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Developing Early-Flowering or Photoperiod-Insensitive Mutants from Rice var. Ayarmin (Machando) by Using Gamma Radiation from ^{60}Co Source

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Abstract: ***Ayarmin is photoperiod-sensitive rice variety which can be grown once a year. It is cultivated in the Ayeyarwady, Sagaing and Mandalay regions in Myanmar and preferred by local customers. In this article, Ayarmin was used to develop photoperiod-insensitive or early-flowering mutants using gamma radiation. Dry seeds of rice, Ayarmin were irradiated with the dose of 200Gy, 250Gy, 300Gy and 350Gy of gamma rays from ^{60}Co source. The main panicles in M_1 generation were bulk-harvested and sown as M_2 generation in summer for the selection of photoperiod-insensitive mutants. In M3 generation, potential mutant lines were sown in the field along with the control plants and early-flowering mutant lines were selected in the monsoon 2016. Eleven mutant lines were obtained in M3 generation; AYM-200B, AYM-200C, AYM-200D, AYM-200E, AYM-200F, AYM-200I and AYM-200J of 200Gy, AYM-250A of 250Gy, AYM-300C of 300Gy, and AYM-350A and AYM-350D of 350Gy. Some of these mutant lines had good quantitative and qualitative characters.***

Keywords: Ayarmin, photoperiod-sensitive rice, photoperiod-insensitive, early-flowering, mutants, gamma radiation

1. Introduction

Rice is one of the most important crops in Myanmar and is grown on about two-thirds of the country's total cultivated area [1]. Myanmar is the world's sixth-largest rice producing country and 61% of its 53 million people depend on agriculture for their livelihood. There are six distinct regions: the western, northern, and eastern mountain ranges; the delta area of the Ayeyarwady and Sittoung rivers; the coastal strips; and the central plain or dry zone. The land area is about 66 million ha, of which only 11 million ha are under cultivation for annual crops. Rice is grown on over 8 million ha [2].

Ayarmin is the rice variety cultivated in the Ayeyarwady, Sagaing and Mandalay regions in Myanmar. Its amylose percent is 25 and it has good eating quality [3]. It is mostly cultivated in Kyaukse, the central plain or dry zone of Myanmar and is preferred by local customers since it has good eating quality but it is very late in maturity and sensitive to photoperiod [4]. Early-maturing variety allows farmers to increase cropping intensity from two to three or four crops of rice per year. So, early-maturing and high-yielding rice varieties are very useful for increasing rice production [5]. Being photoperiod-sensitivity of Ayarmin, long-day treatments can prevent or considerably delay its flowering. Photoperiod or day length is of great importance in the heading of rice plants [6]. For photoperiod-sensitive cultivers, the optimum photoperiod is about 10 h [7]. The heading stage of Ayarmin used to start in November and December (wet season), the day length of those months is about 11 h in this dry zone, Kyaukse [8]. The rice varieties cultivated in dry season head in May and June, in which day length is about 13 h and Ayarmin rice variety can't flower. Although the demand for Ayarmin is high, it can be grown once a year in this region. Photoperiod-insensitive variety can be grown more than one crop per year [9].

Mutation has been successfully employed in breeding of several food crops [10]. It has been used to develop many crop varieties with improved quantitative, qualitative and economic value [11]. Mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars [1].Officially released mutation-derived varieties such as rice, wheat, cotton and sunflower have made a major economic impact in many countries [12]. Realizing the importance of this technique in agricultural development of a country, this research work was initiated in 2014 in Kyaukse, Mandalay region, Myanmar. This study was intended to produce the potential mutant lines with desired early-flowering and photoperiod-insensitive characters through induced mutation.

2. Materials and Methods

Plant material used in the research was the rice variety Ayarmin (Machando). This variety is widely grown cultivar in the Ayeyarwady, Saging and Mandalay regions in Myanmar because of its good eating quality. Pure and healthy dry seeds of Ayarmin were irradiated with the dose of 200Gy, 250Gy, 300Gy and 350Gy of gamma rays from ^{60}Co source at the Department of Atomic Energy (DAE) at Yangon in Myanmar. Non- irradiated seeds were used as control.

The M_1 seedlings were transplanted from the seedbed to the farm using spacing of 15cm×15cm with one seedling per hill. M_1 population is 2400 plants/treatment. Since mutants are mostly recessive, selection was not done in M_1 generation [13]. The main panicle in each plant was bulk harvested in monsoon 2015.

In summer 2016 when the wild type of Ayarmin could not flower, the M_2 seedlings were transplanted from the seedbed to the farm using spacing of 20 cm x 20 cm with one seedling per hill. M_2 population are 2350 plants of 0 Gy (AYM-control), 3300 plants of 200 Gy, 2280 plants of 250 Gy, 2360 plants of 300 Gy, and 2040 plants of 350 Gy. During this period, M_2 plants could be selected for photoperiod-insensitive mutants. Photoperiod sensitivity is an obvious trait and easy to be selected in dry season whereas long day period presented [9]. Then, M3 seeds of mutant lines had been separately harvested as single hill per line. Those photoperiod-insensitive mutants were continuously screened for important agronomic traits.

The M_3 seedlings of the selected mutants were transplanted from the seedbed to the farm using spacing of 20 cm x 20 cm with one seedling per hill. During the monsoon 2016, the early-flowering mutant lines could be selected. Selection was carried out in M_3 generation for traits of agronomic significance such as early-flowering, plant height, panicle length, the effective tiller, filled grains per panicle, thousand grains weight and yield per hill. Their grain qualities were determined for milling quality, milling yield, head rice ratio, grain length, grain shape, amylose content (AC), gel consistency (GC), and gelatinization temperature (GT).Finally, the data were subjected to analysis of variance (ANOVA) using SPSS 16.0 software

3. Results and Discussion

3.1 Studying Plant Heights and Effective Tiller Production in M_1 generation

The In monsoon 2015, it was found that the heights and the numbers of effective tillers per hill were 150 cm and 18 tillers for AYM-control, 153 cm and 6 tillers for 200Gy M_1 plants, 147 cm and 6 tillers for 250Gy M_1 plants, 142 cm and 6 tillers for 300Gy M_1 plants, 124 cm and were 2 tillers for 350Gy M_1 plants at the harvesting time. According to the data, the effective tiller productions are significantly reduced in all treatments. The more the radiation doses increase, the less the effective tillers produce.

3.2 Observation on Yield Component Characters in M_2 generation

The In summer 2016, it was observed that the AYM-control (non-irradiated) plants did not flower while some mutants have completely matured even under the presence of long day period. The life period of rice var. Ayarmin (AYM-control) is 145 days in monsoon and it does not flower in summer [3] but the selected mutant lines are photoperiod-insensitive and completely matured in May and June (summer) 2016. From M_2 population, desirable eleven mutant lines being photoperiod-insensitive were isolated on the basis of agronomic traits. The yield components of potential mutant lines and control plants were presented in "Table 1"

3.3 Observation on Yield Component Characters in M_3 generation

The selected potential mutant lines were evaluated against non-irradiated control plants in M_3 generation. M_3 generation was cultivated during July to December in rainy season. In this generation, some of mutant lines such as AYM-200B, AYM-200D and AYM-200E were flowered 29 days earlier than the control plants. The flowering time of AYM-200F was 27 days earlier and those of AYM-200C and AYM-200J were 24 days earlier than that of the control plants. Among the mutants, potential mutants obtained from 200Gy treatment plants flowered earlier than other mutants obtained from 250Gy, 300Gy and 350Gy treatment plants, except AYM-200I. The other mutants also flowered earlier than AYM-control as shown in Fig.1 and Table 2. Early varieties are less sensitive to photoperiod and will head in the field under the longer days of mid-summer [14].

Figure 1: Field view of control plants and early-flowering mutant lines in M_3 generation (monsoon 2016)

The plant heights of all potential mutants and AYM-control are not significantly different at 0.05 levels according to Duncan's New Multiple Range Test, except AYM-300C and

AYM-350A. Other mutants produce more effective tillers than AYM-control although AYM-200I and AYM-control are not significantly different. AYM-200C and AYM-200D are not significantly different with AYM-control in the comparison of number of filled grains per panicle but yield per hill of both mutants are significantly higher than AYM-control. They also showed the best performance on yield components among the potential mutant lines in M_3 generation. The comparative performance of potential mutant lines for early-flowering with AYM-control on yield components in M_3 generation (monsoon, 2016) were presented in Table 2.

3.4 Physical Characteristics of Rice Grains in M_3 Generation

The mutant lines varied in milling degree ranging from 85.34% to 89.58% while the AYM-control was occurred having 88.93% of milling degree. The results from the Table 3 showed that AYM-350A possessed the highest percentage of milling recovery (69.76%) although the other mutant lines and AYM-control had a lower mean value of total milling recovery. However, both all mutant lines and AYM-control had a higher percentage of head rice ranging from 52.88% to 66.24%. The head rice percentage of the mutant line, AYM-200F was about 10% lower than that in AYM-control and its length/ width ratio (2.6) were also lower than that of AYM-control (2.9). It was shown in Table 3 that all mutant lines and AYM-control were not significantly different in the length/ width ratio, except AYM-200F and they are all medium shape, except AYM-250A (slender).

3.5 Chemical Characteristics of Rice Grains in M_3 Generation

Figure.2 shows that the gel consistency (mm) of mutants was similar with each other although AYM-350A had longer gel length, 39.5 mm. The check variety, Kaw Linn Kyauk Nyin had longest gel length, 100 mm. It was classified as soft groups in gel consistency (61-100 mm). There was no significant difference in gel consistency between AYM-control and all mutant lines. Thus, they were classified as hard category but AYM-350A was medium/hard as shown in Table 4.

The amylose content of Kaw Linn Kyauk Nyin (check variety) was 3.3 %. So, this sticky rice was classified as low amylose category. As shown in Table 4, the amylose content of AYM-control was 25.1% and there were no large differences compared to mutant lines but that of the mutant, AYM-350A was only 21.9 %.

Gelatinization temperature (GT) of Kaw Linn Kyauk Nyin was between 55-69 °C and included in the low GT group. Gelatinization temperature of AYM-350A was between 70-74 °C and included the intermediate GT group, but all other mutant lines and AYM-control were between 75-79 °C. In comparison with AYM-control, all other mutant lines had similar gelatinization temperature and included in the high GT group as shown in Table 4. According to the results, AYM-350A with lower amylose content than other mutants becomes more sticky, fluffy or soft. It had been reported that rice varieties with very low amylose content become very sticky, moist and tender on cooking, whereas varieties with intermediate amylose content become fluffy, soft, moist and tender, and those with high amylose content become fluffy and dry and harden on cooling [15]

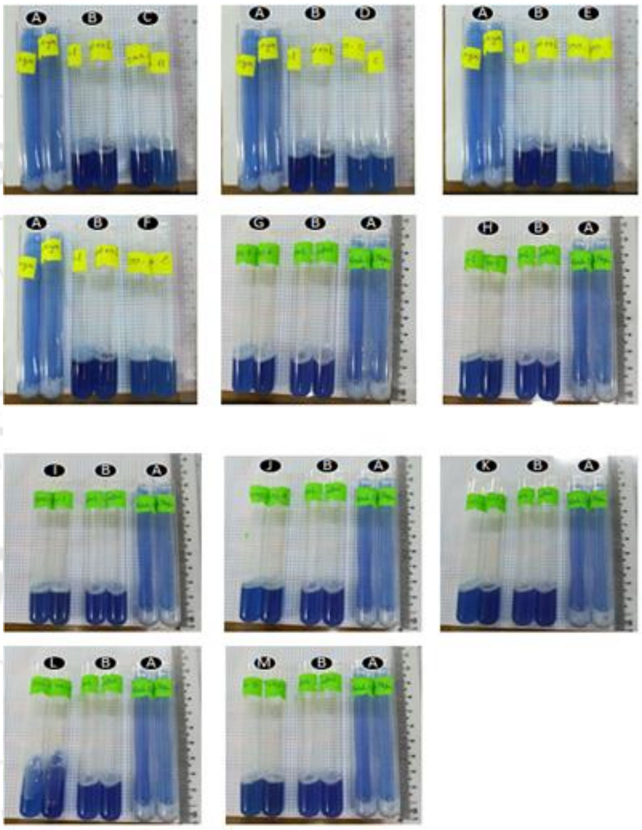

Figure 2: Gel consistency test (A) Kaw Linn Kyauk Nyin (check variety); (B) AYM-control; (C) AYM-200B; (D) AYM-200C; (E) AYM-200D; (F) AYM-200E; (G) AYM-200F; (H) AYM-200I; (I) AYM-200J; (J) AYM-250A; (K) AYM-300C; (L) AYM-350A and (M) AYM-350D.

Table 1: Yield components of potential mutant lines for photoperiod-insensitivity and Ayarmin-control in M_2 generation (summer, 2016)

Potential Mutant Lines	Month of Flowering	Days to 50% Flowering (DAS)	Plant Height (cm)	Effective Tiller	Panicle Length (cm)	Number of Filled Grains per Panicle	1000 Grains Weight (g)
AYM-control	n/a	n/a	155	n/a	n/a	n/a	n/a
AYM-200B	May	104	104	7	26	122	17.01
AYM-200C	June	117	106	6	29.5	160	20.35
AYM-200D	June	127	114	7	25	110	19.21
AYM-200E	June	127	115	10	26	118	18.17
AYM-200F	June	132	122	9	31	227	17
AYM-200I	June	132	128	3	29	132	17.77
AYM-200J	June	132	112	12	25	105	18.87

AYM-250A	May	125	140	12	24.5	148	19.25
AYM-300C	June	127	97	7	22	122	19.37
AYM-350A	June	121	118	13	25.5	110	21.28
AYM-350D	June	135	147	8	26	173	21.68

Table 2: Comparative performance of potential mutant lines for early-flowering with Ayarmin-control on yield components in M_3 generation (monsoon, 2016)

Potential Mutant Lines	Days to 50% Flowering (DAS)	Plant Height (cm)	Effective Tiller	Panicle Length (cm)	Number of Filled Grains per Panicle	Yield per Hill (g)	1000 Grains Weight (g)
AYM-control	112	142.12^{a}	12.42^{d}	23.69^{ef}	126.94^{a}	30.14^{cd}	19.9
AYM-200B	83	138.67^{a}	21.58^{ab}	25.83^{ab}	104.72^{bcd}	38.23^{bc}	17.2
AYM-200C	88	138.83^{a}	20.50^{bc}	26.18^{a}	117.39^{ab}	43.51^{ab}	18.4
AYM-200D	83	142.92^{a}	25.00^{a}	25.62^{abc}	113.32^{abc}	48.72^{a}	17.4
AYM-200E	83	134.83^{a}	17.67^{bc}	24.47^{de}	100.58^{bcde}	30.43^{cd}	16.9
AYM-200F	85	134.75^{a}	18.67^{bc}	24.57^{de}	98.02^{cde}	31.80^{cd}	17.3
AYM-200I	99	143.58^{a}	16.33^{cd}	25.09^{bcd}	101.74^{bcde}	30.81^{cd}	19.1
AYM-200J	88	137.96^{a}	20.58^{bc}	24.78^{cd}	84.84^{e}	34.40^{bcd}	19.7
AYM-250A	90	141.71^{a}	21.00^{abc}	22.17^{g}	90.69^{de}	42.51^{ab}	22.7
AYM-300C	97	113.92^{b}	18.42^{bc}	20.70^{h}	84.87^{e}	30.18^{cd}	19.4
AYM-350A	97	122.33^{b}	17.17^{bc}	22.51^{g}	68.59^{f}	27.22^{d}	23.0
AYM-350D	99	135.17 ^{a}	17.08^{bc}	23.46^{f}	100.82^{bcde}	35.34^{bcd}	20.8
CV%		9.79	30.05	7.82	23.71	33.00	

Means with the same letter in a column are not significantly different at 0.05 level according to Duncan's New Multiple Range Test.

Table 3: Physical characteristics of rice grains from early-maturing mutants and AYM-control in M_3 generation

Samples	% of Milling degree	% of Milling recovery	% of Head rice	Length/Width ratio	Shape	Size (mm)
AYM-control	88.93	68.8	63.52	2.9	Medium	Medium (5.88)
AYM-200B	89.18	64.64	60.24	2.8	Medium	Medium (5.63)
AYM-200C	85.34	62.4	57.2	2.8	Medium	Medium (5.63)
AYM-200D	87.13	63.36	60.8	2.9	Medium	Medium (5.71)
AYM-200E	87.04	62.88	57.28	2.9	Medium	Medium (5.75)
AYM-200F	87.17	64.16	52.88	2.6	Medium	Short (5.29)
AYM-200I	88.04	64.8	60.88	2.9	Medium	Short (5.33)
AYM-200J	88.3	66.4	62.88	2.9	Medium	Medium (5.75)
AYM-250A	86.93	67.6	65.12	3.1	Slender	Medium (6.29)
AYM-300C	86.07	66.24	61.84	2.8	Medium	Short (5.13)
AYM-350A	88.71	69.76	66.24	3.0	Medium	Medium (6.21)
AYM-350D	89.58	68.8	63.76	2.9	Medium	Medium (5.71)

Table 4: Chemical Characteristics of Rice Grains from Early-maturing Mutants, Control and Check Sample in M_3 generation

Samples	Amylose Content (%)	Alkali Spreading Value	Gelatinization Temperature (°C)	Gel Consistency (mm)
Kaw Linn Kyauk Nyin (Check)	3.3	6	55-69 (Low)	100 (Soft)
AYM-control	25.1	3.83	75-79 (High GT)	26.5 (Hard)
AYM-200B	24.1	3.33	75-79 (High GT)	26 (Hard)
AYM-200C	24.9	3.5	75-79 (High GT)	28 (Hard)
AYM-200D	25.2	3.83	75-79 (High GT)	24 (Hard)
AYM-200E	24.6	3.67	75-79 (High GT)	23 (Hard)
AYM-200F	23.7	3.5	75-79 (High GT)	28.5 (Hard)
AYM-200I	23.5	3.5	75-79 (High GT)	26 (Hard)
AYM-200J	24.1	3.5	75-79 (High GT)	28.5 (Hard)
AYM-250A	24.5	3.67	75-79 (High GT)	26 (Hard)
AYM-300C	23.5	3.33	75-79 (High GT)	26.5 (Hard)
AYM-350A	21.9	4.17	70-74 (Intermediate)	39.5 (Medium/Hard)
AYM-350D	23.5	3.83	75-79 (High GT)	26.5 (Hard)

4. Conclusion

In this research, gamma radiation induced eleven potential mutants being photoperiod-insensitive or early-flowering. The highest frequency of photoperiod-insensitive or early-flowering mutants was found in 200Gy. The mutant AYM-300C obtained from 300Gy treatment plants showed the shortest plant type. The lowest amylose contents of the grain were observed in the mutants of 350Gy such as AYM-350A and AYM-350D. In monsoon 2016, it was found that the earliest flowering mutants were AYM-200B, AYM-200D and AYM-200E and they flowered 29 days earlier than the control plants. . Among the mutants, AYM-200C and AYM-200D should be the most selectable mutants because they had good quantitative and qualitative traits. These mutants can be utilized in the breeding programme for elite varieties of the rice. It still needs to study more the selected mutant lines being photoperiod-insensitive or early-flowering obtained from Ayarmin rice variety through gamma radiation. For the objective of early-flowering in Ayarmin (Machando), we should emphasize to do the research by using 200Gy of gamma radiation.

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