials. These lines showed good performance with high yield potency, but long growth durations. To reduce their growth durations, 50 gram seeds of each line were irradiated by 200 Gy of gamma ray at Center for the Application of Isotopes and Radiation Technology, Pasar Jumat, Jakarta. Irradiated M_1 seeds were sown, and twenty day-old seedlings were transplanted to paddy field by planting a single seedling per hole at experimental field, Sawangan, Depok with 20 cm spacing between plants.

Five hundred M_1 plants were harvested individually to obtain the M_2 seeds. Harvesting was conducted by collecting only one main panicle in each M_1 plant. M_2 seeds were sown, and 20 M_2 plants derived from each M_1 plant were transplanted to develop M_2 lines. Selections of early maturity plants were conducted in each M_2 lines and selected plants were harvested individually.

Selected early maturity plants were purified up to M_5 generations, then, selected pure lines were designated as RSKI #. RSKI lines were subjected to yield trials. Breeding scheme of plant materials used in this study can be shown in Fig. 1.

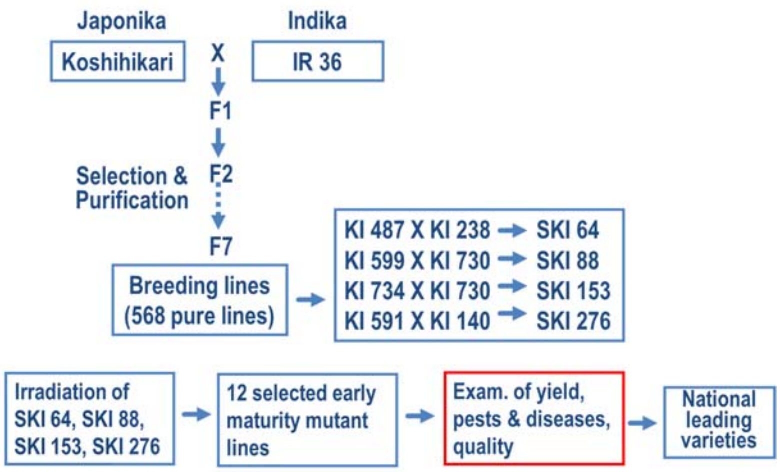


Fig. 1. Breeding scheme of plant materials used in this study.

Yield trials

Yield trials were conducted using 12 selected early maturity mutant lines, check varieties of original line SKI 88, national leading variety Ciherang and national leading early maturity variety INPARI 13. Seedlings were transplanted to plot with plot size of 4 x 5 m and planting spacing of 25 x 25 cm. Experiments were arranged in RBD with 3 replications.

Results and Discussion

In this study, all the F_1 plants derived from crosses of KI lines as in Fig. 1 grew well and selection was conducted in F_2 population with emphasis on agronomical characters. Selected plants were purified and selected pure lines were designated as SKI #. Among selected pure lines, the seeds of SKI 64, SKI 88, SKI 153, and SKI 276 lines were irradiated by 200 Gy of gamma ray. As much as 500 M_1 plants were harvested individually to obtain M_2 seeds. Selection for early maturity was conducted in M_2 lines and 105 selected lines were purified up to M_6 generations. From field observation of 105 lines in rainy growing season of 2012/2013, 12 homogeneous early maturity M_7 mutant lines were selected. These lines were evaluated for yield performance at Pusakanagara Experimental Farm in dry growing season of 2013 and wet growing season of 2013/2014. The results were shown in Table 1.

Table 1. Growth duration and yield of lines/varieties tested.

LINE	DURATION (DAYS)		YIELD (T/HA)	
	DS (2013)*	WS (2013/14)*	DS (2013)*	WS (2013/14)*
RSKI 64-1	98.7 d c	94.3 a	8.88 a b c d	7.70 a b c
RSKI 64-2	99.3 e	97.0 c d e	9.53 a b c	6.71 a b c d
RSKI 88-1	98.0 d	96.3 b c d	8.54 a b c d	6.98 a b c d
RSKI 88-2	94.3 a b	95.0 a b	8.25 b c d	6.51 c d
RSKI 88-3	95.7 c	97.7 d e	8.92 a b c d	7.23 a b c d
RSKI 88-4	96.0 c	96.3 b c d	8.88 a b c d	5.93 d
RSKI 88-5	95.0 b c	95.7 a b c	9.33 a b c	6.51 c d
RSKI 88-6	99.0 d e	97.0 c d e	7.90 d	6.62 b c d
RSKI 88-7	98.3 d e	96.3 b c d	9.74 a	7.93 a b
RSKI 153-1	95.7 c	98.3 e	9.79 a	7.81 a b c
RSKI 276-1	95.0 b c	97.0 c d e	9.75 a	7.70 a b c
RSKI 276-2	93.7 a	100.3 f	7.83 d	5.89 d
SKI 88	117.3 g	123.3 h	9.61 a b	8.12 a
CIHERANG	118.7 h	123.0 h	9.45 a b c	7.48 a b c
INPARI 13	103,7 f	114.3 g	8.16 c d	7.03 a b c d

*) The numbers followed by different characters are significantly different for 5% level.

Table 1 showed that growth durations of mutant lines were ranging from 93.7 to 99.3 days from sowing to harvesting. They were significantly shorter than growth durations of original line SKI 88, national leading variety Ciherang and the early maturity national leading variety INPARI 13. The growth duration of SKI 88 was 117.3 days, Ciherang was 118.7 days and INPARI 13 was 103.7 days.

Yield of mutant lines were ranging from 7.83 to 9.79 t/ha, while SKI 88 was 9.61 ton/ha, Ciherang was 9.45 ton/ha and INPARI 13 was 8.16 t/ha. The yields of ten mutant lines (RSKI 64-1, RSKI 64-2, RSKI 88-1, RSKI 88-2, RSKI 88-3, RSKI 88-4, RSKI 88-5, RSKI 88-7, RSKI 153-1, RSKI 276-1, RSKI 276-2) were not significantly difference with that of original line, SKI 88 and national leading variety, Ciherang, but significantly higher than that of INPARI 13. More over the growth durations of these ten mutant lines were significantly shorter than that of the national leading early maturity, INPARI 13. Performance of mutant RSKI 88-7 and its original plant SKI 88 shown in Fig. 2. Figure 2 shows that the panicles of mutant plants of RSKI 88-7 have appeared while its original plant of SKI 88 has not appeared yet.

Fig. 2. Comparison of early maturity mutant line of RSKI 88-7 and its original line of SKI 88.

To confirm the performance of growth duration and yield of selected lines, these lines were evaluated by growing them at farmer field in Musi Rawas District, South Sumatera and the result sown in Table 2. Table 2 showed that the growth duration of all selected early maturity lines were significantly shorter than that of original line and Ciherang. Even all selected lines were significantly earlier than national early maturity variety INPARI 13. The yields of some selected early maturity lines (RSKI 64-2, RSKI 88-1, RSKI 88-3, RSKI 88-5, RSKI 153-1, RSKI 276-1) were not significantly different from that of national leading early maturity variety INPARI 13. The yields of other selected early maturity lines were significantly lower than that of national leading early maturity variety INPARI 13.

Table 2. Result of yield trial of selected lines in Musi Rawas district.

LINE	GROWTH DURATION (DAYS)*	YIELD (T/HA)*
RSKI 64-1	88.0 g h	5.01 efg
RSKI 64-2	94.0 e	5.81 def
RSKI 88-1	94.7 e	6.0 cde
RSKI 88-2	100,2 d	4.8 fgh
RSKI 88-3	92.7 e f	7.4 b
RSKI 88-4	89.0 f g	4.6 gh
RSKI 88-5	89.3 f g	6.8 bc
RSKI 88-6	91.7 e f g	3.9 h
RSKI 88-7	92.3 e f g	5.2 efg
RSKI 153-1	84.7 h	7.2 b
RSKI 276-1	93.3 e f	7.3 b
RSKI 276-2	112.3 b	5.2 efg
SKI 88	106.1 c	8.7 a
CIHERANG	120,3 a	7.7 b
INPARI 13	100.3 d	6.7 bcd

*) The numbers followed by different characters are significantly different for 5% level.

To further evaluate the yield performances, selected early maturity lines were subjected to yield trials in five locations. The highest average yield in five locations is 8.58 t/ha for RSKI 64-2 and followed by 8.48 t/ha for RSKI 153-1 and 8.48 t/ha for RSKI 276-2, whereas the average yield of an original line SKI 88, a national leading variety Ciherang, and a national leading early maturity variety were only 8.06 t/ha, 8.22 t/ha, and 7.54 t/ha, respectively (Table 3). Multi-location yield trials to reach at least 16 locations as well as other examinations such as pest and diseases, grain quality should be continued as a requirement of variety release in Indonesia.

Table 3. Yield of tested lines in 5 locations of yield trials.

	YIELD (TON/HA)*					
LINE	MATARAM	MURA	SLEMAN	BANTAENG	BNYUWNGI	AVERAGE
RSKI 64-1	8.2 bc	6.5 abc	8.5 abc	6.9 d	7.6 cd	7.54
RSKI 64-2	10.3 a	6.1 bc	10.6 a	7.2 bcd	8.7 abc	8.58
RSKI 88-1	5.8 ef	5.5 c	8.5 abc	6.9 d	7.3 d	7.34
RSKI 88-2	5.7 f	6.5 abc	9.7 ab	7.7 bcd	8.7 abc	7.66
RSKI 88-3	7.8 bc	7.0 abc	7.4 bc	7.1 cd	8.3 abcd	7.52
RSKI 88-4	6.5 def	7.6 ab	9.7 ab	7.2 bcd	8.2 abcd	7.84
RSKI 88-5	6.3 ef	8.1 a	8.2 abc	7.3 bcd	8.1 bcd	7.60
RSKI 88-6	6.7 de	7.2 abc	8.3 abc	7.2 bcd	7.8 bcd	7.44
RSKI 88-7	8.3 b	7.0 abc	7.1 c	8.5 abcd	7.8 bcd	7.74
RSKI 153-1	10.7 a	6.0 bc	6.8 c	10.1a	8.8 abc	8.48
RSKI 276-1	6.0 ef	7.0 abc	7.2 c	7.1 cd	8.2 abcd	7.10
RSKI 276-2	7.7 bc	7.4 abc	8.7 abc	9.3 ab	8.3 abcd	8.28
SKI 88	8.3 b	6.7 abc	7.2 c	9.1 abc	9.0 ab	8.06
CIHERANG	7.7 bc	6.0 bc	8.2 abc	9.9 a	9.3 a	8.22
INPARI 13	7.3 cd	6.1 bc	7.0 c	8.8 abcd	8.5 abcd	7.54

*) The numbers followed by different characters are significantly different for 5% level.

Conclusions

Based on the results it can be concluded as follow;

1. The growth durations of mutant lines were ranging from 93.7 to 99.3 days from sowing to harvesting, they were significantly shorter than those of original line SKI 88, national leading variety Ciherang and the early maturity national leading variety INPARI 13.
2. Yield of most mutant lines were not significantly difference with that of original line, SKI 88 and national leading variety, Ciherang, but significantly higher than that of INPARI 13.
3. To fulfill the requirement of variety release in Indonesia, other multi-location yield trials and other examinations such as pests, diseases and other grain quality examinations should be conducted.

Acknowledgement

Thanks to my colleagues at the Division of Irradiation and Instrumentation CAIRT – NNEA for their helps in seed irradiation, and my colleagues at Plant Breeding Laboratory, CAIRT – NNEA, especially Mr. Carkum, SP., for his excellent technical assistance.

References

BPS 2017. www.bps.go.id

BAPENAS/DEPARTEMAN PERTANIAN/USAID/DAI FOOD POLICY ADVISORY TEAM. 2002. Indonesian food policy program. (Available at www.macrofoodpolicy.com)

CANTRELL, R. P., HETTEL, G. P. 2004. Research strategy for rice in the 21st century. Proceeding of World Rice Research Conference, Tsukuba International Congress Center (Epochal Tsukuba) Tsukuba, Ibaraki, Japan.

SOBRIZAL. 2013. Improvement of yield and grain quality of rice through a wide cross and mutation breeding. Achievement Sub-Project on Composition or Quality in Rice (2007 – 2012), Mutation Breeding Project, Forum for Nuclear Cooperation in Asia (FNCA) March, 2013

SOBRIZAL, ISMACHIN, M. Peluang mutasi induksi pada upaya pemecahan hambatan peningkatan produksi padi. Jurnal Ilmiah Aplikasi Isotop dan Radiasi 2 (2006) 50.

SUSANTO, U., DERADJAD, A. A., SUPRIHATNO, B. Perkembangan pemuliaan padi sawah di Indonesia. Jurnal Litbang Pertanian. 22 (2003) 125.

VEGA, U., FREY, K. J. Transgressive segregation in inter and intraspecific crosses of barley. Euphytica 34 (1980) 875.

Trials on Cross and Mutation Breeding of Rice for Adaptability to Nature Farming

Hirokazu NAKAI*, Yoshiyuki NARA*, Chiharu SUZUKI*, Hiroyasu TABUCHI*, Shigeki NOZAWA** and Yoshihiro HASE**

* Institute for Agriculture, Medicine, and the Environment,
** Takasaki Advanced Radiation Research Institute, National Institutes for Quantum and Radiological Science and Technology (QST)

Summary

We have tried to develop rice varieties, through cross and mutation breeding for adaptability to nature farming in these past more than ten years. We succeeded in selecting breeding lines for adaptability to nature farming, in the hybrid populations from the cross of representative native varieties of Japan, 'Asahi' and 'Kamenoo'. Two breeding lines, namely AKH2 and AKH4, which were of high yield with high eating quality in nature farming, were selected from the cross breeding. However, the selected breeding lines were too late for heading date to be adaptable to wide range of Japan island other than southern part of Japan e.g. Kyushu district. We tried to improve the negative trait for late heading of the breeding lines through the use of induced mutations within five years. This paper refers to the breeding process and discuss on the merit of mutation breeding and on how to use the technique for gaining adaptability to low input sustainable agriculture.

Introduction

Conventional agricultural systems dependent on chemical fertilizer and agricultural chemicals should be converted to low input sustainable agriculture with less amount of chemicals in the situation of serious climate change covered in the world. It is reported that more than 20% of all greenhouse gases, e.g. CO_2, CH_4, and N_2O, might be emitted from the field of agriculture (Nagano et al., 2012, Skinner, 2014). We have tried to perform cross breeding combined with use of induced mutations of rice for adaptability to nature farming, aiming at developing the system of sustainable agriculture. Nature farming is defined in this paper as a farming system that uses only residue of rice plants as the nutrition with no agrochemicals and chemical fertilizer (Okada, 1953). We are reporting a case of the breeding performed in Yunomae-cho, Kumamoto-prefecture, Japan. The AKH2 breeding line was finally selected for high yield and high eating quality in the nature farming condition, from the hybrid population derives from the cross between Japanese native varieties, 'Asahi' and 'Kamenoo'. However, the AKH2 breeding line was found to carry weak points for late heading and long culm. We have thereupon tried to improve the demerits of late heading and long culm through use of induced mutations. Accordingly, some breeding lines with earlier heading date and/or shorter culm length as well as higher yield in the low input conditions were selected. The selected MAKH2 breeding line was noticed as promising new rice variety adaptable to nature farming or low input sustainable agriculture.

This paper refers to the breeding process and discuss on the merit of mutation breeding and on how to use the technique for gaining adaptability to low input sustainable agriculture.

Materials and methods

In 2005, Japanese native varieties, Asahi and Kamenoo were crossed at the Ohito Experimental Farm located in Izunokuni-city, Shizuoka-prefecture located at almost center of the main island of Japan. The 1,638 F_2 plants derived from 16 F_1 plants were grown with planting space of 30 cm × 20 cm in the fields of nature farming of Ohito Experimental Farm in 2007. All the harvested rice straws and 100 kg rice bran were applied to the rice fields as nutritional materials. The 140 plants were selected by eye measurement from all the F_2 plants in the fields. In 2008, 140 F_3 lines originating from the selected F_2 plants were grown and 36 F_3 lines were selected by eye measurement in the field. The selected F_3 lines were measured for wight of grain and eating quality using the measuring instrument (Kett Electric Laboratory, Tokyo, Japan).

Table 1 shows the breeding process performed from 2009 to 2017 in Yunome-town, Kumamoto-prefecture in Kyushu-district, Japan. In 2009, seeds of the selected 36 F_3 lines were sent to a farmer, Mr. SHIIBA, Takema in Yunomae-town, Kumamoto-prefecture to be grown as F_4 lines in the fields of nature farming. In the selection tests, any nutritional matters were never applied to the breeding field.

Ten or less breeding lines were used for adaptation and yield tests from 2013 to 2015, after selection tests up to 2012. In the adaptation and yield tests, the selected 10 or less lines were grown in randomized block design with three replications (60 plants were grown per one line of one block). Grain yield and eating quality were measured as well as main agricultural traits, e.g. culm length, panicle length, number of panicles per plant and so forth. Two breeding lines, AKH2 and AKH4, were finally selected for registering commercial cultivar patent. AKH2 was, in fact, registered as commercial cultivar 'Kumaminori' in 2018.

Table 1. The process of cross breeding of native varieties, Asahi and Kamenoo, performed in the farmer's fields in Yunomae-town, kumamoto-prefecture, Kyushu-district (2009~2017).

Year	2009	2010	2011	2012	2013	2014	2015	2016	2017
Hybrid generation and number of lines grown	F_4 36	F_5 23	F_6 17	F_7 14	F_8 10	F_9 7	F_{10} 6	F_{11} 5	F_{12} 2

However, both breeding lines carry a week point for late heading not to be adaptable to wider range to the northern part in Japan. In such a situation, we tried to improve the demerit of the breeding lines through the use of mutation breeding. In 2013, about 5,000 F_8 seeds of AKH2 and AKH4, were irradiated by gamma-rays of 200 Gy at Japan Atomic Energy Agency (currently Takasaki Advanced Radiation Research Institute, National Institutes for Quantum and Radiological Science and Technology). About 4,000 M_1 plants each of AKH2 and AKH4 were grown in the fields at the Ohito Experimental Farm. Seed fertility was checked using randomly selected 50 M_1 plants. The selection process for early heading of the mutants is shown in Table 2. As shown in the Table 2, after 5 years since the irradiation time, two noticeable mutants were selected from the population of AKH2 in 2018.

Table 2. Selection process for early heading of rice mutants from the irradiated populations of AKH2 and AKH4.

Original line	Year and generation					
	2013 M1	2014 M2	2015 M3	2016 M4	2017 M5	2018 M6
AKH2	4,428N* 48%***	15,840N	119L**	11L	6L	2L
AKH4	3,936N 45%***	14,400N	105L	14L	6L	0L

*N: number of plants. **L: number of lines.

*** average seed fertility for 50 M_1 plants.

Results

Fig.1 shows grain yield and eating quality of the breeding lines, AKH2 and AKH4, which were selected from cross breeding of Asahi and Kamenoo. In the figure, 'Hinohikari' was put as a control because of it being representative leading variety in Kyushu district. As seen in the figure, grain yield of the AKH2 was higher than AKH4 and Hinohikari. As for eating quality, significant difference was not found among the two breeding lines and Hinohikari.

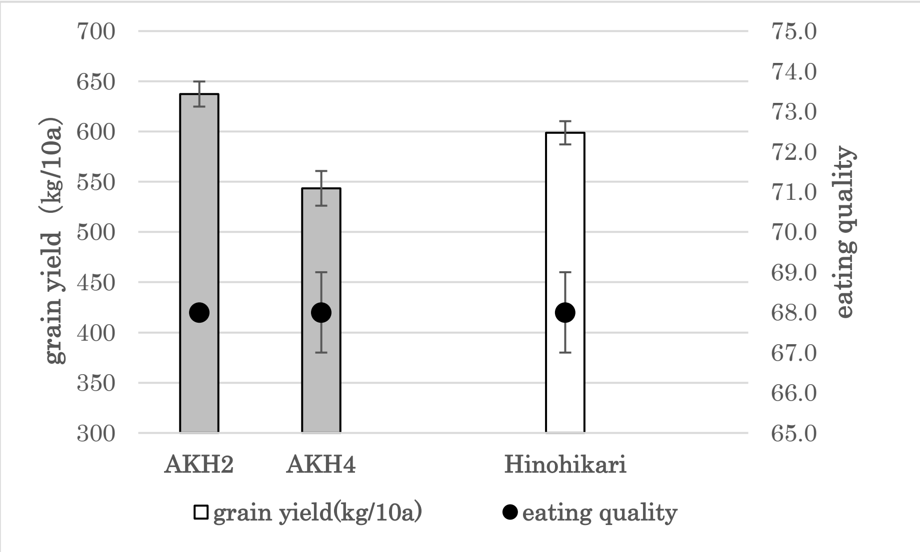


Fig.1. Grain yield and eating quality of the breeding lines, AKH2 and AKH4, in comparison with control (Hinohikari) in the nature farming (from results for adaptation and yield tests in 2014).

We requested some farmers, who were concerned with nature farming in the Kyushu district, to grow the three lines using farm machines. Fig.2 shows one of the results from the farmers on grain yield and eating quality. As seen in the figure, the AKH2 showed the highest yield. Eating quality of rice was clearly higher in the two breeding lines than in Hinohikari, suggesting that eating quality is to be higher in the selected breeding lines than in the popular leading variety, Hinohikari in the Kyushu district. We finally selected the AKH2 breeding line for registering commercial variety in 2018.

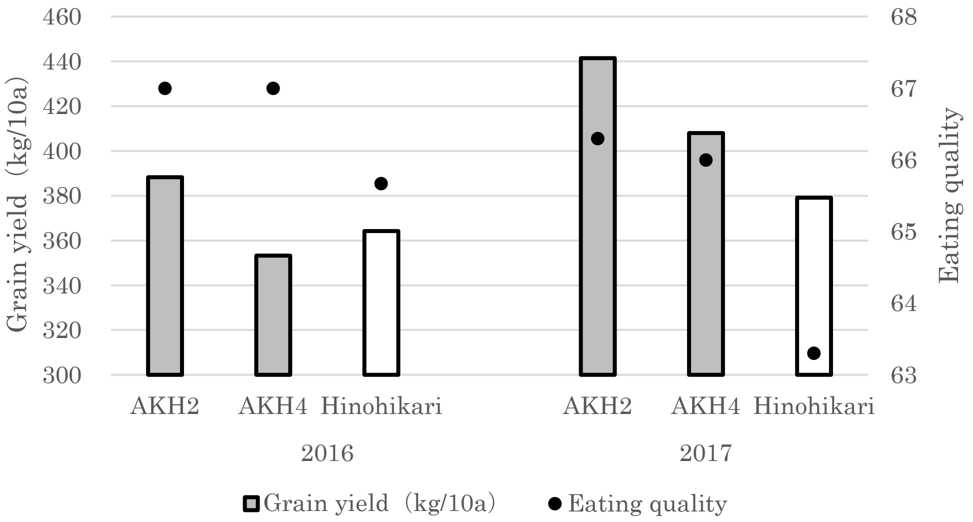


Fig. 2. Grain yield and eating quality of breeding lines, AKH2 and AKH4 selected from the cross breeding in case of being grown by planting machine by a farmer in Kumamoto prefecture in 2016 and 2017.

Fig.3 shows heading date and culm length of the breeding lines of AKH2 and AKH4 comparing with Ashahi and Kamenoo (parent varieties) and Hinohikari (a representative leading variety in Kyushu area). As seen in the figure, heading date of the both breeding lines was almost same as one of the parent varieties, Asahi, which was significantly later in heading date than Kamenoo and Hinohikari. It was noted that heading date of the selected breeding lines was significantly later than that of the local leading variety, Hinohikari. It may be inconvenient for farmers in the local area to grow the breeding lines because of it being late in heading date. Culm length of both breeding lines was almost same as that of the parent varieties, Asahi and Kamenoo. However, it was found that culm length of the breeding lines was significantly longer than that of Hinohikari. We tried to improve the weak point for late heading of the breeding lines to get wider adaptability to grow in northern part of Japan.

Fig.4 shows the frequency distribution for heading date of M_3 mutant lines originating from irradiation of gamma-rays to F_8 seeds of the breeding lines of AKH2 and AKH4. As seen in the figure, the earliest heading date of the mutant originating AKH4 was put in 21~31th in July. Heading date of total 15 mutant lines from both the breeding lines was put in 1 ~10th in September, whereas heading dates of AKH2 and AKH4 were respectively 8th and 6th in September. Heading date of the mutant lines put in 1~10th in September seen in the figure was earlier than that of both the breeding lines, AKH2 and 4. Anyhow, mutant lines put in 1~10th in September were not so practically effective as breeding materials for early heading.

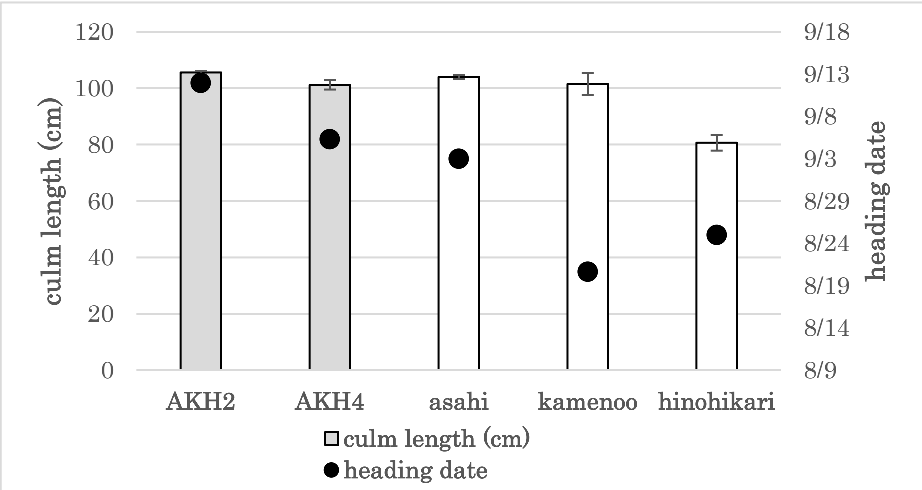


Fig. 3. Heading date and culm length of the breeding lines, AKH2 and AKH4 selected from the cross combination of Asahi and Kamenoo (2014).

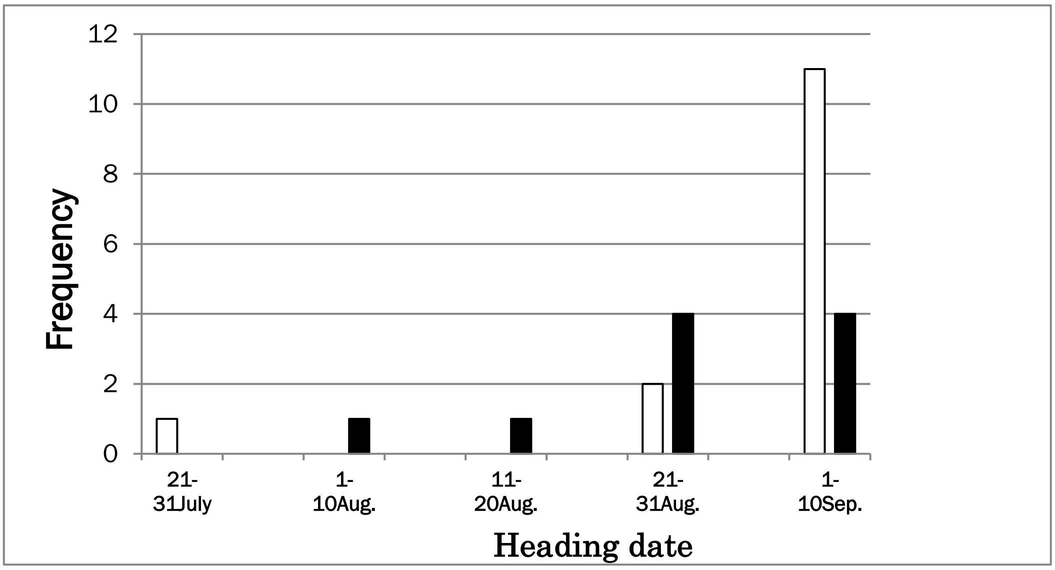


Fig. 4. Frequency distribution of heading date of the mutant lines originating from irradiation of gamma-ray of 20 krad to F_8 seeds of AKH4 (white bar in the figure) and AKH2 (black bar in the figure).

Fig.5 shows culm length and heading date of 6 mutant lines selected as breeding materials for the relevant breeding purpose, which were originating from irradiation of gamma-rays to F_8 seeds of AKH2. As seen in the figure, heading date of all the mutant lines selected was significantly earlier than that of control, AKH2. Heading date (12th in August) of the mutant line, MAKH2, was about one month earlier than AKH2. In case of MAKH2-7, heading date was about one week earlier than the control (AKH2). Culm length of the mutant lines was significantly lower than that of the control, excepting one case of MAKH2-7. We, after all, selected two mutant lines, MAKH2-6 and MAKH2-7, of which heading date are earlier than control, AKH2, with same or more grain yield as the control.

Fig.6 shows plant type of the selected M5 mutant lines, MAKH2-7 and MAKH2-6 comparing with control, AKH2. It was found that MAKH2-7 carried much higher number of panicles comparing with the control, though the culm length was as high as the control. We have finally selected the MAKH2-7 mutant line as a candidate for registering new rice cultivar for adaptability to nature farming or low input sustainable agriculture.

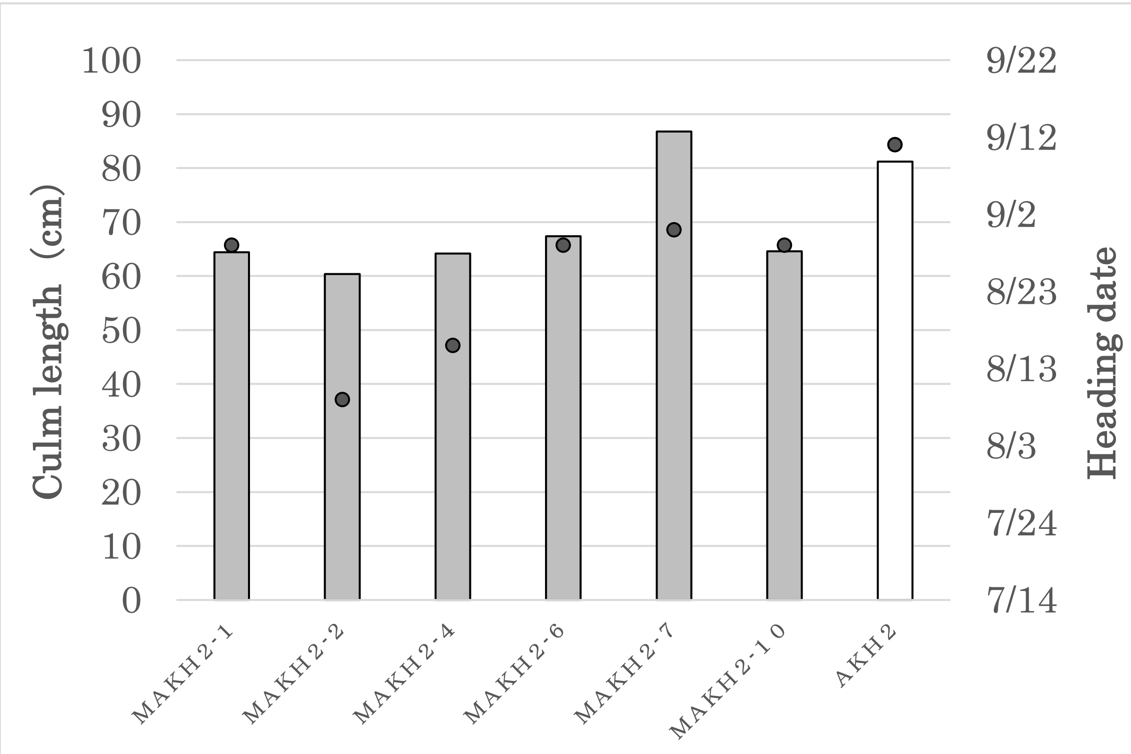


Fig.5. Culm length and heading date of M_4 mutant lines (2017) originating from irradiation of gamma-rays of 20 krad to F_8 seeds of AKH2.

Fig.6. Plant type of the selected mutant lines, MAKH2-7 and MAKH2-6 and control, AKH2.

Discussion

We succeeded in development of new rice variety, Kumaminori, through cross of native varieties, Asahi and Kamenoo, which shows higher grain yield and better eating quality in the nature farming or low input sustainable agriculture. The new variety has been released to farmers to grow in Kyushu district, Japan. However, heading date of the variety is too late to be adaptable to wider range toward northern part of Japan. It is noted that farmers are not to be suffering from lodging due to long culm length of the new variety, because of its strong culm. Farmers are, in general, used to be suffering from lodging of rice in Kyushu district, where is a route of typhoon in autumn season. It was practically proofed for strong culm of the new variety, Kumaminori that plants of the variety were not fell down when the typhoon came in 2008, while the modern leading variety, Hinohikari was done (Fig.7).

We have also succeeded in improving late heading date of the breeding line (AKH2) or new variety, Kumaminori within only five years through use of induced mutations. It is, in general, to take more than ten years in case of cross breeding for completing breeding purpose. In fact, we have taken more than 13 years to develop the new variety, Kumaminori.

Fig.7. Comparison of scenery of the rice fields of Kumaminori in nature farming and Hinohikari in conventional one when attacked by the typhoon in 2018.

The other merit of mutation breeding is to improve a target character without altering other desirable traits of the original variety. It is supposed that the selected mutant line, MAKH2-7 is to be inherited with desirable characters from the original variety, Kumaminori, e. g. high grain yield and eating quality in the condition of nature farming or low input sustainable agriculture. It was especially noted that the original variety, Kumaminori (AKH2) has been proved from epidemiological survey to carry a healing function to atopy and allergic disease. It was found earlier by Hiroshi HASEGAWA that the Yukihikari variety developed in Hokkaido was to carry healing function to atopy or allergic disease, whereas the representative leading variety, Kirara397 in Hokkaido tended to cause the disease (Miura et al., 2003; Hasegawa, 2019).

Representative native variety of Japan, Kamenoo and Ahahi were found to carry healing function to atopy or allergic disease (Sakuma and Komatsu, 2018, The Japan Agricultural News, 13th December 2019). The varietal difference of rice for ability to react to the disease was earlier found to be controlled by gene systems (Monnma, 2005; Sonoyama et al., 2009). The results of epidemiological tests suggest that Kumaminori or the AKH2 breeding line could be inherited healing function to allergic disease from mother varieties, Asahi and Kamenoo. We would expect that the MAKH2-7 mutant line would carry the healing function. Epidemiological tests for allergic disease of the mutant line are in progress. We are now taking a step for registration of the mutant line, MAKH2-7 for commercial variety.

We have found through the breeding of rice adaptable to nature farming that native varieties could be effective materials for breeding of rice for nutritious foods, as well as for adaptability to low input sustainable agriculture. We also would pay attention to the idea proposed by Indonesia and the Philippines in the sub-project "Mutation Breeding of rice for Sustainable Agriculture (2013~2017)" that native varieties can be effective breeding materials for mutation breeding of rice adaptable to low input sustainable agriculture (Nakai, 2018).

In conclusion, we propose that mutation breeding would be useful tool for development of rice varieties for adaptability to low input sustainable agriculture. In addition, we want to suggest that the native varieties be used as effective breeding materials for mutation breeding of rice for adaptability to low input sustainable agriculture, contributing to rehabilitation of the earth environment and human health.

Acknowledgment

We would thank to Mr. Takema SHIIBA for offering rice fields of the breeding and for kind support on the works. We also would like to give our thanks to Nature Farming and Culture Agency of MOA for financial support for the breeding project. We want to express our gratitude to Ms. Aki KOIKE of NSRA for kind support on the trials.

References

NAGANO, Hirohiko; KATO, Shigeru; OHKUBO, Shinnji and INOBUSHI. Emission of carbon dioxide, methan, and nitrous oxide from short- and long-term organic farming Andosols in central Japan, Soil Science and Plant Nutrition 58, 793-801, 2012.

SKINNER, Colin, GATTINGER, Andreas;, MULLER, Adrian;, MADER, Paul, FILESSBACH, Andreas; STOLZE, Mattias; RUSER, Reiner and NIGGLI, Urs. Greenhouse gas fluxes from agricultural soil under organic and non-organic management-Aglobal meta-analysis, Science of The Total Environment, 15, 553-563, 2014.

OKADA, Mokichi. Shizenn Nouhou Kaisetu (in Japanese) ("A commentary on Nature Farming" translated by the author), Eikou Shuppann Co. Ltd., 1953.

MIURA, Takako; MIURA, Shunnsuke; Matsuda, Michio, HASEGAWA, Hiroshi and YANAGIHARA, Tetsuji. Examination of different reaction between rice strains on rice-allergy patients with atopic dermatitis, Arerugi no Rinnshou 23-14, 44-50, 2013 (in Japanese).

Hiroshi Hasegawa, Yukihikari to komearerugi (http://hasekuri.a.la9.jp/yukihikari.htm, 2019/03/20) (in Japanese).

SAKUMA, Masaru and KOMATSU Miyuki. Turuokasi no Sizennnouhou Jissenn ni yoru Kennkouna Machidukuri(in Japanese) (Performance of nature farming for promoting healthy town in Turuoka-city, translated by the author) Koukai Sinnpojiumu Yuukinougyou ・ Sizennnouho kara hirogaru Machidukuri in Tokyo (in apanese)(Symposium on organic・nature farming held in Tokyo, translated by the author) 17 February 2018.

The Japan Agricultural News. 13th December 2019.

MONNMA, Michiko. Characteristics of protein profile of low allergenic rice variety, "Yukihikari", Rep. Nat'l. Food Res. Inst, No69, 7-12, 2005.

SONOYAMA, Kei; OGASAWARA, Toru; GOTO, Haruka; YOSHIDA, Tomoyo; TAKEMURA, Naoki; FUJIWARA, Reiko; WATANABE, Jun; ITO, Hiroyuki; MORITA, Tatsuya; TOKUNAGA, Yoshinari and YANAGIHARA, Tetsuji. Comparison of gut microbiota and allergic reactions in BALB/c mice fed different cultivar of rice, British, Journal of Nutrition 103, 218-226, 2009.

NAKAI, Hirokazu. Project Review on FNCA Mutation Breeding Project in 2017, in FNCA 18th Coordinator Meeting 2018.

Mutation Breeding of Rice for Sustainable Agriculture in Malaysia

Sobri Bin Hussein[1], Abdul Rahim Bin Harun[1], James Mackester Anak Simoli[1], Muhammad Ruzaini Abdul Wahab[1], Shakinah Binti Salleh[1], Faiz Bin Ahmad[1], Phua Choo Kwai Hoe[1], Shyful Azizi Bin Abdul Rahman[1], Ahmad Nazrul A.W[1], Latiffah Binti Nordin[1], Atsushi Tanaka[2], Anna Ling Pick Kiong[3], Koh Rhun Yian[3], Mohd Rafii Bin Yusop[4], Asma Ilyani[4], K, Kogeethavani R[5], Yoshihiro Hase[6], Aki Koike[7], Noorman Affendi M[8], Rahiniza Kamaruzaman[8], Nor'Aishah Hassan[9], Noraziyah Abd Aziz Shamsudin[10] and Norainy Md Hashim[11]

[1]Agrotechnology and Biosciences Division Malaysian Nuclear Agency (Nuclear Malaysia), Bangi 43000 Kajang Malaysia, [2]Kansai Photon Science Institute, National Institutes for Quantum and Radiological Science and Technology (QST), Japan, [3]Division of Applied Biomedical Sciences and Biotechnology, School of Health Sciences, International Medical University, 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia, [4]Plant Breeding & Genetics, Institute of Tropical Agriculture and Food Security, University Putra Malaysia, [5]Malaysian Agricultural Research and Development Institute, [6]Takasaki Advanced Radiation Research Institute, QST, Japan, [7]International Affairs and Research Department Nuclear Safety Research Association (NSRA), [8]MARDI Seberang Perai, Jalan Paya Keladi, Pinang Tunggal 13200 Kepala Batas Pulau Pinang (Malaysian Agricultural Research and Development Institute), [9] Faculty of Applied Science, University Technology MARA, Negeri Sembilan, [10] School of Biosciences and Biotechnology, Faculty of Science and Technology, UKM, [11]Bahagian Perkhidmatan Makmal, Jabatan Pertanian Negeri Kedah. State Department of Agriculture Kedah.

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.

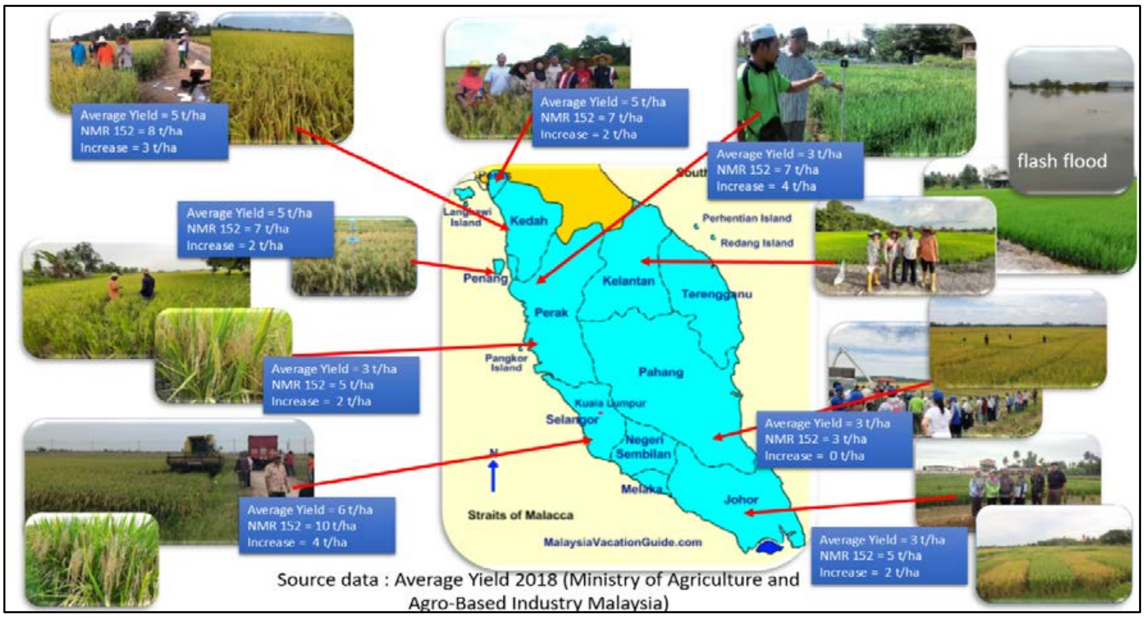


Figure 1: NMR 152 yield increment at different location in Peninsular Malaysia.

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).

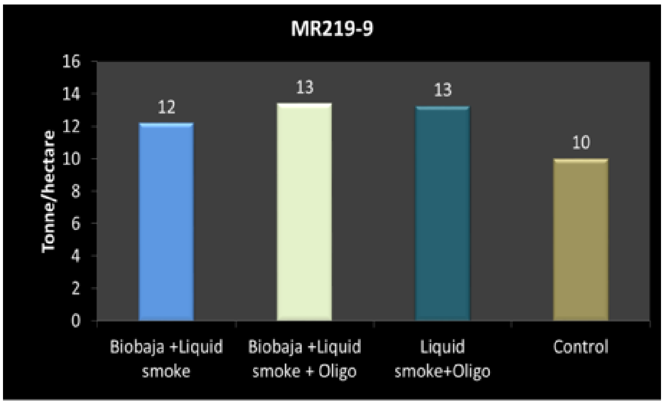


Figure 3a: Effect in yield production on MR219-9/NMR152 using different fertilizer treatment.

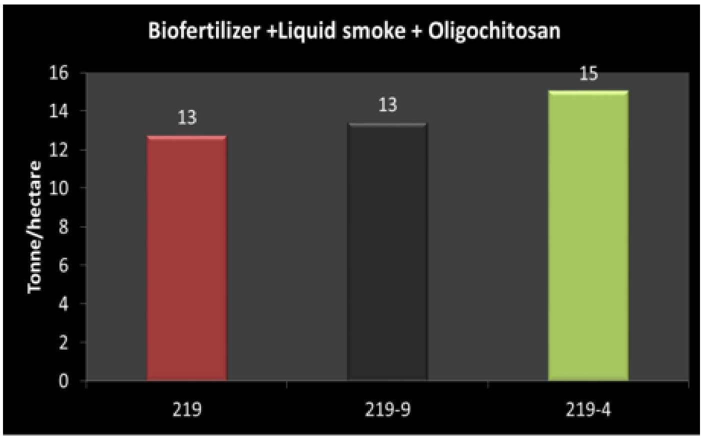


Figure 3b: Effect in yield production using different fertilizer treatment.

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.

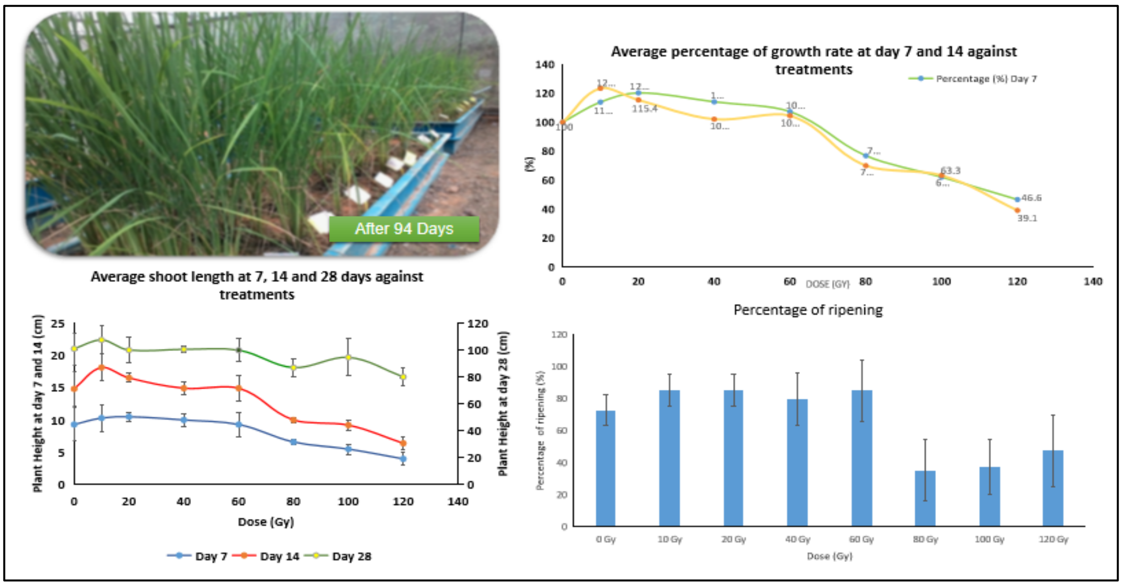
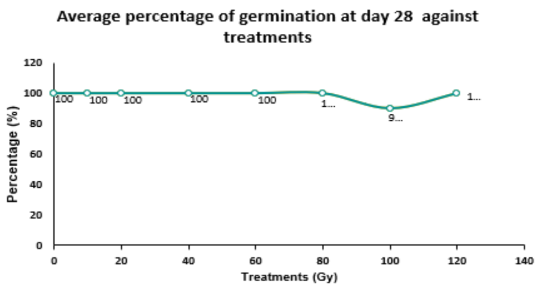
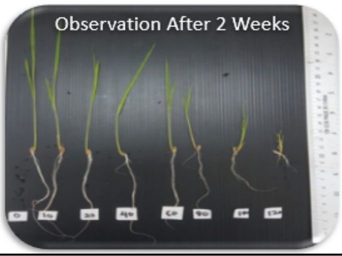


Figure 5: Dose response curves for percentage growth rate, plant height and ripening (Pongsu Seribu2).



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



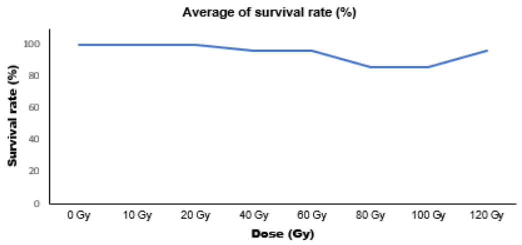


Figure 6: Dose response curves for germination rate, survival rate and spikelet fertility (Pongsu Seribu2).

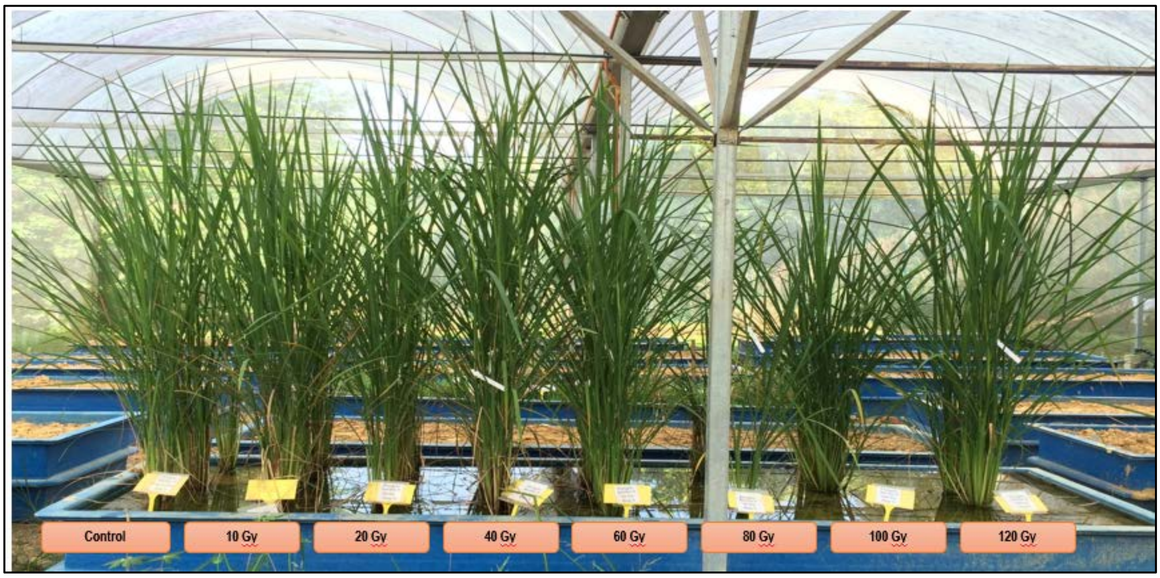


Figure 7: Biological effect of carbon ion on rice (Pongsu Seribu2).

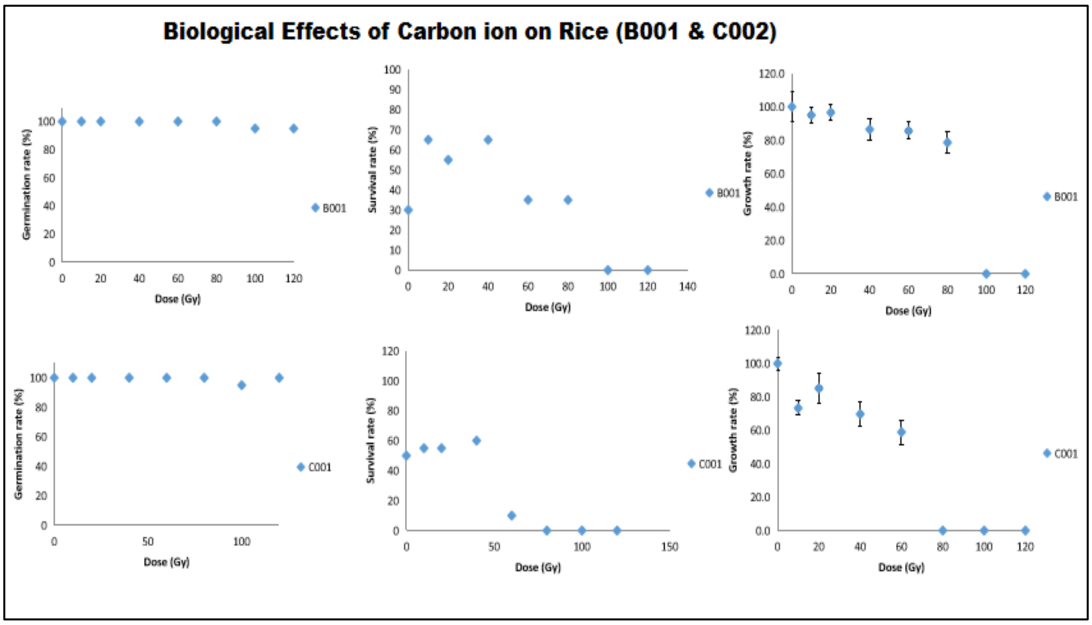


Figure 8: Biological effect of carbon ion on rice (B001 & C002).

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.

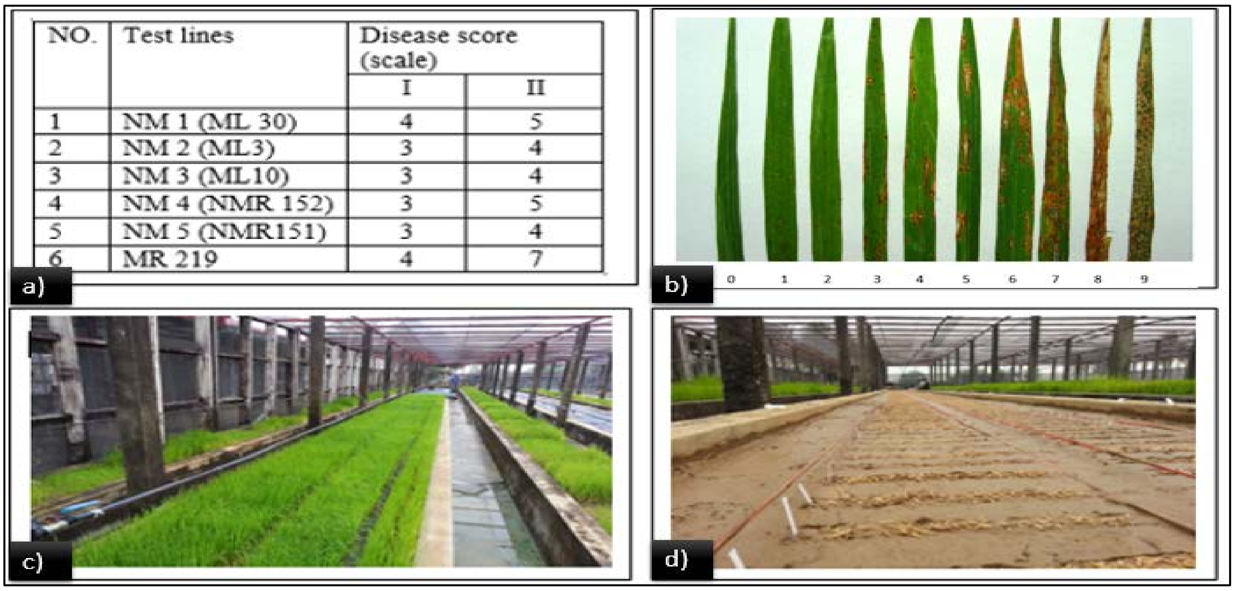
Table 11: Total N uptake and 15N of MR219-9/NMR152/PBR0156 under flooded and non-flooded condition

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	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).

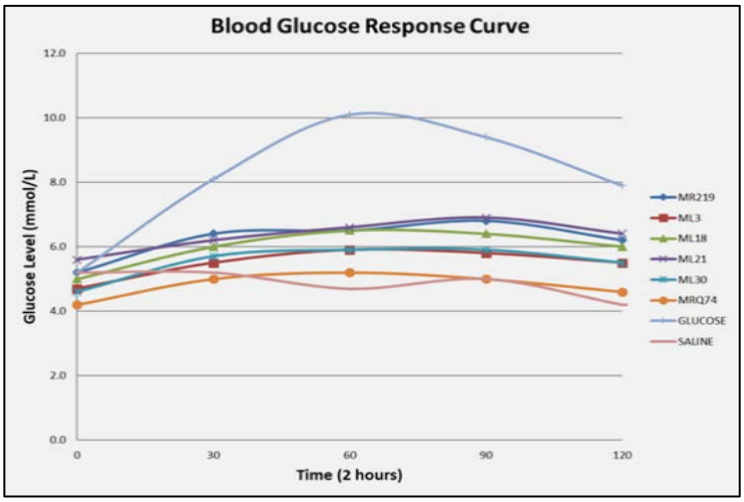


Figure 11: Blood glucose response curves of tested foods at 30minutes intervals

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.

Acknowledgements

The authors would like to thank Ministry of Science, Technology & Innovation (MOSTI) and Malaysian Nuclear Agency for providing the fund to carry out this Sub-Project on Composition or Quality of Rice coordinated by FNCA of Japan. The authors are grateful to the staff at the ion-beam facility at Takasaki Advanced Radiation Research Institute, QST, Japan. Special thanks go to Ms. Aki Koike from NSRA for her considerable patience, liaison and organizational skills that were very instrumental for the success of this project. The author also would like to acknowledge Dato Mansor bin Haji Md Noor from HMN (M) Sdn Bhd for his technical assistance during LVT trial plot and commercialization process. A special thanks also goes to Mr Mohammad Zaidi Shaari from BAYER (M) Sdn Bhd for his assistance in the coating technology.

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.

Improvement of Wheat Productivity Through Mutation Techniques

N. Bayarsukh, Ts Dolgor, B. Ganbaatar
Institute of Plant and Agricultural Science (IPAS), Mongolia

Abstract

The harsh climatic conditions of Mongolia and specific characteristic /late warming soil in the spring and early cold in the autumn/ of the high altitude agricultural zone /late warming soil in the spring and early cold in the autumn/ cause the need for short-maturity varieties. Breeding works are also in the process of selecting new varieties. Breeding of new varieties is moving in this direction as well. In recent years, physical and chemical mutagens have been widely used in the breeding of new wheat varieties and lines with good characters. Early maturity variety of wheat (*Triticum aestivum* L.) developed by induced mutation. New wheat mutant varieties have a high yield capacity and early maturity comparing most of local varieties, selected from hybridization. The mutants had been matured from two to ten days earlier than other varieties.

Introduction

The wheat is dominant crop and cultivated in about 80% of arable land in Mongolia including over 70% in the Central crop production zone, over 20% in the Western zone and 5-8% in the Eastern zone.

Due to the negative impact of climate change the drought frequency increased, carrying capacity of pasture land declined and plant species composition reduced by 2-4 times and yield declined 4-6 times, soil erosion and degradation increased by 7-25 times, soil humus content declined by 37-52% and annually 0.5-1.5 t/ha organic matter decomposed, yield potential of existing crop varieties reduced and pest and disease distribution, frequency and damage increased.

Also, the Western and Khangai mountain cultivation areas located above at the 1500-1900 m above sea level and daily average temperature ranges between 13-18°C in the warmest season of June and July, also the crop duration ranges between 75-110 days. These harsh conditions of the Western and Khangai mountain area allow growing cereals with very short duration.

Existing commercial wheat varieties have not good grain quality, drought tolerance, and disease and pests resistance and cannot sustain stable yield under climate change. Therefore, more new varieties with high stable yield potential and quality are needed in these mountain areas.

The induced-mutation considered useful efficient tool for the improvement specific plant traits like yield, stress tolerance, disease resistance, quality and increase breeding efficiency. Thus, development of early maturity, drought and heat tolerant wheat varieties with potential stable yield under changing climate condition through application of mutation techniques has needed for stable food production in Mongolia.

Materials and Methods

Mutation induction in wheat

The studies carried out at the Institute of Plant and Agricultural Sciences during 2013-2017. We evaluated 10334 mutant lines M_1-M_3 generation in the initial materials field, including 3131 mutant lines originated chemical mutagen treatment. Table 1.

Table 1. Mutants in the initial materials field

Year	Generation /lines			Total
	M_1	M_2	M_3	
2013	394	720	302	1416
2014	1025	750	477	2252
2015	460	1170	660	2290
2016	180	786	1440	2406
2017	-	840	1130	1970
Total	2059	4266	4009	10334

The yield trail of mutant varieties

In the yield trail the 12 registered wheat varieties selected for the test. Three of them mutant and nine are conventional varieties originated from domestic and Russian breeding. Origin of mutant Darkhan-141 variety is gamma ray 18000 rad, but origin of Darkhan-172 mutant variety has derived from chemical mutagen Sodium azide. The varieties grown in the black fallow, with three replications and a plot size was 50 m^2. The crop sown at a rate of 3.5 million seeds per hectare. The soil of the experimental site is Darkhan Uul province (49°28'N, 105°54'E), with pH 6.22, percentage of organic matter 1.35, available N-NO_3 3.5 mg/kg, P_2O_5 3.9 mg/kg^{-1} and K_2O 17.6 mg/ kg^{-1}. The plots harvested with a Wintersteiger harvester. Yield data adjusted to 14% moisture content and the following grain quality parameters were determined. Grain from each plot was dried and a composite 500 g sub-sample taken for quality (duplicate / triplicate) analysis. Protein (percent of dry matter) was calculated by multiplying the corresponding total nitrogen (by Kjeldahl) content by factor 5.7 (ICC 105/2) using a Kjeldahl auto analyzer. The wet gluten quantity extracted from whole meal flour in an automated gluten washer (Single head gluten tester MJ-IIA).

Results and discussion

Experiment on initial materials of wheat mutation breeding

In 2013-2017, the 10334 mutant progenies studied and evaluated by agro-morphological traits and stress tolerance. About 1808 advanced mutant lines selected and used for wheat hybridization breeding material in the wheat breeding program. Table 2.

Table 2. Average yield and yield components of mutants in the M_3 generation

	Plant height, cm	Plant number per m^2	Product able stem per m^2	Seed number per spike	Seed weight per spike	Yield, t/ha
Parents	77.6	166	428	35.7	1.3	4.38
Mutant lines	72.3	182	440	36.7	1.4	4.43
Variance, ±	-5.3	+16	+12	+1.0	+0.1	+0.15

Study of mutants in the yield trails

Maturity: Early and late maturing mutants have frequently induced through mutagenesis and identified easily. Early maturity in cereal crops is one of the useful characteristics for cultivation in cool temperate regions, offering the opportunity to flower frost-free conditions, harvest prior to frost and in drought –prone regions, the ability to produce a viable crop prior to drought conditions.

The heading time and maturity of wheat controlled by the plant's ability to sense via temperature and day length. The growth duration of wheat varieties in the yield trail fluctuated from 78-91 days. The Khalkh gol-1 variety matured very early 78 days, but Darkhan-144 late matured within 91 days.

The mutant variety Darkhan-196 matured in 2-12 days earlier than other varieties. Mutant variety Darkhan-172 matured in 2-10 days earlier than other varieties. Also, these varieties matured in 1-3 days earlier than control Darkhan-131 variety which are suitable in the mountain agricultural area. The mutant variety Darkhan-141 has growth duration of 84 days which is in 1-7 earlier than Darkhan -34, Altaiskaya -100, Altaiskaya -50, Arvin, and Darkhan -144 varieties. Table 3.

<u>Yield and yield components</u>: Stable and high yield potential in specific environmental conditions is the most important objective of Mongolian plant breeding program. Yield is complex trait and influenced by breeding objectives such as plant architecture, maturity, nitrogen utilization efficiency and resistance to biotic and abiotic stresses.

The grain yield of wheat varieties in the yield trail varied from 1.94 to 2.08 t/ha which are higher than the average yield of control varieties of 1.67 to 1.88 t/ha. The highest grain yield (2.08 t/ha) has the variety Arvin, however lowest was the Khalkh gol-1 variety (1.67 t/ha). The mutant variety Darkhan-141 has grain yield of 1.94 t/ha, which is 0.02 t/ha higher than mean yield of varieties. The mutant variety Darkhan-172 and Darkhan-196 have same grain yield (2.02 t/ha), which are higher (0.10 t/ha) than mean of varieties. These three mutants included in high yielding varieties. Table 3.

The thousand kernel weight of wheat varieties fluctuated from 30.5 g to 38.0 g. The highest was the Darkhan-172 mutant of 38.0 g, but lowest was Khalkh gol-1 variety-30.5 g. Thousand kernel weight of mutant variety Darkhan-196 was 31.7 g and Darkhan-141 was 34.6 g.

Table 3. Growth duration and yield of wheat varieties

No	Variety name	Maturity, days	Seed number per spike	1000 kernel weight, g	Yield, t/ha
1.	Khalkh gol-1	78	30	30.5	1.67 d
2.	Darkhan-196 mutant	79	29	31.7	2.02 ab
3.	Karagandinskaya-22	81	31	36.4	1.79 d
4.	Darkhan -172 mutant	81	30	38.0	2.02 ab
5.	Orkhon	84	30	33.2	1.88 c
6.	Darkhan -34	85	32	35.1	2.04 ab
7.	Darkhan -141 mutant	84	29	34.6	1.94 b
8.	Altaiskaya -99	83	31	34.7	1.86 c
9.	Altaiskaya -100	86	30	34.1	1.99 b
10.	Altaiskaya -50	86	26	36.2	1.72 d
11.	Darkhan -166	86	33	36.0	2.08 a
12.	Darkhan -144	91	33	36.9	2.03 ab
	Mean	84	30	34.8	1.92

<u>Grain quality of wheat varieties</u>: Quality parameters of new mutant varieties meet requirements of the national standard for food wheat MNS 0097:2010. The protein content of varieties varied 11.9-13.7%. The Altaiskaya-100 variety grain contained lowest protein 11.9%, however the Darkhan-196 mutant variety contains highest 13.7%. The medium maturity mutant variety Darkhan-141 has good grain quality comparing to other varieties. Protein content of Darkhan-141 mutant variety was 13.0%, it is 0.2-1.1% higher than other varieties, wet gluten (31%) 2.2- 5.7% higher and flour yield (73.0%) 1.7-6.4% higher than other varieties. Bread making quality score of mutant varieties Darkhan-172 and Darkhan-141 were highest (4.3-4.4).

Table 4. Comparing of grain quality of mutants and local varieties

No	Variety name	Protein,	Gluten,	Flour yield,	Bread making score
1.	Khalkh gol-1	13.0	29.4	68.5	4.1
2.	Darkhan-196 mutant	13.7	29.5	68.7	3.8
3.	Karagandinskaya-22	12.3	28.3	69.3	3.9
4.	Darkhan -172 mutant	12.5	28.5	68.0	4.4
5.	Orkhon	12.3	28.2	66.8	4.1
6.	Darkhan -34	12.8	28.1	69.4	4.0
7.	Darkhan -141 mutant	13.0	31.8	73.0	4.3
8.	Altaiskaya -99	12.5	29.6	71.3	3.8
9.	Altaiskaya -100	11.9	27.1	70.9	3.5
10.	Altaiskaya -50	12.2	26.1	67.0	3.7
11.	Arvin	12.3	29.2	66.6	4.3
12.	Darkhan -144	13.1	32.2	68.6	4.3

Fertilizer use effeciency of mutant varieties

We have studied organic and mineral fertilizers influences for mutant variety's yield and grain quality. Analysis of soil samples from planting depth (0-20 cm) indicated low level of available nitrogen and phosphorus, medium in potassium, indicating that the nutrient may be a limiting factor for wheat production in the area. The upper 20 cm (arable layer) contains low (0.86%) organic matter (C), increasing to 1.22% in the subsoil (soil layer 20-40 cm). During the growth stage of wheat the soil nutrients in different treatments had different trend over time. We observed that the application of fertilizers influenced positively to the soil fertility. The use of fertilizers at sowing time, are effective on improvement of seed germination and seedling growth of wheat mutant varieties, especially for Darkhan 141. Field germination of selected varieties increased from 6.3 % to 14.3% under fertilization.

These results showed that interactive effect of varieties and fertilizers had significant effect ($P<0.05$). The highest increased grain yield was recorded in NPK treatment. Application of N-P-K at the rate of 30-20-20 kg/ha to Darkhan 141 had maximum increased (1.22 t/ha or 83.6% compare to control) grain yield. The lowest grain yield (on an average 1.23 t/ha) was recorded in Darkhan 34 standart variety. Darkhan-172 mutant had 43.3% additional yield under the complex mineral fertilizer application. Table 5.

Table 5. Effect of fertilizers on yield of wheat mutant varieties, 2014-2016

Treatments	Darkhan-34		Darkhan 141		Darkhan 172	
	t/ha	Additional yield, %	t/ha	Additional yield, %	t/ha	Additional yield, %
Control	1.15	-	1.46	-	2.03	-
N	1.05	-	1.64	12.3	2.30	13.3
NP	1.27	10.4	2.16	47.9	2.90	42.8
NPK	-	-	2.68	83.6	2.91	43.3
Gumat	1.44	25.2	1.62	11.0	1.98	-
Mean	1.23	17.8	1.91	38.7	2.42	33.1

The fertilization effected positively on grain protein content and increased 0.4-2.79 %. The highest percentage of grain protein (16.3%) and gluten content (36.9) were obtained from combined nitrogen and phosphorus (NP) fertilizer. Table 6.

Table 6. Effect of fertilizers on grain quality of mutant varieties, 2014-2016

Treatments	Darkhan 34		Darkhan141		Darkhan 172	
	Protein, %	Gluten, %	Protein, %	Gluten, %	Protein, %	Gluten, %
Control	12.5	29.2	11.9	29.5	13.0	29.6
N	14.1	30.9	12.6	30.8	13.2	34.0
NP	16.3	36.9	13.0	34.4	12.9	30.1
NPK	-	-	14.2	31.4	13.5	32.8
Gumat	11.9	23.8	11.5	24.6	-	-

Conclusion

The experiment results showed developing new varieties with high yield early maturity and good grain quality through mutation induction. Mutant varieties has very high fertilizer use efficiency.

The new mutant variety Darkhan-172 is registered as promising new early wheat variety for growing in the western and mountain areas in Mongolia.

Acknowledgments

- This work was supported by FNCA "Mutation breeding" project.
- All members of FNCA project for sharing information and experience.
- Prof. Hirokazu Nakai for supervising rice mutation breeding.

References

1. Dolgor.Ts, Results of mutation breeding and future PSARTI Research volume 27, 2009,
2. Maslov.A.B, Mutagenesis in the wide hybridization and polyploidy breeding and genetic experiments Moscow, 1983
3. Maluszynski.M, Micke.A, Sigurbjornsson.B, Szarejko.I and Fuglewicz.A, The uses of mutants for breeding and for hybrid barley, Barley Genetics V: 969-977, 1987
4. Maluszynski, M.; Nichterlein, K.; Van Zanten, L.; Ahloowalia, B. S. Officially released mutant varieties-The FAO/IAEA Database, Mutation Breeding Review No. 12 December 2000. Joint FAO/IAEA Division, Vienna, Austria, ISSN 1011-2618.
5. Shu.Q.Y, Forster.B.P, Nakagawa.H.
   Plant mutation breeding and biotechnology, Joint FAO/IAEA Program Malta, 2012
6. Dolgor.Ts, Bayarsukh.N. Mutation breeding achievements in Mongolia, March 2017; The National nuclear energy project coordinator's meeting, Ulaanbaatar, Mongolia.

Improvement of Traditional Rice Varieties by Gamma Irradiation in the Philippines

Fernando B. Aurigue[1], Ana Maria S. Veluz, Arvin O. Dimaano
and Adelaida C. Barrida[2]
Project Leader: [1] July 2017 onwards; [2] 2013-2015 (retired)

Department of Science and Technology-Philippine Nuclear Research Institute
Commonwealth Avenue, 1101 Diliman, Quezon City, Metro Manila, Philippines

Abstract

Two Philippine traditional rice varieties, namely: Umangan and Native Borie, were used to develop mutant lines that are suitable for organic farming or sustainable agricultural practices of rice production.

Seeds were subjected to acute gamma ray treatment and grown as M_1 generation together with the non-irradiated materials as control. Comparisons were made between plants treated with 200 Gy and 300 Gy, and also with the non-irradiated plants. Early flowering plants with reduced height, more tillers, and higher number of filled grains per panicle were observed from the segregating M_2 generation but no selection was made. M_3 generation was grown from bulked seeds and putative mutant plants with the desired agronomic characteristics were selected based on the results of statistical analysis. The M_4 generation did not show any line that is better than the control in any of the parameters measured.

Apparently, plants with desirable mutated traits were noticed but not isolated early enough. Continuous bulking of seeds and late selection for the desired characteristics showed the importance of following the prescribed methodology. Failure to develop putative mutant lines during the given period (2013-2017) is attributed to the stalling of the project for 1½ years due to lack of personnel, change of workforce, leadership, and priorities in the Philippine government, and seasonal cultivation of rice in the experimental area. With the lessons learned, the current Director of the Institute and Project Leader on mutation breeding of rice have strengthened their partnership or collaboration with the Philippine Rice Research Institute and state universities and colleges that conduct rice improvement programs to use the gamma irradiation facility for developing mutant varieties of rice and other crop plants.

Keywords: gamma irradiation, mutant, rice, traditional rice variety, varietal improvement

Introduction

Rice (*Oryza sativa* L.) is the Philippines' most important staple crop. Rice production is also the main source of livelihood of more than 2.5 million households or about 2.1 million farmers (FAO, 2018). It is very important to the food supply and economy of Filipinos. The country is the eight largest rice producer in the world, accounting to 2.8% of global rice production (FAO, 2018) but the Philippines is the third largest rice importer in 2015, having imported 1.8 million metric tons that year, and continues to be one of the largest importer of rice in the world market to satisfy the domestic demand (Exconde, 2018). The major reason for this imbalance is that the population of the Philippines has gone up to 104.9 million in 2017 and Filipinos have been consuming more milled rice than what is being produced domestically. Per-capita rice consumption was 109.875 kg in 2015 to 2016 (Ordinario & Arcalas, 2018). Domestic consumption of milled rice ranged from 12.85 metric tons in 2013 to 13.25 metric tons in 2017 (USDA, 2019).

According to Crop Statistics of the Philippines, during the period of 2014 to 2018, rice production in the country grew at an average annual rate of 0.3%, from 18.97 million metric tons in 2014 to 19.28 million metric tons in 2017. Likewise, the total area harvested increased from 4.74 million hectares in 2014 to 4.81 million hectares in 2017 or at an average annual rate of 0.4% (PSA, 2018). However, the national average yield for all ecosystems remains low at about 3.87 metric tons per hectare at a relatively high production cost of US$0.24 (PhP12.00) per Kg.

To sustain rice production, other means of increasing productivity must be employed. Organic farming is becoming popular in the Philippines, especially with the organic rice that has increasing demand and commands higher selling price. Additionally, health conscious-Filipinos are looking into options of consuming organically-grown rice and traditional rice varieties (TRVs). Growing number of farmers are planting TRVs mainly because they are more adapted to the ecosystem and do not need much agrochemical inputs as compared to hybrid rice which are highly dependent on agricultural chemicals such as synthetic fertilizers and pesticides (herbicides, fungicides and insecticides).

Another means is to develop mutant lines from TRVs that are highly adapted to existing ecosystems but do not require as much inputs as the more popular and highly promoted hybrid rice varieties. Mutant varieties that are suited to organic agricultural practices are more sustainable for year-round production. The objective of the project is to develop mutant lines with improved agronomic characteristics through gamma irradiation of seeds of two select TRVs.

Materials and Methods

Seeds of two TRVs were obtained from the Philippine Rice Research Institute (PhilRice), Department of Agriculture (DA) in the Science City of Muñoz, Nueva Ecija, Central Luzon. These two rice varieties, namely: 1.) Umangan; and 2.) Native Borie were selected according to their popularity or extent of use in Luzon island, Philippines.

Based on results of previous studies on radio-sensitivity of rice seeds to acute gamma radiation, the seeds were treated with 200 Gy and 300 Gy of gamma rays as the recommended dose levels, while non-irradiated seeds served as the control. Irradiated and control seeds were sown in beds and protected from birds and rodents. Soil samples were collected and analyzed for elemental analysis prior to land preparation. Depending on the results for nitrogen, phosphorus and potassium, the recommended amount of organic fertilizer was incorporated evenly in the soil 3 days before transplanting.

Seedlings were soaked in biofertilizer for 30 minutes before transplanting. A Randomized Complete Block Design (RCBD) with at least three replicates was followed every cropping season. During the second year or at M_2, radiation-modified kappa-carrageenan (RMKC) solution, which has been developed as a plant growth promoter at PNRI from another project, was applied as foliar spray at 100 ppm. Foliar application was done every week for 1 month. On the third year or at M_3, the use of RMKC was modified by increasing the concentration to 200 ppm but applying only three times: 14 days after transplanting (DAT), 35 DAT, and 60 DAT. At M_4, the recommended application rate followed was 200 ppm at 15 DAT, 30 DAT, and 45 DAT.

Proper cultural management was employed during the whole cropping season in all generations of planting. The activities included the maintenance of proper water level at different stages of plant growth, manual control of golden apple snail, installation of nets to prevent birds from eating the grains and trapping of field rats. The same methodologies were followed throughout the conduct of the project (up to M_4 only).

Sowing of seeds of putative mutant plants selected from M_3 generation was delayed for 1½ years. This is largely attributed to a chain of unprecedented events starting off with health trepidation leading to an early retirement in December 2015 for Ms. Barrida as Project Leader. By June 2016, a new President of the Philippines was installed, bringing new national and institutional priorities, and a status quo in the organizational set-up of some projects. Finally, by July 2017, Mr. Aurigue received his designation as the new Project Leader, thereafter resuming the planting, evaluation and selection of putative mutant lines as planned for the project.

Seeds from selected M_3 plants were sown on paddy soil placed in black polyethylene plastic (PEP) bags on July 31, 2017 for M_4 generation plants. The seedlings were transplanted into two rice paddies on August 17 and 18, 2017 following RCBD with three replications. Data for vegetative traits were gathered on November 6, 2017. Necessary cultural practices were employed until harvesting on November 28, 2017. Data gathering on yield parameters were conducted afterwards when the intact seeds have been sun-dried properly with less than 12% moisture content.

Results and Discussion

The M_1 generation of both Umangan and Native Borie flowered earlier (80 DAT) than the control plants. For Umangan, high number of grains per panicle was obtained from 200 Gy with 184.5, followed by 300 Gy with 133.0 (Table 1). For Native Borie, high tillering was observed at 200 Gy with 25.7 followed by the control with 21.3 (Table 2).

Table 1. Agronomic characteristics of non-irradiated and irradiated Umangan at M_1 generation

Dose	Plant height (cm)*	Number of tillers per Plant*	Length of panicle (cm)*	Total number of grains per panicle	Number of filled grains per panicle*	Number of unfilled grains per panicle*
Control	97.55	18.4	22.23	116.7 b	82.6	34.1
200 Gy	101.46	17.5	22.76	184.5 a	101.1	83.4
300 Gy	101.40	19.7	23.43	133.0 ab	102.3	30.3

* not significant

Means followed by similar letter within the same column are not significantly different at 5% level DMRT

Table 2. Agronomic characteristics of non-irradiated and irradiated Native Borie at M_1 generation

Dose	Plant height (cm)*	Number of tillers per plant	Length of panicle (cm)*	Total number of grains per panicle*	Number of filled grains per panicle*	Number of unfilled grains per panicle*
Control	122.33	21.3 b	23.00	188.3	142.7	45.6
200 Gy	121.61	25.7 a	26.86	184.3	123.3	61.0
300 Gy	121.05	17.3 c	25.05	174.3	122.0	52.3

* not significant

Means followed by similar letter within the same column are not significantly different at 5% level DMRT

In the M_2 generation, results for Umangan also showed early flowering at 200 Gy with 70 DAT followed by 300 Gy with 77 DAT. At 200 Gy, there was 7.15% reduction in plant height. Increase in the number of grains per panicle was noted at 300 Gy (Table 3 and Figure 1). Meanwhile, for Native Borie, early flowering at 85 DAT was observed for both 200 Gy and 300 Gy. The control plants took 100 DAT to flower. There was an increase in number of tillers per plant as 16 tillers were counted for 200 Gy and 17 tillers for 300 Gy (Table 4).

Table 3. Agronomic characteristics of non-irradiated and irradiated Umangan at M_2 generation

Dose	Number of Days to Flower	Plant height (cm)	Number of Tillers per Plant	Length of Panicle (cm)	Number of Filled Grains per Panicle	Number of Unfilled Grains per Panicle	100-seed Weight (g)
Control	80	82.93	17.0	22.16	86.0	24	4.24
200 Gy	70	77.00	15.0	21.46	93.0	18	4.51
300 Gy	77	80.26	18.0	21.53	99.0	21	4.47

Table 4. Agronomic characteristics of non-irradiated and irradiated Native Borie at M_2 generation

Dose	Number of Days to Flower	Plant height (cm)	Number of Tillers per Plant	Length of Panicle (cm)	Number of Filled Grains per Panicle	Number of Unfilled Grains per Panicle	100-seed Weight (g)
Control	100	106.33	11.0	25.66	121.0	45	4.11
200 Gy	85	106.66	16.0	26.66	119.0	48	3.91
300 Gy	85	97.53	17.0	25.33	112.0	47	3.70

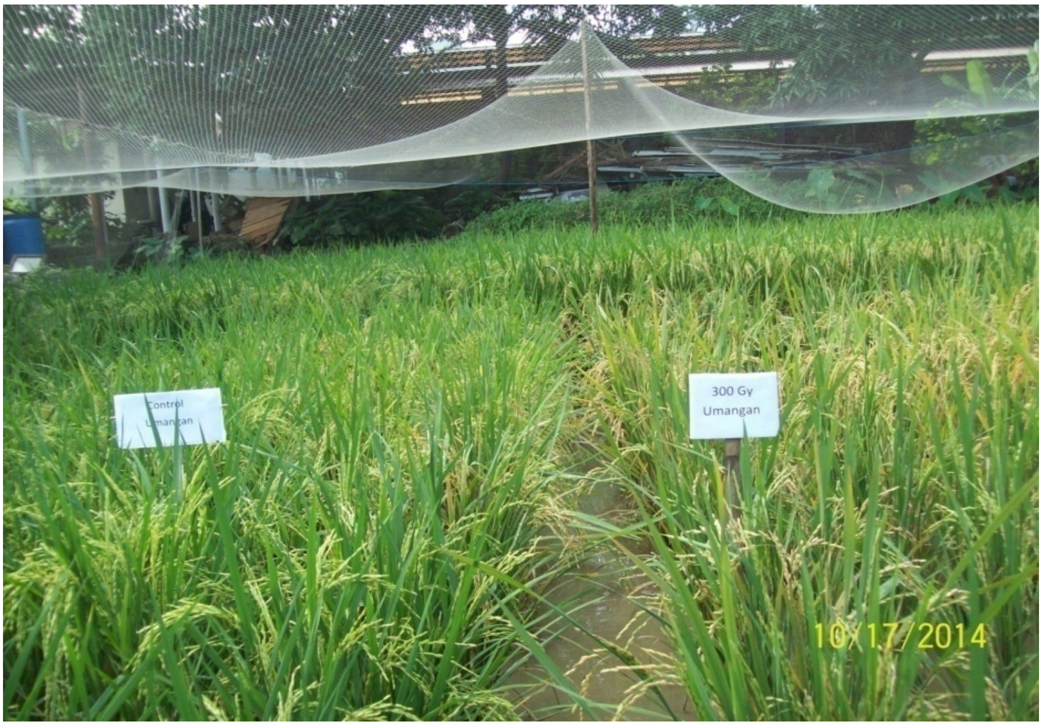


Figure 1. Control and M_2 plants derived from Umangan irradiated with 300 Gy of acute gamma radiation.

In the M_3 generation, the number of days to flowering, number of tillers per plant, and the total number of filled grains per panicle were significantly affected by the treatments for Umangan (Table 5). At 200 Gy, plants flowered 14 days earlier than the control, whereas at 300 Gy plants flowered 11 days earlier than the control. Meanwhile, the number of tillers per plant increased from 11 to 14 for plants derived from both 200 Gy and 300 Gy. The number of grains per panicle obtained at 200 Gy (152 seeds) and at 300 Gy (132 seeds) is comparable to those of the control plants (147 seeds).

In Native Borie, the plant height, number of days to flower, and number of tillers per plant were significantly affected by the treatments at M_3 (Table 6). Plants derived from 200 Gy and 300 Gy flowered 7 days earlier than the control. Reduction in plant height was about 10.46% and 6.46% less than the control at 300 Gy and 200 Gy, respectively. The number of tillers per plant increased by four at 300 Gy (18 tillers) and by two at 200 Gy (16 tillers) compared to the control (14 tillers). Figure 2 shows foliar application of RMKC solution during tillering stage.

Table 5. Agronomic characteristics of non-irradiated and irradiated Umangan at M_3 generation

Dose	Number of days to flower	Plant height (cm)*	Number of tillers per plant	Length of panicle (cm)*	Number of filled grains per panicle	Number of unfilled grains per panicle*	100-seed weight (g)*
Control	92 b	94.66	11.0 b	23.43	121.0 ab	26	4.37
200 Gy	78 a	95.20	14.0 a	23.05	128.0 a	24	4.44
300 Gy	81 a	96.43	14.0 a	23.10	111.0 b	21	4.49

* not significant

Means followed by similar letter within the same column are not significantly different at 5% level DMRT

Table 6. Agronomic characteristics of non-irradiated and irradiated Native Borie at M_3 generation

Dose	Number of days to Flower	Plant height (cm)	Number of Tillers per Plant	Length of Panicle (cm)*	Number of Filled Grains per Panicle*	Number of Unfilled Grains per Panicle*	100-seed Weight (g)*
Control	106 b	109.06 a	14.0 b	27.27	122.33	36.33	3.99
200 Gy	99 a	105.11 ab	16.0 ab	26.93	122.67	49.66	3.84
300 Gy	99 a	98.73 b	18.0 a	26.76	112.67	32.66	4.16

* not significant

Means followed by similar letter within the same column are not significantly different at 5% level DMRT

Umangan

Native Borie

Figure 2. Spraying of radiation-modified kappa-carrageenan solution on two traditional rice varieties at M_3 generation during tillering stage.

In the M_4 generation, there was no significant differences between means for each parameter observed (Table 7). The selected putative mutant lines were similar to their respective control parent. Likewise, results of statistical analysis imply that the yield of selected putative mutant lines was not different from their respective control parent (Table 8). Some of the putative mutant lines growing in the field are shown in Figure 3.

Figure 3. Different putative mutant lines at M_4 generation.

Table 7. Vegetative characteristics of non-irradiated and putative mutant lines of traditional rice varieties at M_4 generation

Line	Plant Height (cm) 80 DAT	Total Number of Tillers	Number of Productive Tillers	Number of Unproductive Tillers	Percent Productive Tillers
Umangan (Control)	104.50	10.4	9.2	1.2	88.5
Umangan (300 Gy) 4	101.60	9.9	7.9	2.0	79.8
Umangan (200 Gy) 8	97.40	8.0	7.5	0.5	93.8
Umangan (300 Gy) 23	103.00	9.6	8.5	1.1	88.5
Umangan (300 Gy) 28	98.40	8.6	7.3	1.3	84.9
Umangan (200 Gy) 39	103.73	10.2	9.0	1.2	88.2
Native Borie (Control)	122.60	8.1	7.0	1.1	86.4
Native Borie (300 Gy) 27-1	111.97	10.9	8.0	2.9	73.4
Native Borie (300 Gy) 27-2	130.80	8.8	8.4	0.4	95.4
Native Borie (300 Gy) 34	112.43	12.5	12.3	0.2	98.4
Native Borie (300 Gy) JF-1	110.10	11.5	11.5	0.0	100.0
Native Borie (200 Gy) JF-2	111.03	10.2	10.2	0.0	100.0

Table 8. Yield parameters of non-irradiated and putative mutant lines of traditional rice varieties at M_4 generation*

Line	Panicle Length (cm)	Number of Branches per Panicle	Number of Filled Grains	Number of Unfilled Grains	100-Seed Weight (g)	Yield (g)
Umangan (Control)	21.88 ab	5.87 ab	45.03 bc	71.93	2.17 b	54.90 abcd
Umangan (300 Gy) 4	21.21 b	5.80 ab	38.03 bc	69.87	2.13 b	51.07 bcd
Umangan (200 Gy) 8	21.34 b	5.83 ab	42.10 bc	69.87	2.17 b	51.63 bcd
Umangan (300 Gy) 23	21.03 b	5.70 ab	36.40 bc	69.50	2.13 b	42.30 cd
Umangan (300 Gy) 28	20.58 b	5.23 b	24.93 c	59.90	2.10 b	25.70 d
Umangan (200 Gy) 39	22.88 ab	6.00 ab	57.13 abc	74.23	2.67 b	67.20 abc

Native Borie (Control)	25.60 a	6.90 a	86.27 a	107.83	9.20 a	94.40 a
Native Borie (300 Gy) 27-1	25.32 a	6.40 ab	69.27 ab	104.20	3.03 b	86.27 ab
Native Borie (300 Gy) 27-2	22.72 ab	5.90 ab	55.33 abc	72.07	2.30 b	57.83 abcd
Native Borie (300 Gy) 34	24.31 ab	6.37 ab	62.20 abc	88.10	2.80 b	80.93 abc
Native Borie (300 Gy) JF-1	23.95 ab	6.20 ab	61.20 abc	86.27	2.77 b	76.60 abc
Native Borie (200 Gy) JF-2	23.52 ab	6.00 ab	59.97 abc	82.20	2.70 b	72.47 abc
F-test	**	**	**	ns	*	**

*Mean of 10 samples

Means followed by the same and/or sharing the same letters within a column do not differ significantly at p≤0.01 (**) or p≤0.05 (*)

ns = not significant

With such findings, no desirable mutant line was developed by gamma irradiation. After reviewing the data generated from M_1 to M_3, and upon investigation of the procedures done in handling the generation advancement, it was found out that seeds derived from the same gamma irradiation dose level were apparently bulked for each variety and no selection of putative mutant plants was conducted prior to M_3 generation.

Summary and Conclusion

The potential to develop mutant lines from two Philippine TRVs, namely: Umangan and Native Borie, that are suitable for organic farming or sustainable agricultural practices of rice production was not tapped at the right time or generation.

Plants derived from seeds subjected to 200 Gy and 300 Gy of acute gamma ray showed desirable characteristics at M_1 and M_2 generations but selection of putative mutant plants was done at M_3 generation which was grown from bulked seeds. The M_4 generation did not show any line that is better than the control in any of the parameters measured.

Due to unforeseen or unexpected events from 2013 to 2017, the project was stalled for 1½ years following the early retirement of the Project Leader without immediate replacement, change in national and institutional leadership and priorities in the

Philippine government, and planting of rice limited to just once a year. Now that no mutant line was obtained for this particular project, partnership or collaboration with PhilRice and state universities and colleges was strengthened to ensure that rice improvement programs through mutation induction by gamma irradiation will be continued until new mutant varieties of rice and other crop plants are developed, registered, and utilized by farmers.

Acknowledgment

The authors are grateful to PhilRice-DA for providing seeds of TRVs. The staff of PNRI Cobalt-60 Multi-purpose Irradiation Facility are acknowledged for treating the seeds with gamma radiation. Science Aides who prepared the paddy, sowed the seeds and transplanted the seedlings, maintained the experimental field, harvested the grains, and assisted in data gathering are all appreciated. They are Mr. Eduardo C. Costimiano, Ms. Rosalie M. Semana, Ms. Dolores M. Lazo (2015), and Mr. John Faustus C. Vidal (2017). Ms. Mary Jane C. Manrique, who assisted in the conduct of the experiments in 2013-2014, is also acknowledged.

References

Exconde, B. 2018. On Rice Production and Consumption Patterns in the Philippines. The Lobbyist. 16 February 2018.

[FAO] Food and Agriculture Organization of the United Nations Regional Rice Initiative. www.fao.org accessed on 19 January 2020.

Ordinario, C. U. and Arcalas, J. E. Y. 2018. Snapshot of rice – consumption data remains grainy as Pinoys grapple with supply, prices. The Broader Look. Business Mirror. 30 August 2018.

[PSA] Philippine Statistics Authority. 2019. Accessed on 19 January 2020.

Simeon, L. M. 2019. PhilRice seeks competitive rice yield. The Philippine Star.

[USDA] United States Department of Agriculture. 2019. indexmundi.com accessed on 19 January 2020.

Application of Mutation Breeding of Submergence tolerance Rice for Sustainable Agriculture, in Thailand

Kanchana Klakhaeng[1], Udompan Promnart[2], Peera Doungsoongnern[2], Kanokporn Boonsirichai[3]

[1] Rice Research and Development Division, Rice Department, Thailand,

[2] Prachin Buri Rice Research Center, Bansang, Prachinburi, Thailand,

[3] Thailand Institute of Nuclear Technology, Ongkharak, Nakhon Nayok, Thailand.

Abstract

The world is facing a food and energy crisis of unprecedented proportions and also climate change, especially the devastating flood in Thailand, which affected at least 25 of the country's 77 provinces and affected 9 million citizens in 2011. Normally in plant genetics and breeding research, mutation is a source of variation. Spontaneous mutation rate is very low, induced mutation have contributed to the discovery and identification of gene function following the completion of genome sequencing project in rice. Therefore, radiation can cause genetics change in living organism to increase significantly. The aims of this project are to improve rice variety with enhanced to tolerance to flood and/or wetland conditions with good grain quality. RD31, a non-glutinous photoperiod–insensitive rice variety was irradiated with 0.44 kGy electron beam due to its several preferable agricultural traits such as high yield and moderately resistance to brown planthopper, bacterial leaf blight, brown spot disease and dirty panicle disease. M_1 plants were planted in dry season 2014. Five hundred panicles were harvested from the main tiller of each M_1 plant. M_2 plants were grown as 1 panicle/row for 500 rows or 10,000 plants in total. M_3 lines were screened for submergence and 317 lines survived with the score of 5-7. M_4 were screened for blast resistance. The result showed that all mutant lines were highly resistance to blast. Some of these lines were screened for submergence and found that 91 submergence tolerant mutant lines were identified. Grain quality were tested and revealed that 16 mutant lines showed 23.22-24.44 % amylose content which were lower than RD31 the original (27.52%). Physical property of all these mutant showed clear chalkiness of endosperm. M_5 mutant lines were grown for observation yield trials and also physical and chemical properties were examined. M_6 mutant lines were grown for intrastation yield trial which were screened for submergence tolerant during 2018. Further work will be carried out before released the new rice variety.

Keywords: Rice, Electron Beam, Flood Tolerance

Introduction

Rice is the principle staple crop of Asia and any deterioration of rice production systems through climate change would seriously impact food security in the world. Climate change has many facets, including changes in long-term trends in temperature and rainfall regimes. The impact of these changing conditions on agriculture are already being seen. Although climate change is a global phenomenon. It will manifest itself as locally variable impacts. Modern rice varieties are not adapted to these conditions and their yield is severely reduced because of high mortality, suppressed tillering ability, reduced panicle size, and high sterility. These are probably the reasons why farmers in affected areas still rely on low-yielding local landraces.

In the past, conventional breeding was used to develop new varieties, but those varieties left breeders unsatisfied still. Mutation breeding can be used to obtain suitable rice varieties which can lead to sustainable agriculture. Therefore, rice can be further improved. The rice improvement by mutation breeding through ion beam and gamma rays irradiation is very useful for creating genetic diversity. The irradiated rice population and its subsequent generations serve as the source of genetic variation for selection or screening according to our objectives. The aims of this project are to improve rice varieties with enhanced to tolerance to flood and/or wetland conditions.

Materials and Methods

Two hundred gram of RD31 recommended rice variety was irradiated with 440 gray electron-beam in 2014. M_1 plants were grown by broadcasting. Five hundred panicles from each main clum were collected as M_2 seeds. M_2 plants were planted as 1 panicle/row for 500 rows or 10,000 plants in total. Agricultural traits, including plant height, number of panicle per hill and maturity of these M_2 plants were observed. Seeds from six plants were collected as M_3 seeds. M_3 plants were screened for submergence tolerance (Appendix 1) and M_4 plants were screened for blast resistance (Appendix 2). The remaining were screened for submergence tolerance, and were examined for grain quality. M_5–M_6 plants were planted for yield trial observation.

Results

RD31, a non-glutinous and photoperiod–insensitive rice cultivar, is one of the certified cultivars for irrigated areas in the central plain of Thailand. It has a relatively high yield and moderate resistance to brown plant hopper, bacterial leaf blight, brown spot disease and dirty panicle disease with a good grain quality.

M_1 plants were planted by broadcasting in dry season 2014. Five hundred panicles were harvested from the main tiller of each M_1 plant to obtain M_2 seeds. M_2 plants were grown as 1 panicle/row for 500 rows or 10,000 plants in total in dry season 2014. (Figure 1). Plant height, number of panicle per hill and maturity of these M_2 plants were observed and recorded (Table 1).

Figure 1. Electron beam irradiated M_2 plants of RD31.

Table 1. Plant height of RD31 and M_2 populations.

Plant groups	Plant height (cm)	
	Height (cm)	No. of plants
RD31	121	20
M_2 of RD31		
-Dwarf	40-54	-
-Semidwarf	55-100	2
-Intermediate-tall	101-120	279
-Tall	121-155	218

The plant height of RD31 was 121 cm. It was classified according to Loresto and Chang (1978) as tall class. Loresto and Chang (1978) used the following criteria to classified plant height in the F_2 populations : 1) tall (121-155 cm), 2) intermediate-tall (101-120 cm), 3) semi-dwarf (55-100 cm), and dwarf (40-54 cm). While Fernandez et al., classified dwarfs into four categories : 1) tall (more than 130 cm), 2) intermediate (110-130 cm), 3) semi-dwarf (80-110 cm), and dwarf (40-80 cm). Upon electron-beam irradiation, the height of M_2 plants ranged from 99-143 cm (Table1 and Figure 2), covering all three height classes, including semi-dwarf, intermediate-tall and tall. The observed frequency were 2, 279 and 218, respectively.

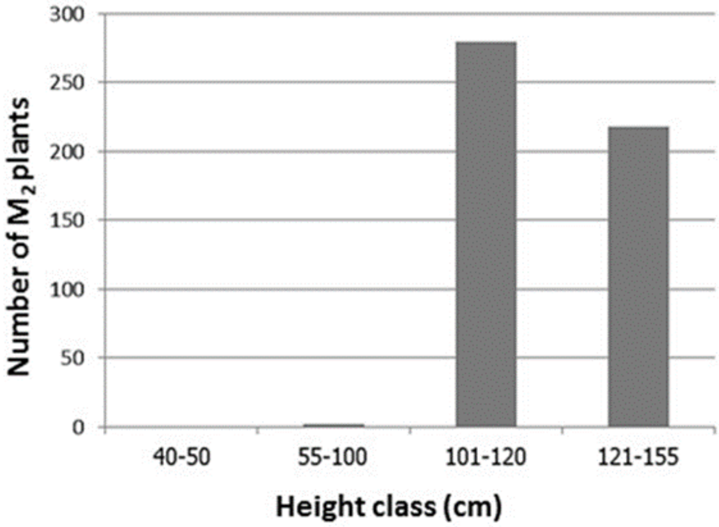


Figure 2. Distribution of M_2 plant height according to the classification of Loresto and Chang, (1978).

Panicle number of each hill was counted as it was a key component of the yield. Yield components refer to the structures of the rice plant that directly translate into yield. The relationship among growth duration, plant stature and grain yield was well explained more than 1,400 years ago (Liu, 1986). Yield components is important for fixing up the character, which plays an ultimate role in influencing the yield. Many reports identified as yield of crops depend on yield components including, the number of panicles per given area, the number of spikelets (potential) grain per panicle, the 1,000 grain weight, and the percent of fill grains per panicle. Upon electron-beam irradiation, panicle number of each hill was counted. Yield components were determined during each rice cycles, first at the vegetative phase (tiller number), second at the reproductive phase (number of fertile tillers and size of panicle sinks) and finally at the grain filling phase (spikelet filling rate). The results showed that M_2 population provided as 5-9 panicle/hill in 176 plants and 10-15 panicle/hill in 323 plants (Table 2).

Table 2. Panicle/hill of RD31 and M_2 plants.

Plants	Panicle/hill	
	Number of panicles	Number of plants
RD31		10
M_2 of RD31	<5	0
	5-9	176
	10-15	323

Normally, maturity of RD31 depends on planting method which is 118 days for transplanting and 111 days for boardcasting. The M_2 plants were grown by transplanting and maturity dates were varying. Approximately 500 M_2 plants with shortening maturity period were selected. Three hundred and thirty-three of them had 110-115 days for maturity; 166 plants with 116-120 days; one plant with 121 days (Table 3). Shortened maturity period was the preferable trait to cope with climate change. The central plain of Thailand frequently encounters a water shortage during the dry season which is follow by a heavy flood during the rainy season. Cultivars with a shortened maturity period are highly desirable because harvesting can be done early before the flooding period. Therefore, two crop will be possible per year before and after the flooding period.

Table 3. Days to maturity of RD31 and M_2 plants.

Plant groups	Maturity (days)	
	Days to maturity	Number of plants
RD31	123	20
M_2 of RD31		
	110-115	333
	116-120	166
	121	1

About 3,000 M_3 lines were screened for submergence (Appendix 1). The plants were submerged under a flash flood condition for 14 days. Only 317 lines survived the flood with the score of 5-7, which were selected for planting in the following season (Table 4, Figure3 and 4).

Table 4. Number of survived M_3 plants with score.

Score	Number of plants
1	-
3	-
5	95
7	222
9	2,680

Figure 3. Submergence tolerant mutants lines from field screening.

Figure 4. Protection of 317 mutant lines from bird damage by nets.

M_4 were screened for blast resistance (Appendex 2). The result showed that all mutant lines were highly resistance to blast (Table 5). Some of these lines were screened for submergence tolerance and found that 91 submergence tolerant mutant lines were identified. Among the biotic factor disease is the most important factor which results in crop losses of $5 billion every year (Asghar et al., 2007). Rice blast caused by *Pyricularia oryzae* Cavara, is one of the most destructive and wide spread diseases (Jin et al., 2000). This disease has caused significant yield losses in many rice growing countries e.g. 75% losses of grains in India (Padmanabhan, 1965), 50% in Philippines (Awoder and Esuruoso, 1975) and 40% loss in Neigeria (Ou, 1985). In Brazil rice blast is considered to be one of the major yield constrains in both irrigated and upland ecosystems (Prabhu and Morais, 1986). However, the use of resistant cultivar is the most economical and environment friendly method for the management of rice blast.

Table 5. Number of M_4 lines which were screened for blast resistance.

Score	Site 1 (PCR)	Site 2 (KHS)
0	305	317
1	11	-
2	1	-
7	-	-
9	-	-

Grain quality were tested and revealed that 16 mutant lines showed 23.22-24.44 % amylose content which were lower than RD31 the original (27.52%) (Table 6). Physical property of all these mutant showed clear chalkiness of endosperm (Table 7).

Table 6. M_4 lines which were examined for percentage of amylose content.

% amylose content	Classification	No. of plants
10-19	Low amylose	-
20-25	Intermediate amylose	16
26-34	High amylose	301

Table 7. Physical property of M_4 lines, chalkiness.

Score	Description	No. of plants
0	None	-
1	Small (less than 10%)	158
2	Medium (11-20%)	147
3	Medium (11-20%)	149
4	Large (more than 20%)	-
5	Large (more than 20%)	-

M_5 mutant lines were grown for observation yield trials during dry season 2017 and physical and chemical properties were examined as shown in Table 8.

M_6 mutant lines were grown for intrastation yield trial during wet season 2018 which were screened blast resistance and screened for submergence tolerant during 30th July 2018 and de-submerge on 13th August 2018 as shown in Table 9.

Further work such as interstation yield trials, farmers field yield trials and adoption from the farmers, nitrogen used efficiency, physical and chemical properties, screening for blast resistant and screening for submergence tolerant will be carried out for more information before released the new rice variety.

Table 8. Observation yield trials and physical and chemical properties of mutant lines during dry season 2017.

No.	Source	Identity	Mat.	Ht.	Grain yield/ kg/rai	Grain size	Brown rice size	Clk.	Amy.	Gel Con.		Gel	AlkD		El.
	#16 WS		(days)	(cm)	ave.	Sh.	Sh.		(%)			temp. (°C)			Ratio
1	3	RD31-B-390-3-B	113	112	608	4.05	3.23	1.88	28.23	38	H	74.5-79	3	H	1.46
2	5	RD31-B-483-6-B	113	123	406	3.93	3.22	1.28	27.90	38	H	74.5-79	3	H	1.48
3	6	RD31-B-389-3-B	110	121	546	4.21	3.32	0.87	27.65	38	H	74.5-79	3	H	1.57
4	8	RD31-B-479-1-B	111	124	319	4.08	3.40	1.59	27.25	38	H	74.5-79	3	H	1.58
5	9	RD31-B-393-2-B	113	126	478	3.91	3.28	1.39	27.58	38	H	74.5-79	3	H	1.54
6	13	RD31-B-472-6-B	113	113	350	3.94	3.22	2.06	28.13	38	H	74.5-79	3	H	1.54
7	14	RD31-B-415-4-B	112	123	98	3.95	3.22	1.87	28.16	38	H	74.5-79	3	H	1.47
8	43	RD31-B-388-5-B	113	118	487	3.94	3.21	1.38	27.68	38	H	74.5-79	3	H	1.57
9	44	RD31-B-298-5-B	112	124	354	4.03	3.40	2.16	27.87	39	H	74.5-79	3	H	1.65
10	46	RD31-B-395-4-B	113	115	132	4.16	3.26	1.94	27.38	38	H	74.5-79	3	H	1.64
11	47	RD31-B-297-2-B	112	113	113	4.19	3.27	2.03	28.23	38	H	74.5-79	3	H	1.61
12	49	RD31-B-331-5-B	112	121	418	3.92	3.41	1.45	29.09	38	H	74.5-79	3	H	1.58
13	50	RD31-B-368-6-B	112	128	234	3.90	3.25	1.40	28.00	39	H	74.5-79	3	H	1.62
14	71	RD31-B-368-4-B	111	116	138	3.93	3.27	0.77	27.58	38	H	74.5-79	3	H	1.63
15	74	RD31-B-369-5-B	113	120	150	4.06	3.33	1.96	27.87	38	H	74.5-79	3	H	1.62
16	75	RD31-B-385-3-B	113	127	395	4.21	3.41	1.56	27.84	38	H	74.5-79	3	H	1.55
17	76	RD31-B-312-6-B	113	123	290	4.11	3.26	1.00	27.90	38	H	74.5-79	3	H	1.53
18	77	RD31-B-449-1-B	113	115	212	4.04	3.26	1.80	28.29	38	H	74.5-79	3	H	1.58
19	88	RD31-B-290-6-B	112	121	525	4.02	3.28	1.94	27.55	38	H	74.5-79	3	H	1.55
20	96	RD31-B-276-4-B	113	123	488	3.98	3.41	2.11	27.87	38	H	74.5-79	3	H	1.65
21	123	RD31-B-314-4-B	114	122	551	4.16	3.29	0.91	28.06	38	H	74.5-79	3	H	1.61
22	134	RD31-B-277-5-B	114	120	437	4.01	3.35	1.88	28.03	38	H	74.5-79	3	H	1.49
ck.		RD31	118	125	420	4.09	3.48	0.74	28.13	38	H	74.5-79	3	H	1.68
ck.		RD49	111	110	409	4.30	3.63	0.63	27.06	38	H	70-74	6	I/L	1.67

Table 9. Intra station yield trial of M6 mutant lines.

No.	Source	Identity	Ht. (cm)	% recov	Mat.	Ht. (cm)	Grain yield/ kg/rai	BI'PCR
	#16 WS	before Sub			(days)	Mat.	ave.	2018
1	3	RD31-B-390-3-B-B-B	70	80	132	129	760	R
2	5	RD31-B-483-6-B-B-B	69	70	133	130	619	R
3	6	RD31-B-389-3-B-B-B	73	70	131	134	615	R
4	8	RD31-B-479-1-B-B-B	67	80	132	133	813	R
5	9	RD31-B-393-2-B-B-B	73	70	133	134	650	R
6	13	RD31-B-472-6-B-B-B	72	70	132	131	698	R
7	14	RD31-B-415-4-B-B-B	71	70	131	129	679	R
8	43	RD31-B-388-5-B-B-B	71	80	132	131	854	R
9	44	RD31-B-298-5-B-B-B	69	65	132	134	662	R
10	46	RD31-B-395-4-B-B-B	68	70	132	130	692	R
11	47	RD31-B-297-2-B-B-B	71	65	132	128	514	R
12	49	RD31-B-331-5-B-B-B	70	65	133	131	522	R
13	50	RD31-B-368-6-B-B-B	71	80	134	130	865	R
14	71	RD31-B-368-4-B-B-B	72	70	133	129	623	R
15	74	RD31-B-369-5-B-B-B	70	70	134	132	632	R
16	75	RD31-B-385-3-B-B-B	68	70	133	131	589	R
17	76	RD31-B-312-6-B-B-B	72	70	134	132	535	R
18	77	RD31-B-449-1-B-B-B	71	70	134	129	661	R
19	88	RD31-B-290-6-B-B-B	72	70	134	131	673	R
20	96	RD31-B-276-4-B-B-B	70	80	134	133	711	R
21	123	RD31-B-314-4-B-B-B	71	65	130	129	522	R
22	134	RD31-B-277-5-B-B-B	71	65	131	129	488	R
ck.		RD31	73	30	134	133	151	R
ck.		RD49	74	20	135	127	79	R

Disscussion

IRRI released the first high-yielding modern rice cultivar, the IR8, for the tropical irrigated lowlands in 1966 (Chandler, 1969). Since then, IRRI has developed many high-yielding rice varieties such as IR36, IR60 and IR72 (Peng and Khush, 2003). New plant type (NPT) was developed at IRRI in the late 1980s. The NPT was designed based on the results of simulation modeling. The proposed NPT has a low tilling, few unproductive tillers, 200-250 grains per panicle, a plant height of 90-100 cm, thick and sturdy clumps, dark green and erect leaves, vigorous root system with 100-130 days for maturity (Peng *et al.,* 1994)

Under flooding condition, the oxygen and carbon dioxide level are reduced. Photosysthesis and cellular respiration are also affected. Xu and Mackill (1996) reported a major gene that control the ability of rice to tolerate submergence condition, which was named *Sub-1*. Then, several studies have shown that plants with *Sub-1* gene could survive under a fully submerged condition for two weeks. Quantitative trait locus (QTL) analysis revealed that submergence tolerance is controlled by a single major QTL on chromosome 9 along with a number of other minor QTLs (Xu and Mackill. 1996; Nandi *et al.,*1997; Toojinda *et al.,*2003).

Acknowledgements

The authors would like to thank IAEA and FNCA for technical cooperation and technical assistance. The project was fund by Rice Department and Thailand Institute of Nuclear Technology.

Appendix 1. Screening for submergence tolerance.

Seeds were germinated and subsequently planted in the row in the experimental field for 75 cm long and 25 cm wide. FR13A variety was used as tolerant check, IR42 variety as susceptible check and the original parental variety were planted after every tenth row for comparison.

Fourteen days after germination, water level was raised to 10 cm. In the 30th days, the water level was suddenly increased to 150 cm and maintained for 14 days as a flash flood condition. All were observed once a week for their performance under flooding condition. After 14 days of flooding or susceptible check died, the water was drained out, to allow the remaining plants recover for 14 days. Then, visual scoring for recovering ability was carried out according to Standard Evaluation System for Rice (IRRI, 1996)(Table 1a).

Table 1a. Standard Evaluation System for Rice, scoring submergence tolerance.

Index	Description	Scale
1	Minor visible symptom of injury	100
3	Some visible symptom of injury	95-99
5	Moderate injury	75-94
7	Sever injury	50-54
9	Partial to complete death	0-49

Appendix 2. Screening for blast resistant.

Seeds of each mutant lines were soaked and after two days sprouted seeds of each lines were sown in a single row on a raised bed of the disease nursery plot. The length of each row sown was 50 cm and after every two test rows/lines there was a row of a susceptible of KDML 105 The row were kept 10 cm apart. The disease nursery was also bordered around with two lines of susceptible. The nursery was watered on 5th day of transplanting and the chopped leaves were spread over the entries of the nursery when the seedling were 10 days old. At three week stage, the test entries of the nursery were also sprayed with already prepared inoculum of *P. oryzae* as described by Khan et al ., (2001) by adjusting the spore suspension to 1x106 spores/ml the nursery was continuously sprayed with tap water to maintain the humidity. Normal agronomic practice were followed and data of disease were recorded three weeks after spray inoculation by using rating scale of IRRI (1996).

Appendix 2b. 0-9 grade disease rating scale used for screening for blast nursery.

Grade	Disease severity	Host response
0	No lesion observed	Highly Resistant
1	Small brown specks of pin point size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesion are mostly found on the lower leaves	Moderately Resistant
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately Resistant
4	Typical susceptible blast lesions; 3 mm or longer infecting less than 4% of leaf area.	Moderately Susceptible
5	Typical susceptible blast lesions of 3 mm or longer infecting 4- 10% of the leaf area.	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area.	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area.	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesion of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

References

Asghar, A.H., Rashid, M. Ashraf, M. H. Khan and A.Z. Chaudhry. (2007). Improvement of basmati rice, against fungal infection through gene transfer technology. Pak. J. Bot. 39 (4): 1277-83.

Awodera, V.A. and O.F. Esuruoso. (1975). Reduction in grain yield of two rice varieties infected by rice blast disease in Nigeria. Nigerian Agric J., 11: 170-3.

Chandler R.F. (1969). Plant morphology and stand geometry in relation to nitrogen. P265-285.

Fernandez, F., Vergara, B.S., Yapit, N. and Garcia, O. (1979). Growth stages of the rice plant, pp.17-18. In Rice Production Training Series. IRRI, Los Banos, Philippines.

Liu, Z. (1986). Genetic and morphological studies of selected semi-dwarf rice. M.S. thesis, University of the Philippines at Los Banos, Philippines.

Lorest, G.C. & Chang, T.T. (1978). Half-diallel and F2 analyses of culm length in dwarf, semidwarf and tall strains of rice (Oryza sativa L.). Bot. Bull. Academia Sinica 19, 87-106.

Nandi, S., Subudhi, P.K., Senadhira, D., Manigbas, N.L., Sen-Mandi, S., & Huang, N. (1997). Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotypeing. Molecular and General Genetics 255, 1-8.

OU, S.H. (1985). Rice Diseases. CAB International Mycological, Institute Kew, Survey, UK.

Padmanabhan, S.Y. (1965). Estimating losses from rice blast in India. In the rice blast disease: Johnan Hopkins Press, Baltinoie, Msryland. 203-221.

Peng, S., & Khush, G.S. (2003). Four decades of breeding for varietal improvement of irrigated lowland rice in the International Rice Research Institute. Plant Prod. Sci. 6, 157-164.

Peng, S., Khush. G.S., & Cassman, K.G. (1994). Evolution of the new plant ideotype for increased yield potential. Pp 5-20 *In* Cassman KG. (ed.) Breaking the yield barrier. IRRI, Los Banos, Philippines.

Prabhu, A.S. and O.P. Morais. (1986). Blast disease management in upland rice in Braziil. In progress in upland Rice Research, In Proceedings of the 1985 Jakarta Conf. : 382-283.

Toojinda, T., Siangliw, M., Tragroonrung, S., & Vanavichit, A. (2003). Molecular genetics of submergence tolerance in rice : QTL analysis of key traits. Annals of Botany 91, 243-253.

Xu, K., & Mackill, D.J. (1996). A major locus for submergence tolerance mapped on rice chromosome 9. Molecular Breeding 2, 219-224.

Mutation Breeding of Rice for the Sustainable Agriculture in Vietnam

Le Huy Ham, Vo Thi Minh Tuyen, Nguyen Thi Hong

Agriculture Genetics Institute, Vietnam

Introduction

In Vietnam, rice plays an important role for national food security and political stability. Rice also has a direct effect on social security because it is consumed by nearly 89 million of the total population and an important source of income for more than 90 million people living in agricultural and rural areas. Rice is the country's main crop, accounting for more than 90% of total cereal productivity. Rice production in 2015 reached 45 million tons, and it is 0.3% higher than in 2014. Vietnam is one of the top 5 countries that studies reveal will be severely impacted by climate change. A rise in sea level of 1 meter will inundate about 5,000 square kilometers (km^2) of the Red River Delta and 15,000–20,000km^2 of the Mekong River Delta, reducing total rice production in Vietnam by about 5 million tons. Bad harvests, natural calamities, floods, and pests and diseases will also occur more often. Ensuring domestic food security has thus become a national objective that requires long-term strategies and policies, especially for the protection of the agricultural land area. Vietnam does not only seek to achieve domestic food security but it also plays an important role in the international rice market, and consequently, in the food security of the international community.

Despite of a decrease in rice land area, rice production has been rising due to rapid yield growth. This in turn has been driven by irrigation and land development, together with technological change. In 2009, Vietnam had nearly 7.4 million hectares (ha) of rice lands, which declined to 2 million ha in 2015 (Ministry of Agriculture and Rural Development, 2015). However, rice productivity increased from 39,5 million tons in 2009 to more than 45 million tons in 2015. This was a remarkable achievement due to the application of advanced science and technology such as the introduction of different rice varieties, new production models, and an efficient irrigation system. Post harvest losses have also been reduced greatly due to the mechanization of rice harvesting and drying, and soil improvement.

Mutation Breeding in Vietnam

At present, about 15% of rice production area is covered annually by mutant varieties in Vietnam. Great achievement by the use of nuclear techniques and related biotechniques. More than 55 mutant varieties were developed, in which most of varieties are cereal crops, especially rice.

The used materials were local lines (Cuom, Chiem bau, Tam thom, Nang thom, Nang huong, Te etc.) and the varieties presently used in the crop productivity (C4-63, A8, CR203, Khang dan, IR64, IR50404, Bac thom). The treatment methods were dry seeds with different radiation doses (Gamma ray, X-ray with 80, 100, 150, 200, 250 Gy, useful doses were 100, 120, 200 Gy); the others were germinated seeds with radiation doses of 20, 30, 40, 80 Gy, useful doses were 30, 40, 60 Gy).

Since the 1990s, the Agricultural Genetics Institute (AGI), Vietnam Academy of Agricultural Sciences (VAAS), has been engaged in R&D activities and contributed much to the technology transfer to the agricultural sector of Vietnam. Mutation breeding is one of the major fields of institute in crop improvement, in which the biggest accomplishments have been achieved in the development of mutation varieties for crops production. Before 2000, objective of mutation breeding is often quantity of yield variety. One very interesting example from AGI is DT10 mutant variety which has been certified as national variety in 1990 due to the high performance, good tolerant to harmful condition. This variety is available in rice more than 25 years and now this is still disseminating in some specific location. This variety covers the area around 1.0 mil. ha in agriculture production. After success of mutant variety DT10, scientists from AGI released series of mutant varieties (20 mutant varieties) and satisfied production demand.

In the South Vietnam, mutation induction has been carried out mostly in Cuulong Rice Research Institute and Institute of Agriculture South Science (IASS). These institutions have also succeeded in the mutation breeding by the use of nuclear techniques. Over the past decade, the institutes have developed eight excellent mutant varieties with high yielding, improved quality, disease resistance, tolerance to pest and lodging resistance. One of these excellent mutant varieties (VND9s-20) which has been certified as national variety in 1999 became one of the top five varieties for rice production. It covered 300 thousand ha per year in south of Vietnam, due to its high yielding, good quality and tolerance to brown plant hopper. Not only has this variety been widely cultivated in the low lands, but also expanded to the high lands and remote mountain areas, where poor farmers are benefiting from growing it.

The great support from the Vietnamese Government makes the application of nuclear techniques in food and agriculture so successful. Due to the excellent performance of the mutant rice varieties mentioned above, some of these mutant varieties were adopted for the national strategy program of "Eradicate hunger and alleviate poverty" in different areas, particularly for central highland region, where there are many ethnic minorities living in remote mountain areas. Most of the eight mutant varieties have shown out-standing performance under local production conditions.

Release of new mutant rice for large scales of production

Recently, beside of yield varieties, scientists has been concentrating creation of new variety having good performance on quality (aroma, protein, amylose content, component of quality), as well as tolerance to harmful condition of environment such as salinity, cold or high temperature, drought, lodging variety and so on. Mutation breeding is powerful tool for rice breeding improvement.

During 2008 - 2017, groups of rice breeders continue effective collaborations for pure line selections under field trials of rice mutants and their crossings with high quality and tolerance in Red River Delta (North Vietnam) and Mekong River Delta (South Vietnam). There are ten outstanding new varieties have been certified as new varieties and ongoing projects to enlarge mutation varieties in different provinces (Table 1).

Khang dan mutant variety has been transferred the trading rightcopyright from AGI to the seed company since 2008 after it was certified as national variety. With some advantages, the area covered with this variety is around 300 thousands ha/year in north Vietnam. In 2012, the seed company is rewarded the national prize for Khang dan mutant variety due to the leading area for agriculture production of this variety.

ĐB5, ĐB6 mutant varieties have high yield, good tolerant to pest and disease. OM 2496 has high productivity, aroma and salinity tolerance. Nam dinh 5 variety has Quality, aroma and high yield. PD2 is glutinous rice and photo sensitiveness. It has high yield and aroma. P6 mutant has short growth duration (85-90 days in summer season) and high temperature tolerant. Mutant rice varieties DT39 Quelam, CNC11 have improved in productivity for high yield, better quality and tolerant to bacterial leaf blight. Moreover, DT39 gave high nutrition content such as iron, zinc, Kali and Maggie in comparison to origin variety.

Mutant rice varieties DT39 Quelam and CNC11 has been transferred the trading rightcopyright from AGI to the seed company.

Table 1. Leading mutant varieties in rice production in Vietnam (duration 2008-2015).

No	Variety	Year of certify	Dominant characters	Area of production/year (ha)
1	Khangdan mutant	2008	High yield, good tolerant to pest and disease	300,000
2	ĐB5	2008	High yield, good tolerant to pest and disease	5,000
3	ĐB6	2008	High yield, good tolerant to pest and disease	5,000
4	OM 2496	2009	High productivity, Aroma, Salinity tolerance	3000
5	PD2	2010	Glutinous rice, photo sensitiveness, high yield, aroma	10,000
6	P6 mutant	2011	Short growth duration 85-90 days in summer season, high temperature tolerant	15,000
7	Nam dinh 5	2012	Quality, aroma, high yield	3,500
8	ĐB15	2012	High yield, good tolerant to pest and disease	600
9	DT39 Que lam	2013	Quality, high protein, high yield, resistant to leaf blight	800
10	CNC11	2015	Quality, high protein, high yield, resistant to leaf blight	300

Applications of gamma rays irradiation and marker assisted selection for improving of rice varieties for the sustainable agriculture.

Climate change creates adverse conditions for rice production in rice growing regions. For adaptation to mentioned challenges, cultivation of varieties resistant to biotic and abiotic stresses is required.

In present, the application of irradiated techniques and biotechnology in rice breeding has focused on some major traits, such as high yield, short duration, good quality, bacterial leaf blight resistance, blast resistance and salt tolerance.

In 2013, dry seeds of three varieties were irradiated with 300 grey of Cobalt-60 gamma rays. Among them, BT62.1 was resistant to bacterial leaf blight (carrying *Xa7, Xa21* genes), P5.3 was resistant to blast (carrying *Piz* gene), and BT3.1 was salt tolerant variety (carrying *saltol* gene). All of these varieties have low yielding. Purpose of irradiation is improvement of yield. M1 and M2 plants of these varieties were evaluated for mutant characteristics. We expect to get the mutant lines not only have resistance to diseases but also have high yield, short duration, and good quality.

In 2014, M3 and M4 progenies of these varieties were used for marker assisted selection (MAS) and inoculation in the green house to select elite lines resistant to bacterial leaf blight resistance, blast resistance and salt tolerance. Twenty M4 mutant lines (from M4.1 to M4.20) retained as short duration, good quality, bacterial leaf blight resistance, blast resistance or salt tolerance as original varieties but they have the higher yield than the original ones.

In 2015, these lines are evaluated the agronomic and biological traits as well as resistance characters in field condition (narrow scale). The results showed that: 6 promising mutant lines were selected (2 mutant lines showed high resistance to the BB pathogen, 2 lines resisted the BL pathogen and 2 lines showed high salt tolerance in the field. The promising mutant line, DT80 carrying *saltol* gene and can withstand salinity of 0,6%. DT80 was evaluated in salinity field of the Nam Dinh province in spring season 2015.

In 2016, DT80 mutant line was evaluated the agronomic and biological traits as well as tolerant characters in field condition (narrow scale) and send the National testing Center. Beside of this activity, promising lines should be evaluated in different local conditions for evaluation of wide adaptability of new variety and evaluate the response of the farmer. The result of this evaluation indicated that DT80 mutant variety have improved in productivity for high yield (Potential yield: 6,8 - 7 ton/ha), better quality (amyloze contents: 14%), short growth duration (105-110 days in summer season), medium plant height (112 cm), Wide adaptation and easily cultivation. This new mutant variety were evaluated with some major pests and disease and the evaluation in the field showed that DT80 were affected by these biotic and abiotic stresses in the majority of harmful levels from 1-3 points.

DT80 was sent to The National Testing Center for Crops to test for Value of Cultivation and Use of crop (VCU) 3 seasons and Distinctness, Uniformity and Stability of crop (DUS) 2 seasons.

DT80 mutant variety has been transferred the trading right from AGI to the Thanh hoa seed company in November, 2016. In 2017 it registered as new variety by MARD.

Results of irradiation ion beam on rice breeding

Carbon ion beam have been recently considered as potential mutagens. A characteristic feature of ion beams is their ability to deposit high energy on a target, densely and locally. Rice seeds were treated with Carbon ion radiation (40, 60, 80 and 100 Gy) by AVF-Cyclotron at the Japan Atomic Energy Research Institute (JAERI), Takasaki, Japan and were planted and selected at the Agricultural Genetics Institute. There are some results of carbon ion Beam irradiation up to now.

Table 2. Overview irradiation data.

Varieties	Time	Dose
Bac thom and Khang dan	6/2010	40Gy and 60Gy
CMBT and BLBT	3/2013	40Gy and 60Gy
DT80, DT82, DT86, T5, TBR2 and RVT6	1/2015	60Gy and 80Gy
BC/6 and P12	6/2015	40Gy, 60Gy, 80Gy and 100Gy
ĐB2, KN6, BH9	6/2016	40Gy, 60Gy, 80Gy and 100Gy

Results of carbon ion beam irradiation on Bac thom and Khang dan

There are many types of variations in M2 generation from irradiated Bacthom and Khang dan. Four promising mutants were selected from Khang dan in which two lines came from dose of 40Gy and two other mutant lines were derived from dose 60 Gy. Most of the mutant lines express higher yield than the original variety and better resistant to pests and diseases. However, the objective of the project is to increase yield and to obtain good quality rice variety, therefore the research is concentrated in Bacthom variety. Selection process was conducted according to better agronomic traits; short grow duration, high yield and purity of mutant lines in the M4 and M5 generations. The mutant lines were grown and evaluated similar to the M5 and M6 but in the expanded testing area and perform in some different locations to assess purity, growth and development capacity, as well as yield and resistance to pests and diseases. The promising lines will be chosen for further research. Result indicated in the table 1 and 2 below. In M7 generation of Bacthom mutant variety showed that grow duration of some mutant lines (M7-2, M7-6 and M7-8) were similar to control; meanwhile some mutant line indicated short grow duration than the origin such as M7-4; M7-3. In M7 population, purity of 3 mutant lines M7-3, M7-4 and M7-5 were in level 1 better than the origin in level 5 as well as M7-2, M7-6 and M7-8. However, purity level of mutant line can be improved by selection process in further generation. Panicle length is also one of component of yield performance of variety. Most of mutant lines have panicle length that is longer than the control of Bacthom variety. That mean of most mutant lines may have higher yield compare to original Bacthom variety.

Table 3. Biological and agricultural characteristics of 6 Bacthom mutant lines in M7 (summer season 2013).

Lines	Growth duration (day)	Plant height (cm)	Flowering duration (points)	Plant hardness (points)	Panicle length (cm)	Purity (points)
BT (Cont.)	107	108.0	5	3	21.5	5
M7-2	107	115.6	5	3	25.8	5
M7-3	104	114.4	5	1	23.6	1
M7-4	102	117.2	5	3	24.5	1
M7-5	105	108.2	5	1	22.1	1
M7-6	106	109.6	5	3	22.4	5
M7-8	108	102.8	5	3	24.2	5

- Plant hardness: *1-Very hard, 3-Hard, 5-Medium hard, 7-Weak hard, 9-Weak hard.*
- Flowering duration: *1-Focus (≤ 3 days), 5-Medium (4-7 days), 7-Long (≥7 days)*
- Purity: *1-High (different trait ratio <0,25%), 5-Medium (different trait ratio: 0,25-1%), 9-Low (different trait ratio > 1%).*

With regards to the grain yield component of mutant lines in the M7 generation showed a significant difference compare with the control for full seed per panicle the highest were obtained at M7-4 and M7-8 lines and showed significant difference from other lines (table 2). This resulting in potential yield of M7-4 and M7-8 were higher than that of original and other lines with 9.63 tons/ha and 12.48 tons/ha. These other mutant lines performed grain yield higher than original variety except M7-6 which was 5.34 ton/ha. However, one elite mutant to be selected will not only depend on the yield performance character but also on the cooking quality and aroma characteristic of the variety.

Table 4. The grain yield components of 6 mutant lines of mutant Bacthom in M7 (Summer season 2013).

Lines	No. of panicle /hill	No. of seeds /panicle	No. of full seed /panicle	Weight of 1000 seeds (gr)	Potential yield (ton/ha)
BT (Cont.)	5.1	140.5	130.9	19.6	5.50
M7-2	5.3	177.6	159.3	22.1	7.84
M7-3	5.2	182.2	160.4	19.8	6.94
M7-4	5.8	212.4	197.7	20.0	9.63
M7-5	6.0	132.7	119.3	19.5	5.86
M7-6	5.5	127.3	118.5	19.5	5.34
M7-8	6.2	257.9	236.1	20.3	12.48

Cooking quality is main objective of the breeding improvement. Six mutant lines of Bacthom were tested in room condition for aroma, softness, stickiness, and taste to evaluate the quality of selected mutant lines in comparison to original variety.. The result of this experiment indicated that M7-2; M7-3; M7-6 and M7-8 have cooking qualities similar to the original variety Bacthom, meanwhile M7-4 and M7-8 indicated dry and hard when cooked even M7-4 and M7-8 but have highest grain yield.

Results of carbon ion beam irradiation on CMBT and BLBT

Ion beam irradiation 3/2013: The variable new mutant lines were screened and evaluated in M2 generation. (selected lines with short growing period, medium plant height, tillering ability, a number of seeds per panicle is higher than that of variety control. Specially, selected lines reveals small tillering angle, this is characteristic need to be improved from original lines). Selection process was conducted according to better agronomic traits; short grow duration, high yield and purity of mutant lines in the M3 and M4 generations.

Screening of mutant lines in M5 and M6 generation. In this period, agrobiological characteristics and tolerance traits is being tested in the field conditions (narrow scale). MAS was applied to identify the target genes. The prospective lines in M5 generation to be continued in varietal testing system for assessment of the agro -biological characteristics: field grain yield and grain quality towards approval as national varieties. Five mutant lines (two from CMBT, three from BLBT) with high potential yield and good agronomic traits were selected

Based on agronomic traits in fields, test in greenhouse and MAS in lab, 14 promising mutant lines in M5 generation were selected: 5 mutant lines from CMBT and 9 mutant lines from BLBT.

The results showed that 4 promissing lines (BL.1,2,6,7) have a growth duration is shorter and number of fulled seed is much higher than that of control. So that these lines obtained the higher yield than control variety (Table 9).

The results showed that 2 promissing lines (CM.3,4) have a growth duration is shorter and number of fulled seed is much higher than that of control. So that these lines obtained the higher yield (7.5 ton/ha) than control variety (Table 5).

In 2015 spring season, the total of 6 promissing mutant lines were obtained. These line will be selected in 2016 for evaluating the agronomic traits and resistance to pests and diseases.

Table 5. Agronomic traits and yield potential of BB mutant lines in M5 from BLBT (Spring season, 2015).

N_o	Mutant lines	Duration of growth (days)	Height of plant (cm)	Number of panicle/ plant	Panicle exertion (cm)	Number of fulled seed	Weight of 1000 grains (gr)	Potential yield (ton/ha)
Cont.	BLBT	130	109	6.5	2.3	184.7	22.3	5.7
1	**BL.1**	129	95	8.0	3.0	219.0	19.8	6.9
2	**BL.2**	128	95	8.0	3.2	236.7	20.1	7.2
3	BL.3	132	95	5.0	4.2	167.7	19.5	6.5
4	BL.4	130	95	7.0	3.0	192.3	20.2	5.9
5	BL.5	127	100	7.0	0.8	180.0	21.2	5.6
6	**BL.6**	125	100	7.0	1.7	257.7	21.5	7.5
7	**BL.7**	130	105	6.5	0.6	276.9	21.0	7.1
8	BL.8	129	100	5.8	0.9	242.0	21.4	6.1
9	BL.9	125	102	6.0	0.5	261.3	21.4	6.4

Table 6. Agronomic traits and grain yield components of Salinity tolerance mutant lines in M5 from CMBT (Spring season, 2015).

N$_o$	Mutant lines	Duration of growth (days)	Height of plant (cm)	Number of panicle/ plant	Panicle exertion (cm)	Number of fulled seed	Weight of 1000 grains (gr)	Potential yeild (ton/ha)
Cont.	CMBT	128	111	6.0	2.5	159.5	22.2	5.5
1	CM.1	128	105	8.0	3.5	183.0	20.3	5.9
2	CM.2	130	105	8.0	1.3	183.3	20.1	6.4
3	**CM.3**	129	100	8.0	-0.5	248.0	20.8	**7.5**
4	**CM.4**	130	100	8.0	-0.7	251.0	20.5	7.1
5	CM.5	129	100	7.0	5.7	145.3	21.3	6.7

Table 7. Agronomic traits and grain yield components of promising mutant lines (summer season, 2016).

N$_o$	Mutant lines	Duration of growth (days)	Height of plant (cm)	Number of panicle/ plant	Panicle exertion (cm)	Number of fulled seed	Weight of 1000 grains (gr)	Potential yeild (ton/ha)
Cont.	BLBT	105	110	5.5	2.3	145.7	22.5	5.3
1	BL.1	102	98	5.8	3.0	179.0	20.0	6.5
2	BL.2	108	100	6.1	3.2	186.7	20.1	6.8
6	BL.6	105	102	6.8	2.7	217.5	21.3	**7.5**
7	BL.7	103	110	6.5	**0.3**	176.9	21.0	6.1
3	CM.3	107	108	7.0	**0**	178.0	20.5	6.5
4	CM.4	105	109	6.5	**0.2**	175.0	20.6	6.1
Cont.	CMBT	128	109	6.0	2.5	159.5	22.0	5.5

The results of evaluating the agronomic trait in the field (Table 11) showed that these lines have a shorter duration, with higher production in comparison with control. Three mutant lines: BL7, CM.3, CM.4 were high yield, but their panicle exertion is very short (0-0.3cm). This reason make these lines can increase higher empty seed ratio for reducing the yield. Out of which, the promissing BL6 line revealed hard stem hardness, can resistance with abiotic and biotic stresses. Next, these lines will be adapted in different rice ecosystem to production expanding and sent to The National Testing Center for Crops to test for Value of Cultivation and Use of crop.

Table 8. Summary results of carbon ion beam irradiation (From 1/2015).

Variety	**Dose**	**M1** Sterility	**M2** variation	**M3** variation	**M4** (mutant lines)	**M5** (Promissing line)
DT80	60Gy	83%	15	25	5	0
	80Gy	86%	25	37	8	0
DT82	**60Gy**	**89%**	**10**	**15**	**12**	**8**
	80Gy	91%	20	18	5	0
DT86	60Gy	91%	26	20	8	0
	80Gy	91%	30	18	9	4
	100Gy	95%	18	24	9	6
T5	**60Gy**	**87%**	**9**	**16**	**12**	**7**
	80Gy	88%	15	21	5	0
TBR2	60Gy	87%	29	32	9	0
	80Gy	88%	5	0	0	0
RVT6	60Gy	85%	31	25	5	3
	80Gy	92%	45	18	6	3
Total			**278**	**269**	**93**	**31**

Results of carbon ion beam irradiation from 2015 to 2017

The variable new mutant lines were screened and evaluated from M2 to M5 generation. We selected lines with short growing period, medium plant height, tillering ability, a number of seeds per panicle is higher than that of variety control. The results in table 7 showed that: 278 mutant individual in M2 generation were selected in 2015. In 2016, 269 mutant individual in M3 generation and 93 mutant lines in M4 generation were selected. In 2017, based on agronomic traits in fields, 31 promising mutant lines in M5 generation were selected: 18 promising lines from 60 Gy, 7 promising lines from 80 Gy and 6 lines at 100 Gy.

In 2016, 56 mutant individual in M2 generation were selected with short growing period, medium plant height, tillering ability, a number of seeds per panicle is higher than that of variety control. In 2017, Set of 102 mutant lines were obtained in M3 generation, 39 mutant lines in M4 generation were selected. The results showed that (Table 8): 17 mutant lines were selected from 60 Gy, 7 mutant lines from 80 Gy and 15 mutant lines at 100 Gy.

Table 9. Summary results of carbon ion beam irradiation (From 6/2015).

Time irradiation	**Variety**	**Dose**	**M1** Sterility	**M2** variation	**M3** variation	**M4** (mutant lines)
6/2015	BC/6	40Gy	75%	0	0	0
		60Gy	91%	15	26	17
		80Gy	91%	5	12	0
		100Gy	95%	3	9	0
	P12	40Gy	68%	5	0	0
		60Gy	91%	12	18	0
		80Gy	91%	10	23	7
		100Gy	95%	6	14	15
Total				**56**	**102**	**39**

Table 10. Summary results of carbon ion beam irradiation (From 6/2016).

Variety	**Dose**	**Germination***	**M1** Sterility	**M2** variation
BH9	40Gy	100%	75%	
	60Gy	100%	91%	31
	80Gy	99%	91%	23
	100Gy	97%	95%	14
KN6	40Gy	100%	65%	
	60Gy	98%	91%	11
	80Gy	100%	91%	22
	100Gy	100%	95%	24
ĐB2	60Gy	100%	91%	
	80Gy		91%	
	100Gy		95%	20

From irradiated time 6/2016: 145 mutant individual in M2 generation were selected in 2017. Among that: 44 mutant lines were selected from 60Gy, 45 mutant lines from 80Gy and 58 mutant lines from 100Gy.

The promising mutant line with some improved traits such as: light aroma, softer endosperm, good tillering ability, big and longer seed size, more grain number per panicle....comparing to original variety. Thus, our research was focused on database of 5 target genes regulated these features as in table below:

Through sequence and BLASTN, total of 24 point mutants were identified in target genes (as in table).

Table 11. Results of study to clarify mutation related to high yield and good quality Material of study.

No.	Name	Features
1	Original variety (DT82)	BLB resistance, low yield, tillering ability (4-5 tillers), plant height (113-115cm), dark green leaf, small seed, dark yellow husk seed
2	Ppromising line (60Gy)	BLB resistance, improved yield, better tillering (8-10 tillers), plant height (120 cm), light green leaf, better quality, big and long seed, light yellow husk seed

Table 12. Target gene information.

No.	Gene name	Regulation	Gene features
1	Os08g0424500	Betaine aldehyde dehydrogenase, Rice fragrance	Chr.8 (21703754:21707360), including 13 exons
2	Os06g0133000	Glutinous endosperm	Chr.6 (1929936:1935488), including 15 exons
3	Os03g0171600	Leaf senescence, seed size and grain number	Chr.3 (4284298:4286750) including 1 exon
4	Os07g0261200	Grain number, plant height, and heading date 7	Chr.7 (9173610:91755560) including 2 exons
5	Os03g0203200	Shoot branching	Chr.3 (5895977:5897579), including 2 exons

Table 13. Identified point mutations in target genes.

Gene	Number of mutant	Location of mutants
Os08g0424500	6	Exon 1 (40, 41, 42, 47, 48); exon 12 (121)
Os06g0133000	6	Exon 3 (33, 34, 35, 70); exon 4 (15); exon 9 (115)
Os03g0171600	5	426, 615, 621, 1017, 1263
Os07g0261200	4	Exon 1 (73, 253); exon 2 (32, 36)
Os03g0203200	3	Exon 2 (24, 98, 401)
Total	**24**	

Conclusion

- The dose of 60 Gy is effective for screening good trait mutants
- To be need further trial to assess the efficiency of dose 80Gy and 100Gy
- Mutations conducted from ion beam were almost point mutations

Work plan

- Testing promising lines in different ecological zones
- Continue screening mutation lines to select the good and stable lines
- Repeat the irradiation with range of doses to performed the most effective dose for mutation breeding.

Conclusion

Successful story of mutation plant breeding in Vietnam is contributed from political support by Vietnamese government for mutation plant breeding research such as international organization such as FAO/IAEA, through project IAEA/VIE/05/13/14 from 1997-2003 and Ministry of science and Technology Foundation was supported by Program: Applied research and development of energy technologies; code: KC05.09/11-15 from 2012-2015. Beside of this from 2008, Vietnam became official member of Forum for Nuclear Cooperation in Asia (FNCA). Scientists from different institutions have chance to go abroad and attend training course, scientific symposium and workshop, or to exchange information and experiences with scientists working in mutation breeding.

It's clear that mutation induction nowadays is one of powerful tool for crops improvement in Vietnam. Mutant rice varieties from institutions contribute to increase of rice performance and productivity in recent years. This is confirmed that Vietnam Government has been concentrating research and development of the activities on nuclear techniques in agriculture. Beside of that farmers also benefit from scientific research activities.