Mutation breeding for improvement of aromatic rice mutant by using ion beam irradiation

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Abstract. One of the effective methods in plant breeding is the mutation breeding. Recently new techniques in mutation breeding has risen by using heavy ion beam irradiation. The local rice variety, Pare Bau was used to study the effect of heavy ion beam for aromatic rice improvement. Dry seeds of rice were irradiated at dose of 10 Gy Argon ion. Selected mutants from M2 were planted on M3 generation to check their stability in growth performance and early flowering. Results show that one M3 mutant line showed significantly lower plants height and larger percentage of fertile grain in comparison to control. Therefore, these results indicate that heavy-ion irradiation may provide useful genetic resources for high yield breeding on early generation of mutant line.

1. Introduction
Rice (Oryza sativa L.) is one of the world’s most important staple food crops. It is grown in 111 countries ranging from the flood plains of Bangladesh to the Himalayan foothills of Nepal and from the rain forests of Indonesia to desert plains of Australia [1]. Indonesia is an agricultural country which is located on the equator, it has a tropical climate and rich in biodiversity, including rice. Rice breeding has contributed the increase of rice production.
Almost every region in Indonesia has local rice varieties that have been cultivated through many generations. One of local rice in Indonesia is aromatic rice variety “Pare Bau”. This variety is one in the high land of Toraja, South Sulawesi, Indonesia. Indonesian local varieties generally have long panicles, few tillers, high posture with rounded and hard-to-fall seeds, broad and long leaves, insensitive photoperiod, and intermediate to high amylose content. Each variety adapted well to the area where the plant originated. The taste of rice was according to the tastes of the local people and had a specific aroma. The characters of local rice cause low yield.

Before the 1970’s, most Indonesian farmers used local rice varieties with their distribution covering a narrow area according to different environmental conditions, and even since the release of modern rice varieties, such as PB 5, Pelita 1-1, PB-26, PB-36, and other varieties such as for planthopper resistant, distribution of local rice varieties become increasingly narrow and pushed to the highlands (up land race), dry land, and some rainfed areas that have not been planted with superior varieties. This resulted in the occurrence of genetic erosion, requiring strong efforts to preserve local rice for the erosion recovery [2].

In general, plant breeding program consists of establishment of the population variability, selections, evaluation and testing of the selection results. Establishment of populations and their variability were gained through hybridization [3], mutation [4], varietal introduction [5] or explorations [6].

Indonesia first released the rice mutant variety in 1982 under the name of Atomita-1, and since then 14 varieties of rice mutant have been released [7]. Plant mutation breeding has effectively changed a few traits without altering other traits that are preferred. This is certainly very useful for improving plant varieties [8].

One of plant mutation breeding methods is using heavy ion beam irradiation [9]. In rice, mutants produced have been useful for genetic and physiological assessments of yield-limiting factors [10]. Many mutant genes controlling important traits like plant height, tiller number and panicle length have been cloned and characterized at the molecular level [11,12].

Through our mutation breeding program, it is expected to produce a variety of early flowering, new high yielding rice cultivars and other characters the support the improvement of rice quality and competitiveness. The objectives of the present research are to study the effect of ion beam irradiation on local aromatic rice variety “Pare Bau” and to evaluate early mutant varieties which have the same characteristics of the original Pare Bau.

2. Materials and methods

Aromatic rice variety ‘Pare Bau’ was used for this study. Dry seeds were irradiated by Argon ion with irradiation dose of 10 Gy at the RIKEN Nishina Center, Wako, Saitama, Japan. Irradiated seeds were referred to as the M₁ generation. The M₁ seeds were germinated and sown in a seedling plots and grown in a green house for 3 weeks. Twenty-one-day-old seedlings were used for transplanting to the rice field in a two-lane row system which was prepared two weeks before planting. Each line has 50 seedlings with planting distance at 30 x 30 cm. One seedling was planted per planting hole. Since mutants are mostly recessive, selection was not done in M₁ generation and panicles were collected from all M₁ plants which has 50 seeds each panicle.

Seeds from the primary panicles of M₁ were harvested for M₂ seeds. M₂ plants of each treatment were sown by using single plant per hill with non-irradiated control. The primary panicles of M₂ plants form each treatment were collected for M₃ generation and sown in a tray by single plant per hill. The selected mutants were evaluated in the summer cultivating season of 2018 (April-August).

Planting conditions at vegetative and generative phases were presented in Figure 1 and 2. The snails attack was controlled by drying the fields for a week and removing the snails manually. By this way the snails would be difficult to move from one area to another to minimize the spread and plants damage. In addition, we installed plastic sheets 50 cm tall around the rice fields to prevent mouse from entering into the fields. While during the generative phase pests were controlled with insecticide
sprays, while the birds were controlled manually by covering the planting area with nylon net (1 x 1 cm hole).

Figure 1. Field condition (a) Sowing, (b) Planting, (c) Nylon net covering.

Means of the variables for each mutant line were calculated. The number of plants used as samples were 10 plants each line. The parameters used were days to flowering, plant height, number of tillers, panicle length, and percentage of fertile grain.

Relationship between agronomic characters was analyzed using simple correlation analysis. Analysis was taken based on average data in each line. Simple correlation analysis was computed using computer software package of SPSS.

3. Results and discussion

Days to flowering is important agronomic trait, controlled by different endogenous genetic factors as well as environmental signals [13]. The results showed that the mutant lines flowered 4-8 days earlier than the controls (Table 1). The mutant line which has the earliest flowering is PB-A-12.2.12 lines (Figure 2).

There were reduction for some plant height of mutant. The mutant lines used had a plant height in the range of 151.4-160.0 cm, while wild type was 156.8 cm tall. The plant height of PB-A.8.1.5 is significantly shorter than control. Based on result of Morita et al., [14] that ion beam irradiation can reduced plant height of rice.

Number of tillers and panicle length didn’t have any significantly differences between mutant and the wild type. All mutant lines have percentage of fertile grain in the range of 52.4 - 67.1%, while control was 32.2%. The percentage of fertile grain per panicles of mutant line PB-A 8.1.5 were significantly higher than the control. However, fertile grain weight is a quantitative trait, greatly influenced by environmental fluctuations.

Table 1. Average and Standard deviation of days to flowering, plant height, number of tiller, panicle length, and percentage of fertile grain in local aromatic-rice (“Pare Bau” in Toraja, Indonesia)

| Line          | Days to Flowering (Day) | Plant Height (cm) | Total tiller (No.) | Panicle Length (cm) | Rate of fertile grain (%) |
|---------------|-------------------------|-------------------|--------------------|---------------------|---------------------------|
| Control       | 128 ± 1.4               | 157 ± 3.4         | 9 ± 2.1            | 32.7 ± 2.6          | 32.2 ± 16.7               |
| PB-A.5.3.36   | 124 ± 2.3               | *                 | 152 ± 7.7          | *                   | ss                        |
| PB-A.5.3.45   | 121 ± 1.4               | **                | 154 ± 6.7          | **                  | ss                        |
| PB-A.6.1.12   | 123 ± 1.9               | **                | 160 ± 4.8          | **                  | ss                        |
| PB-A.8.1.5    | 123 ± 1.0               | **                | 151 ± 6.2          | *                   | ss                        |
| PB-A.12.2.12  | 120 ± 1.0               | **                | 159 ± 4.1          | **                  | ss                        |

* denotes significant difference between mutant and control at p < 0.05.
** denotes significant difference at p < 0.01.
Each value is the average ± standard deviation (n=10). * Significant difference between each line and control was estimated by T-test. * p<0.05. Not significant (p>0.05)

Table 2. Correlation Coefficient among morphological characters in five aromatic mutant lines.

| Days to Flowering | Plant Height | Total Tillers | Panicle Length | Percentage of Fertile Grain |
|-------------------|--------------|---------------|----------------|-----------------------------|
| Days to Flowering | 1            | -0.465<sup>ns</sup> | -0.873<sup>ns</sup> | 0.046<sup>ns</sup> | -0.316<sup>ns</sup> |
| Plant Height      | 1            | 0.628<sup>ns</sup>  | -0.747<sup>ns</sup> | 0.035<sup>ns</sup>  |                  |
| Total Tillers     | 1            | -0.272<sup>ns</sup> | 0.057<sup>ns</sup>  |                  |                  |
| Panicle Length    | 1            | 1              | 0.166<sup>ns</sup>  |                  |                  |

<sup>ns</sup>= non-significant; **=Correlation is significant at the 0.01 level (2-tailed).

Correlation analysis of characters can be used as tool for indirect selection. From the result in Table 2, there were no correlation between characters for all mutant lines. It may be expected that the characters were controlled by different gene.

Figure 2. Appearance of control and five mutant M<sub>3</sub> lines and control at days to flowering

4. Conclusions
From the findings of present research, it has been concluded that mutant induced by heavy ion beam has earlier flowering than control and larger percentage of fertile grain. This indicates that the treatment with ion beam irradiation can improve plant characters as desired.

Acknowledgement
The author would like to express deepest gratitude to Directorate General of Resources for Science, Technology and Higher Education, Indonesia, Ministry of Education and Culture for the scholarship.

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