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.

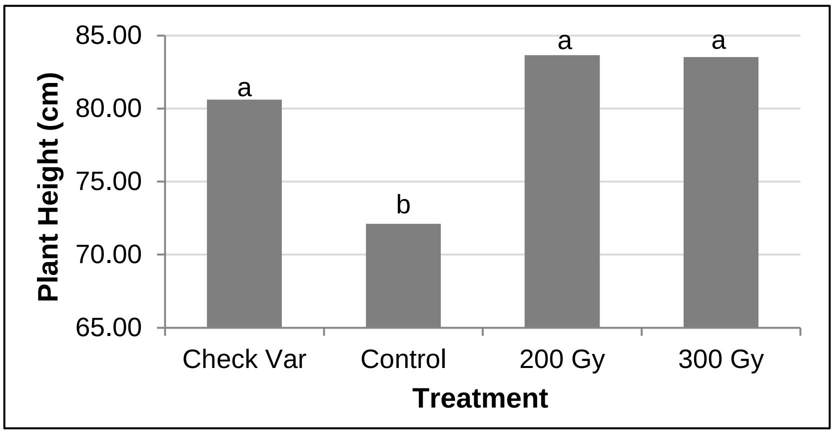

Figure 1. Plant height at maturity as affected by gamma irradiation at M_7 generation (bars with the same letters are not significantly different at 5% level based on DMRT)

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.

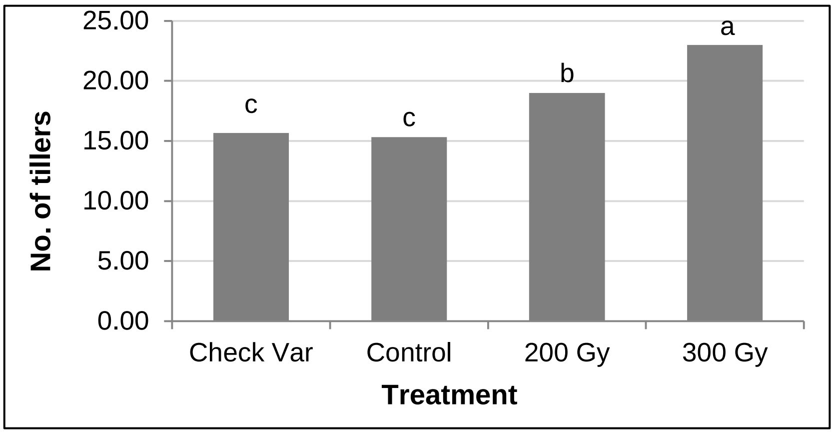

Figure 2. Number of tillers per plant as affected by gamma irradiation at M_7 generation (bars with the same letters are not significantly different at 5% level based on DMRT)

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

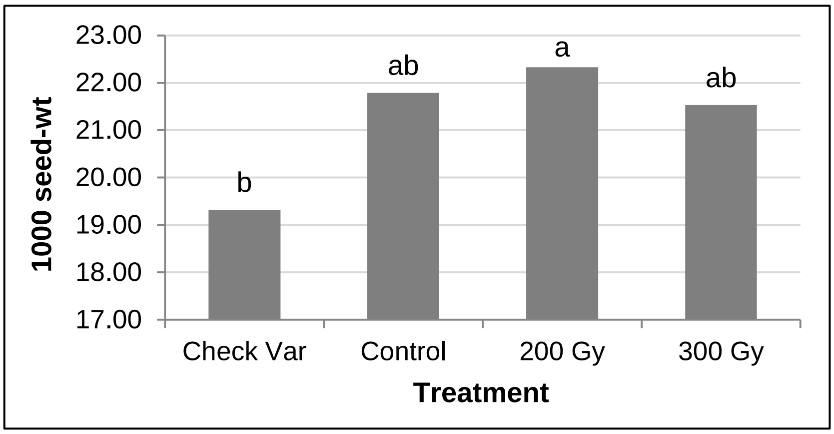

Figure 3. 1000 seed - weight (g) as affected by gamma irradiation at M_7 generation (bars with the same letters are not significantly different at 5% level based on DMRT)

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

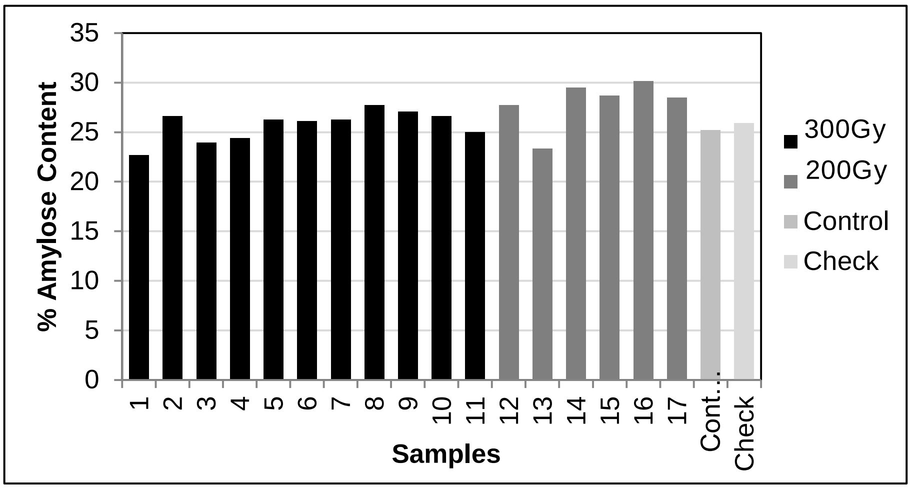

Figure 4. Percent amylose content using the quantitative method of analysis

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

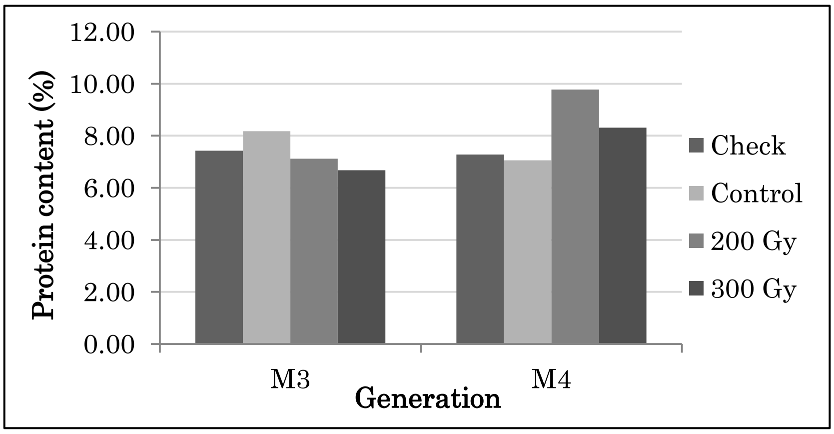

Figure 5. Protein content at different treatments in two generations

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.