FNCA／MEXT
Technical Material

Achievement
Sub-Project on Composition or Quality in Rice
(2007 – 2012)

Mutation Breeding Project

Forum for Nuclear Cooperation in Asia (FNCA)

March, 2013

This report was prepared by the Nuclear Safety Research Association (NSRA) as a part of activities on Forum for Nuclear Cooperation in Asia (FNCA), organized by MEXT.

Foreword

Rice is the most important crop not only in Asia but also in the world. In fact, 90 % of all the production of rice in the world is produced in Asia. One report also states that almost 60 percent of all the population in the world eats rice, whereas about 30 percent eats wheat. Farmers or the relevant scientists, especially in every Asian rice- producing country, have made great efforts directed at increasing rice yield. In addition, people have recently been interested in the quality or composition of rice, in view of an aspiration for health and life. Considering these situations, in FY2007 we took up rice as materials to perform mutation breeding in the sub-project on "Composition or Quality Improvement in Rice", to be finished in FY2012.

It must be noted that important agronomic traits, e.g. grain yield and resistance to various environmental stresses, were kept in mind to be improved in this sub-project, aiming mainly at improving the quality or composition of rice. The contents of amylose, protein, fiber, vitamin A, anthocyanin, resistant starch and phytin were treated in terms of the quality or composition of rice. The breeding purposes were completed by every member country depending on their own needs. It was our primary concern on whether or not the results obtained from the project could be realized for technology transfer to society as well as those being of scientific worth.

I am pleased to state that we have obtained a lot of fruits worthwhile from both the practical and fundamental points of view in the sub- project. It is noted that a new mutant variety, DT 39, has been certified by the Ministry of Agriculture, Vietnam, to be released in January 2013. This variety of rice is of high yield with high protein content as well as resistance to bacterial leaf blight, sheath blight and brown plant hopper. Attention must also be paid to the fact that the variety was developed just within the term of the sub-project for 5 years, while it might have taken at least 10 years when using the conventional breeding method.

This book may prove that many mutant lines have been selected, in every member country, for high yield and/or resistance to various environmental stresses, e.g. diseases, insects, drought and so forth, as well as the improved quality or composition of rice. I want to say that some of the mutant lines obtained will be officially released as new commercial varieties in the near future.

In 2009, a workshop for training on how to use ion beams was held in TIARA, JAEA. With the workshop as a start, a system for the utilization of ion beams has been established for the member countries to perform the mutation breeding of rice efficiently through the use of ion beams as well as gamma-rays. The fruits of the trials for the utilization of ion beams may also be found in this book.

It is worth emphasizing that, during the performance in a series of FNCA mutation breeding sub-projects, strong merits of mutation breeding have been confirmed especially on the following three points: (1) to improve target traits without altering the other ones, (2) to develop new genes, (3) to effectively improve target traits on plants of vegetative propagation, e.g. fruits. This must be a good chance to now recognize the strong points of mutation breeding to be promoted for moving forward the establishment of sustainable agriculture, in the crucial situations of earth environments and food problems. I would be extremely appreciative, if the breeders or researchers concerning agriculture could be encouraged by the fruitful results of the sub-project presented in this book so as to move forward in the future.

March 2013

Hirokazu NAKAI
Project Leader of Japan, Mutation Breeding Project, FNCA
Professor Emeritus, Former Vice President,
Shizuoka University

Content

1. Bangladesh

High Yielding mutants with shorter life cycle selected in rice irradiated with carbon ion beam

A. N. K. Mamun, A. K. Azad[1], M. H. Kabir, P. K. Roy, M. R. Islam and M. T. Jahan, M. A. Azam[1], M. L. Hakim, & G. Ahmed

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

1.1 Summary

To investigate the possibility of induced fixed and stable mutants in M_1 generation of rice in which, one hundred and fifty seeds of the highly photoperiod sensitive and tall *indica* type rice cv. Ashfal and popular variety BRRI dhan29 were irradiated with different doses of carbon ion beams from the Radiation Applied Biology Division, Quantum Beam Science Division, Japan Atomic Energy Agency, Japan. In case of BRRI dhan29 and Mutant of BRRI dhan29, it was grown during Boro season, 2012 and in the case of Ashfal M_1 was grown during long day length at Boro (December-May) season and M_2 during short day length at Aman season (July–December) in 2009 whilst M_3 once again grown during Boro season in 2010. Nine M_1 hills in which, 2 from each of 40, 120 and 160Gy, and 1 from each of 60, 80 and 200 Gy were headed even under long day in Boro season despite none of the parent plants of Ashfal headed during Boro season. Of the heading of M_1 plants that had the plant height as the parent that could not produce any seed. Interestingly only the shorter plant height that capable to produced seeds. The M_2 progenies took 85 days and 110 days for heading and maturity respectively, during short days in Aman season. In contrast, the parent Ashfal headed after 7 November and matured at 152 days. In M_3 generation, the progenies bred true as in M_2 for heading, maturity and plant height. It was further confirmed that the Ashfal parent and the mutants had similar 1000-grain weight. Interestingly in case of mutant of BRRI dhan29 showed higher yield and early maturity compared to the parent of BRRI dhan29. Finally, it could be concluded that it is possible to induce stable mutant(s) with photoperiod insensitive and shorter plant height and also higher yield and early maturity traits in rice treated by 40 to 200Gy dose range of carbon ion beams in M_1 of *indiac* rice cv. Ashfal and popular rice variety in Bangladesh BRRI dhan29.

Key words: rice, indica type, ion beams, mutation, photoperiod sensitivity, fixed M_1 mutant.

1.2 Introduction

Rice (*Oryza sativa* L.) is a short day plant (Vergara and Chang, 1985). Almost all rice cultivars matured in a shorter time when they were grown under a short photoperiod (about 10 h) than under a long photoperiod (about 14 h). The degree of photoperiod sensitivity greatly varies among cultivars. In Bangladesh, rice is being grown in three seasons in a year; Aman season (July to December), Boro season (December to May) and Aus season (March to June). Ashfal is a local T.aman (July to December) cultivar being cultivated in the coastal south western part of Bangladesh from time immemorial. It is highly photoperiod sensitive with long duration and tall height cultivar. It is 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). Ashfal cannot be grown in Boro (long day) season as it is highly photosensitive. It often becomes lodged even in Aman season due to its tall culm which cause yield losses to a considerable extent.

Recently, ion beam irradiation has been appeared to utilized 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-M_4 generations (Azad *et al*., 2010; Hamid *et al*., 2006; Shamsuzz*aman et al*., 1998; Azam and Uddin, 1999). But it has been reported that it is possible to isolate fixed mutants even in M_1 generation of heavy ion irradiated sweet pepper (Honda *et al*., 2006).

In this study, we aimed to describe the possibility to get fixed M_1 mutant(s) with photoperiod insensitive, short day-length and shorter plant height from local T.aman Ashfal and also to get higher yield and early maturity from BRRI dhan29 in rice by ion beam irradiation.

1.3 Materials and Methods

Dehusked seeds of Ashfal rice and BRRI dhan29 were exposed to 26.7 MeV/n carbon ions with different doses of 0, 10, 20, 40, 60, 80, 100, 120, 160 and 200 Gy in January 2009 at Japan Atomic Energy Agency (Takasaki, Gunma, Japan). One hundred and fifty seeds were used for each irradiation dose. The irradiated seeds were allowed to germinate in petri dishes. Seedlings were transplanted on February 11, 2009. Single seedlings were transplanted into each hill. Spacings were maintained between rows and hills are of 20 cm and 15 cm respectively. All plants were grown under natural environment at the Bangladesh Institute of Nuclear Agriculture (BINA) head quarters' farm Mymensingh, Bangladesh. The day length at the time of heading in Boro (December to May) season is between 12 - 13 h whilst that in Aman season (July- December) is 10 - 11 h. Fertilizers were applied at a rate of N-90 Kg, P-18 Kg, K-63 Kg, S-10 Kg and Zn-1.60 Kg/ha in the forms of urea, tripple super phosphate, muriate of potash, gypsum and zinc sulphate equally in all plots. 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. Heights were measured randomly from ten M_1 plants of each irradiated dose at 3 weeks after transplanting. M_2 seeds were harvested individually from three fertile M_1 plants at 5 months after transplanting. The M_2 seeds were spread on a seed bed on July 16, 2009. Survived seedlings were transplanted on August 17, 2009 and they were grown during Aman season. 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. Plant heights, number of effective tillers per plant and panicle length were measured at the time of maturity with randomly selected 5 competitive plants. M_3 seeds were harvested separately from each M_2 plant.

M_3 plants were grown as plant progeny during next Boro (long day) season (December 2009 to May 2010). Data were recorded as the same as described in M_2. Grain characteristics such as length (L) and width (W) of unhusked and husked grain were recorded in October 2012. The mutants derived from 140 Gy dose were excluded previously that are not included.

1.4 Results

Seeds of Ashfal irradiated with carbon ions were grown during Boro (long day) season in 2009. Survival rate and plant height was determined to examine the effect of carbon ion irradiation over the plant growth cycle. Survival rate of the irradiated seeds were significantly decreased at 60Gy and remains gradually at higher doses (Table 1). Seedling height also gradually decreased with the application of increased rate of irradiation dose. The height of the seedlings were reduced almost half compared to unirradiated seeds and it was started at 80Gy and gradually goes to at higher doses. Since the cultivar Ashfal is a highly photoperiod sensitive in which, none of the control plants headed during the Boro season. However, we have found nine M_1 plants headed under the same growing conditions (Table 1).

Table 1. Effects of carbon ion irradiation on survival rate, seedling height and heading in highly photosensitive rice cultivar Ashfal grown in Boro season

Dose (Gy)	Number of seeds sown	Number of survived plants	Survival rate (%)	Seedling height* (cm)	Number of headed plants	Number of fertile plants
0	150	147	98.0	23.5 ± 0.4	0	–
10	150	122	81.3	23.0 ± 0.7	0	–
20	150	141	94.0	21.7 ± 0.4	0	–
40	150	121	80.7	19.2 ± 0.8	2	1
60	150	94	66.0	15.5 ± 0.2	1	0
80	150	15	10.0	13.5 ± 1.5	1	0
100	150	9	6.0	12.5 ± 0.5	0	–
120	150	6	4.0	11.6 ± 1.3	2	0
160	150	11	7.3	12.9 ± 1.1	2	1
200	150	3	2.0	12.3 ± 2.0	1	1

* Mean ± SE of seedling heights at 3 weeks after transplanting were shown.

It was confirmed that photoperiod sensitivity was genetically altered in the fertile M_1 plants in which, M_2 seeds were harvested from them and grown during next Aman (short day) season in 2009. Seedlings were raised in seed bed with 301, 650 and 246 M_2 seeds which were derived from the 40, 160 and 200Gy treated with carbon ion beam respectively. Germination rate was appeared very low which probably due to damage of soft embryo tissue caused by ion beam or the effect of seed dormancy occurred or the effect of both. Of which 9, 126 and 18 M_2 seedlings were transplanted to the field. The parent Ashfal headed within 122 days and matured within 152 days after sowing in the field. In contrast, M_2 progenies derived from 40 and 200Gy doses of carbon ion beams and 200Gy has taken almost 85 and 110 days to heading and maturity respectively. The number of effective tillers per plant was observed 13 in Ashfal whilst it ranged from 4 to 13 in M_2 progenies (Table 2). The panicle length was found similar to Ashfal in all M_2 progenies studied. These facts suggests that the photoperiod insensitive phenotype of the three fertile M_1 plants was inherited to all M_2 progenies without any segregations.

Table 2. Characteristics of M_2 progenies grown in Aman season and M_3 progenies grown in Boro season

Mutant line	**M_2 generation grown in *aman* season, 2009**			**M_3 generation grown in *boro* season, 2010**		
	Plant height (cm)	**Number of effective tillers/plant**	**Panicle length (cm)**	**Plant Height* (cm)**	**Number of effective tillers/plant***	**Panicle length* (cm)**
RM(1)-40(C)-1-1	75	10	21	75.6 ± 1.0	10.0 ± 0.6	22.6 ± 0.8
RM(1)-40(C)-1-2	73	11	23	77.8 ± 1.7	8.2 ± 0.7	22.8 ± 0.5
RM(1)-40(C)-1-3	77	5	23	79.0 ± 0.7	9.0 ± 0.6	22.4 ± 0.5
RM(1)-40(C)-1-4	73	10	19	77.0 ± 1.8	8.4 ± 1.0	22.8 ± 0.6
RM(1)-40(C)-1-5	76	7	24	76.8 ± 2.0	9.2 ± 0.7	22.8 ± 0.2
RM(1)-40(C)-1-6	72	7	21	74.0 ± 1.3	9.0 ± 0.8	22.8 ± 0.5
RM(1)-40(C)-1-7	74	7	21	81.0 ± 1.7	11.8 ± 0.6	23.0 ± 1.7
RM(1)-40(C)-1-8	81	6	22	–	–	–
RM(1)-40(C)-1-9	91	13	28	83.4 ± 5.1	11.6 ± 1.4	26.2 ± 1.6
RM(1)-200(C)-1-1	68	13	26	70.6 ± 2.0	9.2 ± 1.1	23.0 ± 0.4
RM(1)-200(C)-1-2	64	10	22	65.9 ± 0.7	6.0± 0.7	21.6 ± 0.7
RM(1)-200(C)-1-3	68	6	23	67.4 ± 2.4	7.8 ± 0.9	23.2 ± 0.7
RM(1)-200(C)-1-4	67	8	22	69.4 ± 4.8	8.6 ± 0.9	24.6 ± 1.4
RM(1)-200(C)-1-5	66	5	22	66.0 ± 1.4	6.2 ± 0.5	22.6 ± 0.9
RM(1)-200(C)-1-6	69	8	21	67.8 ± 1.2	7.8 ± 0.6	22.4 ± 0.7
RM(1)-200(C)-1-7	61	6	20	69.8 ± 1.6	6.6 ± 0.7	22.4 ± 0.5
RM(1)-200(C)-1-8	62	9	22	67.6 ± 2.0	8.6 ± 0.5	22.6 ± 0.4
RM(1)-200(C)-1-9	71	7	22	70.2 ± 0.7	7.6 ± 0.4	22.0 ± 0.4
RM(1)-200(C)-1-10	56	5	19	69.4 ± 1.6	9.8 ± 0.6	22.6 ± 0.5
RM(1)-200(C)-1-11	58	7	22	69.4 ± 1.1	7.4 ± 0.2	22.2 ± 0.5
RM(1)-200(C)-1-12	64	6	21	69.2 ± 1.2	9.6 ± 1.7	21.8 ± 0.7
RM(1)-200(C)-1-13	75	6	23	69.4 ± 1.0	11.0 ± 0.9	22.6 ± 0.2
RM(1)-200(C)-1-14	66	9	21	69.6 ± 1.0	8.8 ± 0.5	23.2 ± 0.9
RM(1)-200(C)-1-15	63	7	21	67.0 ± 0.8	8.4 ± 0.9	22.6 ± 0.8
RM(1)-200(C)-1-16	56	4	16	70.6 ± 1.9	10.6 ± 1.0	22.6 ± 0.7
RM(1)-200(C)-1-17	61	8	21	65.0 ± 1.5	10.2 ± 0.8	22.8 ± 0.5
RM(1)-200(C)-1-18	48	4	20	70.6 ± 2.0	9.2 ± 1.1	23.0 ± 0.4
Ashfal (parent)	148	13	24	–	–	–

*Mean ± SE of 5 plants, RM indicates Rice Mutant; 40(C) and 200 (C) indicate irradiation with 40

For further confirmation of photoperiod insensitivity of mutant lines, M_3 plants derived from the each M_2 plants were grown during next Boro (long day) season in 2010. Interestingly none of the parental Ashfal was found to be headed in this season whilst all mutant lines headed almost 90 days after sowing and matured within 119 days after sowing. Days to heading and maturity were found slightly longer than in the case of M_2 generation where that were grown during Aman season. It was observed that in M_2 generation, the plant height of mutant lines were markedly shorten than that of Ashfal (Figure 1).The mutant lines from 40Gy were slightly shorten in plant height than that of mutant lines from 200Gy and it was found consistent with the plant height of each mutant line observed in M_2 generation.

Length (L) and width (W) of unhusked and husked grain and their ratio altered significantly in mutants over the parent Ashfal (Table 3). Length of unhusked and husked grain increased significantly in all the mutants except the line RM(1)-160(C)-1. In RM(1)-160(C)-1, it was found to decreased significantly. In contrast, width of unhusked and husked grain decreased significantly in all mutants whilst ratios of length and width (L/W) of unhusked and husked grain increased significantly. Finally, 1000-grain weight remained unchanged in most of the mutants except 3.

Table 3 .Grain characteristics of eight mutants along with parent Ashfal

Mutant/variety	Grain length, L (mm)	Grain breadth (mm)	L: W	1000-Grain weight (g)
RM(1)-200 (C)-1-1	9.4 (7.0)	2.4 (2.0)	3.92 (3.50)	24.0
RM(1)-200 (C)-1-3	8.9 (7.0)	2.4 (2.0)	3.71(3.50)	23.9
RM(1)-200 (C)-1-9	9.1 (7.0)	2.3 (2.2)	3.96 (3.18)	22.5
RM(1)-200 (C)-1-10	9.4 (7.2)	2.4 (2.0)	3.92 (3.60)	24.0
RM(1)-200 (C)-1-13	8.5 (6.8)	2.3 (2.0)	3.70 (3.40)	23.8
RM(1)-200 (C)-1-17	9.3 (7.0)	2.4 (2.0)	3.88 (3.50)	24.2
RM(1)-200 (C)-1-18	9.4 (7.0)	2.4 (2.0)	3.92 (3.50)	22.6
RM(1)-160 (C)-1	7.5 (5.8)	2.8 (2.2)	2.68 (2.64)	20.5
Ashfal	7.9 (6.2)	3.0 (2.6)	2.63 (2.38)	24.2
SE	0.2 (0.2)	0.1 (0.1)	0.18 (0.15)	0.4

Figures in parenthesis indicate grain characteristics from husked grain.

Preliminary yield trial with M_6 high yielding mutant lines of boro rice

Seedlings of 10 mutant lines derived from BRRI dhan29 by irradiated its seeds with carbon ion beams in Boro season along with unirradiated BRRI dhan29 were transplanted on 22 January 2012 at 41 days after seed sowing. The experiment followed RCB design with 3 replications. Seedlings were transplanted at 15cm distances within rows of 20cm apart. A unit plot size was 3.0m × 2.0m. Recommended doses of fertilizers, cultural and intercultural operations were also followed as and when necessitated. Data on plant height, days to maturity; number of effective tiller, panicle length, filled and unfilled grains panicle^{-1} and yield plot^{-1} were recorded. Maturity was recorded plot basis while plant height, effective tiller number, panicle length, filled and unfilled grains/panicle was recorded from hills per plot at harvesting time. Grain yield was recorded from $1m^2$ areas which was latter converted to yield/ha at 14% moisture condition of grain. Moisture data was recorded with a grain moisture meter. Finally, the recorded data were subjected to proper statistical analyses following the design used and are presented in Table 4.

It appears that the mutants and the check variety differed significantly for yield and yield attributing traits except panicle length (Table 4). The mutants took 147 to 157 days to mature whilst their parent BRRI dhan29 took 159 days. The mutant RM (2)-40 (C) -3-1-7 took the shortest period only 147 days for maturity. Filled grains was significantly the highest in the mutant RM(2)-50 (C) -2-1-1 and it also had the highest grain yield at 14% moisture content of 8.58t/ha, which was 1.35 t/h more than BRRI dhan29. This higher yield of this mutant was attributed to its considerably longer panicle length and significantly higher number of filled grains/panicle (Table 4).This mutant together with those with higher filled grains/panicle, longer panicle length, statistically as par yield and shorter maturity period would be put into advance yield trial in the next Boro season.

Table 4. Yield and some yield attributing traits of 10 mutant lines derived from BRRI dhan29 with carbon ion beam irradiation along with unirradiated BRRI dhan29 at BINA farm, Mymensingh

Mutant/variety	**Days to maturity**	**Plant height (cm)**	**Effective tiller (no.)**	**Panicle length (cm)**	**Filled grains panicle^{-1} (no.)**	**Unfilled grains panicle^{-1} (no.)**	**Grain yield/ha (t)**
RM(2)-40 (C) -1-1-1	151	112.27	6.93	26.53	139.13	27.00	7.02
RM(2)-40 (C) -1-1-10	149	96.30	8.20	27.00	119.33	23.87	6.93
RM(2)-40 (C) -1-1-7	149	95.53	8.60	25.87	113.60	26.13	5.80
RM(2)-40 (C) -4-2-5	149	91.67	11.40	25.40	109.13	10.87	6.90
RM(2)-40 (C) -4-2-7	159	115.00	8.80	25.73	94.33	18.07	6.53
RM(2)-40 (C) -4-2-8	157	117.60	6.47	28.20	140.60	30.80	6.34
RM(2)-40 (C) -1-1-5	149	112.87	7.93	25.33	136.67	21.07	7.30
RM(2)-40 (C) -3-1-7	147	108.80	7.33	25.80	116.47	21.40	7.10
RM(2)-40 (C) -4-2-2	157	103.07	9.33	25.20	103.73	24.73	6.88
RM(2)-50 (C) -2-1-1	155	115.40	7.80	27.00	169.00	22.87	8.58
BRRI dhan29	159	97.50	10.40	24.57	127.75	22.65	7.23
LSD(0.05)		9.47	1.53	NS	31.42	6.76	0.72

NS- not significant

1.5 Discussion

Heavy ion irradiation has been attaining an important to the mutation breeder as it produced rare mutant(s). In view of this, seeds of Ashfal rice and BRRI dhan29 were irradiated with different doses of carbon ion beams and was grown in Boro season. Three of nine headed plants were found fertile and the rest six were exhibited sterile in case of Ashfal. Since the Ashfal is a highly photoperiod sensitive cultivar and none of the control plants headed during Boro season. These results suggest that photoperiod sensitivity was altered by carbon ion irradiation in a few M_1 plants. Plant heights of M_2 progenies were found significantly shorten than Ashfal and this is also observed consistent with the fact that the fertile M_1 plants had shorter plant height than Ashfal. This might be due to the fact that the fertile mutant lines shifted from vegetative phase to reproductive phase earlier than parental Ashfal and it is caused by ion beam irradiation. It has been reported that one dominant and one recessive genes 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 M_1 plants homozygous monogenic recessive for photoperiod insensitivity. These facts leaded M_1 plants to bred true in M_2 and M_3 generations. The M_2 progenies from 160Gy were omitted from these evaluations because they were prone to lodging for weak culm although they had shorten plants. Moreover, these progenies had shorter panicle with smaller grains and lower number of seed grains. The mutants derived from 40Gy dose also did not continue after M_3 generation because of their inferior performance. The grain characteristics such as size and shape although most of the mutants altered but grain weight mostly remained unchanged as the parent. This also confirmed that the M_1 plants that mutated as photoperiod insensitive with shorten plant height were not contaminated with other variety. If it was contaminated then 1000-grain weight must differed significantly with Ashfal and the contaminants. The mutant of BRRI dhan29 showed higher yield and early maturity compared to parent of BRRI dhan29 which could be due to the useful effect of carbon ion beam.

Hence, it could be concluded that it is possible to induced fixed and stable mutant(s) in M_1 generation of *indica* type rice Ashfal and BRRI dhan29 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 heavy ion beams irradiation has unique properties that induce fixed mutation even in M_1 generation. In other mutational studies showed that mutants are usually detected in M_2 and M_3 generations and thus need to handle numerous plants to screen. If mutants can be screened in M_1 generation, the number of plants that must be handle is dramatically decreased. Therefore, further studies are needed to investigate whether other crop species could induce fixed mutant(s) in M_1 generation.

1.6 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, Japan Atomic Energy Agency (JAEA), Ms. Aki Koike, Nuclear Safety Research Association (NSRA) Dr. Liu Luxiang, RCA Coordinator of China for their kind help and cooperation.

1.7 References

Azad MAK, Hamid MA, and 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.

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

Bonilla PS, Dvorak J, Mackill D, Deal K, Gregorio G (2002). RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Philippines Agric. Sci*. 85(1):64-74.

Gregorion GB, Senadhira DRD, Mendoza RD, Manigbas NL, Roxas JP, Guetra CQ (2002). Progress in breeding for salinity and associated abiotic stresses in rice. *Field Crops Res.* 76:91-101

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.

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.

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

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 M_1 plant selection. *Euphytica* 152:61-66.

Islam MM (2004). Mapping salinity tolerance genes in rice (*Oryza sativa* L.) at reproductive stage. Ph.D. Thesis, University of the Philippines Los Banos, College Laguna, Philippines.

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

Niones JM (2004). Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near isogenic line. M.Sc. thesis, University of the Philippines Los Banos, College Laguna, Philippines.

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.

Salil KB, Islam MM, Emon MR, Begum SN, Siddika A, Sultana S (2007). Identification of salt tolerant rice cultivars via phenotypic and marker assisted procedures. *Pakistan J. Biol. Sci*.10(24):4449-4454.

Shamsuzz*aman* 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.

Vergara BS, Chang TT(1985). The flowering response of the rice plant to photoperiod. The International Rice Research Institute, Los Banos, Laguna, Philippines.

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.

2. China

FNCA Mutation Breeding Project
Sub-Project on Composition or Quality in Rice

J. Luo, P. Hu, S. Tang, G. Jiao and G. Shao

National Rice Improvement Center,
China-IRRI Joint Research Center on Rice Quality and Nutrition,
China National Rice Research Institute

2.1 Introduction

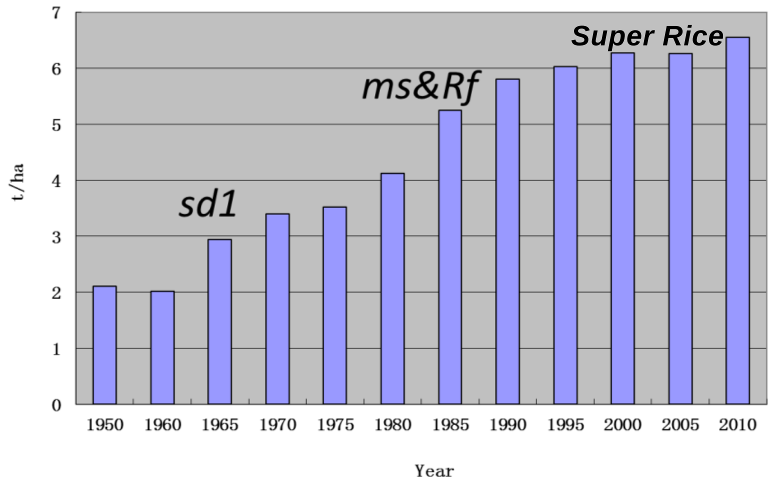
Rice (*Oryza sativa L.*) is one of the most important crops in the world and is a staple food crop for at least 65% of the population of China. The performance of rice sector in terms of production and yield had been very impressive in the most of last six decades. The wide adoption of semi-dwarf varieties expedited the rice yield per unit from 2t/hm^2 in1960s to the level of 3.5t/hm^2 in 1970s. However, the increase of rice production due to the development of semi-dwarf varieties still could not match the rapid increase in population. Exploitation of heterosis in rice became the important choice for getting more rice. Since 1949, the year of founding of the Peoples' republic of China, the rice production has continued to increase along with the rapid growth of the Chinese population. China's total population reached to 1.37 billion people in 2011 and has increased 148.5% compared with the population in 1949. Meanwhile rice yield per unit and total output increased 254.0% and 313.2% as 6.69 t/hm^2 and 200.8 million tons respectively in 2011. However there is only 11.7% increase about the rice sowing area during this period and the rice sowing area is 30.0 million hm^2 in 2011.

Hybrid rice has played an important role in demand of food supply in China. China has made a great effort to increase its rice yield through exploiting the genetic resources of rice. The performance of rice sector in terms of production and yield had been very impressive in the most of last five decades. Hybrid rice that has a yield advantage of 10-20% over the conventional varieties was developed and commercially grown in 1976, which resulted in the increase of yield to over 6.0 t/hm^2. The area under hybrid rice had been increased from 0.14 million hm^2 in 1976 to about 18 million hm^2 in recent years, more than 60% of the total rice planting areas in China (Table 1). In some regions, like Jiangxi and Sichuan province, more than 90% of their rice varieties belong to hybrid rice. Several thousands of hybrid rice varieties were released in the past 50 years. Until now total 96 super rice varieties were confirmed by Ministry of Agriculture (MOA) and were released to the farmers since MOA initiated the super rice breeding program in 1996. The planting area of super rice occupied 25% of total rice planting area and reached 7.3 million hm^2 in 17 provinces in 2011 (Figure 1). These hybrid rice varieties already made important contribution for food security in China.

Table 1. Yearly area of hybrid rice and percentage of the total rice area in China in 1976-2010

Year	Hybrids planting area (m hm^2)	% of total rice area
1976	0.14	0.4
1978	4.34	12.6
1982	5.62	17.0
1986	9.00	27.9
1990	13.62	41.2
1997	17.73	55.8
1999	16.55	52.9
2002	15.48	54.9
2005	19.01	65.5
2008	18.45	63.1
2010	18.05	60.2

Figure 1. Increase of yield in rice through genetic improvement in China



With the great progress of hybrid rice research, the general quality of hybrid rice, especially in terms of head rice rate, chalk-ness and the amylose content etc., has dramatically improved. According to the results in South China Rice Regional Trial in1998- 2004, comparing with the state standard for rice quality, the mean amylose content in every type of hybrids belongs to high quality rice (>3rd grade). In regard to head rice rate (>3rd grade), over 60% single *indica* and late *indica*, over 90% single-season late japonica hybrids reached the standard respectively (Table 2). However, among the 560 hybrid rice combinations developed in 1998-2004, 106 of them reached state standard of the 3rd grade of high quality rice. So the coordination of comprehensive quality is very important to hybrid rice breeding. Hybrid rice breeding technology is different from that for inbred rice breeding. Currently, the most popular male sterility system is the cytoplasmic male sterility (CMS, popularly known in China as the three-line system). This utilizes three different lines, namely a cytoplasmic male sterile line (A line), a maintainer (B line), and a restorer (R line). The quality of B and R line is the key to determine the quality of hybrid rice regarding to three-line system.

Table 2. Performance of hybrids in some quality items in South China Rice Regional Trial in 1998-2004

Type*	Head rice rate (%)		Chalkness (%)		Amylose content (%)		Over 3rd grade	
	Mean	% hybrids reaching the standard	Mean	% hybrids reaching the standard	Mean	% hybrids reaching the standard	No. of hybrids	%
EI (Y)	42.7	21.1	17.3	11.3	19.4	52.1	1	1.4
SI (Y)	57.9	81.0	9.9	30.0	20.7	76.2	44	21.0
LI (Y)	53.9	64.8	9.3	37.3	20.2	85.9	30	21.1
SJ (Y)	70.7	93.1	4.5	58.6	15.5	82.8	17	58.6
EI (S)	48.9	44.4	14.1	8.3	20.6	45.8	0	0.0
LI (S)	62.5	97.2	5.7	55.6	20.8	69.4	14	38.9
Total		66.3		29.6		71.8	106	18.5

*EI(Y or S)— Early season *indica* rice (Yangtze River or south China)

LI(Y or S)— Late season *indica* rice (Yangtze River or south China)

SI&J (Y) — Single *indica* or *japonica* rice (Yangtze River)

2.2 Objective of the Project

The main objective is to obtain ideal CMS lines and restorers with good quality including adaptive amylose content and to get the optimum irradiation doses of heavy ion beam for early super rice Z7 and late-season inbred rice W2 to create efficient mutant.

2.3 Materials and Methods

Two experiments were carried out in this project during 2008-2010.

2.3.1 Experiment 1 Rice mutation breeding for hybrid rice with high yield and good quality

(1) Mutation breeding of CMS lines

Zhong 3A is a good new indica CMS line with good-grain quality and high disease resistance ability, developed from crossing Zhong 9A to Zhenong996/Jin 23B and successively backcrossing its filial male sterile plants to Zhenong/Jin 23B. It showed stable and complete male sterility, vigorous stigma with high exsertion rate, good disease resistance ability, high combining ability and high outcrossing rate. It had tall and thick stems with good lodging resistance. It was technically identified in Zhejiang Province in September 2005. However, most of the hybrids crossed with Zhong 3A showed high amylose content (>22%). The amylose content of Zhong 3B is 20.1%. Therefore we expect to decrease amylose content of the CMS lines in order to obtain hybrid rice with low amylose content (15-20%) by mutation breeding. In 2008, seeds of Zhong 3B were irradiated with 350Gy and 400Gy from Co-60 gamma irradiator in Hangzhou. Ten M4 lines of Zhong 3 B (maintainer) with low amylose content were selected for further backcross with CMS lines in 2010.

(2) Mutation breeding of restorers

AVF-cyclotron, TIARA, JAEA

Nineteen restorers including CR63, CR84, CR1577 had been collected and irradiated with 250Gy from Co-60 gamma irradiator in Hangzhou in 2006. Six crosses as CR1577(Mutant)/R501, CR1577(Mutant)/R725, Yangdao6/CR1577(Mutant), Shuhui527/CR63(Mutant), CR63(Mutant)/Minghui86, R207/CR84(Mutant) were made and harvested for restorer selection in winter of 2008. Two restorers (ZZ-2 and 07HY-1580) were collected and irradiated with 350Gy and 400Gy from Co-60 gamma irradiator in Hangzhou in 2008. For each treatment 2500 seeds were used. The main objective is to obtain ideal restorers with good quality including adaptive amylose content. Two mutant lines (M_4) from a restorer as CR84 were crossed with CMS lines as Nei 2A, Nei 5A, Nei 6A,, 618A and Zhong 3A respectively in winter of 2009. These hybrids showed good appearance quality, weak potential ability of high yield in summer of 2010. 79 lines of F4 generations were obtained from six combinations between mutant lines with the other restorers through agronomic traits selection. These restorer mutants showed adaptive amylose content. Some of mutants were used as restorer parent for further utilization.

2.3.2 Experiment 2 Seed Irradiation Test by Ion Beam for Rice Mutation Breeding

Through the FNCA cooperation project we initiated heavy ion beam irradiation technology for mutation breeding research in 2010. Two batch of hulled rice seeds were irradiated by the heavy ion beam of the TIARA, JAEA. Two rice cultivars as Z17 and W2 were used. One is a super-high-yielding early-season i*ndica* cultivar; another is a good-quality late-season *indica* cultivar.

The dry seeds of Z17 and W2 were irradiated by carbon ion by the doses of 10, 20, 40, 60, 80, 100, 120Gy. About 150 seeds were packed with plastic Petri dish for each experiment per test-dose. In addition more dry seeds of Z17 were irradiated by carbon ion by the doses of 30, 40, 50Gy respectively, 400 seeds for each irradiation. After irradiation, biological effects were be observed by measurements of the germination rate, survival rate, growth rate and ripening rate. Sowing date, transplanting date and harvesting date was June 10^{th}, July 9^{th} and Oct 12, 2010 respectively. Method of measurement is same to J. Hidema's experiment. After irradiation, the seeds were placed on wet filter paper in petri dish and kept at 30^{o}C for 2 days and then the seeds were planted in plastic-tray containing sterile field soil and placed in the control environmental greenhouse (12hr photoperiod, day/night 27^{o}C/27^{o}C). The germination rate was measured at 2days after planting. The survival rate was measured by counting the viable plants at 2 and 3 weeks after germination. The growth rate (the ratio of the value for plant length of each irradiated plant to the value for plant length of un-irradiated plant) was measured, 30 days after sowing in the control environmental greenhouse. The percentage of grain ripening was measured at 4 months after sowing (maturity stage).

2.4 Results and Discussion

Along with the irradiation dosage increasing, the germination rate, survival rate, growth rate, ripening rate decreased in different extent. Ion beam made some damages to rice seed (Figure 2, 3) . Regarding to Z17, when the irradiation dosage is up to 50Gy, the survival rate and ripening rate make a sharp decrease. When the dosage is down to 20Gy, there is no big difference in all investigated data. Ripening rate significantly decreased at doses of up to about 60Gy, but panicle number significantly increased at doses of up to about 100Gy (Table 3,4, Figure 4, 5). The sterile and low seed setting plants probably kept tillering under water condition in the field. The survival rate of W2 after irradiation decreased sharply (Figure 6).

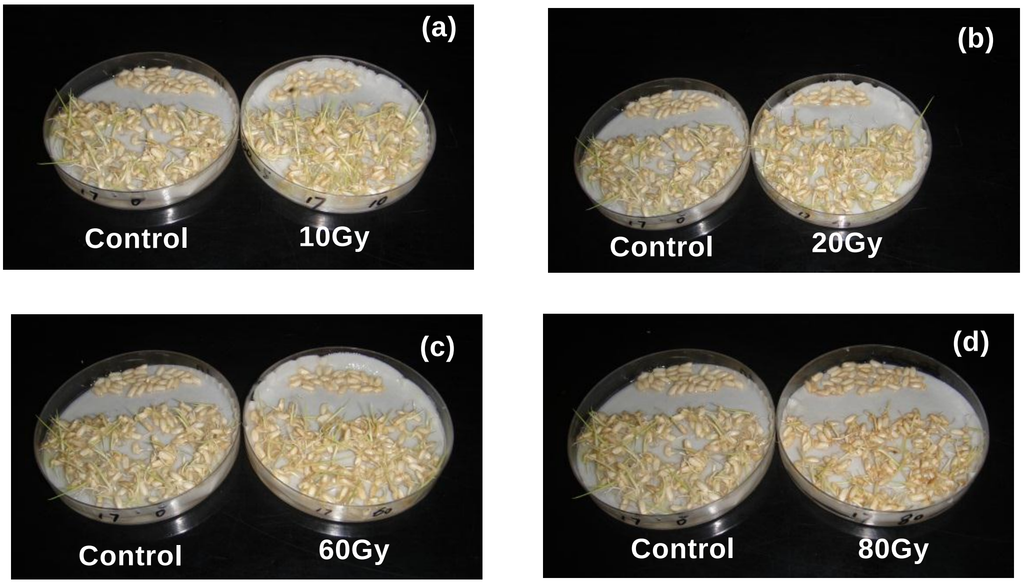


Figure 2. Effect of different dose of ion-beam irradiation of Z17 at day 2.
(a)Germination rate at dose (0Gy and 10Gy), (b)Germination rate at dose (0Gy and 20Gy), (c)Germination rate at dose (0Gy and 60Gy), (d)Germination rate at dose (0Gy and 80Gy).

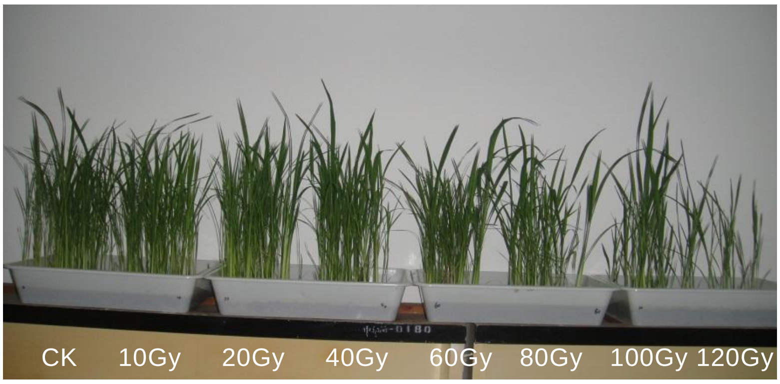


Figure 3. Emergency features of Z17 seedling in a phytotron.

Table 3. Z17 Performance after ion-beam irradiation

Dose (Gy)	Germination rate (%)	Survival rate (%)		Plant Height* (cm)	Growth rate (%)	Percentage of ripening (%)
		2weeks after germination	3weeks after germination			
0 (Control)	87.25	63.09	50.34	30.33	100.00	74.20
10	83.39	63.76	47.65	29.67	97.85	73.30
20	76.16	51.66	35.10	30.19	99.55	61.70
40	73.83	51.01	33.56	30.04	99.05	51.20
60	70.34	46.21	32.41	26.32	86.79	32.40
80	73.51	43.05	26.49	27.09	89.32	13.20
100	72.85	33.11	17.88	28.15	92.82	25.70
120	67.11	20.81	10.74	26.59	87.69	12.80

*: Measured at 30 days after sowing in the control environmental greenhouse.

Table 3 showed the effects of carbon-ion beams on plant height, panicle number, seed number and ripening rate in rice plant. Ripening rate significantly decreased at doses of up to about 60Gy, but panicle number significantly increased at doses of up to about 100Gy. The sterile and low seed setting plants probably kept tillering under water condition in the field.

Table 4. Agricultural traits average performance of Z17 M_1 generation

Dose (Gy)	Plant height (cm)	Panicle number (no./plant)	Seed number (no. /panicle)	Ripening rate (%)
Control	94.1	8.35	150.65	74.2
10Gy	93.6	9.30	150.86	73.3
20 Gy	93.0	8.40	152.24	61.7
40 Gy	93.5	9.85	150.90	51.2
60 Gy	95.0	9.70	170.62	32.4
80 Gy	89.9	9.15	168.08	13.2
100 Gy	90.2	11.25	158.06	25.7
120 Gy	87.9	11.00	164.17	12.8

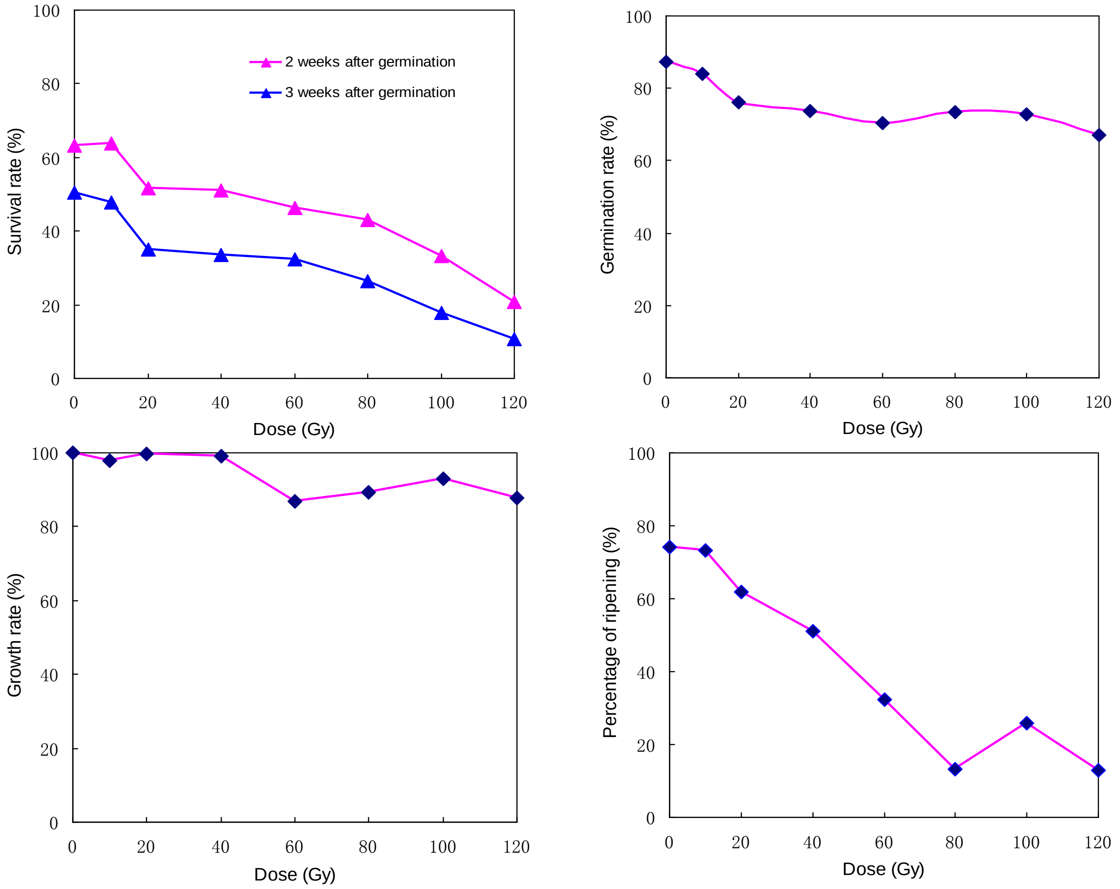


Figure 4. Z17 Dose-response curve for germination rate, survival rate, growth rate and percentage of ripening.

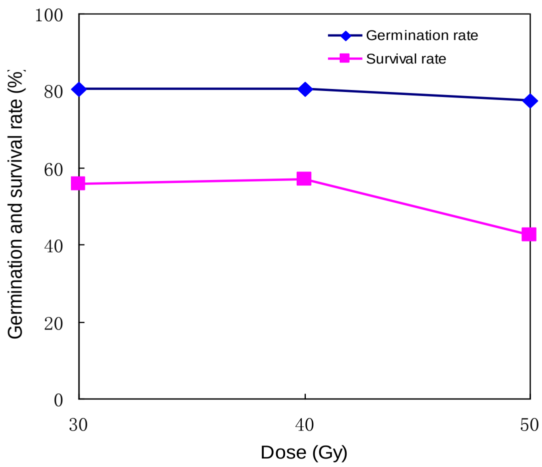


Figure 5. Z17 Dose-response curve (30, 40, 50Gy) for germination rate, survival rate.

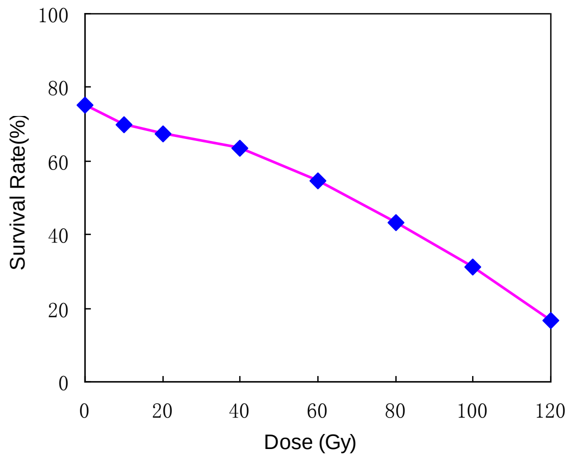


Figure 6. W2 Dose-response curve for survival rate.

2.5 Conclusion

Hulled seeds of Z17, local super rice cultivar, was irradiated with 7 different doses (10, 20, 40, 60, 80, 100, 120Gy) by the heavy ion beam of the TIARA, JAEA in 2010. These materials with 0 doses as control were cultivated in summer of 2010. The germination rate, survival rate, growth rate and percentage of ripening were investigated. These results suggested that the optimum irradiation doses of heavy ion beam for Z7 might be between 30 to 50Gy doses.

2.6 Acknowledgments

The authors would like to thank Japan Atomic Energy Agency (JAEA) for providing the ion-beam facilities. This research was supported by China Atomic Energy Authority.

2.7 References

Cao L-Y, Zhan X-D, Chen S-G, Feng Y, Wu W-R, Shen X-H, Cheng S-H, Breeding Methodology and Practice of Super Rice in China. ***Rice Science***, 2010, 17(2): 87-93

Cheng S-H, Cao L-Y, Yang S-H, Zhai H-Q. Forty years' development of hybrid rice: China's experience. ***Rice Science***. 2004, 11, 225-230.

Cheng S-H, Zhuang J-Y, Fan Y-Y, Du J-H, Cao L-Y. Progress in Research and Development on Hybrid Rice: A super-domesticate in China. ***Annals of Botany,*** 2007, 100: 959–966.

Luo J, Tang S-Q, Jiao G-A, Hu P-S. Breeding of Good Quality and Resistance Indica CMS Line Zhong 3A. ***Hybrid Rice***, 2007, 22(1)：13-14

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

Yuan L-P. Strategy of hybrid rice breeding. ***Hybrid Rice***, 1987, (1):1-3.

3. Indonesia

Improvement of Yield and Grain Quality of Rice through a Wide Cross and Mutation Breeding

Sobrizal

Center for the Application of Isotopes and Radiation
National Nuclear Energy Agency

3.1 Abstract

To meet the Indonesian domestic demand for both rice grain quality and quantity, high variability of pure lines derived from a cross of Indica rice variety, IR36 and Japonica rice variety, Koshihikari was constructed. Among these lines, KI237 line was treated by gamma ray irradiation to remove its undesirable characters. KI237 shows good performance with high yield potency and red phericarp, but susceptible to lodging because of tall plant stature. To reduce the plant height, 50g seeds of this line were irradiated by 200Gy of gamma ray at Center for the Application of Isotopes and Radiation Technology, Pasar Jumat, Jakarta, Indonesia. Selections and purifications were conducted in M_2 and M_3 generation, finally, one dwarf and four semi-dwarf M_3 mutant lines were selected. Based on the segregation analysis in M_2 and M_3 generation, it was concluded that the semi-dwarf of RKI237-1-17 mutant line was controlled by single recessive mutated gene. For breeding purpose, based on their agronomical performances ten semi-dwarf lines were selected and subjected to yield and other character evaluations. The results of multi-location yield trials showed that the highest average yield of multi-locations yield trials in five locations was 7.39t/ha for RKI199 and followed by 7.24t/ha for RKI241, while average yield of chiherang, a national leading variety is only 6.56 ton/ha. Out of ten lines, three lines are still with brown phericarp and the phericarp color of other lines change to white. These ten lines have various amylose contents, ranging from 13.41% to 20.83%. Various amylose contents and phericarp colors would be useful as genetic resources for improvement of rice grain quality to serve the wide preference variability of Indonesian consumers. To fulfill the requirement of variety release in Indonesia, other multi-location yield trials as well as other examinations such as pests, diseases and other grain quality examinations should be conducted.

Key words: yield, grain quality, intra-specific cross, mutation

3.2 Introduction

Rice is a steeple 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 1.3%, Indonesian rice production should be increased. This can be achieved by introducing the high yielding varieties to the farmers.

Recently, the demand for superior grain quality increased for national markets in Indonesia. Rice consumers in Indonesia exhibit wide preference variability and consumption patterns because of archipelagic nature of the country (Toquero 1991). Consumers in Medan, Padang and Makassar, for example, prefer non-sticky cooked rice, while consumers in Java prefer sticky and soft-cooked rice. Nowadays, consumers have become more discriminating in terms of rice quality due to higher incomes and better life quality. Indonesian rice consumers are now willing to pay higher price for better quality desired. These imply requirement of technology generation for better quality.

The primary components of rice grain quality include milling efficiency, appearance, cooking and edibility characteristics, and nutritional quality (Li et al. 2004). Generally, the milling efficiency is determined by head rice yield; appearance is determined by grain length, grain width, width-length ratio, and translucency of endosperm; cooking and edibility characteristics are determined by amylose content of endosperm, gelatinization temperature, and aroma; nutritional quality is determined by protein, oil, phytic acid, and micro nutrient contents.

IR36 is an Indica improved rice variety, tolerance to various pests and diseases, and adapted well in almost all Indonesian rice growing areas. Recently, IR36 rice variety is almost not grown in Indonesian because of its poor in cooking and edibility characteristics. Whereas, Koshihikari is a very popular Japonica variety and known having good cooking and edibility with low amylose content, but it cannot grow well in Indonesia due to photoperiod sensitivity. By using these unique germplasms as well as mutations ten rice promising lines with high yield and grain quality have been constructed. The objective of this research is to develop high yielding and grain quality of rice varieties.

3.3 Materials and Methods

To increase the genetic variability of breeding materials, IR36 and Koshihikari were cross, and the F_1 plants obtained from this cross were self-fertilized up to F_5 generation. F_5 lines were developed from 100 F_2 plants by pedigree method with 10 plants per line for each generation, and finally the size of F_5 population became 100,000 plants. Selection for agronomical and other major characters was performed in F_5 populations. Selected plants were purified up to F_7 or F_8 generations, then, selected pure lines were designated as KI #. KI lines are used as basic materials in further breeding program.

Among these lines, the seeds of KI237 line were treated by gamma ray irradiation. This line shows good performance with high yield potency and red pericarp, but susceptible to lodging because of tall plant stature. To reduce the plant height, 50g seeds of this line were irradiated by 200Gy 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 20cm 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. Observations of semi-dwarf plants were conducted in each M_2 lines before harvesting. The M_2 lines showed segregation between normal and semi dwarf plants was harvested individually.

In the next growing season, 80 plants derived from each M_2 plant of selected M_2 lines were planted to develop M_3 lines. Observations of segregation between semi-dwarf and normal plants were conducted in each M_3 lines. The χ^2 test in segregated M_3 lines was performed to examine fitness of the frequencies of the semi-dwarf plants against expectation from Mendelian segregation.

Selected semi-dwarf plants were purified up to M_5 generations, then, selected pure lines were designated as RKI #. RKI lines were subjected to yield trials, amylase content, and other examinations. Breeding scheme of plant materials used in this study can be shown in Fig. 1.

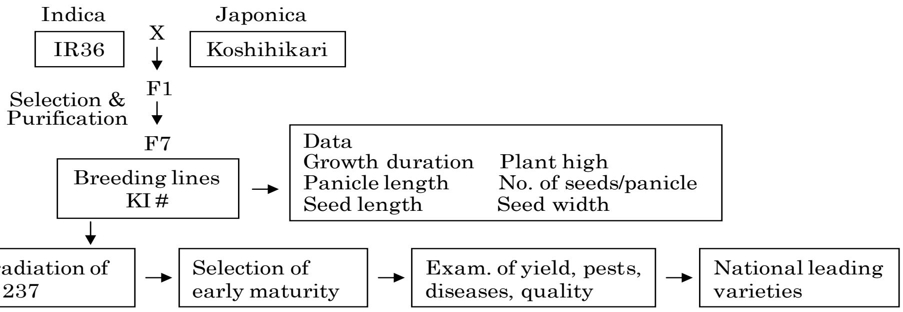


Figure 1. Breeding scheme of plant materials used in this study.

3.4 Results and Discussion

In this study, all the F_1 plants derived from cross of IR36/Koshihikari grew vigorously but their pollens as well as seeds were semi-sterile. The fertilities were improved gradually from early to the next generations. Selection was conducted in F_5 population with emphasis on agronomical characters, seed fertilities, and pest and disease tolerances in the field. Of the 100,000 F_5 plants, 568 plants were selected and they were purified up to F_7 or F_8 generation to construct 568 fixed lines. Selected pure lines were designated as KI #.

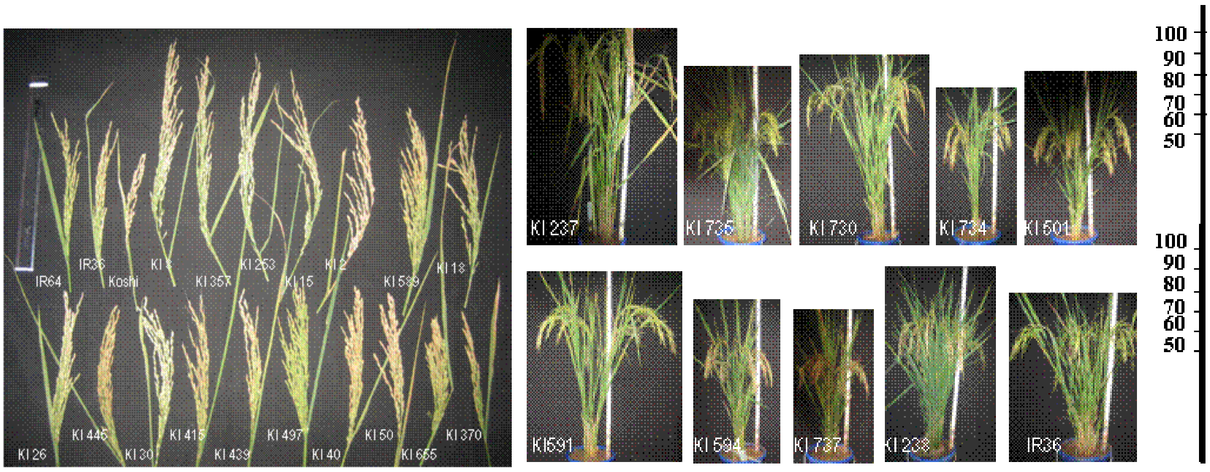


Fig. 2. Variability of selected pure lines derived from a cross of Koshihikari and IR36.

Figure 2 shows varying features of KI lines. Variations were shown in many characters such as plant height, growth duration, number of tillers, flag life, panicle length, number of seeds per panicle, seed length, seed width, and others. For rice breeding program, some of these lines showing good performance were directly subjected to yield trials and several others were irradiated by gamma ray to induce mutations for amelioration of one or two undesirable characters, or crossed each other to gather some desirable characters in one individual.

Among these KI lines, the KI237 was selected to improve by mutation breeding because this line showed good yield potency but so tool that susceptible to lodging. To reduce the plant height of KI237, its seeds were irradiated by 0.2kGy gamma ray. As much as 500 M_1 plants were harvested individually to obtain M_2 seeds. Selection for dwarf and semi-dwarf were conducted in M_2 lines. Out of 342 M_2 lines, each M_2 line consisted of 20 plants, 5 lines segregated for plant height. In this study, since the original of these lines is KI237, the plants having the same height as RKI237 were grouped as normal plants. Among the segregated lines, the line of RKI237-1 segregated for 8 semi-dwarf and 12 normal plants. The semi-dwarf plant reached 60 – 62% of plant height of original plant KI237 at the mature stage (Fig. 3A). It was also compared the length of internodes, panicle, and seed between these two plants (Fig. 3B; Fig. 4; Table 1). In rice, internodes elongation starts from the bottom at the panicle initiation stage. All internodes of semi-dwarf plant were shortened in comparison with the original pant. The elongation of the upper internodes was weakly inhibited. The retardation of the 1^{st} (uppermost) internodes was 24%, moreover, the retardation of panicle and seed length was only 10% and 2%, respectively. The elongation pattern of the internodes in this mutant was almost the same as *sd1* (Dee-geo-woo-gen), the original parent of the first release modern rice variety (Itoh *et al*. 2004).

To perform the genetic analysis of this semi-dwarf character, the segregated M_2 line of RKI 237-1 was harvested individually and 80 plants derived from each harvested M_2 plant were planted to generate the M_3 lines. Four M_3 lines derived from normal M_2 plants (RKI 237-1-2, RKI 237-1-3, RKI 237-1-8 and RKI 237-1-12) and two M_3 lines derived from semi-dwarf M_2 plants (RKI 237-1-1 and RKI 237-1-17) were selected and use them for analysis of the mutated gene(s). It was observed that all M_3 plants derived from RKI 237-1-1 and of RKI 237-1-17 M_2 plants showed semi-dwarf stature, all M_3 plants derived from RKI 237-1-2 and of RKI 237-1-8 M_2 plants showed normal stature, and the other two M_3 lines derived from normal M_2 plants, RKI 237-1-3 and RKI 237-1-12 segregated for 57 normal and 22 semi-dwarf and 59 normal and 21 semi-dwarf plants, respectively. The segregation ratios between normal and semi-dwarf plants in both RKI 237-1-3 and RKI 237-1-12 M_3 lines fitted well to Mendelian expected segregation 3 : 1 (Table 2). These results suggested that the semi-dwarf character in these lines was controlled by a single recessive gene. This gene was tentatively designated as sd^{237-1}.

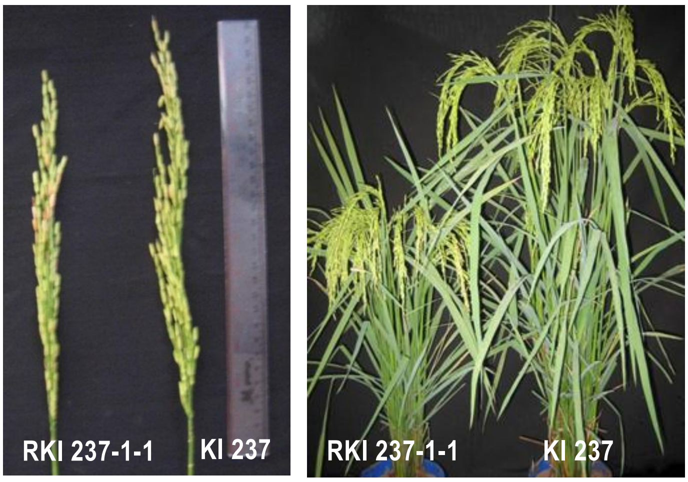


Fig. 3. Morphological characterization of semi-dwarf mutant RKI237-1-1 and its original plant, KI237.

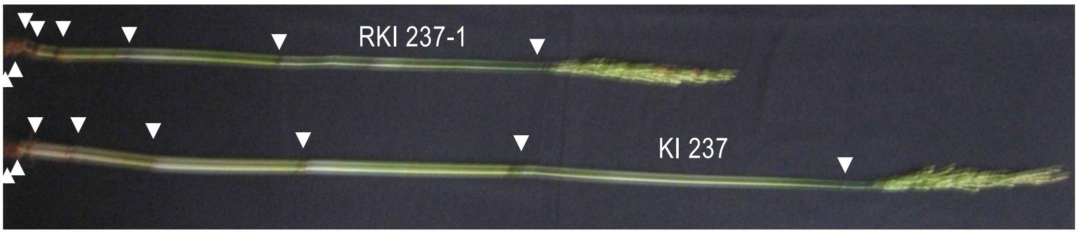


Fig. 4. Comparison of culm elongation between semi-dwarf mutant RKI237-1 and its original line KI237.

Table 1. The length of internodes, panicles, and seeds of KI237 and its semi-dwarf mutant.

Line	Length of internodes, panicle, and seed (cm)								
	IN-I	IN-II	IN-III	IN-IV	IN-V	IN-VI	IN-VII	Panicle	Seed
RKI 237-1	0,3	0,5	1,5	3,0	7,5	21,5	34,0	28,0	10,3
KI 237	0,7	1,0	4,5	10,5	20,0	28,0	44,5	31,0	10,5
PIL (%)	42.9	50.0	33.3	28.6	37.5	76.8	76.4	90.0	98.1

PIL = Percentage of internodes length of RKI 237-1 mutant over the original plant.
IN = Internodes

In rice, At least 7 semi-dwarfing genes have been identified by classical genetic analysis (Nagato and Yoshimura 1998), some of them, such as *sd1* on chromosome 1 and *d35* on chromosome 6 have been isolated and have been extensively analyzed to elucidate the regulatory mechanisms of plant growth and development (Spielmeyer et al. 2002; Itoh et al. 2004). Even though the performance of rice mutants carrying $sd^{237\text{-}1}$ gene differed from that of rice mutant carrying *sd1* or *d35*, the allelic test between $sd^{237\text{-}1}$ gene and other semi-dwarfism genes are necessary to clarify whether the $sd^{237\text{-}1}$ is a new identified gene or not. Chromosome mapping concerning the $sd^{237\text{-}1}$should also be conducted in near future to locate the $sd^{237\text{-}1}$ gene on rice chromosome linkage map.

Table 2. Segregation of normal and semi-dwarf plants in M_3 lines.

M_3 line	Type of F_2 plant	Normal	Semi-dwarf	χ^2 (3:1)
RKI 237-1-3	Normal	57	22	0.34 ^{ns}
RKI 237-1-12	Normal	59	21	0.08 ^{ns}
RKI 237-1-2	Normal	80	0	-
RKI 237-1-8	Normal	80	0	-
RKI 237-1-1	Semi-dwarf	0	80	-
RKI 237-1-17	Semi-dwarf	0	80	-

^{ns} non significant at 0.1% level.

Semi-dwarfism is a valuable trait in crop breeding, because it increases lodging resistance and decreases damages due to wind and rain. During the green revolution, in the second half of the 20th

century, a rice semi-dwarf variety, IR8, enabled dramatic yield increases and help to avert predicted food shortages in Asia (Khush 2001). At the same time, a dominant wheat semi-dwarf cultivar, *Rht1* as well as *Rht2*, facilitated a burst in productivity and lead to the wheat green revolution (Evans, 1998). The original of $sd^{237\text{-}1}$ is KI237, a pure selected line derived from Indica / Japonica cross. This line showed high yield potency, but susceptible to lodging. The $sd^{237\text{-}1}$ mutant improved lodging resistance without significantly changing its major characters. This mutant could be used as a genetic resource for the improvement of KI237 line through back-cross breeding as well as be developed further in breeding program directly to be new high yielding mutant varieties.

Recent developments of gene transfer technology have enormous promise for improvement of plant productivity; however, there is a lack of available new genes which can be transferred to current high-yielding varieties. In other words, there are no genes that have been identified which can contribute to world crop production as much as *sd1* (DGWG) in rice, and *Rht1* as well as *Rht2* in wheat (Maluszinki 1998). Since the elongation pattern of the internodes of $sd^{237\text{-}1}$ mutant was almost the same as *sd1*, the $sd^{237\text{-}1}$ mutant gene can be used with *sd1* simultaneously to avoid genetic vulnerability without reducing yield.

To evaluate the yield performances, ten selected semi-dwarf pure lines were subjected to yield trials in five locations. The highest average yield in five locations is 7.39t/ha for RKI199 and followed by 7.24t/ha for RKI241, whereas the average yield of Ciherang, a national leading variety is only 6.56t/ha (Table 3). The lowest average yield is 6.54t/ha for KI237 line, an original line of mutant lines. Multi-location yield trials will be continued in next growing season to reach at least 16 locations as a requirement of variety release in Indonesia.

Table 3. Multi-locations yield trials in Pusakanegara, Landak, Purbalingga, Pariaman and Banyuwangi.

Line	Yield (ton/ha) *)					
	Pskngr	Lndk	Prblg	Prmn	Bywgi	Average
RKI-124	6.80 abc	4.95 a	6.80 d	7.30 ab	8.85 a	6.94
RKI-130	6.14 c	5.15 a	7.40 c	7.25 a	8.85 a	6.96
RKI-131	6.92 ab	4.64 a	6.45 de	6.75 abc	8.68 a	6.69
RKI-198	7.15 a	5.52 a	6.10 ef	7.20 ab	9.21 a	7.04
RKI-199	7.12 a	5.60 a	7.85 b	7.15 a	9.21 a	7.39
RKI-211	6.63 abc	7.77 a	6.02 f	6.63 dc	9.55 a	7.32
RKI-237	6.18 c	5.52 a	7.75 bc	6.80 abc	8.85 a	7.02
RKI-240	5.40 d	6.35 a	7.95 b	6.43 d	9.03 a	7.03
RKI-241	6.94 ab	5.54 a	8.75 a	6.97 abc	7.99 a	7.24
RKI-245	6.39 bc	5.44 a	7.75 bc	7.50 a	8.51 a	7.11
KI-237	4.51 e	6.27 a	6.50 de	7.00 abc	8.42 a	6.54
Ciherang	6.52 abc	4.54 a	5.95 f	7.10 ab	8.69 a	6.56

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

The amylosa content of tested RKI lines are varied largely, ranging from 13.41% to 20.83%. The amylose content of rice is recognized as one of the most important determinants of eating and cooking quality (Bao et al. 2002). The lines having various content of amylose should be useful as genetic resources for improvement of grain quality to meet the wide preference variability of consumers. The amylosa contents of RKI199 and RKI241 are 17.16% and 15.27%, respectively (Table 4). The color of phericarp is white for RKI199 and brown for RKI241 (Fig 5). If the yield of RKI199 and RKI241 is stable in next multi-location yield trials these lines can be recommended to release as new leading varieties in Indonesia. To fulfill the requirement of variety release in Indonesia these data should also be completed with other examinations such as pests, diseases and other grain quality examinations.

Line	Amylose Content (%)	Endosperm color
RKI-124	20.83	White
RKI-130	19.89	White
RKI-131	20.69	White
RKI-198	17.35	White
RKI-199	17.16	White
RKI-211	14.17	Brown
RKI-237	14.25	White
RKI-240	13.41	White
RKI-241	15.27	Brown
RKI-245	16.71	Brown
KI-237	24.36	Brown
Koshihikari	16.90	White
Ciherang	18,00	White

Tabel 4. Amylose contents and phericarp color of selected RKI lines.

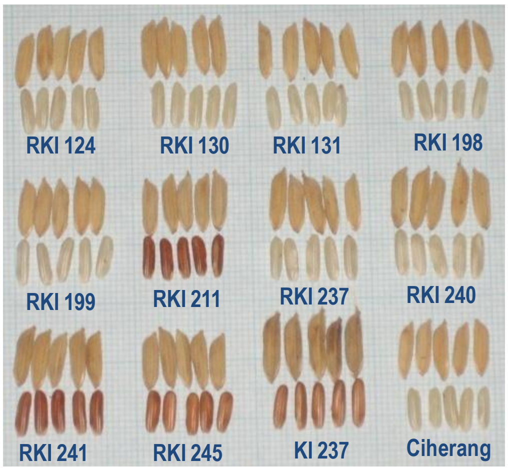


Fig. 5. Grain rice performances of selected lines

3.5 Conclusion

Based on the results it can be concluded as follow;

1) Large genetic variation was observed in fixed lines derived from a wide cross of IR36 and Koshihikari. These lines can be used as basic materials in breeding program.
2) Semi-dwarf character of RKI237-1 line was control by a single mutated gene, this gene was tentatively designated as $sd^{237\text{-}1}$.
3) Out of ten selected lines, three lines are still with brown phericarp and the phericarp color of other lines change to white.
4) These lines have various amylose contents, ranging from 13.41% to 20.83%.
5) The highest average yield was 7.39t/ha for RKI199 and followed by 7.24t/ha RKI241.
6) 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.

3.6 Acknowledgement

Thanks to my colleagues at the Division of Irradiation, Electro-mechanic 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.

3.7 References

Bao, J.S., Sun, M., Corke, H. 2002. Analysis of genetic behavior of some starch properties in indica rice (*Oryza sativa* L.): thermal properties, gel texture, swelling volume. Theor. Appl. Genet. 104:404-413.

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

Evans, L. T. 1998. Fidding the ten billion. Plant and Population Growth. Cambridge University Press. Cambridge.

Itoh, H., Tatsumi, T., Sakamoto, T., Otomo, K., Toyomasu, T., Kitano, H., Ashikari, M., Ichihara, S. and M. Matsuoka. 2004. A rice semi-dwarf gene, Tan-Ginbozu (D35) encodes the gibberellin biosyinthesis enzyme, ent-kaurene oxidase. Plant Moleculer Biology 54: 533-547.

Khush, G. S. 2001. Green revolution: the way forward. Nature Rev. Genet. 2: 815-822.

Li, J., Xiao, J., Grandilo, S., Jiang, L., Wan, Y., Deng, Q., Yuan, L., McCouch, S.R. 2004. QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa*) and African (*O. glaberima* S.) rice. Genome 47:697-704.

Maluszinski, M. 1998. Crop germplasm enhancement through mutation techniques. In Rutger, J. N., Robinson, J. F. and R. H. Dilday (eds). Proceeding of the International Symposium on Rice Germplasm Evaluation and Enhancement. Stuttgart, Arkansas, USA, p. 74-82.

Nagato, Y. and A. Yoshimura, 1998. Report of the committee on gene symbolization, nomenclature and linkage groups. Rice Genet. Nwsl. 15: 13-

Spielmeyer, W., Ellis, M. H. and P. M. Chandler. 2002. Semidwarf (sd-1) “green revolution” rice, contains a defective gibberellin 20-oxidase gene. Proc. Natl. Acad. Sci. USA 99: 9043-9048.

Taquero, Z.F. 1991. Consumer demand for rice grain quality in rice grain marketing and quality issues. Selected paper from the International Research Conference. 27-31 August 1990, Seoul, Korea. IRRI, Manila. p. 37-46.

4. Japan

Creation of Materials for Breeding Amylose Library of Primary Rice Varieties

M. Nishimura

Institute of Radiation Breeding
National Institute of Agrobiological Sciences

4.1 Introduction

Low-amylose rice holds promise for increased use in applications such as aseptically packed rice. The Institute of Radiation Breeding has raised mutant strains of rice varieties such as Norin 8 and Reimei with various amylose mutations, and has provided many of the breeding mother plants of existing low-amylose varieties. The institute is also working on raising isogenic lines of Koshihikari that relate to the low-amylose genes of these varieties. The purpose of this research is to induce mutations by means of gamma rays and other radiation to create near isogenic lines (NILs) with amylose-content gradients of about 2% from primary rice varieties, Koshihikari and Hitomebore.

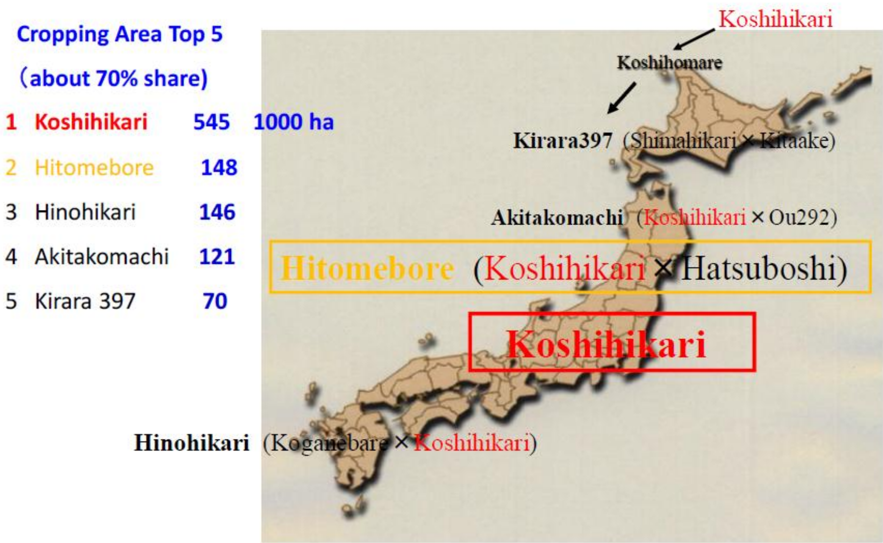


Figure 1. Main rice varieties in Japan

Figure 2. Low-amylose varieties for pre-cooked rice

4.2 Materials and Methods

(1) Creation of Koshihikari near isogenic lines (NILs) using existing amylose mutants

(2) Selection of amylose-content mutants from primary varieties such as Koshihikari and Hitomebore.

(3) Evaluation of amylose mutants

Cultivate under various conditions to evaluate the phenotype of the amylose library created in the project.

Table 1. Specific research methods and other actions taken throughout the research term

Year	Research objective	Research method	Expected achievements
2006	Raise Koshihikari NILs relating to amylose	Use existing amylose variants	Broadened amylose variants
2007-2010	Screen amylose variants from primary varieties	Use mutagens such as γ rays , ion beams and EMS treatment	Creation of amylose library
2011-2012	Evaluate amylose library	Evaluate amylose library under various cultivation conditions	Completion of amylose library

4.3 Results and Discussion

4.3.1 Creation of Amylose Library of Koshihikari

We created the amylose library consisted of mutants backcrossed with original variety, Koshihikari. Furthermore, we continued to screen amylose variants from Koshihikari. Consequently, we obtained NILs consisted of $F_{3\text{-}5}$ lines backcrossed with Koshihikari and newly selected mutants to be added to this library induced by EMS treatment and gamma-ray irradiation. We developed amylose library consisted of 29 lines. As a result, we completed raising amylose library consisted of 29 mutant strains with amylose-content gradients of about 2% (Fig.1). Amylose contents of mutants and NILs were determined by absorption spectrophotometry method in 2011. In 2012, we will try the evaluation of the response of amylose content of NILs to the temperature during grain-filling period.

Amylose content (%)

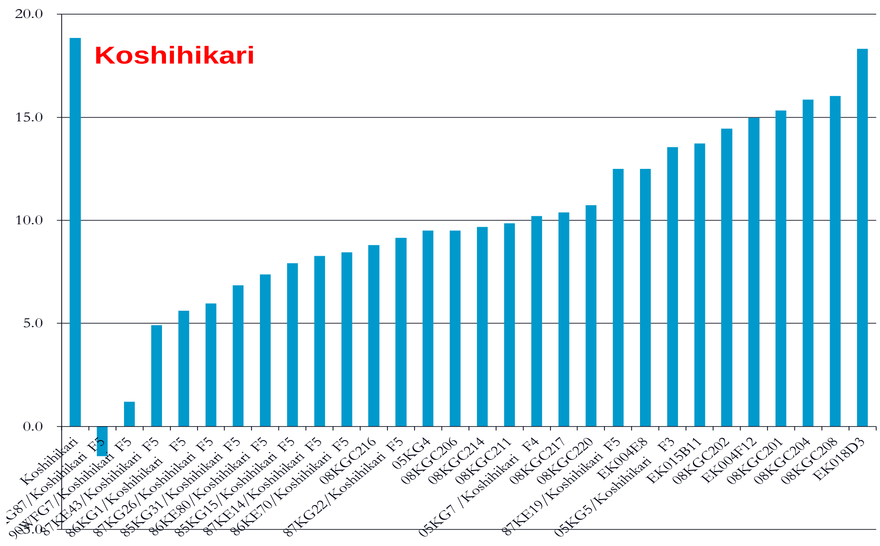


Figure 3. Koshihikari NILs relating to amylose content in 2011.

4.3.2 Selection of new mutants and raising NILs with low amylose content from Hitomebore

Hitomebore NILs were created by backcrossing with an original variety and ten mutants with waxy or low amylose contents were newly selected. The generations of these mutants are M_7 to M_{10} in 2013.

Ion beam irradiation was conducted at the AVF cyclotron in the Takasaki Advanced Radiation Research Institute of the Japan Atomic Energy Agency. The ion particles used were $^{12}C^{5+}$ (220 MeV), $^{12}C^{6+}$ (320 MeV), and $^{4}He^{2+}$ (100 MeV). Gamma-ray irradiation was conducted at the Institute of Radiation Breeding using a 10 Gy/h dose rate.

We obtained NILs consisted of 12 $F_{5\text{-}6}$ lines backcrossed with Hitomebore, No.2 variety in Japan and newly selected 10 mutants to be added to this library induced by ion beam and gamma-ray irradiation. In culm length, some lines of NILs were shorter than original variety in spite of backcrossed by Hitomebore. Amylose contents of mutants and NILs were determined by absorption spectrophotometry method in 2011.

Amylose content (%)

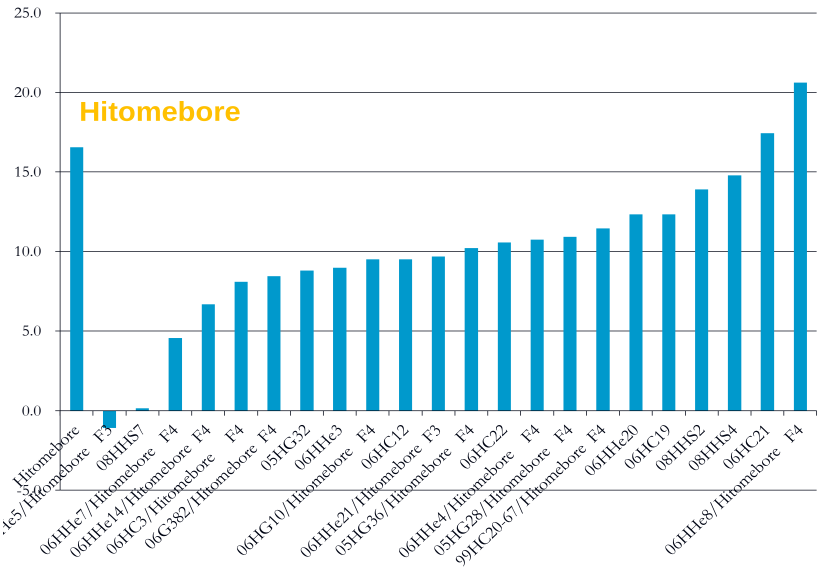


Figure 4. Hitomebore NILs relating to amylose content in 2011.

4.3.3 Analysis of temperature response of low amylose mutant strains

The mutant strains developed from parental Koshihikari were cultivated a in a double cropping system (planted on May 16 and transplanted on June 14 in 2012) with various grain-filling temperatures. The fully matured grains were evaluated for endosperm amylose content.

In general, the temperature in August of 2012 was higher than that of 2011, therefore, amylose content of mutant strains were relatively lower. As shown in Fig.5, the strains with relatively low amylose content (around 5%) were stable throughout the range of tested temperatures. On the other hand, the strains with an amylose content around 10% were classified into two types of stable and sharp response to the temperature. There are mutant strains that have an amylose content of about 12%, which is less than that of standard non-glutinous varieties. Some of them have a relatively stable temperature response and they could be promising candidates for the further breeding material.

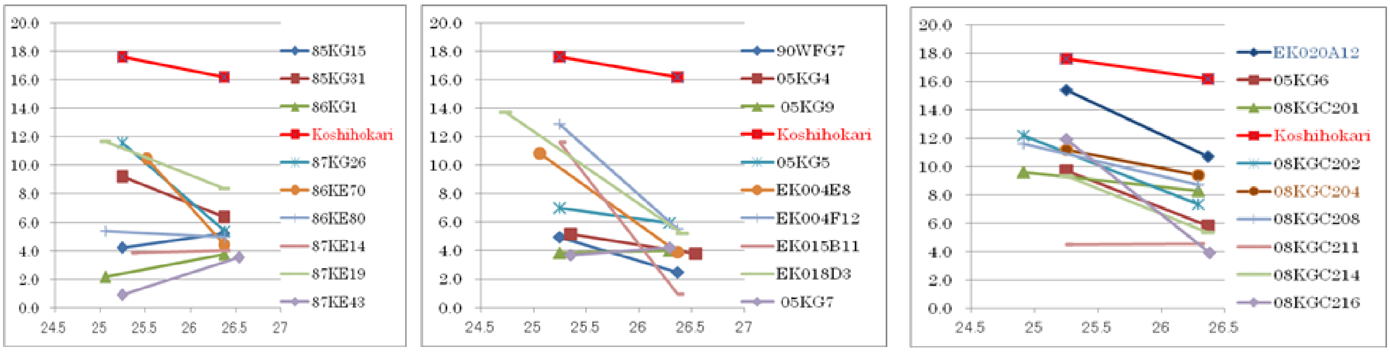


Figure 5. Response of amylose content to the temperature in Koshihikari NILs in 2012.
(vertical scale: amylose content %, horizontal scale: mean temperature during 20 days after heading)

4.4 Conclusion

We completed amylose library consisted of Koshihikari and Hitomebore NILs with amylose-content gradients of about 2%. They will be used for the genetic analysis in the new MAFF project from 2013. We also analyzed the temperature response of low amylose mutant strains.

5. Korea

Molecular characterization of high level of VitE accumulating rice mutant induced by *in vitro* mutagenesis

J.E. Hwang, D.S. Kim, S.-Y. Kang

Radiation Breeding Research Team, Advanced Radiation Research Institute,
Korea Atomic Energy Research Institute

5.1 Introduction

Vitamin E is an important constituent of the human diet (Evans and Bishop, 1922). Vitamin E is an essential lipid-soluble nutrient that consists of eight tocochromanols in nature, which can be separated into two groups, α-, β, -γ- and δ- tocopherols and four corresponding unsaturated derivatives, α-, β-, γ- and δ-tocotrienols. Tocopherols help maintain membrane structure and integrity (Falk and Munne Bosch, 2010), act as antioxidants and free radical scavengers (Tappel, 1962; Behl and Moosmann, 2002), and perform other nonantioxidant functions related to signaling and transcriptional regulation (Ricciarelli et al., 2002). Both tocopherols and tocotrienols occur in plants, as well as in photosynthetic microbes such as *Synechocystis*, with the highest concentration in seeds. The seeds of most plants have significant amounts of γ- tocopherol, whereas leaves have predominately α- tocopherol. Tocotrienols, however, are not as prevalent in plants as are tocopherols, but they are the primary form of vitamin E in the seeds of most monocot species (Padley et al., 1994) and fruits of some dicots (Aitzetmüller, 1997).

In the case of tocochromanol biosynthesis pathway, seven genes coding for tocochromanol biosynthesis enzymes have been identified and characterized in Arabidopsis and rice (Mene-Saffrane and DellaPenna, 2010). This pathway diverges at the step where polyprenyl side chain is attached to homogentisic acid (HGA) by homogentisic acid phytyl transferase (VTE2) or homogentisic acid solanesyl transferase (HST) (Mene-Saffrane and DellaPenna, 2010). The first committed step in the biosynthetic pathway is the prenylation of homogentisic acid with phytyldiphosphate to form 2-methyl-6-phytylbenzoquinol (MPBQ). The overall tocopherol composition is determined by the combined activities and substrate specificities of the homogentisate prenyltransferase (VTE2), tocopherol cyclase (VTE1), and two methyltransferase enzymes (VTE3 and VTE4) present in a given tissue (Jo and Hyun, 2011). The action of the cyclase directly on MPBQ leads to the formation of δ-tocopherol. By contrast, ring methylation at position R_2 by the first methyltransferase, MPBQ methyltransferase (VTE3), leads to 2,3-dimethyl-5-phytylbenzoquinol and the subsequent action of tocopherol cyclase leads to the formation of γ-tocopherol. The second methyltransferase, γ -tocopherol methyltransferase (VTE4), performs ring methylation at R_3, converting the γ – and δ isoforms to the α-and β-isoforms, respectively. Tocotrienols are formed when geranylgeranyldiphosphate is used in place of phytyldiphosphate as the side chain precursor.

In this study, T1001-1 was isolated from *in vitro* mutagenized population by ionizing radiation and shown to have increased VitE contents. To study the molecular mechanism of VitE biosynthesis, we identified rice genome encodes seven VitE biosynthetic enzyme and we analyzed its expression patterns. The results of DNA sequencing analysis demonstrate that the *OsVTE2* promoter and genomic sequences contain mutated region. In addition, we showed that the mutant confers retarded seedling growth during the early seedling growth stage in rice. These observations suggest that the mutation of the *OsVTE2* might affect a Vitamin E biosynthesis.

5.2 Materials and methods

5.2.1 Plant materials and growth conditions

The wild-type rice cv. Dongan embryo culture had been irradiated with gamma rays of 30–120 Gy generated from a ^{60}Co gamma irradiator (150 TBq of capacity; ACEL, Nordion, Ottawa, ON, Canada) at the Korea Atomic Energy Research Institute. The mutant cell lines were regenerated on medium containing 0.5 mM 5MT. Regenerated plants from 5MT-resistant calli were genetically stabilized through consecutive generations of self-pollination and the homozygous M10 progenies were obtained as reported elsewhere (Kim et al., 2004).

For seedling growth test, seeds were sown on 1/2 MS medium containing Murashige and Skoog salts (Duchefa, Haarlem, Netherlands), 3% Sucrose and 0.7% phyta-agar (Duchefa, Haarlem, Netherlands) under 16 h light/8 h dark cycle at 24^{o}C.

5.2.2 HPLC analysis

Tocopherols and tocotrienols were determined using HPLC (Sykam system S1211). Resolution of vitamin E species was achieved using an Agilent Eclipse LC-Si column (4.6 mm length, 5 μm) and a solvent system consisting of methanol:water (95:5, v/v) with a flow rate of 1.5 ml min^{-1}. Sample components were detected and quantified by fluorescence with excitation at 292 nm and emission at 330 nm. A sample volume of 10 μl was injected for the chromatographic analysis. Peaks of α-tocopherol, β-tocopherol, γ-tocopherol, α-tocotrienol, β-tocotrienol and δ γ-tocotrienol, δ-tocotrienol were identified by comparing their retention times with commercially available authentic standards. Pure tocopherols and tocotrienols were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Cayman Chemical (Ann Arbor, MI, USA), respectively. The content of each form was calculated based on standard curves of external standards.

5.2.3 Semi quantitative RT-PCR analysis

Total RNA was isolated using Trizol reagents according to the manufacturer's recommended protocols (GibcoBRL, Cleveland, OH). 1 μg of total RNA was reverse transcribed in Power cDNA Synthesis Kit (Intron Biotech Inc., Sungnam, Korea) for 60min at 42°C using 1 μg oligo(dT)15 primers. 1 μl of cDNA was used for PCR amplification with each gene specific primer (Table 1). The resultant RT-PCR products were electrophoresed and analyzed on a 2.0% (w/v) agarose gel after staining with ethidium bromide.

5.2.4 Western-blot analysis

The OsVTE2 expression pattern was determined by western blot analysis using the anti-OsVTE2 polyclonal antibody (1:5,000 dilution), followed by the addition of peroxidase- conjugated goat anti-rabbit IgG (1:5,000 dilution) according to the manufacturer's guidelines. Hybridization to protein bands was detected using the SuperSignal West Pico Trial Kit (Thermo, USA)

5.3 Results and Discussion

5.3.1 HPLC detection of tocopherols and tocotrienols in rice grains

Rice mutant lines in the M_{10} generation and the control (Dongan byeo) were grown in the same filed under normal cultural conditions, and mature seeds were harvested for determination of vitamin E composition. Normally, the β- and δ-isomers usually appear in trace amounts, while α-tocopherol and γ-tocotrienol are the most abundant isomers. In our experiments, we also found that it was difficult to detect δ-tocopherol. Therefore, this isomer was not included. In rice seeds, the major tocopherol found is α-tocopherol, and amount of β-tocopherol and γ-tocopherol are relatively low (Fig. 1). As a result, the contents of α-tocopherol increased to 42% in T1001-1 mutant seeds than that of the controls. HPLC analysis showed that α-tocotrienol and γ-tocotrienol were the major tocotrienols present in seeds of mutants and control. However, we found that there was no significant change in the content of each tocotrienol (Fig. 1). In the T1001-1 C mutant line, the γ-tocotrienol content was increased to 14% than in the control. The content of VitE (total of tocopherol and tocotrienol) was increased to 26% in T1001-1 c mutant seeds. Tocotrienols, like tocopherols, are also potent antioxidants (Serbinova et al., 1991), but they are less readily absorbed than α-tocopherol (Kamal-Eldin and Appelqvist, 1996). Thus, high accumulation of α-tocopherolin T1001-1 mutants provides effective protection against oxidative damage.

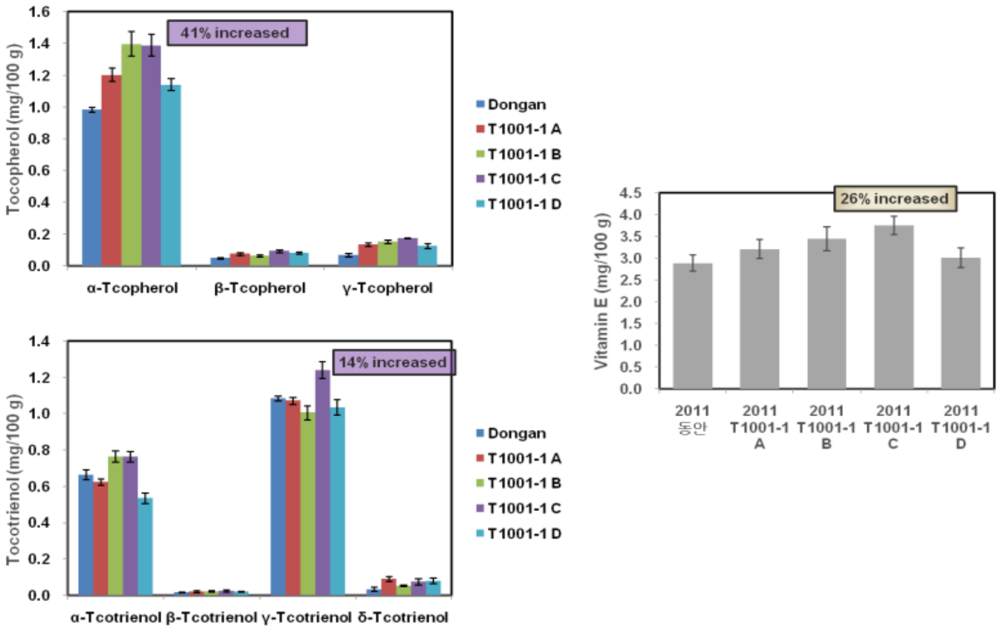


Figure 1. Total and individual tocochromanol accumulation in seed of the Dongan and T1001-1.

Tocopherol and tocotrienol composition of the seed of rice was determined by HPLC. The total VitE content was 26% increased in the T1001-1 mutant seeds compared with Dongan.

5.3.2 Phenotypic analysis of T1001-1 mutants

As T1001-1 mutants seedling grew slowly, we compared seedling growth rates in mutant and control plants (Fig. 2B). The primary root elongation pattern of T1001-1 mutant was not different from that of control (Fig. 2C). Sattler et al. (2004) reported that *vte2* mutants exhibited seedling growth in Arabidopsis. Thus, these T1001-1 mutant phenotypes have possibility by increasing of VTE2 expression.

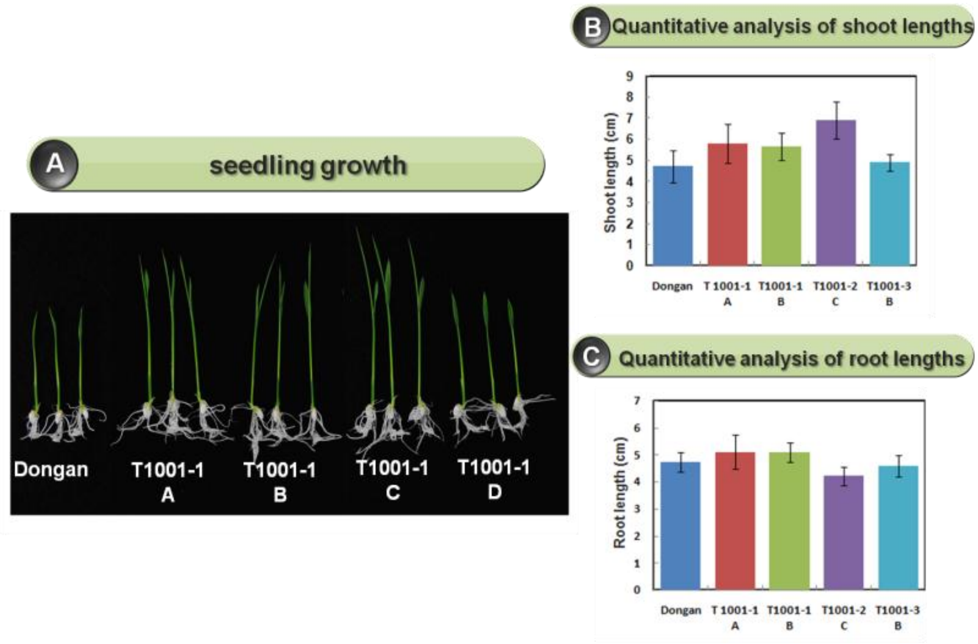


Figure 2. Comparison of seedling growth in mutant and control plants.

(A)The early seedling growth differences between the control (Dongan) and mutant lines (T1001-1) for 7-days were determined by measuring the shoot and root lengths. (B) Quantitative analysis of shoot lengths of 7-day-old seedlings from each mutant line. (C) Primary root length of 7-day-old control and mutant line seedlings.

5.3.3 Expression of tocochromanol biosynthesis genes

To study the molecular mechanism of VitE biosynthesis, we identified rice genome encodes seven VitE biosynthetic enzyme and we analyzed its expression patterns. Semi quantitative reverse transcription analysis revealed that seven VitE biosynthesis genes were expressed at different levels in four T1001-1 mutant lines. Interestingly, *VTE2* transcripts were highly accumulated in the T1001-1 C (Fig. 3), whereas the expression of *VTE3* was decreased in T1001-1 lines (Fig. 3). This result indicates that accumulation of VtiE in T1001-1 mutants is controlled by different expression of *VTE2* and *VTE3* transcript.

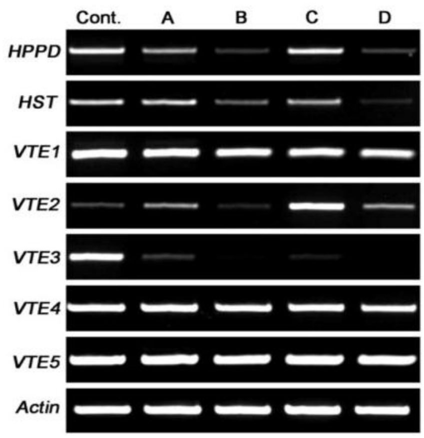


Figure 3. Transcript levels of VitE biosynthesis genes in rice plants.

Expression profiles of 7 rice VitE biosynthesis genes in Dongan (Cont.) and mutant lines (T1001-1). *Actin* gene was used as loading control.

5.3.4 Sequence analysis of VTE2 and VTE3

Fig. 2 showed dramatic change of *VTE2* and *VTE3* expression in T1001-1 C mutant. Therefore, *VTE2* and *VTE3* genes were sequenced in both the control and T1001-1 C mutant. The A nucleotide at position -245 is exchanged by a G nucleotide and the C nucleotide at position -106 is changed by T of the VTE2 promoter in the T1001-1C mutant compared to the wild-type sequence (Fig. 4). The position -245 mutation region was reported ZFP-TFs (zinc finger transcription factors) binding site and -106 mutation region existence-box-*cis* element. ZFP-TFs were reported to upregulate the expression of the endogenous *Arabidopsis* γ-tocopherol methyltansferage (GMT) gene (Van Eenenaam et al., 2004). The presence of these motifs indicates that *VTE2* may be regulated by ZFP-TFs binding *cis*-acting elements. Genomic sequence of *VTE2* was exchanged in only intron region, therefore, there was no effect at amino acid sequence. In the T1001-1 C mutant line, we observed a G to A base change at position 662 and an added C of VTE3 promoter but not found important *cis*-elements (Fig. 4). Sequencing of the *VTE3* genomic DNA sequence was not different. These results indicated that increasing of *VTE2* transcript expression was controlled by VTE2 promoter sequence mutation in accumulation of tocochromanol.

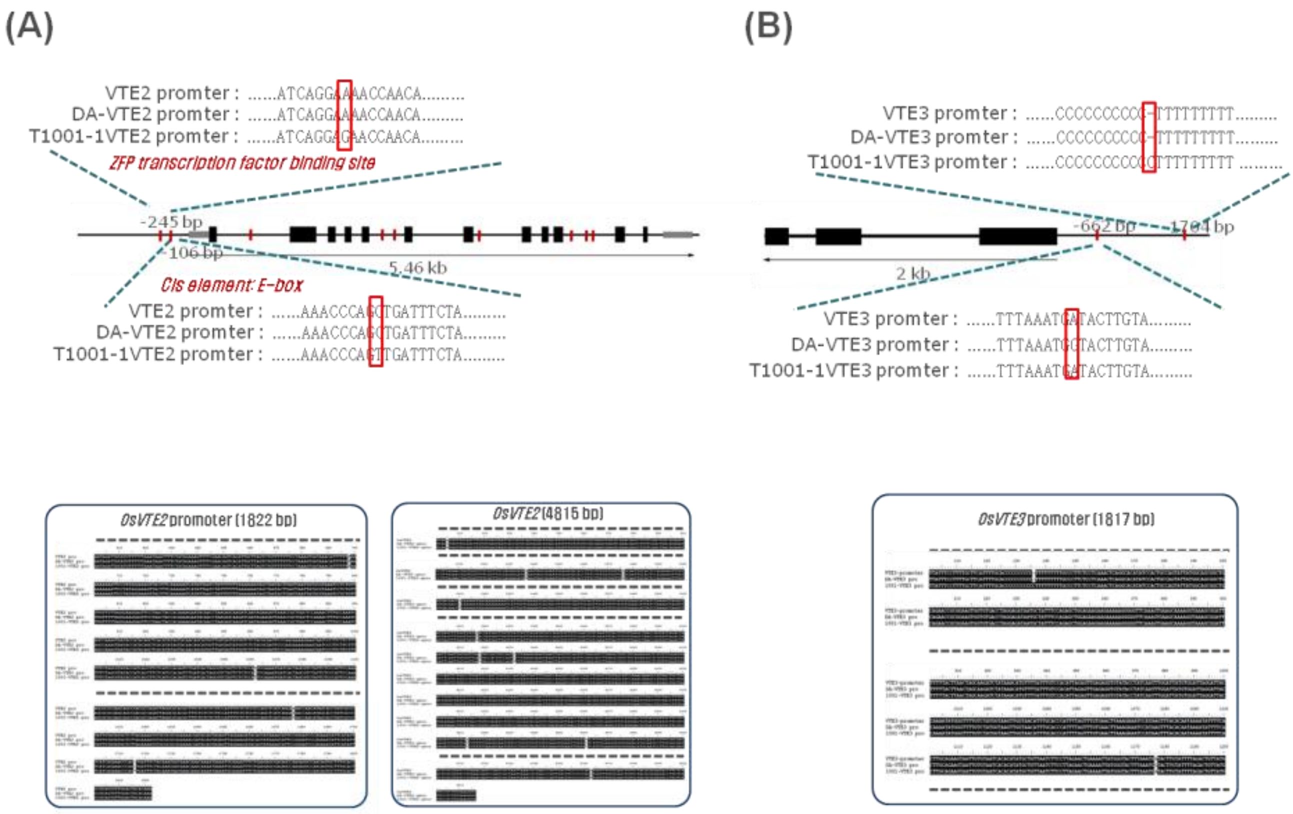


Figure 4. Comparison of the promoter and genomic DNA sequences from Dongan (DA) and T1001-1 mutant with the related region of *VTE2* (A) and *VTE3* (B).

Number of the DNA sequence is shown in the upper margin. The empty region at sequences indicates the different sequence in DA and T1001- 1C mutant.

5.3.5 Protein expression of VTE2

The VTE2 expression pattern was determined by western blot analysis using the prediction 4 of anti-VTE2 polyclonal antibody. To precisely define the expression pattern of VTE2, we studied the expression levels of VTE2 protein by western blot analysis (Fig. 5). These results are similar with the expression patterns of *VTE2* transcript that was accumulated more extensively in T1001-1 mutant than Dongan byoe in protein level.

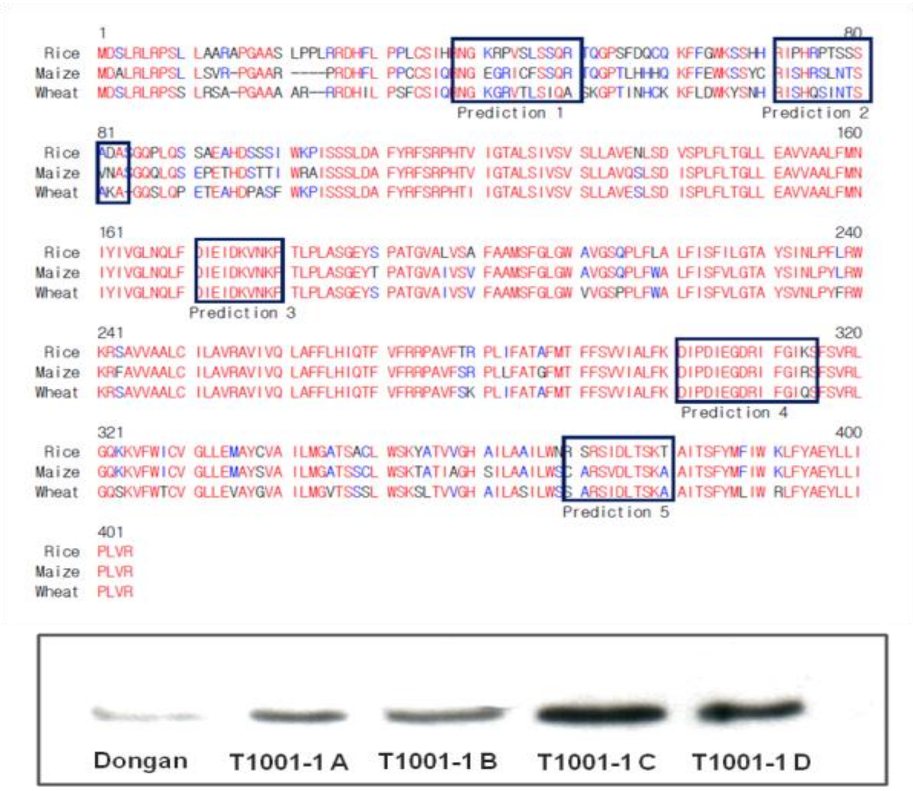


Figure 5. Western blot analysis of VTE2 in T1001-1 mutants and Dongan byeo.

Total proteins from seedling of Dongan or T1001-1 mutants were isolated and subjected to western blot analysis.

5.4 References

Aitzetmüller K (1997) Antioxidative effects of carum seeds. J Am Oil Chem Soc 74:185

Behl C and Moosmann B (2002) Oxidative nerve cell death in Alzheimer's disease and stroke: Antioxidants as neuroprotective compounds. Biol Chem 383: 521–536

Evans HM and Bishop KS (1922) On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science 56: 650–651

Falk J, Munne-Bosch S 2010 Tocochromanol functions in plants: antioxidation and beyond. J Exp Bot 61: 1549–1566

Gunstone FD, HarwoodJL, Padley FB (eds) The lipid handbook, 2nd edn. Chapman & Hall, London, pp 127–130

Kamal-Eldin A, Appelqvist LA (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671–701.

Padley FB, Gunstone FD, Harwood JL (1994) Occurrence and characteristics of oils and fats. In: Sen CK, Khanna S, Roy S (2006) Tocotrienols: vitamin E beyond tocopherols. Life Sci 78:2088–2098

Ricciarelli R, Zingg JM, and Azzi A (2002) The 80th anniversary of vitamin E: Beyond its antioxidant properties. Biol Chem 383: 457–465

Tappel AL (1962) Vitamin E as the biological lipid antioxidant. Vitam Horm 20: 493–510

6. Malaysia

Application of Mutation Techniques and Biotechnology for Minimal Water Requirement and Improvement of Amylose Content in Rice

[1]Rusli Ibrahim, [1]Abdul Rahim Harun, [1]Sobri Hussein, [2]Abdullah Mat Zin, [3]Sariam Othman, [4]Marziah Mahmud, [4]Mohd Rafii Yusof, [1]Siti Hajar Mohd Nahar, [4]Zarifth Shafika Kamaruddin, [5]Ana Ling P.K

[1]Agrotechnology & Biosciences Division, Malaysian Nuclear Agency, Ministry of Science, Technology & Innovation (MOSTI), Bangi, Selangor; [2]Universiti Malaysia Terengganu, 21030 Kuala Terengganu; [3]Malaysian Agriculture Research and Development Institute (MARDI), Serdang, Selangor; [4]Universiti Putra Malaysia (UPM), Serdang, Selangor; [5]Department of Bioscience, Faculty of Engineering and Science, University Tunku Abdul Rahman, Kuala Lumpur

6.1 Introduction

Rice is the staple food crop in Malaysia. 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 rice imports. 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). Thus, rice will be the crop most affected by water scarcity as it depends most heavily on irrigation. Therefore, there is an urgent need to develop rice varieties adapted to water stress and still produce acceptable yield and posses all the other beneficial traits.

Water supply is affected by the loss of watersheds due to deforestration and soil erosion. There is severe depletion of valuable groundwater resources as water is taken up for agricultural and industrial purposes. Some varieties of rice can be grown without irrigation, however their growth totally depend. 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. It offers the possibility of inducing desired attributes that either cannot be expressed in nature or have been lost during evolution.

Heavy ion beams, such as carbon ions, are more effective in plant for inducing mutations compared with electron beam (Hidema *et al.*, 2003). Novel mutant 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 (Mohamed *et al.*, 1988) but none was targeted for adaptive traits per se. Water shortage has become the bottleneck of Malaysia's food security. The development of water-saving rice varieties to decrease water consumption in rice production is inevitably a major goal in agriculture research. Thus, to achieve long-term food security and sustainable development in Malaysia, 'Water-saving or drought-resistance' (WDR) rice varieties are urgently needed.

Drought is one of the most important limiting factors in more than 65% of paddy fields in Malaysia where super rice varieties cannot perform well under drought stress. Therefore, the development and production of drought-tolerant rice varieties, to stabilize and improve the production levels in the low-middle-yielding fields, is needed. 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 programmes. The objectives of this study, therefore, 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 such as amylose content.

6.2 Materials and Methods

6.2.1 Mutation Induction

Seeds of popular local variety MR219 were irradiated with 300Gy of gamma radiation from the ^{60}Cobalt source at Malaysian Nuclear Agency in 2008. Irradiated seeds were sown at Mardi Research Station in TanjungKarang, Selangor. The M_1 seedlings were transplanted into the field with 25cm x 25cm planting distance. Ten thousand M_1 seedlings were planted to produce M_2 seeds and a total of 5,250 plants were selected from which 2 panicles per hill were randomly harvested from each hill.

For ion beam irradiation, four rice cultivar, MR219, Q74, MR211 and PongsuSeribu 2 were irradiated in Takasaki Japan. MR219 and Q74 were irradiated on 20th Jan 2009 for sensitivity test. A total number of 100 seeds per dose (0, 10, 20, 40, 60, 80, 100, 120, 160, 200 Gy) of MR219 and MRQ74 were irradiated with ion beam in Takasaki Japan. The seeds were evaluated at Malaysian Nuclear Agency. The shoulder dose for MRQ74 was observed at 120Gy. The optimum doses of these cultivars were identified and the second irradiation was done on 16 Jun 2009 whereby 1,500 seeds of each cultivar were irradiated with doses of 60 and 80Gy. Meanwhile Seeds of MR211 and PongsuSeribu were irradiated on 5th Feb. 2010 for sensitivity test.

A total of 1,500 M_1 seeds of MR219 and MRQ74 irradiated with 60 and 80Gy were raised at Malaysian Nuclear Agency green house. Only M_2 seeds of MR219 with 80Gy were planted in Agriculture Department experimental Plot, TgKarang Selangor. About 10,000 of M_2 individual plants of MR219 were planted. About 5% of M_2 populations were selected for further screening in M_3 including analysis of amylose content.

6.2.2 Green-house screening

Green-house screening was done using cement troughs filled with paddy soils. A water float control was constructed to control the level of water from time to time by adjusting it in order to attain the saturated water regime as require in the study. M_2 seeds of MR219 were hand seeded at a distance of 4cm between and within rows. Flooded water level was maintained at 4cm for 7 days after seeding to allow for good establishment of seedling. Then after, water was gradually drained to provide stress to the plants. However, the water level was again raised to 4cm above soil surface at 21 and 45 days after sowing. During this time, fertilizer was applied at the rate of 60:40:30 kg/ha. The trough remained flooded about 7 days after fertilization to allow optimum distribution of fertilizer and after which the water level was again lowered to 5cm from soil level to provide additional stress to the water. Second stress was done at 30 - 40 DAS where the water level was reduced to 10cm from soil level and finally at 41 – 50 DAS until maturity, the water level was finally reduced to 15cm from soil level. At maturiy, a total of 500 panicles with good filled grains were selected for field screening.

Figure 1. Greenhouse screening of mutant lines of MR219 irradiated with gamma rays for minimal water requirement using water simulation technique.

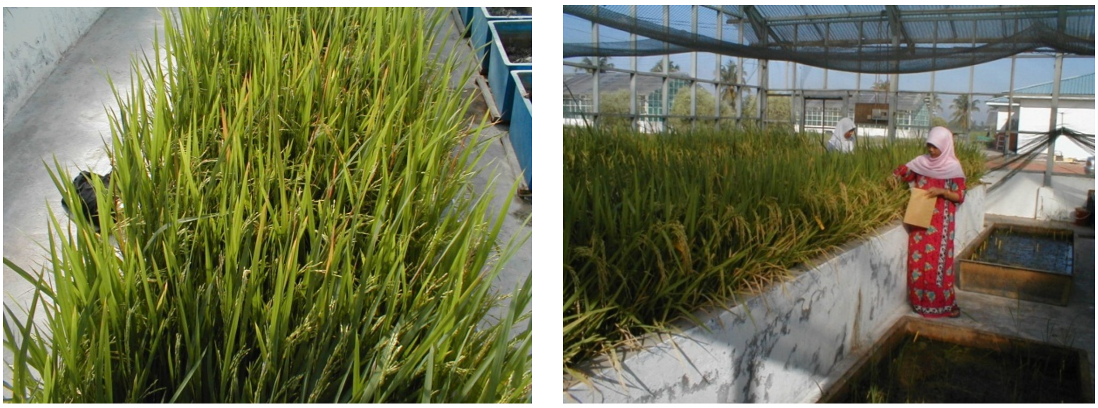


Figure 2. Selection of mutant lines of MR219 irradiated with gamma rays tolerant to minimal water requirement using water simulation in the greenhouse

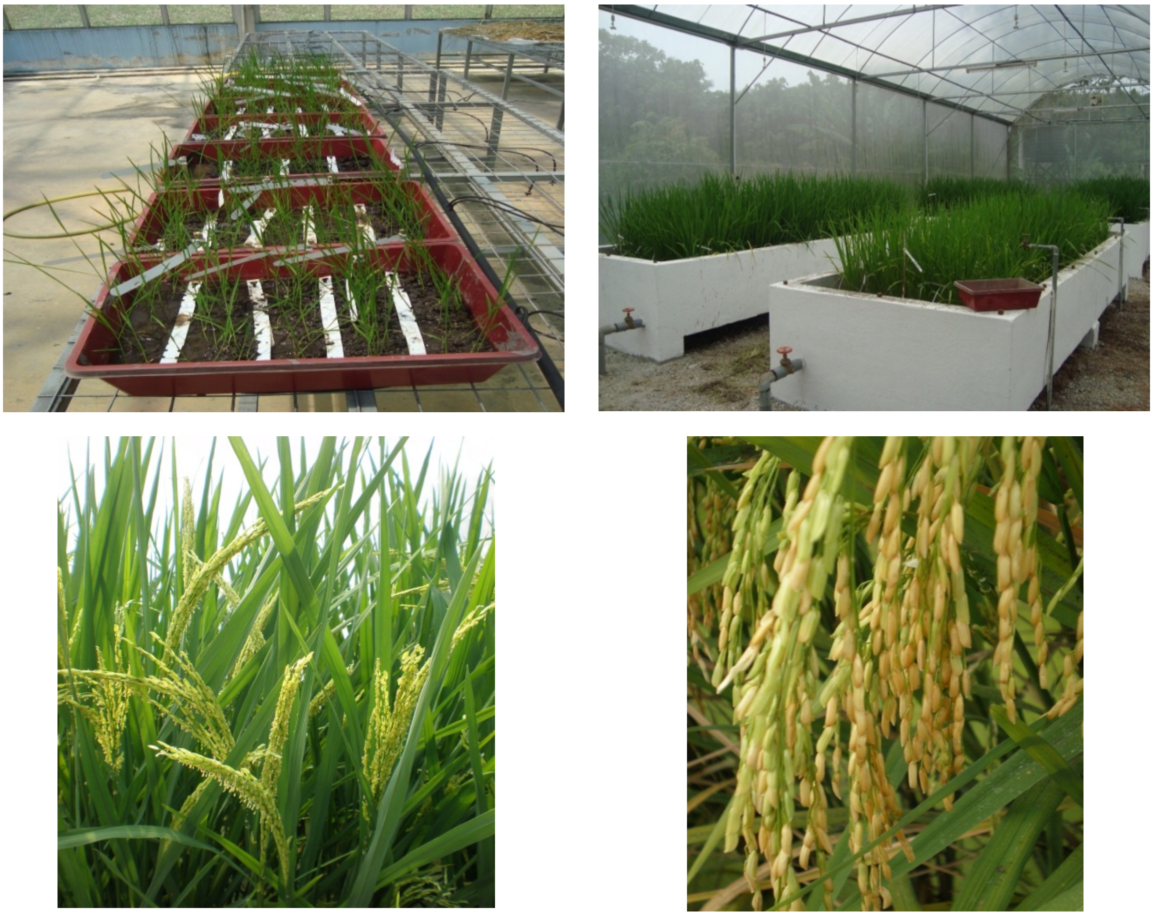


Figure 3. M_1 plants of MR219 and Q74 irradiated with 60 and 80 Gy of ion beam planted at Malaysian Nuclear Agency green house.

6.2.3 Field Screening

For field screening, the plot lay-out was constructed to simulate precise water stress regime. The screening procedure (stress treatment, fertilization methods and etc) was similar to the green house screening but 14 days old seedlings were transplanted instead. The seedlings were transplanted to the field with planting distance of 10cm x 10cm to make up for 16 plants per entry. A total of 38 lines were planted. Flooded field condition was maintained until 7 days after planting to allow for seedling establishment prior to water stress treatment at different stages of plant growth ie. at 41 - 50, 60 - 70 and 80 - 99 days after transplanting. During stress treatment the water level was maintained at 15 cm below soil level. The 1st fertilizer application (100:40:30) applied was done at 21 days after transplanting and the 2nd fertilizer application was done at 53 days after transplanting. Sampling and harvesting was done at 85% ripening. Important agronomic traits were recorded at maturity from 5 plants, sampled at random from each plot.

After several series of selection and fixation, 12 potential lines with the required adaptive traits were recovered at M_4 generation. However, only two potential lines designated as MR219-4 and MR219-9 were selected for further testing under several stress environments. Selection was mainly based on the percent filled grain because under water stress environment, it is the most sensitive indicator of tolerant lines.

6.2.4 Yield Trials

MR219-4 and MR219-9 were evaluated for yield and adaptation in MADA under saturated and flooded conditions. The aim was to study the interaction effects of rice variety and water management on yield and yield components such as panicles/sq m, spikelets/panicle, percent filled grains and the 1000-grain weight. The lines were also evaluated under aerobic soil condition in MARDI Seberang. The same mutants were evaluated for adaptation and yield under flooded conditions in KETARA, Besut, Terengganu.

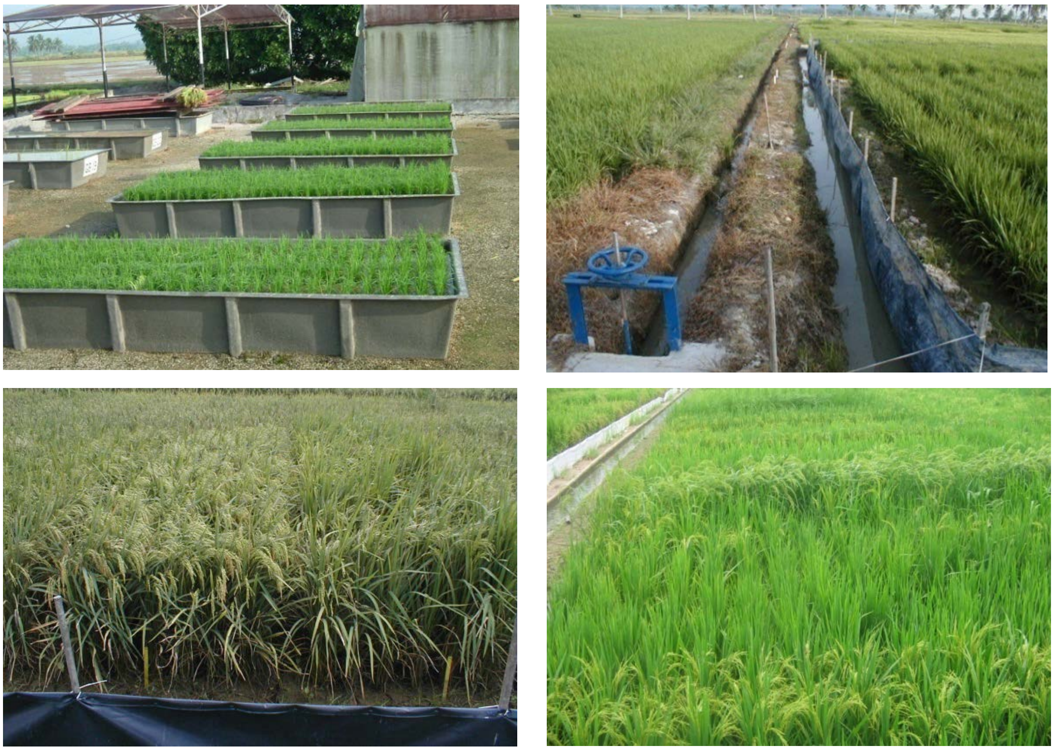


Figure 4. Field screening and selection of advanced mutant lines of MR219 irradiated with gamma rays for minimal water requirement using water simulation technique.

Figure 5. Field planting of M_3 mutant lines of MR219 irradiated with 80Gy ion beam at Tanjong Karang, Selangor Experimental Station

6.2.5 Analysis of Amylose Content

To determine the apparent amylose percentage a standard curve using amylose from potato was applied. The method described by Juliano (1971) was used to make a standard curve and amylose-iodine solution with rice flour was prepared manually by grinding rice grains in mortar and pestle. Then the samples were sieved with 45μm aperture. Using this method, 0.04g of standard amylose was weighted and put into a volumetric flask. 1ml of 95% ethyl alcohol was added and gently mixed to spread the flour. 9ml of 2 N Naoh were added to the mixture. The standard solution was then transfer to magnetic stirrer and stir for 10 minute after adding distilled water to 100ml. The absorbance of the solution was measured at 620nm using a spectrophotometer, setting blank at zero absorbance. Data obtain were recorded and standard solutions against amylose content were plotted.

For the analysis of amylose content in a test sample, a portion (10g) from each of the sample was reserved. Seeds from individual panicle were harvested and a portion was subjected to analysis of amylose content. Apparent amylose content determination was performed using a near-infrared reflectance spectrophotometer According to this procedure, 0.1g of rice flour was transferred into a 100ml dry volumetric flask and mixed with 1ml of 95% ethyl alcohol. 9ml of 2 N Naoh were added to the mixture. The test solution was then transfer to magnetic stirrer and stir for 10 minute after adding distilled water up to 100ml. In another 100ml volumetric flask was added in 70ml distilled water, 2ml of 1N glacial acetic acid, 2ml iodine solution and 5ml aliquot of the test solution. The solutions were added distilled water to the volume of 100ml and let stand for 10 minute.

In the presence of amylose, a blue-black color will be observed. The intensity of the color can be tested using a spectrophotometer which reflects the concentration of starch present in the solution. The test solutions were measured using a spectrophotometer at 620nm by setting the absorbance of blank solution at zero. The blank solution was prepared by adding 2ml glacial acetic acid, 2ml iodine solution and distilled water were adjusted to 100ml. The amylose percentage was evaluated with the standard curve.

Methodology for determination of amylose content
(By Juliano, 1971)

Method for determination of amylose content

1. Sample preparation (de-husking & grinding)
2. Weight 0.1g rice flour into 100ml dry Erlenmeyer flas
3. Dissolve in 1 ml 95% ethanol and 9 ml 2M Sodium Hydroxide (NaOH)
4. Boil the solution mixture for 10 min at 95ºC
5. Homogenize the solution and add distilled water till 100ml
6. Transfer 5ml of the sample aliquot into a new 100ml dry Erlenmeyer flask
7. Add 2ml 1M Acetic acid and 2ml Iodine solutions
8. Add distilled water till 100ml
9. Measure the absorbance of amylose-iodine complex at wavelength 620nm

6.3 Results and Discussion

6.3.1 Radiosensitivity Test of MR219 irradiated with gamma irradiation

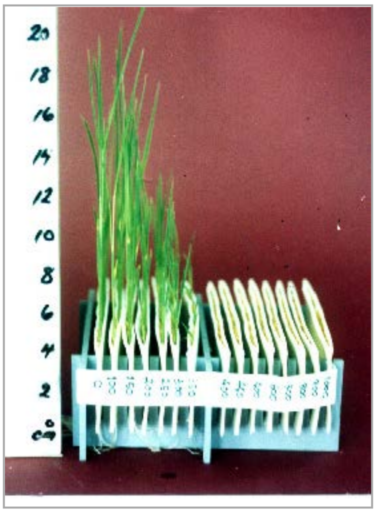


Figure 6. Radiosensitivity test of MR219 using sandwiched blotter technique

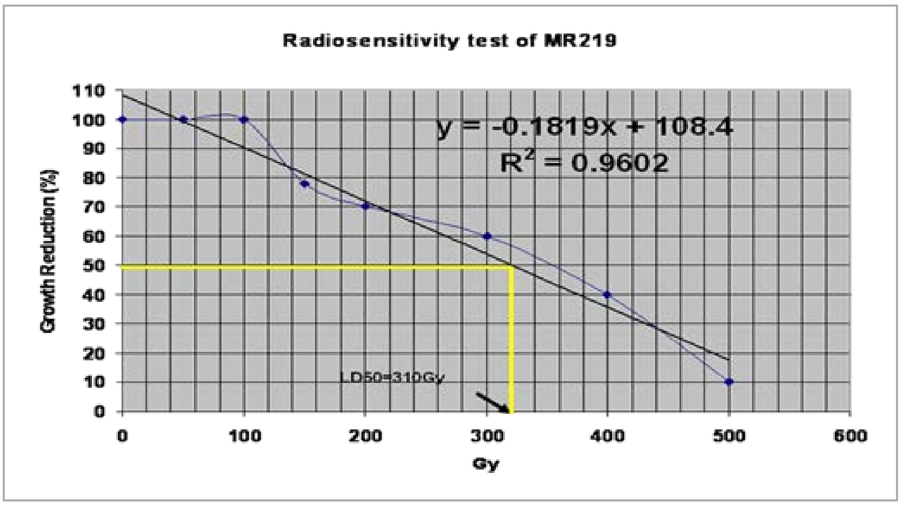


Figure 7. LD50 of MR219 irradiated with gamma rays was calculated to be 310Gy

6.3.2 Dose-response curves of MR219 irradiated with different doses of with ion beam

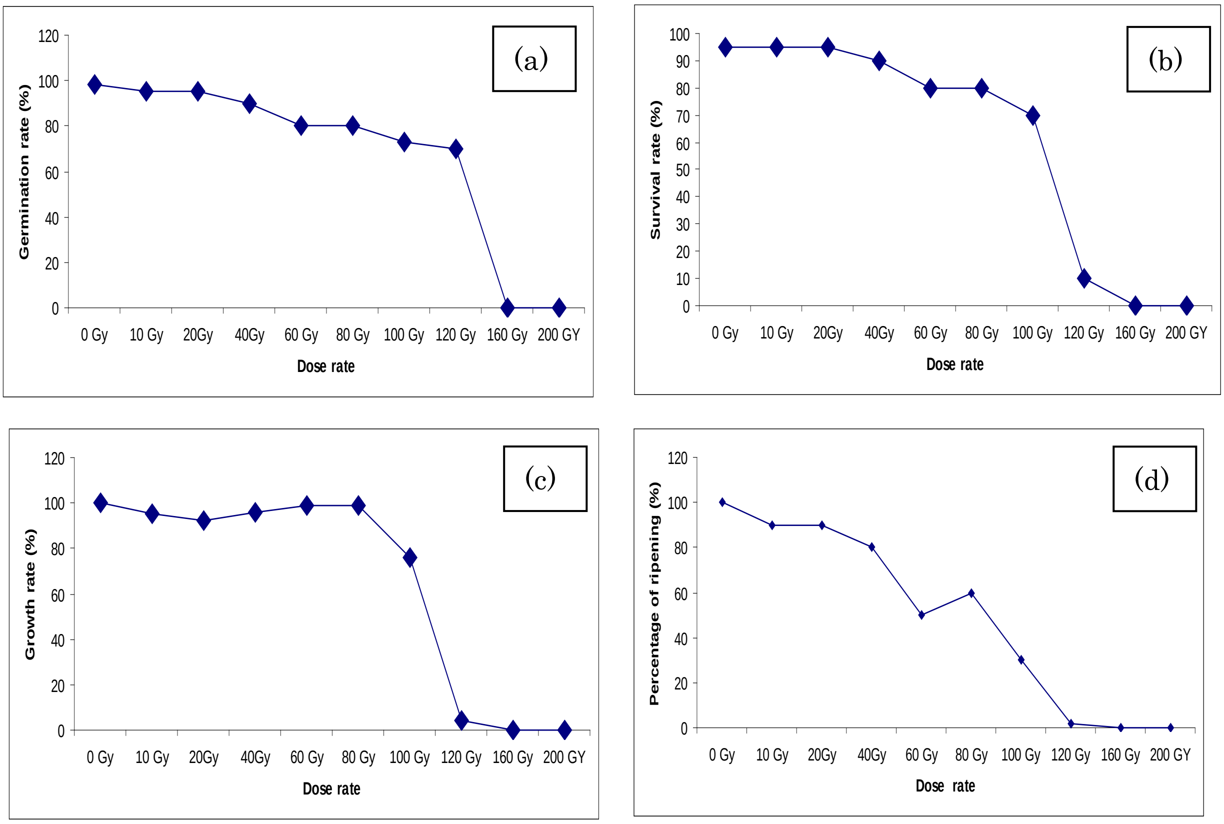


Figure 8. Dose-response curves of MR219 irradiated with different doses of with ion beam: (a) germination rate, (b) survival rate with different dose, (c) growth rate and d) percentage of ripening

6.3.3. Germination rate of MRQ74 irradiated with ion beam

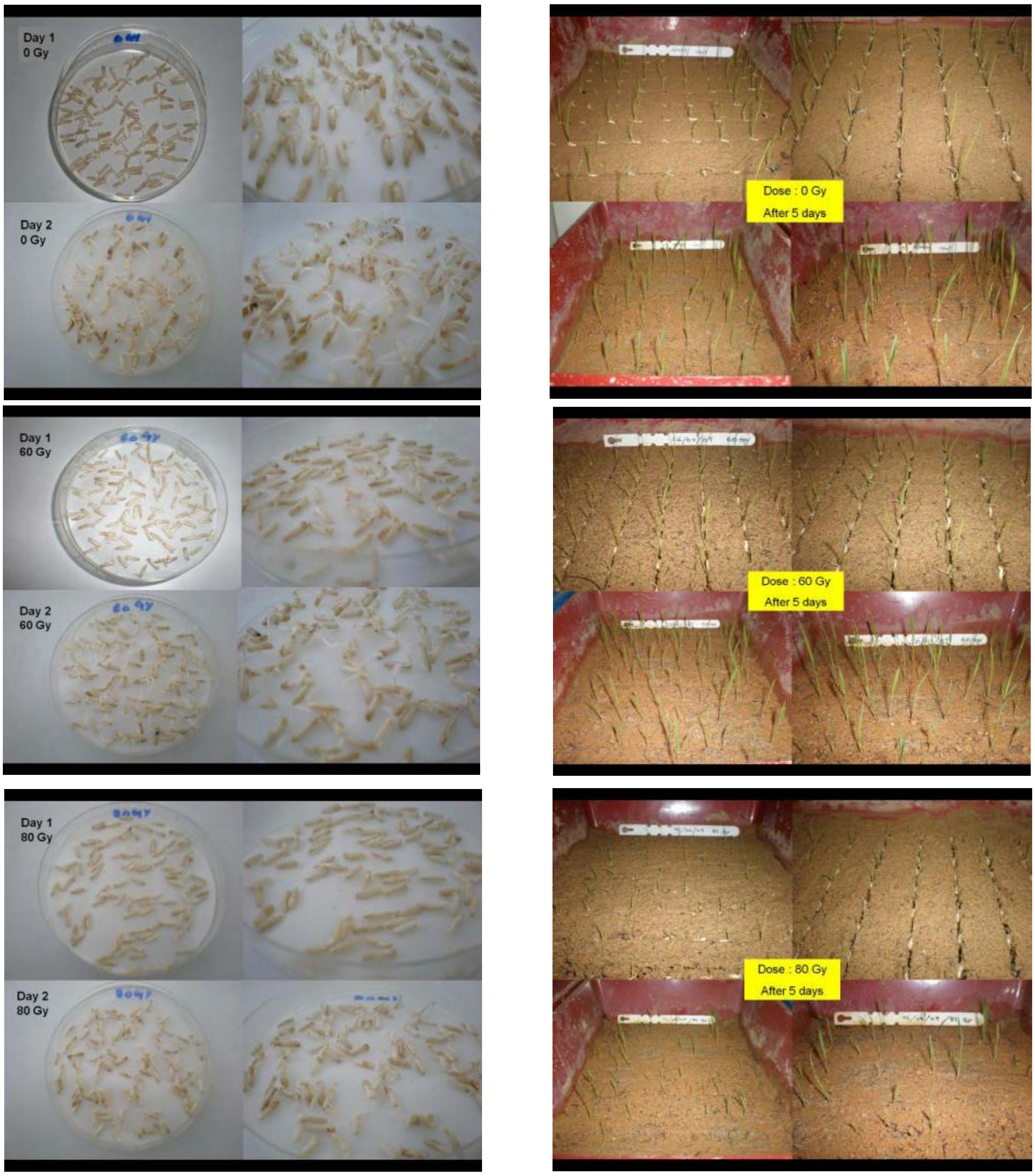


Figure 9. Germination rate of control and irradiated seeds of MRQ74 at day 1, 2 and 5

6.3.4 Plant type

The general morpho-agronomic and genetic evaluation traits of mutants and parent are as tabulated in Table 1. Panicle length, flag leaf width, leaf width are longer or broader than the parent. Similarly, the culm number for both mutants were comparatively or more than the parent.

Table 1. Morpho-agronomic characteristics of advanced mutant lines compared to its parents.

Plant Characters	MR219-4	MR219-9	MR219	Difference Over parent
Flag leaf length(mm)	34.2	36.4	40.1	lower
Flag leaf width (mm)	13.7	16	14.3	Higher(MR219-9)
Leaf length(cm)	41.3	40.6	41.4	Lower
Leaf width (mm)	15.3	12.3	13.7	Higher(MR219-4)
Culm length (cm)	74.3	75	72	Higher
Culm number (No)	15	13	14	Higher(MR219-4)
Culm diameter (mm)	3.61	3.62	3.62	same
Blast Reaction	Resistant	Resistant	Resistant	same
BPH Reaction	Resistant	Resistant	Resistant	same
Culm Strength	Strong	Strong	Strong	same
Panicle length	28.2	28.4	26.4	Higher

6.3.5 Yield and yield components

Yield trial in MADA, has indicated that the yields of MR 219-4 and MR219 -9 were comparable to the yield of MR 220. However, lines MR 219-4 and MR219-9 had significantly higher percentage of filled grains and 1000-grain weight than MR 220 (Table 2). There was no significant interaction effect of rice variety and water management on yield and yield components. Rice grown under saturated condition yielded as high as rice grown under normal flooded condition. Similarly, the yield components (panicles/sq m, spikelets /panicle, percent filled grains and the 1000-grain weight) did not differ significantly between flooded and saturated conditions. However, rice plants are significantly taller (how many percent) when they are grown under saturated than flooded condition.The growth and yield performance of MR 219-4 are similar to that of MR 219-9.The lines were also evaluated under aerobic soil condition (sprinkler-irrigation) under dry land regime in MARDI SeberangPerai and performed satisfactorily. Grain yield as high as 6.3t/ha for MR219-4 and 3.4t/ha for MR219-9 were recorded under aerobic condition.

Table 2. Yield and yield components of MR219-4 and MR219-9 in Muda (direct seeding method)

Treatment	Yield (kg/ha	Yield component				Plant height (cm)
		Panicle /sq.m	Spikelets/panicle	Percent filled grain	1000-grain wt (g)	
Saturated	6480 a	503 a	107 a	68.2 a	28.4 a	97.5 a
Flooded	6799 a	500 a	104 a	70.2 a	28.3 a	95.1 b
MR 219 – 4	6542 a	447 a	107 a	73.3 a	29.0 a	97.1 a
MR 219 – 9	6460 a	512 a	99 a	77.7 a	28.9 a	98.6 a
MR 220	6815 a	517 a	109 a	61.7 b	28.1 b	95.4 a
Water Management x variety	ns	ns	ns	ns	ns	ns

Performance of yield components and yield for KETARA are as tabulated in Table 3. Both mutants yielded lower than MR219 but they were better than MR219 for some traits such as grain width, percent filled grain, early maturation.

Table 3. Growth and yield performance of MR219-4 and MR219-9 in KETARA (direct seeding method).

Parameters	MR219-4	MR219-9	MR219	Difference Over parent
Panicles /plant	6.5	7.4	9	lower
Plant ht	100.1	100.4	103.4	lower
No. of tiller/hill	9	8	9	lower
Percent Filled Grain	78.1	87.7	73.4	higher
1000 grain wt(gm)	30.3	30.3	28	higher
Grain Length(mm)	9.69	9.69	9.86	lower
Grain width (mm)	2.38	2.41	2.35	higher
Panicle length	n.a	n.a	n.a	n.a
Yield (kg)	5259	6000	6444	lower
Maturity	110	112	116	lower

Table 4. Yield performance of MR219-4 and MR219-9 at different field condition.

Field condition	**MR219-4**	**MR219-9**
Normal flooded conditions	4.8 t/ha	5.5 t/ha
Saturated soil	5.9 t/ha	6.8 t/ha
Flooded condition	7.2 t/ha	6.1 t/ha
Aerobic soil condition	6.3 t/ha	3.4 t/ha

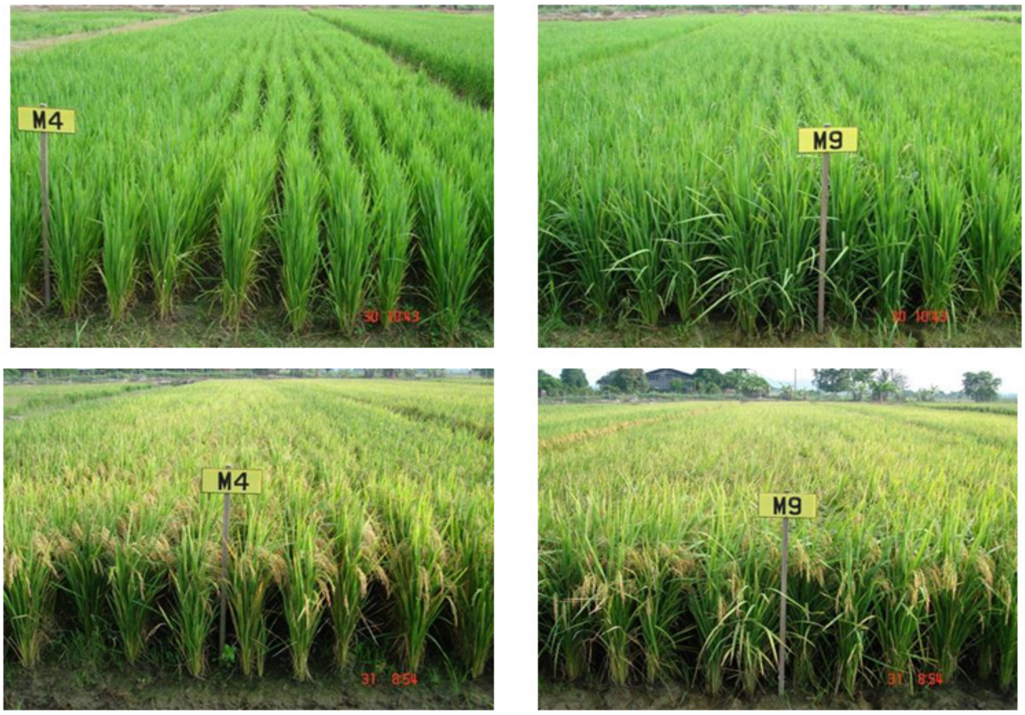


Figure 10. Evaluation of yield performance of advanced mutant lines MR219-4 and MR219-9 under low water requirement

Figure 11. Field Screening of mutants lines under simulated water stress condition at MARDI Seberang.Perai

Figure 13. Advanced mutant of MR219-4 under water stress (aerobic) condition

To produce a variety of rice mutants induced by carbon-ion beams (12C+6; 320 MeV), it is important to investigate the effects of the carbon-ion on several biological end points such as germination, survival, ripening and subsequent mutations in rice (Hidema *et al.*, 2003). For MR219 which had been irradiated with ion beam, a total of 31 mutant lines were selected in M_3 generation based on their agronomic traits. However, physico-chemical characteristics were not significantly different from the parental variety except for amylose content which showed high variation among all lines. Based on overall data recorded, mutant line ML21 had the greatest performance in term of yield. Further screening should be carried out in M_4 to select for potential mutant lines which are resistance to blast disease and high yielding.

6.3.6 Analysis of amylose content

Traditional Malaysian rice varieties, such as Mahsuri and PongsuSeribu with 6 months of maturity period have high amylose content of 25.4% and 22.2% respectively in comparison to low amylose content of glutinous rice (13.3%), Carreon (13.3%), Columbia (16.6%). Original parental cultivar MR219 has intermediate amylose content (20.0%), whereas MRQ74 has high amylose content (30.0%). Mutant lines from MR219 irradiated with gamma rays have higher amylose content (22.5 to 23.7%), whereas mutant lines irradiated with ion beam have low amylose content (MR219/I2 - 18.0% and MR219/I12 - 18.6%). Parental variety MRQ74 with high amylose content (30.0%) after irradiation with ion beam was able to produce mutant lines with low amylose content (MRQ74/8 - 21.4%, and MRQ74/26 - 22.8%). This shows that ion beam irradiation is much more effective than gamma rays in producing lower amylose content.]

Table 5. Amylose content of Standard Rice samples

Standard rice samples	% Amylose
Koshihikari (Japonica rice	17 (low)
MR219 (Local indica variety)	20 (intermediate)
Mahsuri (Traditional indica variety)	25 (high)

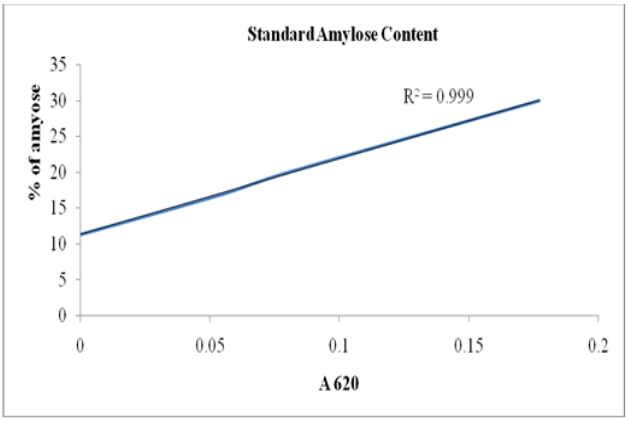


Figure 14. Standard curve of amylose using UV-VIS Spectrophotometer

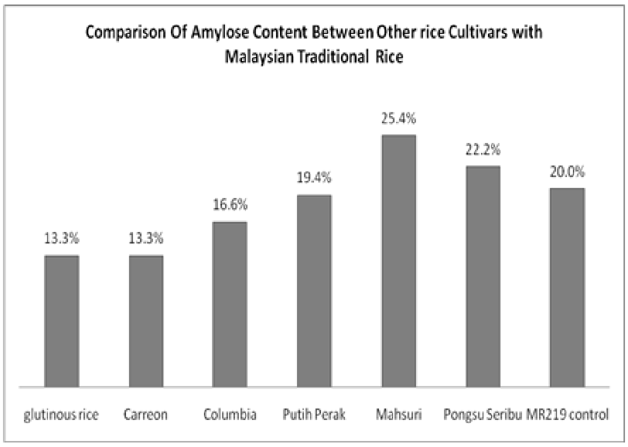


Figure 15. Comparison of amylose content between other popular rice cultivars with Malaysian traditional rice

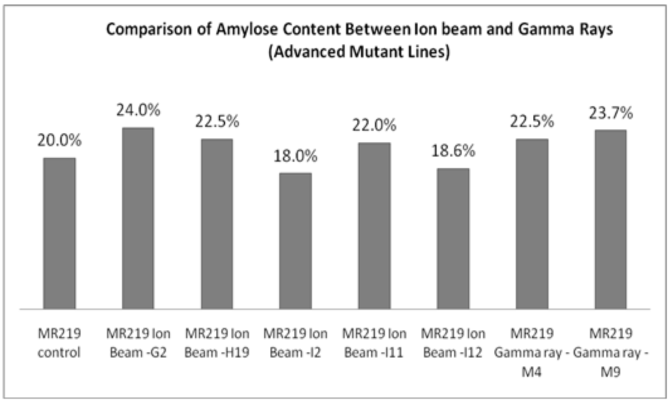


Figure 16. Comparison of amylose content between ion beam and gamma rays

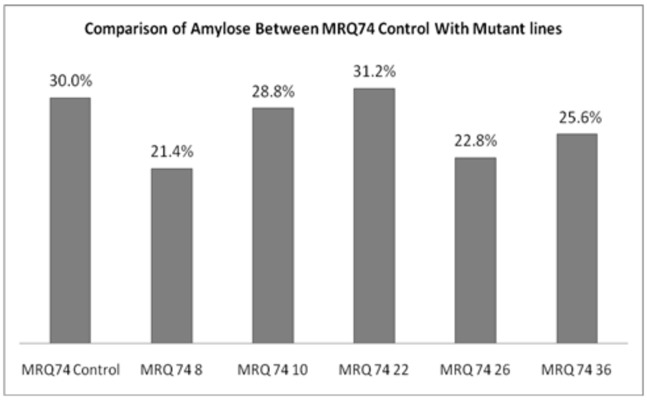


Figure 17. Comparison of amylose content between MRQ74 control with mutant lines

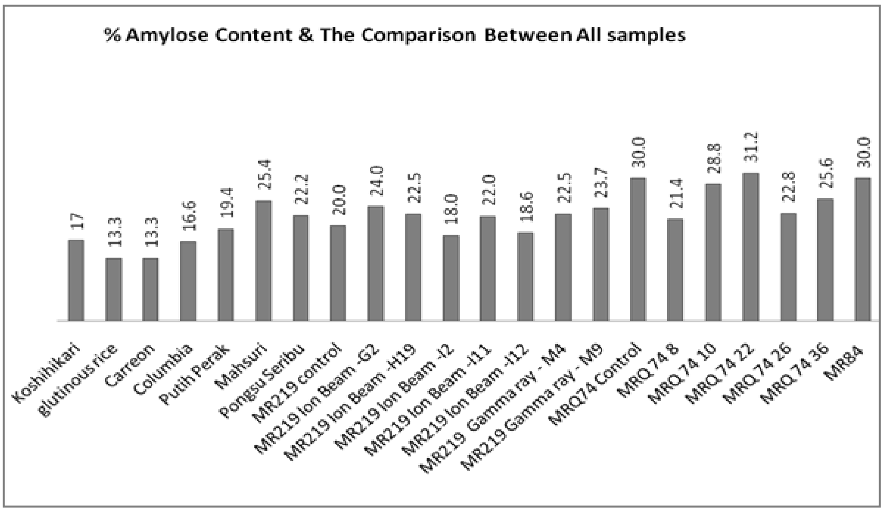


Figure 18. Percentages of amylose content and comparison between all samples

6.4 Conclusion

Main objectives of FNCA Plant Mutation Breeding Sub-Project on Composition or Quality in Rice are to select potential mutant lines of high yielding which are tolerance to minimal water requirement and improvement of amylose content. Under flooded condition, mutant line MR219-4 recorded a yield of 7.2t/ha and MR219-9 recorded 6.1t/ha compared to 5.8t/ha from the parental variety MR219. Under saturated condition, MR219-4 recorded 5.9t/ha whereas MR219-9 recorded 6.8t/ha of yield performance. The most striking observation made was when both mutant lines were tested under aerobic condition and mutant line MR219-4 performed much better than MR219-9, recorded a yield of 6.3t/ha in comparison to MR219-9 with 3.4t/ha. This shows that gamma irradiation was able to induce potential mutant lines suitable for minimal water condition with improved yield. These two advanced mutant lines will be further evaluated for stability in yield performance and other agronomic traits before they can be registered as new mutant varieties to be released to the farmers. In terms of improvement of quality of rice with respect to amylose, ion beam is more effective than gamma rays in producing lower amylose content. Mutant lines from MR219 irradiated with gamma rays have high amylose content (22.5 to 23.7%), whereas mutant lines irradiated with ion beam have low amylose content (18.0 - 18.6%). Similarly, when MRQ74 seeds with high amylose content of 30.0% were irradiated with ion beam, mutant lines recorded lower amylose content (21.4 - 22.8%). Further screening and selection in M_4 to M_6 generation will be carried out to select for potential mutant lines with high yield and resistance to blast disease.

6.5 Acknowledgement

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 for the contributions of Dr. Atsushi Tanaka from Japan Atomic Energy Agency (JAEA) for providing ion-beam irradiation facilities. Special thanks go to Ms. Aki Koike from NSRA whose considerable patience, liaison and organizational skills were very instrumental for the success of this project.

6.6 References

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.

Hase, Y., Tanaka., Baba, T. and H. Watanabe (2000). Plant Journal 24, 21-32;

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.

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

Mohamad, O., MohdNazir, 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

Varieties through the Use of Induced Mutations inMalaysia.Plant Mutation Reports. 1: 27-34.

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

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

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.DOI 10.1007/s11738-009-0456-3.

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, AlorSetar, Kedah, Malaysia

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

7. The Philippines

Grain Quality Improvement in Rice (*Oryza sativa* L.) through Induced Mutation Breeding

A. C. Barrida, F. G. Rivera and A. O. Dimaano

Agriculture Research Section, Atomic Research Division,
Philippine Nuclear Research Institute (PNRI)

7.1 Abstract

Gamma and ion beam irradiations were used for improving the grain quality and agronomic characteristics of the Philippine rice variety IR72. Among the selections from gamma irradiated plants, four mutant lines with intermediate amylose content were selected at 300Gy and one line at 200Gy using the quantitative method. Increased protein content was obtained on plants irradiated with 200Gy gamma rays.

Agronomic characteristics that were significantly affected by gamma and heavy ion beam irradiations were plant height at maturity, number of tillers per plant, and 1000 seed-weight. Lines that are early maturing, short, high tillering, and with long panicle were selected on both irradiation methods used.

7.2 Introduction

Rice (*Oryza sativa* L.) is the main staple food crop in the Philippines. It is also the main source of livelihood of more than 5 million farmers all over the country (Manila Bulletin, 2007). In 2011, the average rice yield per hectare for the first half improved to 3.8tons (or 76 cavans of 50kilos each). This is roughly three cavans more than last year's average yield of 3.64 tons per hectare (DA, 2011). Rice production in the Philippines is important to the food supply in the country and economy. The country is the 8th largest rice producer in the world, accounting for 2.8% of global rice production (FAO, 2011). However, the country was also the world's largest rice importer in 2010 (Reuters, 2011).

When more rice become more available and countries achieved rice sufficiency, grain quality becomes an important breeding objectives and consideration by consumers both in the domestic and international markets (Juliano and Duff, 1991). Physical properties such as length, width, translucency, degree of milling, color and age of milled rice are grain quality indicators. The amylose content of rice starch is the major eating quality factor while protein content is an index of nutritional value. In general, grain quality and quality preferences vary across rice growing countries and regions. Filipinos prefer translucent, well milled, long grain rice with aroma and minimal broken grains which is soft on cooling (Manila Bulletin, 2005).

The International Rice Research Institute (IRRI 1973) was able to breed a rice variety with 18-23% more protein than the check variety IR8 without any significant difference in grain yield. The grain quality can be altered by gamma radiation. In Malaysia, rice mutant PS 1297 was developed with good agronomic characteristics but with higher amylose content of 28.4%. Hag et al. (1971) successfully used gamma radiation to obtain three rice mutants having higher protein content than their parents. The quality of protein in rice is one of the best among the cereals but its content of 7%

is one of the lowest (Manila Bulletin, 2005).

Given the importance of grain quality characteristic of rice for creating and stimulating demand, the Forum for Nuclear Cooperation in Asia (FNCA), has initiated a cooperative project entitled "Quality Crop Breeding in Rice (*Oryza sativa* L.)" among member countries (Bangladesh, China, Indonesia, Japan, Korea, Malaysia, Philippines, Thailand and Vietnam). Such cooperative project endeavors to use mutation breeding to widen the genetic variability for quality characteristics and produce quality rice which is acceptable in each participating country.

7.3 Objectives

1) To develop mutants with desirable agronomic traits and physico - chemical attributes.

2) To develop mutants with good eating quality, high protein and low to intermediate amylose contents.

7.4 Economic Impact

Development of high yielding mutants with low to intermediate amylose and high protein content will open new market that will generally benefit the farmers. High income consumers pay higher premiums for high quality rice, encouraging more hectarage to be planted to quality varieties. If these are widely adopted, producers will benefit by retaining quality rice for home consumption and having a wider domestic market for their product. In addition, a country exporting rice will benefit from quality improvement thus expanding the potential export market.

7.5 Methodology

7.5.1 Study I - Mutation induction by gamma and ion beam irradiations for improving the agronomic characteristics and grain quality of IR72

The study was conducted at the Philippine Nuclear Research Institute (PNRI) screen house and experimental fields. The variety used was IR72, high yielding but with poor grain quality. Radiosensitivity test was done to determine the optimum dose of gamma rays and heavy ion beam for irradiating IR72. For gamma irradiation, seeds were irradiated with 100, 200, 300, 400, 500, and 600Gy at the PNRI Gamma Irradiation Facility. Immediately after irradiation seeds were sown in the seedbed. Data on percentage emergence, percentage survival and seedling height were taken after two weeks. After the M_1 generation and the results of analysis for amylose and protein contents of M3 seeds, 200 and 300Gy were selected based on the rate of mutations exhibited. These doses were used for planting the succeeding generations for further selection of mutants. The control and the check variety IR 64 were grown for comparison.

Ion beam irradiation was conducted at the Takasaki Advanced Radiation Research Institute of the Japan Atomic Energy Agency Takasaki, Japan. Dehulled rice seeds were irradiated with 10, 20, 40, 60, 80, 100, 120, 160, and 200Gy using the AVF-cyclotron. Radiosensitivity test was also done inside the screenhouse to determine the optimum dose. In planting the M4 generation, 20 and 40Gy were selected based on the improved agronomic traits of the plants in the previous generations.

In field experiments the same methodology was employed both on gamma and ion beam-irradiated plants. The experiments were conducted with three replications using the Randomized Complete Block Design (RCBD). Seedlings were transplanted in the field after three weeks after sowing at a distance of 20 cm x 20cm. Only one seedling was planted per hill. Replanting of missing hill was done one week after transplanting. Proper cultural management was observed on the whole growing season. Split application of fertilizer was done and based on the result of soil analysis. The first application was made before transplanting and the second one was done before panicle initiation. Spraying of pesticide and weeding were done whenever necessary. A desired water level was maintained for optimum growth of the plant. Sampling and harvesting were done at 85% ripening. Plant sampling was done randomly and individual plants were selected based on agronomic characteristics. At harvest, agronomic and yield data were recorded. Yield components such as number of tillers, length of panicle, number of grains per panicle, number of filled and unfilled grains were also determined.

7.5.2 Study II – Methods for Determination of Amylose and Protein

7.5.2.1 Determination of Amylose

Screening of amylose was done from M_3 to M_6 generations using the iodine staining method. This quick method of screening rice for amylose content was provided by the counterpart from Thailand. The method was agreed upon by the working group, and the project leaders of each participating member state. In the qualitative method, those seeds with pink to colorless in color after staining were considered to have low amylose content and those with blue or dark purple in color was high amylose. On the other hand, the quantitative determination of amylose content of rice grain was analyzed using the modified method being used by the Laboratory Services Division of the Bureau of Plant Industry, Department of Agriculture (DA-BPI) and the simplified method of estimating amylose by Juliano. This procedure was taken from rice grain quality evaluation procedures of IRRI.

Rice was pulverized into flour, weighed and an ethanol was added to wet the sample. A 1 N solution of sodium hydroxide was then added to gelatinize to starch and was stand overnight for 18-24 hours or was placed in a water bath and boiled for 10 minutes. The gelatinized sample was made to volume and an aliquot of the solution was transferred to another volumetric flask. Acetic acid (1 N) and iodine solution were added to form the deep blue colored starch - iodine complex. The colored developed in the sample was read in a UV-VIS spectrometer at 620nm wavelength against several concentrations of known potato amylose standard following the same above mentioned treatment/procedure made in the sample.

7.5.2.2 Determination of Protein

A modified semi-micro Kjeldahl method was used in the determination of protein in milled rice. The organic nitrogen is converted to ammonia which reacts with the excess sulfuric acid forming ammonium sulfate during digestion. A salt mixture of sodium or potassium sulfate, copper sulfate and selenium or a readily available kjeltabs was used as catalyst to hasten the rate of decomposition reaction. The solution is then made alkaline with 40-50% sodium hydroxide prior to steam distillation. The ammonia being liberated during the process of distillation was trapped in a boric acid containing receiver followed by titration using standardized 0.1 to 0.2 N sulfuric or hydrochloric acid. Total nitrogen of the milled samples was computed based on the volume and normality of the acid used. The moisture of the rice samples was corrected and percentage protein was calculated by multiplying the factor 6.25.

7.6 Results and Discussion

7.6.1 Selection and evaluation of mutant lines with improved agronomic characteristics

Planting of the selected putative mutants lines with improved agronomic traits and with low to intermediate amylose content was done continuously for further confirmation and evaluation. There was a significant increase in plant height at 200 and 300 Gy as shown in Fig. 1.

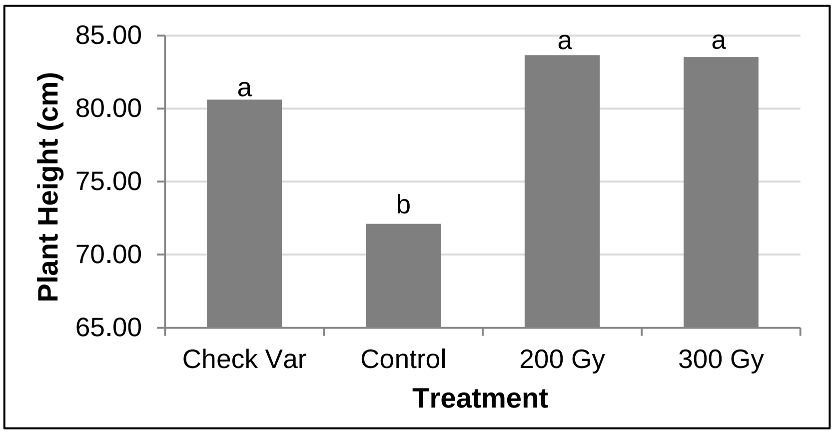


Figure 1. Plant height at maturity as affected by gamma irradiation at M_7 generation (bars with the same letters are not significantly different at 5% level based on DMRT)

The number of tillers per plant was significantly affected by gamma irradiation. (Fig. 2) There was a 33.34% increase at 300 Gy and 19.3% increase at 200 Gy compared to the control and check variety.

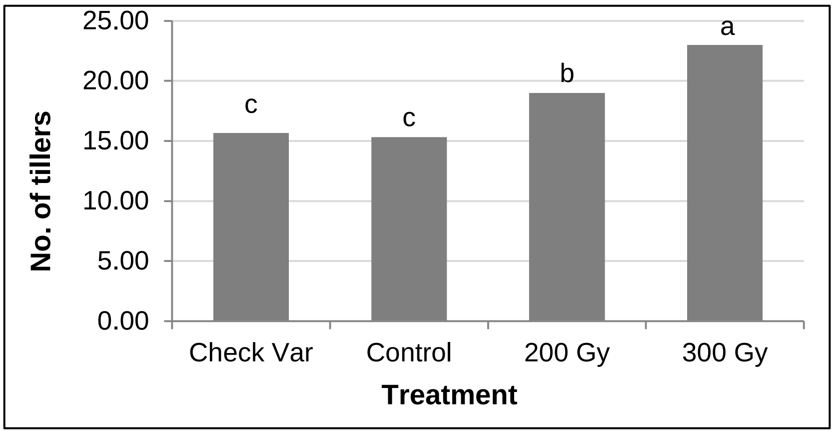


Figure 2. Number of tillers per plant as affected by gamma irradiation at M_7 generation (bars with the same letters are not significantly different at 5% level based on DMRT)

The weight of 1000 seed (g) increased at 200Gy. The control and 300Gy obtained similar weight as presented in Fig.3.

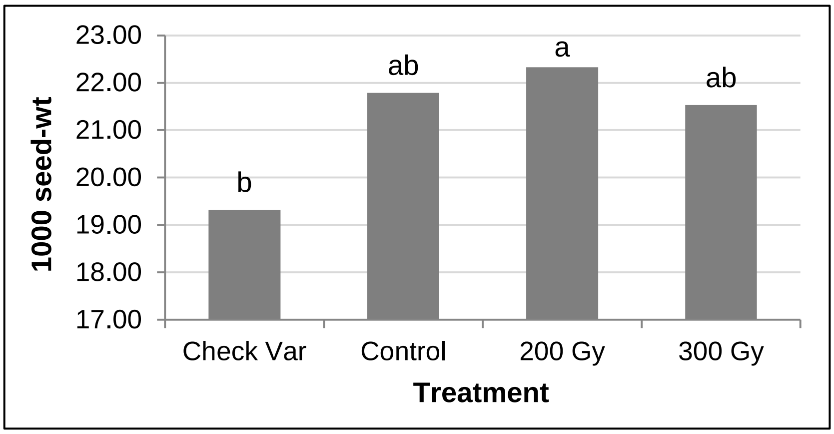


Figure 3. 1000 seed - weight (g) as affected by gamma irradiation at M_7 generation (bars with the same letters are not significantly different at 5% level based on DMRT)

The number of days to flower was not affected by the treatments. However, the control took longer to flower with 97 days as compared to irradiated plants with 90 days. Likewise, the length of panicle (cm), number of grains per panicle and filled grains were not significantly affected by the treatments (Table 1).

Table 1. Agronomic data at M_7 generation

Treatment	**Days to flower**	**Length of panicle (cm)**	**Grains/ Panicle**	**Filled Grains/ panicle**
Check	93	23.20	124	78
Control	97	22.05	121	90
200 Gy	90	24.60	121	93
300 Gy	90	23.00	116	91

7.6.2 Selection of mutant with low to intermediate amylose content

The analysis of amylose content using the qualitative or the iodine staining method of M_6 seeds irradiated with 200 and 300Gy gamma rays showed that at 200Gy, 12 out of 153 lines analyzed had low to intermediate amylose, while at 300Gy only one out of 127 lines had low to intermediate amylose. Those with low amylose content were considered promising mutant lines. The control and the check variety have high amylose content (Table 2).

Table 2. Lines at M_6 generation with varying amylose content

Treatment	No. of Lines analyzed	No. with Low to Intermediate Amylose	No. with High Amylose
Check	10	0	10
Control	10	0	10
200 Gy	153	12	141
300 GY	127	1	126

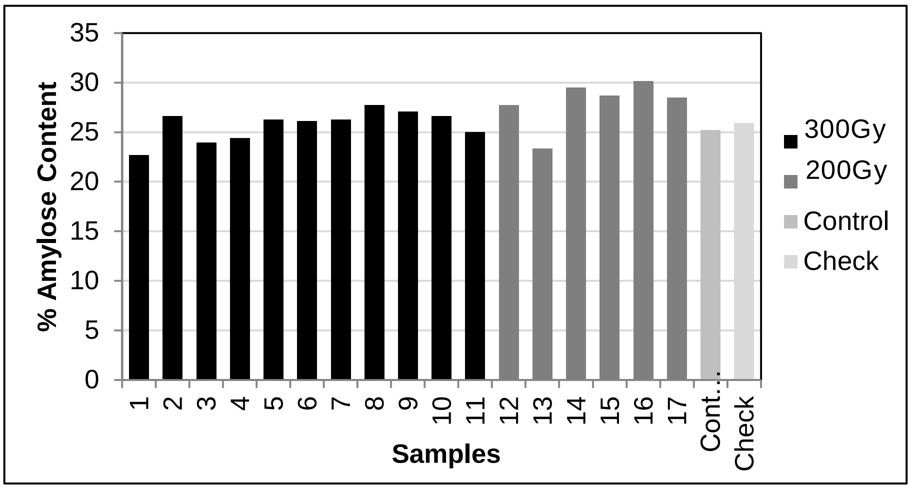


Figure 4. Percent amylose content using the quantitative method of analysis

For the M_7 generation, quantitative method of analysis for amylose content (Fig. 4) was applied for the mutant lines selected in the previous generation and to confirm the result of qualitative method. Result showed that four lines at 300Gy and only one line at 200Gy had intermediate amylose content. The control and check variety have high amylose contents.

7.6.3 Selection of lines with high protein content

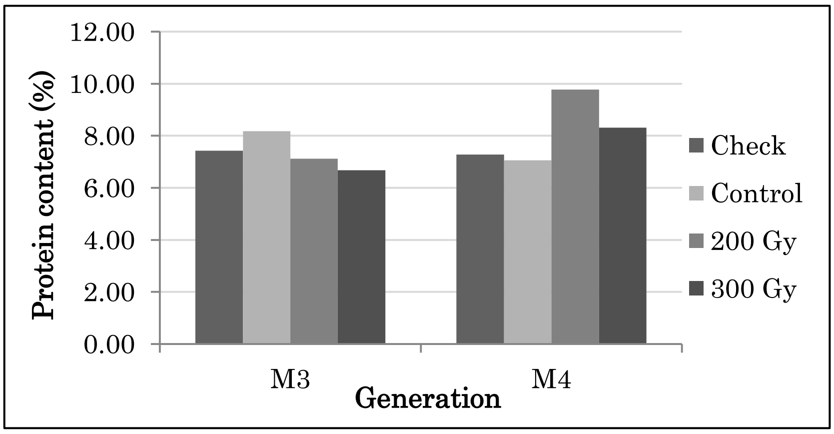


Figure 5. Protein content at different treatments in two generations

Fig. 5 shows higher protein content at 200 and 300Gy in the M_4 generation. The increase in protein content was 31.11% higher at 200Gy and 8.58% higher at 300Gy compared to the control. The control and check variety had similar protein content.

7.6.4 Heavy Ion Beam Irradiation for the Selection of Mutant with Improved Agronomic Characteristics

Irradiation with heavy ion beam at 20 and 40Gy yielded plants that were early maturing (one week earlier than the control), short, high tillering and with long panicle. The agronomic data are given in Table 3.

The plant height at maturity was significantly affected by the treatment wherein the plants irradiated with 40Gy was the tallest followed by 20Gy with 83.13 and 78.36cm. The control was the shortest with 72.11cm.

The number of tillers per plant significantly increased at 20 and 40Gy and was not significantly different from each other at 23.00 and 24.00, respectively. At 40Gy, plants were 30.43% tiller than the control, while at 20Gy 33.33% tiller than the control. Some authors reported that the mutation frequencies per unit dose were higher with ion beams than with gamma rays, thus indicating that ion beam had a higher mutation effectiveness than gamma rays, which confirms the result of previous studies, (Fujii et al. 1966, Mei et al. 1994). Similar result was also obtained by Sobrizal et. al (2009) that, based on the point of seed fertility of M_1 plants 20 Gy is the best dose. In addition, Kang (2009) suggested that the proper irradiation doses of heavy ion beam for cultivar "Ilpum" might be between 20 to 30Gy doses. The weight of 1,000 seeds was significantly higher at 40Gy with 22.67gram. However, there was no significant difference between 20Gy and the control. As in gamma irradiated plants, the number of days to flower, panicle length, seed per panicle and number of filled grains per panicle were not affected by heavy ion beam irradiation.

Table 3. Agronomic traits at M_4 generation as affected by ion beam irradiation

Treatment	Plt. Ht.at Maturity (cm)	Days to flower	Number of tillers/ Plant	Panicle Length (cm)	Seed/ panicle	Filled Grains/ panicle	1000 seed wt. (g)
Control	72.11 c	97	16 b	22.05	121	90	19.68 b
20 Gy	78.36 b	90	24 a	22.99	118	79	21.94 b
40 Gy	83.13 a	90	23 a	22.30	109	85	22.67 a

In a column, means with the same letters are not significantly different at 5% level based on DMRT.

7.7 Future Plans

The project will not just end by simply producing mutant lines with intermediate amylose content. The mutant should be planted continuously to determine if the improved characteristics are already stable. Likewise, yield testing of the mutant is very important to find out if the yield is not affected by the alteration of amylose. In addition, determination of amylose on those plants irradiated with ion beam and the use of molecular technique for the confirmation of the mutants will also be undertaken.

7.8 Conclusion

Gamma and ion beam irradiations showed similar effects for inducing mutations in plants as exhibited by the results obtained in this study. Both methods of irradiations gave significant results on some agronomic traits of the plants. Likewise, the grain quality specifically the amylose and protein contents were also significantly affected by gamma irradiation.

7.9 Acknowledgement

The authors wish to thank the FNCA for this project on grain quality improvement in rice and the sponsors especially the government of Japan, NSRA, MEXT, Dr. Tanaka for irradiating the rice seeds using ion beam and the PNRI Irradiation Facility Services Unit. The authors gratefully acknowledge the support of Ms. Glenda B. Obra, Head of Agricultural Research Section of PNRI, Mr. Eduardo C. Costimiano for assisting in the whole implementation of the project, and Mr. Fernando B. Aurigue who did the editing of the manuscript.

7.10 References

Department of Agriculture - August 10, 2011 press release.

FAO Stat. FAO Statistics. Retrieved 30 March 2011.

Fujii, T., M. Ikenaga and J.T. Lyman, 1966. Radiation effects on Arabidopsis thaliana, II. Killing and mutagenic efficiencies of heavy ionizing particles. Radiat. Bot. 6 : 297 - 306.

Haq, M.S., N. Choudhury and M.M.Rahman. 1970. Breeding for high protein content of rice through induced mutations. STI/PUB/258. IAEA, Vienna, Austria.

Hag, M. S., Chindhurg, N., and Rahman, M. M. Breeding for high protein content and quality of rice through induced mutation. Atomic Energy Center: Dacca, Pakistan (cited in Plant Breed. Abst. 1971, 41(3):4436).

IRRI. 1973. Improvement of the protein content of rice. In: Annual Report for 1972. Int. Rice Res. Inst.: Los Baios, Philippines.

Juliano, B.O. and Duff, 1991. Rice grain quality as an emerging research priority national rice breeding programs in rice grain marketing and quality issue. IRRI, Philippines.

Kang Si-Yong. 2009. Seed irradiation test by heavy ion beam for rice mutation breeding. Paper presented in the 2009 FNCA Workshop on Mutation Breeding. September 21-24, 2009. Hangzhou, China.

Kikuchi, H.1994. Improving the eating quality of rice using low amylose mutants. Gamma Field

Symposia 33:18.

Manila Bulletin, 2005. The rice Filipinos like to eat: Physico-chemical basis. Sept. 4, 2005.

Manila Bulletin. 2007. NGOs Opposed Entry of GM rice into the country Manila Bulletin 410: 27 page B-12.

Mei, M., H. Deng, Y. Lu, C. Zhuang, Z. Liu, Q. Qiu, Y. Qiu and T.C. Yang. 1994. Mutagenic effects of heavy ion radiation in plants. Adv. Space Rec.14: 363-372

Parpia, H..A.B.1970. Bridging the protein –calorie gap. STI/PUB/258. IAEA, Vienna, Austria

Reuters. 28 January 2011. Retrieved 30 March 2011.

Science Daily (Feb. 19, 2010)

Sobrizal, Carkum, Winda Puspitasari, Yulidar and Nana Supriatna.2009. Improvement of diah suci variety by ion beam irradiation. Paper presented in the 2009 FNCA Workshop on Mutation Breeding. September 21-24, 2009. Hangzhou, China

Tanaka, S. and Y. Takagi. 1970. Protein content of rice mutants Improving plant protein by nuclear techniques. STI/PUB/258. IAEA, Vienna, Austria.

8. Thailand

Rice Mutation Breeding for Various Grain Qualities in Thailand

S. Taprab, W. Sukviwat, D. Chettanachit, S. Wongpiyachon and W. Rattanakarn

Bureau of Rice Research and Development, Rice Department,
Ministry of Agriculture and Cooperatives, Thailand

8.1 Introduction

Rice breeding through mutagenesis in Thailand had been continuously done for long period of time. Recommended varieties of mutated glutinous rice (RD6) and RD 15 an earlier matured mutant of KDML 105 were released. They are photoperiod sensitive varieties that can be grown once a year in Thailand. RD10 was later a glutinous mutated variety released. It was a photoperiod insensitive variety that can be grown more than one crop per year. Those released mutants were purposed for higher yield per unit area. Rice breeders are consequently carrying on mutation breeding for better grain quality. We reviewed previous mutation breeding activities in Thailand prior to FNCA Mutation breeding project. The results showed some success in improving grain qualities of various purposes. Seeds of recommended varieties were widely used for mutation induction through gamma ray and fast neutron irradiations. We have joined FNCA Sub-project on improvement of composition or quality in rice since 2007. Using nuclear technique to induce mutation in rice for various grain qualities was our breeding program proposal. We have just started by mutation induction by gamma ray irradiation in 2008. We have not joined the Ion-beam mutation breeding project.

Collaborated with FNCA Mutation Breeding Project, five years plan of rice mutation breeding for improvement of grain quality in Thailand started in 2007. Main objective is to develop mutants with various grain qualities such as amylose content, protein content and low phytic acid. Two groups of varieties used for mutation induction are low amylose content varieties (KDML105 and RD15) and high amylose content varieties (SPR1 and CNT1) including one international check variety, IR64. Gamma ray with 200 and 300Gy, and fast neutron with 20 and 30Gy are used for irradiation. KDML105 and RD15 are supreme grain quality aromatic rice in Thailand contain about 15% amylose. They can be grown only one crop a year due to their photoperiod sensitivity. Mutants with various level of amylose content retained excellent physical grain quality with good cooking quality (aroma) and photoperiod insensitivity are intended to be obtained. KTH17, SPR1 and CNT1 are high yielding varieties and high amylose content of about 27%. They have very good physical grain quality. Irradiated grains of those varieties were screened for low amylose content and low phytic acid. IR64 was used for international check variety of the FNCA mutation breeding project.

8.2 Materials and Methods

Rice seeds of varieties: KDML105, RD15, KTH17, SPR1, CNT1 and IR64

Mutation induction: -200 and 300Gy of Gamma ray irradiation,
-20 and 30Gy of fast neutron irradiation

Grain analyses: -IRRI’s standard protocol for amylose content analyses,
-SDS-PAGE technique (Laemmli, 1970) for protein and its component analyses,
-Screening technique developed by Victor Raboy (2005) for low phytate analyses

Mutant selection: -Pedigree selection method

Seeds had been irradiated and M_1 obtained in Wet season 2007. M_2 plants of KDML105 and RD15 had been grown in Dry season 2008 where their wild types could not flower. During this period, M_2 plants could be selected for photoperiod-insensitive mutants. Then, M_3 seed of 200 mutant lines had been separately harvested as single hill per line. Photoperiod sensitivity is an obvious trait and easy to be selected in dry season whereas long day period presented. Those 200 photoperiod-insensitive mutants were continuously screened for important agronomic traits and to be analysed for amylose content. They were again tested for photoperiodism, tillering ability and maturity in Dry season 2009. A half of single hill harvested M_4 seeds had been grown in Wet season 2009 and another half of seed had been analyzed for amylose content. In wet season, flowering time of the mutants were earlier than their wild types but not much differ in plant height. Expected higher amylose content with aroma would be identified from those mutants. While, lower amylose mutants would be used for breeding germplasm and for special purposes. Selected M_5 mutants had been grown in Dry season 2010 and M_6 and M_7 seeds had been grown in Wet season 2010 and 2011. In 2008, we developed simple technique for amylose content determination on breeding lines and distributed to FNCA members.

While amylose content was being analyzed, we developed a technique for protein analysis. Protein extraction technique reported by Iida et al. (1993) and Tanaka et al. (2004) had been modified and tested for indica rice protein analysis. Extracted proteins such as globulin, albumin, prolamine and glutelin from KDML105 and Koshihikari had been identified using SDS-PAGE technique (Laemmli, 1970).

In case of low phytic acid mutants, 2 mutant lines are going to be tested for bioavailability in artificial intestine digestion. New irradiated populations were screened for low phytic acid mutants.

8.3 Results

Wet Season 2010 and Dry Season 2011

8.3.1 Protein component analyses

Using SDS-PAGE technique adapted from Laemmli (1970) for protein and its component analyses to determine total protein, albumin, globulin, glutelin and prolamine of KDML105 and Koshihikari. The result showed clear bans of albumin, globulin and glutelin but not of prolamine (Figure 1). We could distinguish KDML105 from Koshihikari by their pattern of protein components on SDS-PAGE algarose gel (Figure 2).

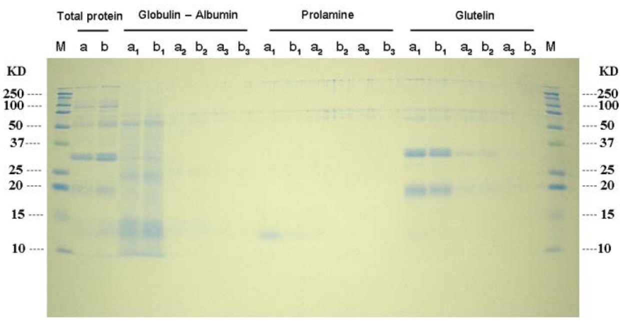


Figure 1. SDS-PAGE analyses on total protein, globulin, albumin, prolamine and glutelin of rice variety, KDML105.

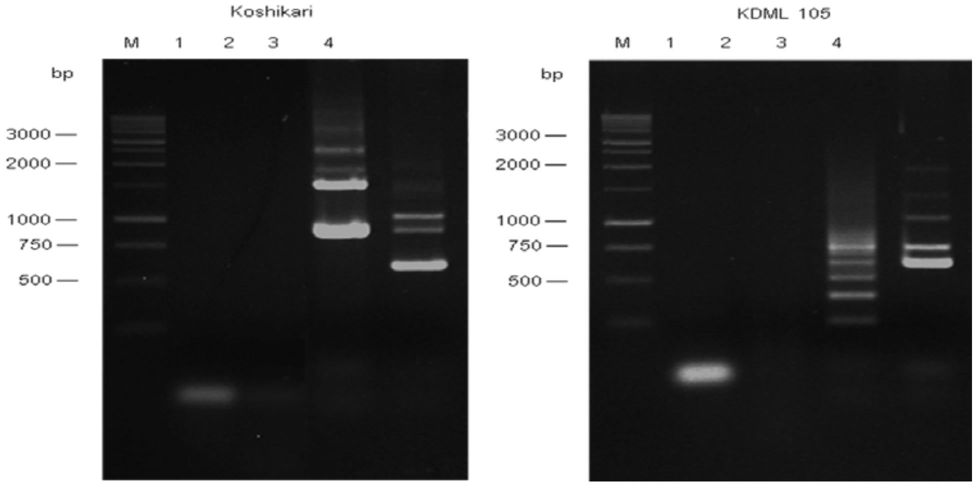


Figure 2. Agarose gel electrophoresis of Koshihikari and KDML 105, lane M, DNA marker (GeneRuler™ 1 kb DNA Ladder, Fermentas), lane 1, the PCR product amplified from genomic DNA with the primer set F2 and F8, lane 2, the nested RT-PCR product amplified with the primer set F2 and F8, lane 3, the PCR product amplified from genomic DNA with the primer set FC and F5, lane 4, the nested RT-PCR product amplified with the primer set FC and F5.

Molecular cloning of Glu-B from KDML105 was done by Nested reverse transcriptase-mediated PCR. The cDNA was made from total RNA of 20 days seedling using RetroTools® Two Step Kit (BIOTOOLS B&M LABS., S.A.) and random hexamers (pd$(N)_6$)as primers for 1st – Step Reverse Transcription (RT). The second round F2(5’-GTTTTGGAACGTTAATGCCCATAG -3’) -F8(5’-ATGAGCACCAAAAGATCCAC-3’) primers were used to amplified the read through LCC1 and the PCR product amplified from LCC1 genomic DNA with the primer set FC (5’-CTCCTAGATATCAACAACAGAC -3’) and F5(5’- AGTTGTTGCTCTATATGTCTTCGACT-3’). Then the PCR product was process to insert in pGEM®-T Easy(Promega, USA). Nucleotides sequences analysis of Glu-B product were done by ABI 3130 automatic sequencer (Applied Biosystems) using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Results show the nucleotide sequences with 600 bps are homologous to *Oryza sativa* glutelin precursor (GluB4) gene exon 1 through 4 and complete cds.

8.3.2 Mutant lines selection

Since 2008, mutant lines with photoperiod insensitiveness had been achieved. In the year 2010, two hundreds M_5 mutant lines of both KDML105 and RD15 derived from 2008 had been analyzed for amylose content and protein components. Low amylose mutant lines had been achieved from KTH17 which is originally high amylose content local variety. Various amylose content mutant lines from original low amylose content varieties KDML105 and RD15 had been obtained. Some of those mutants contained lower and higher amylose content than did of their wild types. 293 mutant lines of RD15 showed wide variation of amylose contents from 10.58 – 28.08 % (Figure 3). 241 mutant lines showed less variation of amylose contents than did of RD15, from 11.96-16.02 % (Figure 4). 191 mutant lines of KTH17 showed wide variation of amylose contents from 13.30 – 29.98 % (Figure 5). Protein contents of those mutant lines are being analyzed.

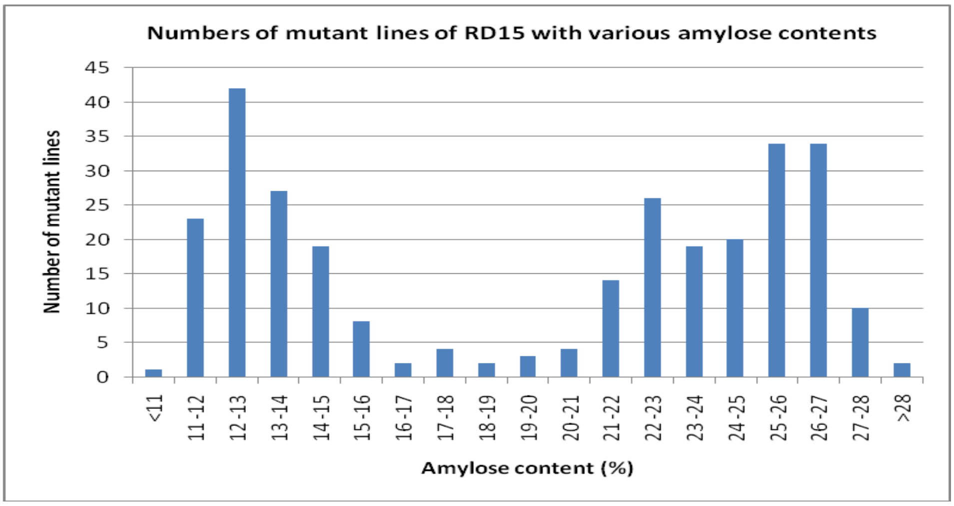


Figure 3. Number of mutant lines of RD15 with various amylose contents.

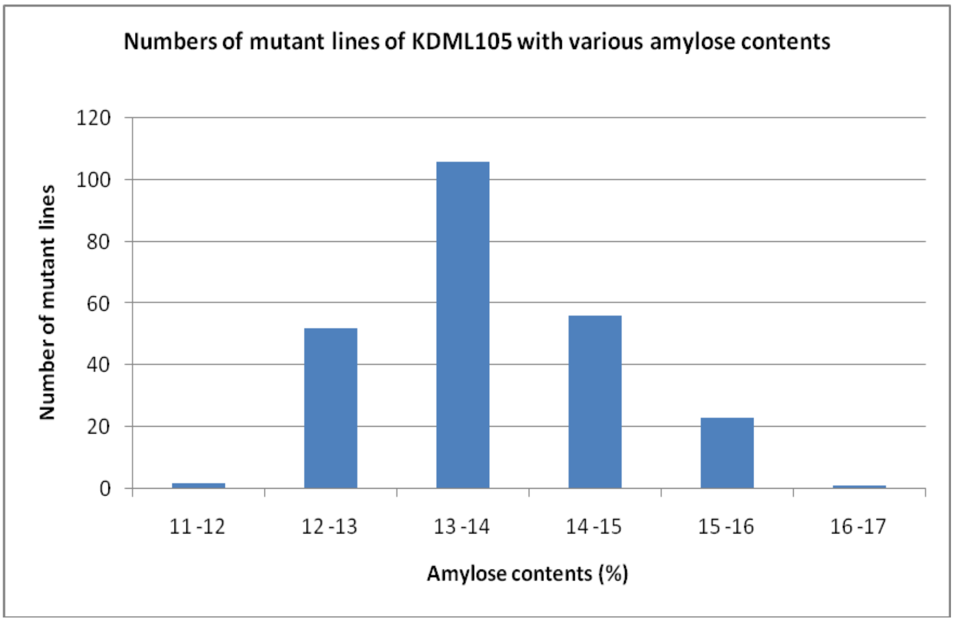


Figure 4. Numbers of mutant lines of KDML105 with various amylose contents.

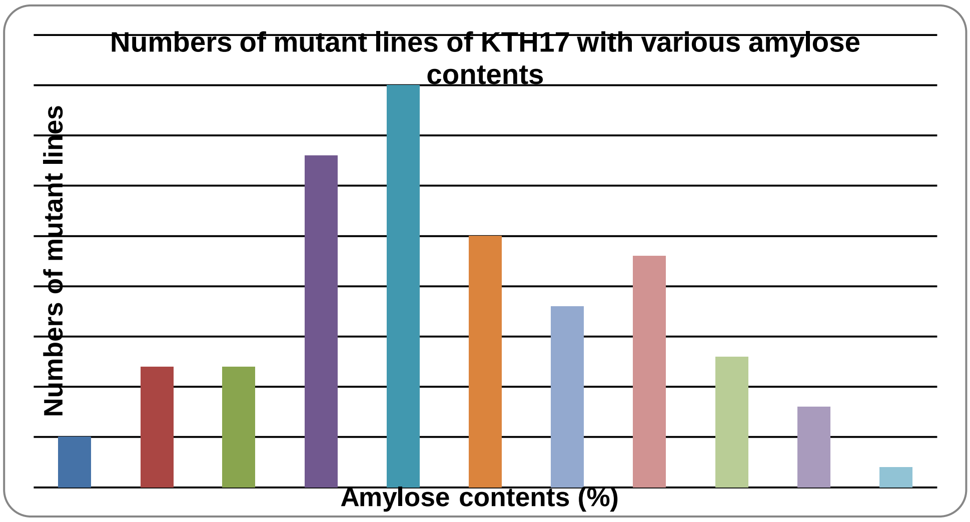


Figure 5. Numbers of mutant lines of KTH17 with various amylose contents.

When photoperiodism and dosages of gamma ray were considered, it was found that 20Kr (200Gy) and 30Kr (300Gy) induced different frequency of mutation on amylose content. Photoperiod sensitive mutants of RD15, KDML105 and KTH17 mostly contained lower amylose than wild types did. From RD15, photoperiod sensitive mutants derived from 20Kr irradiation contained lower amylose while 30Kr mutants contained slightly higher amylose (Figure 6). Highest frequency of 20Kr mutants of RD15 was 45 lines with 12% amylose content while 30Kr mutants was 43 lines with 13% amylose content. From KDML105, photoperiod sensitive mutants derived from 20Kr and 30Kr were similar distribution to RD15 mutants. But 20Kr created wider range of amylose with 1 line contained 18% amylose while 30 Kr had highest amylose of 15%. Both 20Kr and 30Kr had highest frequency at 13% amylose content with 45 and 61 lines, respectively (Figure 7). Similarly, high amylose variety KTH17 had been also induced lower amylose mutants. KTH17 wild type contained 27-28% amylose while its mutants derived from both 20 Kr and 30 Kr contained lower amylose. Their highest frequencies are 24 and 16 lines at 23% amylose content (Figure 8). In contrast, photoperiod insensitive mutants showed different distribution from photoperiod sensitive mutants. From RD15, lower dose of 20Kr induced higher amylose content than higher dose of 30Kr. It produced very higher amylose content up to 28% mutants compared to its 15% amylose wild type. It had highest frequency at 25% amylose with 32 mutant lines. The dose of 30Kr induced lower amylose to the lowest of 10% amylose content. Its highest frequency was 40 mutant lines at 12% amylose content (Figure 9).

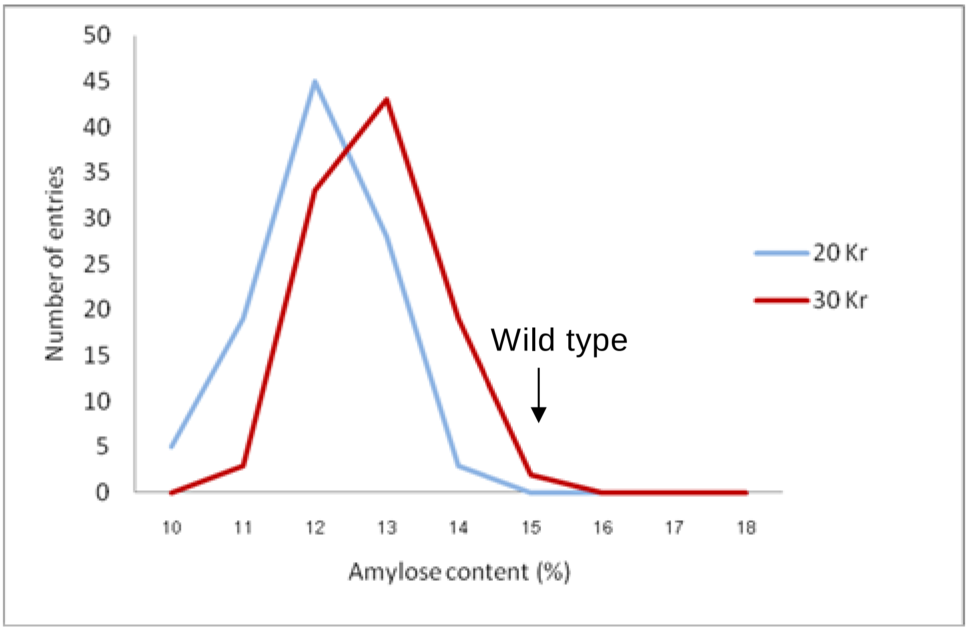


Figure 6. Amylose content (%) of RD15 photoperiod sensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

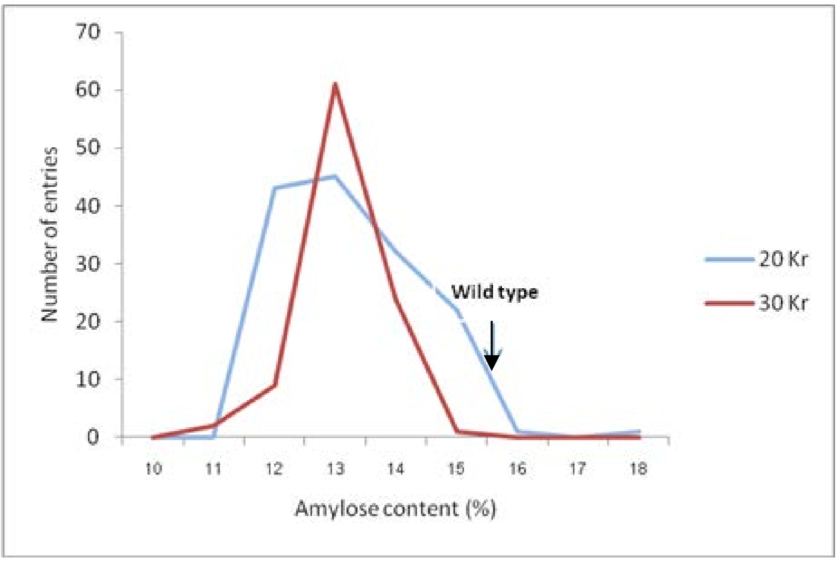


Figure 7. Amylose content (%) of KDML105 photoperiod sensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

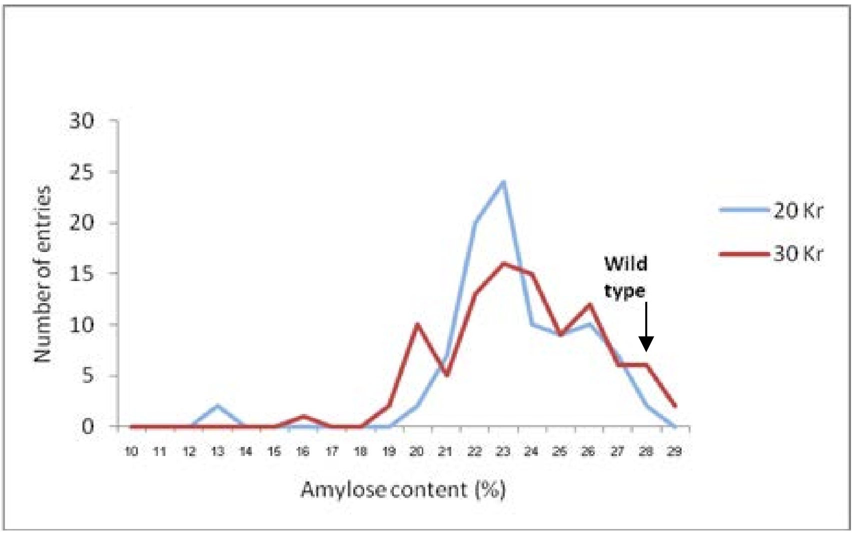


Figure 8. Amylose content (%) of KTH17 photoperiod sensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

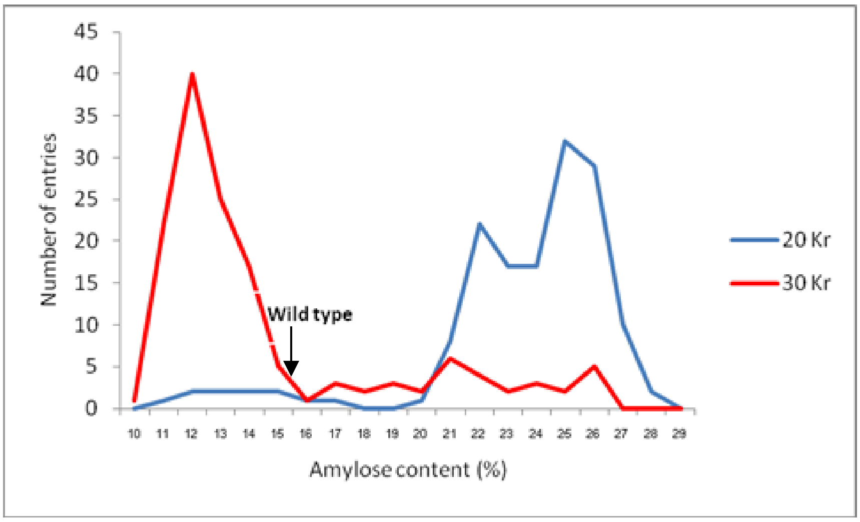


Figure 9. Amylose content (%) of RD15 photoperiod insensitive mutants irradiated with Gamma Ray 20Kr and 30Kr

8.3.3 Grain analyses of mutants

Twenty five M_7-mutant lines of KDML105 and RD15 had been randomly selected to be analyzed on grain physical properties and chemical quality. Grain size had been measured on brown rice in length, width and thickness. Grain shape was determined by Length/Width ratio (L/W). The ratio of L/W that was greater than 3.0 indicated slender grain shape. Visually observation on chalkiness, pericarp color and hull color had been also practiced. Means and standard deviations of mutants and wild types were shown in Table 1.

Table1. Grain physical properties of 25 M_7 mutants of KDML105 and 25 M_7 mutants of RD15 compare to its wild type.

Designation	Size of brown rice (mm)			L/W ratio	Chalkiness	Pericarp color	Hull color
	Length	Width	Thick				
M7-KDML105 mutants	7.16 ± 0.08	2.05 ± 0.02	1.68 ± 0.03	3.34 ± 0.05	dull	White	Straw
M7-RD15 mutants	7.46 ± 0.09	2.14 ± 0.02	1.75 ± 0.03	3.48 ± 0.04	dull	White	Straw
KDML105 (wild type)	7.40	2.12	1.76	3.49	dull	White	Straw
RD15 (wild type)	7.49	2.11	1.74	3.55	dull	White	Straw

It had been found that all mutants shown dull endosperm as well as their wild type. Chalkiness could not be identified in these samples. We have an experience that aromatic rice especially KDML105 and RD15 shown dull endosperm when grown in acid soil. Since our experimental field was acid sulfate soil, dull endosperm occurred. All mutants had white color pericarp and straw color hull as well as their wild types. Mutants of KDML105 had shorter grain length (7.16mm) than their wild type (7.40mm) and shown slightly bold shape (3.34 L/W) compared to their wild type (3.49 L/W). Mutants of RD15 shown almost same grain length but they were wider and thicker that gave smaller L/W ratio. This indicated that mutants of RD15 had bigger grain size than their wild type (Table 1).

Grains from 25 mutants of KDML105 and 25 mutants of RD15 that had been divided to be analyzed on physical properties were synchronously analyzed on chemical quality. Amylose content, Gel consistency, Alkali spreading value, Elongation ratio were important chemical quality that had been analyzed. Total protein (%) was analyzed by Kjaldal Method. Aroma or odor of milled rice had been tested by human sensory test. Aroma expressed in 4 levels of sensory test are 0 = odorless (not aroma), 1 = mild aroma, 2 = aroma and 3 = strong aroma. 2-Acetyl-1-pyrroline (2 AP) (Fig 10) was also analyzed through gas chromatography. **2-Acetyl-1 pyrroline** is a pyrroline that is 1-pyrroline in which the hydrogen at position 2 is replaced by an acetyl group. It is an aroma and flavor compound present in jasmine rice and basmati rice. It is responsible for the 'popcorn' aroma in a large variety of cereal and food products. It is one of the key odourants of the crust of bread and considered to be responsible for the cracker-like odour properties. In bread, it is primarily generated during baking but amounts are influenced by ingredient composition and fermentation conditions.

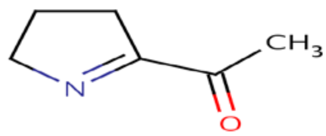


Figure 10. Structural formula of 2-Acetyl-1-Pyrroline

It found that M_7 mutants of KDML105 had low amylase content similar to their wild type. They contained amylose within range of 15.03 to 16.06% of amylose or 15.50≠0.2% in average, while their wild type contained 15.42% amylose. In contrast, M_7 mutants of RD15 contained higher amount of amylase than did of their wild type. The mutants had wider range of amylose content. Their amylase content varied from 15.40 to 27.79% while their wild type contained 15.55% of amylase. Out of these 25 mutants, there were three mutants that contained 15.40, 15.87 and 15.93% of amylose. Mutants of RD15 had harder gel than mutants of KDML105 and their wild type. Mutants of KDML105 had softer gel than their wild type. Alkli spreading value of all mutants was almost not differed from their wild type as well as elongation ratio. Total protein content of mutants averagely higher than their wild type in both KDML105 and RD15. But it was still in normal level of those varieties. Unfortunately, we could not find low protein content mutants in these samples. Mutants of KDML105 contained 9.1% while those of RD15 contained 9.8% protein. Aroma tested by smelling shown different levels of aroma scented between KDML105 and RD15 mutants. Mutants of KDML105 had strong aroma as well as their wild type with score of 3. Mutants of RD15 showed aroma in score 2 of sensory test level as well as their wild type. When 2-acetyl-1-pyrroline had been detected it was found that all mutants of KDML105 contained 2 AP in higher amount than their wild type in both milled rice and brown rice. Brown rice and milled rice of KDML105 mutants contained 2.00 and 2.32ppm of 2 AP, respectively. Their wild type KDML105 contained 2.06 and 2.24ppm of 2 AP in its brown rice and milled rice. We could detect 2 AP in only 3 mutants of RD15 that contained low amylase content. The mutants of RD15 those contained high amylose were not aroma with 2 AP detection, but sensory test detected aroma from those mutants. It would be an expression of other volatile compound. One mutant with 15.87% amylase content showed 2.18 and 2.16ppm 2 AP in its brown rice and milled rice which was almost similar to its wild type (2.99 and 2.06ppm 2 AP) (Table 2). It was very rare opportunity to find aromatic rice with high amylose content in our breeding material. This will be new chance for us to produce various products from our aromatic mutants for world market.

Table 2. Chemical quality of M7 mutants of KDML105 and RD15 compare to their wild type

Designation	amylose (%)	Gel[1] (mm)	Alkali	E.R.[2]	Protein (%)	Aroma[3]	2 AP[4] (ppm) Milled Rice	2 AP[4] (ppm) Brown Rice
M7-KDML105 mutants	15.5±0.2	72.9±3.9	6.9±0.28	1.6±0.03	9.1±0.39	2.7±0.48	2.32±0.35	2.00±0.28
M7-RD15 mutants	25.6±3.8	54.0±9.9	7.0±0.0	1.7±0.03	9.8±0.62	2.0±.00	0.1±0.43	0.1±0.44
KDML105 (wild type)	15.42	69	7.0	1.66	8.77	3	2.24	2.06
RD15 (wild type)	15.55	71	7.0	1.66	9.24	2	2.06	2.99
	Gel[1]	= Gel consistency			Aroma[3]	= aroma by sensory test		
	E.R.[2]	= Elongation Ratio			2 AP[4]	= 2 Acetyl-1-pyrroline detection		

In other purpose, low phytate mutant lines achieved from SPR1 are being evaluated on their grain yield and other agronomic characters. Forty eight mutant lines of low phytic SPR1 are going to be examined their protein content.

8.4 Conclusion

Gamma ray irradiation can be applied for mutation induction in rice. Dosages of 20Kr (200Gy) and 30Kr (300Gy) showed advantage on mutation induction for grain quality improvement. Thai rice varieties namely, SPR1, KTH17, KDML105 and RD15 had been irradiated by 20Kr and 30Kr of gamma ray to induce mutation for various grain qualities such as phytic acid, amylose content and protein and its compositions. Amylose content had been analyzed using standard method for rice developed by IRRI. Globulin, albumin, prolamine, and glutelin were detected using SDS-page analyses. Low phytate mutants had been screened using technique developed by Raboy (2004). Low amylose mutant lines had been achieved from KTH17 which is originally high amylose content local variety. Various amylose content mutant lines from original low amylose content varieties KDML105 and RD15 had been obtained. Some of those mutants contained lower and higher amylose content than did of their wild types. Photoperiod sensitive mutants from RD15, KDML105 and KTH17 had lower amylose content than did of their wild types. Both 20Kr and 30Kr induced similar distribution of mutants in case of photoperiod sensitive. Among photoperiod insensitive mutants derived from RD15, 20Kr induced higher amylose while 30Kr induced lower amylose than its wild type. M_7 mutants shown bigger grain size but shorter length than did of their wild types. Aromatic rice with low amylose content obtained from KDML105 mutants. Newly found aromatic rice with high amylose content derived from RD15 mutants could not be detected on its 2-acetyl-1 pyrroline compound. Those mutants might contain other volatile aromatic compound.

9. Vietnam

Breeding New Rice Variety DT39 Quelamby Gamma Ray

Bang. D. T. T. Nhung N. T. H., Duc N. M. and Hung N. P.

Institute of Agriculture Genetics

9.1 Introduction

In 2012, Vietnam rice export reached 7.7 millions of tons and overcomes Thailand and becomes the biggest country for rice export in the world. However, price of Vietnamese rice in international market is often lower than of Thailand's because the quality is lower. Vietnam government conducts many breeding projects for rice quality improvement. One of the most effective methods for rice quality improvement is mutation breeding by irradiation of Gamma rays ^{60}Co source combined with selection process. Generally, mutation breeding by irradiation of Gamma rays has many advantages compared to other breeding methods such as improvement of one or some characteristics while maintaining the rest characteristics of variety. In Vietnam, rice variety Bacthom No.7 has very good cooking quality, easily cultivated, relatively short growth duration, stable grain yield potential but poor resistance to bacterial leaf blight. That's why, Bacthom No.7 is not widely cultivated in many locations, especially in summer season.

For the above reasons and also as a follow up on the sub-project on composition or quality in rice under the FNCA, we carried out the project "Improvement of Bacthom No.7 by irradiation of dried seeds with gamma rays of ^{60}Co source".

Aims of research: Creation of new rice variety with good cooking, high quality, easily cultivated, short growth duration, high and stable grain yield, and good resistance to bacterial leaf blight.

9.2 Materials and Methods

3,000 dry seed at 13% moisture content was used for irradiation by gamma ray ^{60}Co source at the range doses of 150, 200 and 250Gy in the K hospital, Hanoi. The experiment was started in summer season 2008.

9.3 Results and Discussion

9.3.1 Selection Process

9.3.1.1 M_1 generation: All main panicles in M_1 generation were harvested to develop M_2 population.

9.3.1.2 M_2 generation: At the dose of 150Gy, there were no differences observed. However, from 1,753 individual plants harvested from 200Gy, the frequency of variation observed was around 3%. Mutants with changed characteristics consist of morphological traits, growth duration, tillering ability, hard stem, seed size and so on. From those variations observed, 112 mutant lines were harvested individually for M_3. At 250Gy, obtained variations included high sterility ratio and short plant height. However, most of these variations were not useful for breeding, therefore all plants from this dose were not included in the next experiment.

9.3.1.3 M_3 generation: Single plants were cultivated and evaluated separately, each plant is considered as one family. Selection was continued to discard unfavorable lines for example less seed, less panicle, or sterile plants and so on. Elite individuals in M_3 were selected to develop promising rice lines in M_4 generation. There were some elite individuals with better economic characteristics compared to control variety namely BT15-1, BT15-2, BT15-3 BT20-1, BT20-2, and BT20-4 with long seed, good cooking quality, high grain quality, and resistant to bacterial leaf blight. BT20-2 and BT20-2 have higher 1,000 seeds weight, 25.89g and 31.73g, respectively.

9.3.1.4 M_4 - M_6 generation: From 4 promising lines in M_5, one outstanding lines M_6-20.3 was selected and showed no segregation, hard stem, long seed, stable grain yield potential, and good resistant to bacterial leaf blight. Line M_6-20.3 was named DT39 Quelam and further evaluated in narrow scale for agronomic and biological trait by author. Beside of that DT39 Quelam was officially sent to the National Testing Center for Crop to test for **Value of Cultivation and Use of crop** (VCU) from M_6- M_8 generation (during 2011- 2012).

9.3.2 Evaluation Process

9.3.2.1 Evaluation results of author

Evaluation experiment was carried out in order to compare yield performance and agronomic trait with the control Bacthom 7 in both seasons. The result showed that the yield in spring season was higher than summer season in both varieties, and DT39 Quelam had higher yield than Bacthom 7 in both season.

Table 1: Evaluation agro-biological characters and yield of DT39 Quelam by author (Spring and summer 2010 in Tuliem, Hanoi)*

Variety	Growth duration (days)	Plant height (cm)	No panicle/ plant	Panicle length (cm)	Number seed/ panicle	Seed set ratio (%)	Yield (tons/ha)
DT39 Quelam	Spring: 132	102,2 ± 0,30	5,5 ± 0,28	30,2 ± 0,24	162,3 ± 6,13	89,5 ± 0,32	**6,3** ± 0,31
	Summer: 105	112,2 ± 0,22	5,0 ± 0,30	28,5 ± 0,27	147,3 ± 6,05	88,2 ± 0,25	**5,9** ± 0,30
Bacthom Control	Spring: 134	98,6 ± 0,27	5,2 ± 0,22	22,3 ± 0,30	152,5 ± 5,76	87,5 ± 0,31	**5,6** ± 0,26
	Summer: 106	107 ± 0,32	5,2 ± 0,30	20,6 ± 0,23	140,2 ± 6,20	82,3 ± 0,28	**5,0** ± 0,31

*Transplanting 1 seedling per hill, 45 hills per square meter.

The results in the above table showed that mutant line DT39 Quelam and Bacthom 7 have the same growth duration. Panicle length of DT39 Quelam is longer than Bacthom 7 and number of seeds was also higher. The resulting grain yield of DT39 Quelam was higher than that of Bacthom 7.

Table 2 shows the main characteristics of DT39 Quelam and Bacthom 7.

Table 2: Some main characteristics of mutant DT39 Quelamand control Bacthom 7

	Characteristics	Bacthom 7 (control variety)	DT39 Quelam (mutant variety)
1	Growth duration (days) - Spring - Summer	 130-135 105-108	 130-135 104-106
2	Plant height (cm)	106	110
3	Tilerring ability	Medium	Medium
4	Leaf colour in harvest	Ligh yellow	yellow
5	Flag Leaf type	short, narrow, incline	short, narrow, arrect
7	Stem hardness	Hard	Harder
8	Flowering duration (days)	5	5
9	No. of panicle/hills	5.4	5.5
10	Panicle length (cm)	25.2	28.2
12	No. of seeds/panicle	140	155
13	Abort grain ratio (%)	12-15	12-17
14	Awn	Absent	Short and partly
15	Weight of 1000 seeds (gram)	19.2	23.8
16	Seed type	Small, short (2.0-7.2 mm)	Small, length (2.2-9.3 mm)
17	Husk color	Light brown	Dark brown

The data in table 2 showed that mutant variety DT39 Quelam7 was not so much different from Bachom 7 in 17 phenotype characters. In some main characters such as length of panicle, grain weight of 1,000 seeds, number of seeds per panicle DT39 Quelam was higher than Bacthom 7. DT39 Quelam had harder stem, darker husk color than Bacthom 7.

9.3.2.2 Evaluation results of The National Testing Center for Crop (NTCC)

The National Testing Center for Crops (NTCC) is the unique office that is responsible for independently evaluating and testing new varieties in Vietnam before release.

DT39 Quelam had been sent to NTCC for testing and evaluation in three continuous seasons (summer season 2011, spring and summer season 2012).

Table3: Assessment for agronomic traits of DT39 Quelam variety by NTCC *(Source: NTCC)**

Seasons	Variety	Seedling vigor (points)	Flowering duration (points)	Plant hardness (points)	Plant height (cm)	Growth duration (days)
Summer 2011	BT7 (control)	5	5	3	112.8	106
	DT39 Quelam	5	5	1	120	105
Spring 2012	BT7 (control)	5	5	1	101	139
	DT39 Quelam	1	5	1	106	140
Summer 2012	BT7 (control)	5	5	1	108.5	107
	DT39 Quelam	5	5	1	112.8	104

**points: from 1 to 9 means best to worst*

The result of NTCC for Crop for three continuous seasons (Table 3) showed that generally, DT39 Quelam and Bacthom 7 (BT7) have the approriate growth duration for rice production in many locations in spring season and shorter in summer season, 104-105 days (DT39 Quelam), 106-107 days (Bacthom 7). In spring season 2012, seedling vigor of DT39 Quelam was point 1 (cold tolerant) better than Bacthom 7, point 5. Plant height of DT39 Quelam was higher than Bacthom 7, flowering duration was similar in both varieties.

Table 4 induced that grain weight of DT39 Quelam was higher than Bacthom 7 in three tests. But the data also showed the disadvantage of DT39 Quelam. The abort ratio in three test was higher than Bacthom 7 and the purity (point 1, 5 and 3 for each season) was lower than Bacthom 7 (point 1) in three tests. However, field puritication index of variety could be improved by selection proccess.

Evaluation of 1,000 grain weight of DT39 Quelam and Bacthom 7 (table 5) showed clearly different between two varieties. 1,000 grain weight of origin variety, Bacthom 7 was from 18.5g to 19.2g meanwhile that of DT39 Quelam was from 23.2g to 25.2g in three seasons. It may result that the yielding performance of DT39 Quelam is often higher than Bacthom 7 (table5).

Table4: Variety purity and other traits   *(Source: NTCC)**

Seasons	Variety	Purity (points)	No.panicle per hill	No. Seeds per panicle	Abort ratio (%)	Grain Weight of 1,000 seeds (g)
Summer 2011	BT7	1	5.2	141	17.6	18.5
	DT39 Quelam	1	5.0	147	22.0	23.2
Spring 2012	BT7	1	5.2	152	13.0	18.6
	DT39 Quelam	5	4.9	148	16.6	24.8
Summer 2012	BT7	1	5.6	139	11.9	19.2
	DT39 Quelam	3	5.6	145	22.5	25.2

*points: higher means better.

Table5: Yield performance evaluation in rice production trials *(Source:* NTCC*)Unit: tons/hectare*

Season	Variety	Location												Aver.
		Hưng Yên	Hải Dương	Nghệ An	Thái Bình	Thanh Hoá	Vĩnh Phúc	Hòa Bình	Hà Tĩnh	Hải Phòng	Tuyên Quang	Bắc Giang	Điện Biên	
Summer 2011	BT7	5.47	5.18	-	3.79	4.28	5.07	-	4.81	6.24	4.99	-	-	4.98
	DT39 Quelam	5.67	5.39	-	3.83	4.13	5.59	-	5.15	6.60	5.59	-	-	**5.23**
Spring 2012	BT7	5.23	3.87	6.00	3.56	4.81	5.10	5.73	4.76	-	-	-	-	4.88
	DT39 Quelam	5.47	4.24	5.57	3.41	5.67	6.13	5.63	5.23	-	-	-	-	**5.17**
Summer 2012	BT7	5.79	5.74	-	3.90	4.83	3.47	4.97	-	-	-	5.77	5.37	4.98
	DT39 Quelam	6.06	6.00	-	5.03	5.44	4.60	4.67	-	-	-	6.73	6.20	**5.59**

In three continuous seasons of the test for value of cultivation and use, the mutant variety gave higher yield compare to Bacthom 7. In summer 2011, DT39 Quelam gained 5% compare to Bacthom 7. Especially at 6/8 location trail test in summer 2012, the yield of DT39 Quelam reached average 5.59 t/ha higher by 12.24% while that of Bacthom 7 was 4.98 t/ha.

For the rice production in northern part of Vietnam, Bacthom 7 variety is mainly grown in spring season, because this variety often gives high yield, good quality and moreover but it is very sensitive to bacterial leaf blight in summer season. Therefore, the cultivation area is significant decreased even the quality is accepted for consumption. The result from the NTCC indicated that new mutant variety DT39 Quelam could be used for summer season.

Table6: Resistant ability for pests and diseases *(Source: NTCC)*

Seasons	Variety	Blast		Sheath blight	Bacterial Leaf blight	Brown blight	Stem borer	Leaf roller	Brown hopper
		In leaf	In panicle						
Summer 2011	BT7	-	-	0-1	3-5	-	1-3	0-1	0-1
	DT39 Quelam	-	-	0-1	1-3	-	0-1	1-3	1-3
Spring 2012	BT7	0-1	-	1-3	0-1	0-1	0-1	0-1	0-1
	DT39 Quelam	0-2	-	1-3	0-1	0-1	0-1	0-1	1-3
Summer 2012	BT7	1-3	0-1	1-3	1-3	1-3	1-3	1-3	1-3
	DT39 Quelam	1-2	0-1	1-3	1-3	0-1	0-1	1-3	1-3

*points: smaller means better.

Vietnam is tropical country, where pests and diseases appear every year. So, resistance to pests and diseases in rice is a very important criterial standard for commercial production. The data from table 6 indicated that the resistance to pests and diseases in the field condition for blast, bacterial leaf blight, brown blight, and stem borer of DT39 Quelam was similar to Bacthom 7. Especially, in summer season 2011, DT39 Quelam showed resistance to bacteria leaf blight at 1-3 point while Bacthom 7 showed it at 3-5 point.

Table7: Grain quality evaluation of DT39 Quelam *(Source: NTCC, 2012)*

Variety	Milled rice ratio (%)	Amylose Content (%)	Husked rice length (mm)	Rice ratio of length/width	Collagenic temp.	Gel Durability	Protein content (%)
DT39 Quelam	71.25	17.82	6.47	3.09	(70 – 74°C)	medium	9.10
BT7	69.40	15.65	5.59	2.83	(70 – 74°C)	soft	8.21

Evaluation for the quality according to phenotype of milled and cooked rice indicated that DT39 Quelam had some advantage characters (table 7), and amylose content (17.82%) was higher than Bacthom 7 (15.8%). In this case, the higher amylose content leads to the better quality because low amylose content results in sticky cooked quality. In this experiment, after mutation induction protein content was increased from 8.21% of Bacthom 7 to 9.10% in DT39 Quelam. The most important character of new mutant variety DT39 Quelam is that the cooked rice is soft, not so sticky, high protein and with light aroma.

Table8: Testing for cooking quality of DT39 Quelam *(Source:* NTCC, 2012*)*

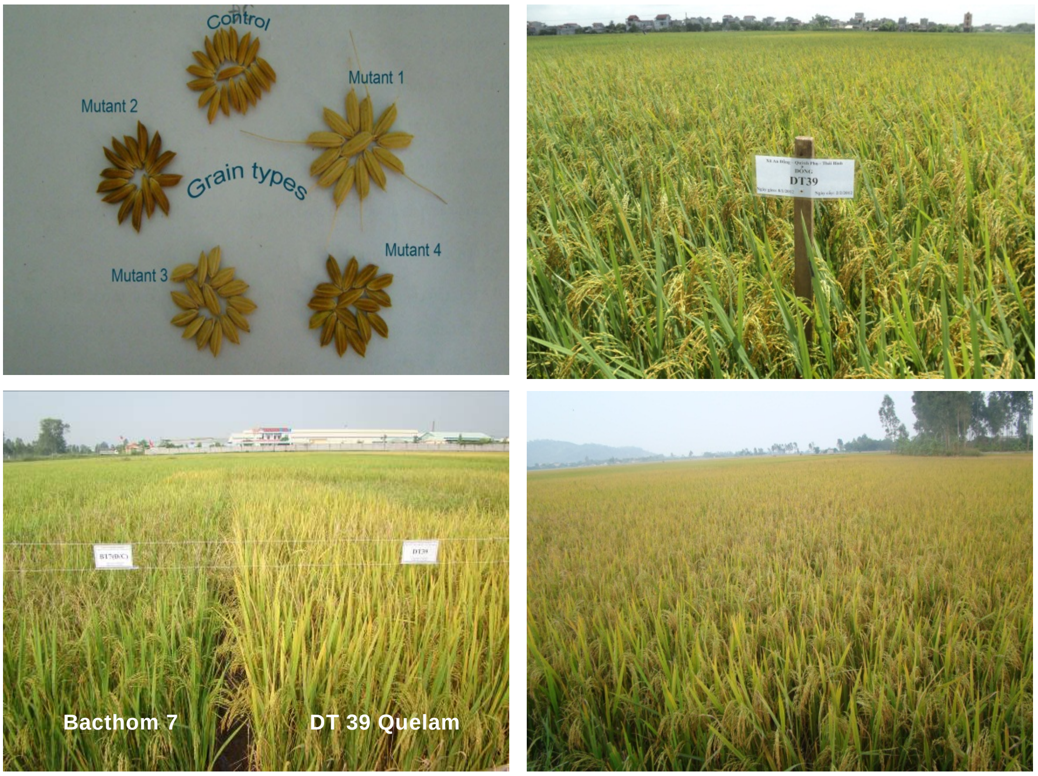
Variety	Aroma	Softness	Stickness	Whiteness	Polish	Attractive
DT39 Quelam	1	4	4	5	3	3
Huongthom 1	2	3	4	5	3	2
Bacthom 7	2	4	4	5	3	3

**points:*

- Aroma: bigger means better; - Softness: smaller means harder

- Stickiness: bigger means sticker ; - Attractive: bigger means better cooking

Test for cooking quality of DT39 Quelam was carried out by committee consisted of 7 persons who worked independent evaluation. The result showed that in most indexes, DT39 Quelam was similar to Bacthom 7 except for aroma which was lower in DT39 Quelam. Due to irradiation treatment, aroma character of Bacthom 7 was decreased in DT39 Quelam.



Photos of DT39 Quelam in experiment and exhibition field

9.4 Conclusion

1) New rice variety DT39 Quelam was created by gamma ray irradiation of cobalt source at the dose of 200Gy from Bacthom 7.
2) Main characteristics of DT39 Quelam: Grow duration in spring season: 130-135 days, summer season: 104-105 days; good tolerance to main pest and disease. Good cooking quality, high yield, hard stem, high protein, better resistance to bacteria leaf blight and better cold tolerance compared to the original variety.

3) DT39 Quelam has been planting in some provinces in northern part of Vietnam in more than 87 ha. In most location of trial field, the mutant variety gave higher yield around 5-14% from the original variety.

9.5 Acknowledgement

The authors wish to express sincerely thank to the IAEA and FNCA for its long time supports on our rice mutation breeding programs and my attendance at the annually FNCA work shop 2009-2013. I also wish to thank Dr. Atsushi Tanaka, Dr. Hirokazu Nakai and Ms. Aki Koike for their kind hospitality and help me during the meeting.

9.6 References

Bang Dao ThiThanh et al., 2008, new rice variety, DT38 selected sucessfully by gamma ray irradiation, poster at International Symposium on Induced Mutation in Plant organized by FAO/IAEA 12-15 August, 2008-Vienna, Austria.

Bang Dao ThiThanh et al. 18-19 August 2011, Primary results of application of carbon ion beam and gamma ray for rice beeding improvement. The IX National symposium on Nuclear Science and Technology, Phanrang-Vietnam

ProccedingInternational Symposium on Induced Mutation in Plant, FAO/IAEA 12-15 August, 2008-Vienna, Austria

Recently progress of rice mutation breeding and germplasm in China. Plant breeding report, Vol.1 No.1; 2006 pag 4

THINH DO KHAC et al., 2008. Socio-Economic impacts of mutant rice varieties in Southern Vietnam, reported at International Symposium on Induced Mutation in Plant organized by FAO/IAEA 12-15 August, 2008-Vienna, Austria.

Yu Zengliang, Introduction to ion beam Biotechnology, 2006, P 183-199