

## **Improvement of Traditional Rice Varieties by Gamma Irradiation in the Philippines**

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### **Abstract**

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

Seeds were subjected to acute gamma ray treatment and grown as M<sub>1</sub> generation together with the non-irradiated materials as control. Comparisons were made between plants treated with 200 Gy and 300 Gy, and also with the non-irradiated plants. Early flowering plants with reduced height, more tillers, and higher number of filled grains per panicle were observed from the segregating M<sub>2</sub> generation but no selection was made. M<sub>3</sub> generation was grown from bulked seeds and putative mutant plants with the desired agronomic characteristics were selected based on the results of statistical analysis. The M<sub>4</sub> generation did not show any line that is better than the control in any of the parameters measured.

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

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

## Introduction

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

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

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

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

## Materials and Methods

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

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

Seedlings were soaked in biofertilizer for 30 minutes before transplanting. A Randomized Complete Block Design (RCBD) with at least three replicates was followed every cropping season. During the second year or at M<sub>2</sub>, radiation-modified kappa-carrageenan (RMKC) solution, which has been developed as a plant growth promoter at PNRI from another project, was applied as foliar spray at 100 ppm. Foliar application was done every week for 1 month. On the third year or at M<sub>3</sub>, the use of RMKC was modified by increasing the concentration to 200 ppm but applying only three times: 14 days after transplanting (DAT), 35 DAT, and 60 DAT. At M<sub>4</sub>, the recommended application rate followed was 200 ppm at 15 DAT, 30 DAT, and 45 DAT.

Proper cultural management was employed during the whole cropping season in all generations of planting. The activities included the maintenance of proper water level at different stages of plant growth, manual control of golden apple snail, installation of nets to prevent birds from eating the grains and trapping of field rats. The same methodologies were followed throughout the conduct of the project (up to M<sub>4</sub> only).

Sowing of seeds of putative mutant plants selected from M<sub>3</sub> generation was delayed for 1½ years. This is largely attributed to a chain of unprecedented events starting off with health trepidation leading to an early retirement in December 2015 for Ms. Barrida as Project Leader. By June 2016, a new President of the Philippines was installed, bringing new national and institutional priorities, and a status quo in the organizational set-up of some projects. Finally, by July 2017, Mr. Aurigue received his designation as the new Project Leader, thereafter resuming the planting, evaluation and selection of putative mutant lines as planned for the project.

Seeds from selected M<sub>3</sub> plants were sown on paddy soil placed in black polyethylene plastic (PEP) bags on July 31, 2017 for M<sub>4</sub> generation plants. The seedlings were

transplanted into two rice paddies on August 17 and 18, 2017 following RCBD with three replications. Data for vegetative traits were gathered on November 6, 2017. Necessary cultural practices were employed until harvesting on November 28, 2017. Data gathering on yield parameters were conducted afterwards when the intact seeds have been sun-dried properly with less than 12% moisture content.

## Results and Discussion

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

**Table 1.** Agronomic characteristics of non-irradiated and irradiated Umangan at M<sub>1</sub> generation

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

\* not significant

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

**Table 2.** Agronomic characteristics of non-irradiated and irradiated Native Borie at M<sub>1</sub> generation

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

\* not significant

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

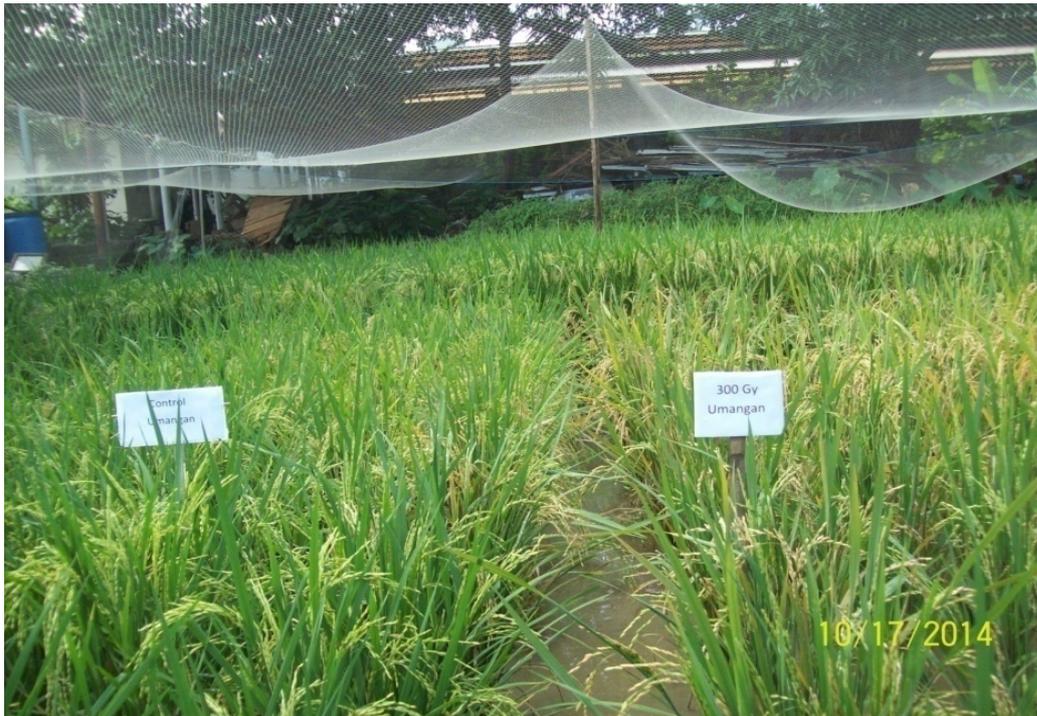
In the M<sub>2</sub> generation, results for Umangan also showed early flowering at 200 Gy with 70 DAT followed by 300 Gy with 77 DAT. At 200 Gy, there was 7.15% reduction in plant height. Increase in the number of grains per panicle was noted at 300 Gy (Table 3 and Figure 1). Meanwhile, for Native Borie, early flowering at 85 DAT was observed for both 200 Gy and 300 Gy. The control plants took 100 DAT to flower. There was an increase in number of tillers per plant as 16 tillers were counted for 200 Gy and 17 tillers for 300 Gy (Table 4).

**Table 3.** Agronomic characteristics of non-irradiated and irradiated Umangan at M<sub>2</sub> generation

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

**Table 4.** Agronomic characteristics of non-irradiated and irradiated Native Borie at M<sub>2</sub> generation

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



**Figure 1.** Control and M<sub>2</sub> plants derived from Umangan irradiated with 300 Gy of acute gamma radiation.

In the M<sub>3</sub> generation, the number of days to flowering, number of tillers per plant, and the total number of filled grains per panicle were significantly affected by the treatments for Umangan (Table 5). At 200 Gy, plants flowered 14 days earlier than the control, whereas at 300 Gy plants flowered 11 days earlier than the control. Meanwhile, the number of tillers per plant increased from 11 to 14 for plants derived from both 200 Gy and 300 Gy. The number of grains per panicle obtained at 200 Gy (152 seeds) and at 300 Gy (132 seeds) is comparable to those of the control plants (147 seeds).

In Native Borie, the plant height, number of days to flower, and number of tillers per plant were significantly affected by the treatments at M<sub>3</sub> (Table 6). Plants derived from 200 Gy and 300 Gy flowered 7 days earlier than the control. Reduction in plant height was about 10.46% and 6.46% less than the control at 300 Gy and 200 Gy, respectively. The number of tillers per plant increased by four at 300 Gy (18 tillers) and by two at 200 Gy (16 tillers) compared to the control (14 tillers). Figure 2 shows foliar application of RMKC solution during tillering stage.

**Table 5.** Agronomic characteristics of non-irradiated and irradiated Umangan at M<sub>3</sub> generation

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

\* not significant

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

**Table 6.** Agronomic characteristics of non-irradiated and irradiated Native Borie at M<sub>3</sub> generation

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

\* not significant

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



Umangan



Native Borie

**Figure 2.** Spraying of radiation-modified kappa-carrageenan solution on two traditional rice varieties at M<sub>3</sub> generation during tillering stage.

In the M<sub>4</sub> generation, there was no significant differences between means for each parameter observed (Table 7). The selected putative mutant lines were similar to their respective control parent. Likewise, results of statistical analysis imply that the yield of selected putative mutant lines was not different from their respective control parent (Table 8). Some of the putative mutant lines growing in the field are shown in Figure 3.



**Figure 3.** Different putative mutant lines at M<sub>4</sub> generation.

**Table 7.** Vegetative characteristics of non-irradiated and putative mutant lines of traditional rice varieties at M<sub>4</sub> generation

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

**Table 8.** Yield parameters of non-irradiated and putative mutant lines of traditional rice varieties at M<sub>4</sub> generation\*

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

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

\*Mean of 10 samples

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

ns = not significant

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

### Summary and Conclusion

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

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

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

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

### **Acknowledgment**

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