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Induction and Selection of Early Maturing Less Shattering Mutations in Rice, by Gamma Irradiation

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ABSTRACT. This study was undertaken to induce and select early maturing and less shattering mutants in cold tolerant rice variety Pd 85-3, by Gamma irradiation.

Preliminary investigations showed a LD_{50} value of 32 kR and most effective doses as 30 and 35 kR Gamma rays. The first generation after irradiation (M_1) was planted at the Central Agricultural Research Institute, Gannoruwa in 1988/89 Yala, with 5000 plants per treatment. The M_2 population was planted at Pussellawa Rice Breeding Station at a cooler climate with 50,000 plants per treatment.

M_1 generation showed a reduction in germination, tiller number and plant height. A high sterility (mean more than 50%) and lower than 50% survival rate was observed. Agronomic studies revealed that irradiation had caused higher variation in plant characteristics and the mean values were adversely affected. In the M_2 generation 121 mutants with less than 10% shattering were selected. Seventy nine of them had less than 25% sterility showing cold tolerance. The other 42 were less tolerant to cold. Among these, 14 mutants matured 20 to 25 days earlier than the control and six of the early mutants were also less sterile showing cold tolerance.

The other favourable characters observed in these mutants are short stature and higher total number of seeds. Nineteen dwarf and 93 semidwarf mutants were found. A high total number of seeds (more than

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1000) were observed in 9 mutants and 34 mutants had between 501 to 1000 seeds. All these mutants are to be further selected in the next generations.

INTRODUCTION

Increase in rice production during the last two decades has been achieved mainly by breeding high yielding varieties. However, the rice yields in adverse environments such as low temperature areas in the up and mid country regions of Sri Lanka are low. Elevations exceeding 600 m are particularly liable to cold damage (Peiris and Jayawardena, 1985). Approximately 8,100 ha of paddy lands are affected annually by cold (Herath, 1965). These areas are mostly confined to Nuwara Eliya, Badulla, Kandy and a small part of Matara districts. The yield reduction due to cold in Sri Lanka is 30 - 60% in elevations of 900 - 1200 m and 20 - 30% in elevation of 750 - 900 m (Kaneda, 1972).

The low temperature effects on rice are low germination, stunted root shoot growth of seedlings, delayed heading, incomplete panicle exertion, uneven maturity, high spikelet sterility and a prolonged maturity period (Kaneda and Beachell, 1974). All these factors ultimately cause reduction in grain yields, which is about 2.0 t/ha in these areas.

Critical temperature for cold injury is below 20°C (Satake, 1976). The most sensitive growth period in rice is the young microscopic stage which appears 10 days before anthesis (Satake and Hayase, 1970). The coldest months in these areas are from December to February. Flowering during *Maha* season coincides with this period causing high sterility. Although old varieties like *Hathyal*, *Muhudukiriyal* and *Kalu Heenati* are tolerant to cold they give low yields. The new improved varieties are susceptible to cold (Dissanayake *et al.*, 1985).

Therefore, it is necessary to breed suitable cold tolerant high yielding varieties. The rice breeders in the Central Agricultural Research Institute Gannoruwa, Peradeniya had undertaken this task and bred a promising variety Pd 85 - 3 (Nadin/H_7), which is able to produce significantly high yields (4.5 t/ha) under low temperature conditions (Peries *et al.*, 1986). Two draw - backs of this variety are high grain shattering levels (32%) and a longer maturity period (150 to 155 days). Therefore, the main objective of this study was to induce low shattering and early maturing mutants in Pd 85-3 while, retaining its cold tolerant ability.

Induction of mutants using gamma rays as the mutagenic agent was used in this experiment. The main reasons for using mutation breeding, was the ability of mutagen to alter one or two characters without changing the whole genotype of a well adapted variety and the relatively short time period (3 - 3½ yr) taken for the breeding programme (Sigurbjornsson and Micke, 1974). Breeding mutants is widely used in the world, producing over 1300 varieties in recent years (Anonymous, 1989_a).

Therefore, the present study was undertaken to induce and select early maturing and less shattering mutants in the cold tolerant rice variety Pd 85-3 by gamma rays.

MATERIALS AND METHODS

Preliminary investigations were carried out at the Central Agricultural Research Institute (CARI) Gannoruwa in 1987/88 *Maha* to find out the most effective LD_{50} value (which cause 50% lethality in the first generation after irradiation).

The LD_{50} value was found by an estimated method (Gaul, 1963). Pure viable seeds of Pd 85-3 which had a moisture content of 13% was irradiated with 15, 20, 25, 30, 35 and 40 kR Gamma rays by the Cobolt 60 unit at Gannoruwa. The experimental design used was the completely randomised block design with four replicates. The seeds were sown in rows and the distance between two rows was 2 cm. Plants were kept under green house conditions. The seedling height and root length were measured in uprooted 10 days old seedlings where the first leaf had ceased growth. The LD_{50} was calculated as that which caused 20-30 % growth reduction of 10 day old seedlings.

The experimental procedure used in this mutation breeding programme is shown in the following diagram:

Preliminary Investigations (LD_{50} Value) —— Irradiation ——> M_1 generation (5000 plt/trt)

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Selected mutants <—— Selection —— M_2 generation (50,000 plt/trt)

M_1 population (1^{st} generation after irradiation) was planted at CARI Gannoruwa in 1988/89 *Yala*. About 1½ kg of seeds per treatment were irradiated with 30 and 35 kR Gamma rays and their germination was tested. The treated seeds along with the control a were sown in rows in an upland nursery. Seedling height of 500 plants per treatment and the control were measured after 21 days. Seedlings were transplanted in the field using one plant per hill at a spacing of 15 x 25 cm in 3 m wide plots. Two lines (40 plants) of Pd 85-3 were planted as the control after every 25 rows of treated plants. Altogether 5000 plants of 30 and 35 kR treatments were planted.

The plant height and tiller number of 500 randomly selected plants of treated and control populations were scored at every two weeks. An agronomic study of these plants was done at maturity, recording column height, panicle number, thousand grain weight, shattering and sterility percentages. No selection was done in this generation. All the plants were harvested individually and stored in a cool dry place until planting.

The M_2 population (2^{nd} generation after treatment) was grown at the Rice Breeding Station, Pussellawa in 1989/90 *Yala*, where plants were grown in a cooler climate. The M_1 seeds were sown in lines in an upland nursery from May to June 1989. As the M_2 population was very large, sowing and transplanting were done in batches, to make handling easier. Seedling height of 1000 plants per treatment and the control were measured in 21 days. These seedlings were transplanted in the field as 3 row, 2 row and 1 row progenies (60, 40 and 20 plants respectively) according to the availability of plants in each line. Spacing and transplanting were the same as for M_1. Approximately 50,000 plants per treatment were planted. Air and water temperature were recorded throughout the study at Pussallawa. Field observations of any visual variation were recorded.

Selection was done in the M_2 generation. The plants which flowered early were tagged with dated waxed labels. At maturity, first the early flowering plants and then the others were hand selected for shattering according to Standard Evaluation System for rice (Anonymous, 1980). The panicles were firmly grasped and pulled over the whole length to collect the shattered seeds. The plants with less than 10% shattering were selected and characteristics such as plant height, maturity period, total number of seeds, shattering and sterility percentages were recorded. Plants which had cold susceptible characters such as incomplete panicle exertion, irregular maturity, sterile white panicles, panicle tip degeneration and late maturity were discarded.

One hundred and twenty one mutants selected were categorised according to the standards given below;

a) Trait – Shattering %, sterility % and maturity period (Anonymous, 1989_b)

b) Trait – Plant height (Anonymous, 1980)

c) Trait – Total number of seeds
According to the variation found in the selected mutants.

All the selected seeds were cleaned and stored in a cool dry place.

RESULTS AND DISCUSSION

The preliminary investigations revealed that the estimated LD_{50} value was 32 kR. Therefore, the most effective doses were 30 and 35 kR Gamma rays for variety Pd 85–3. Similar results were obtained by Sharma (1985) where the LD_{50} value for irradiated rice ranges from 25 to 40 kR Gamma rays, with an average value of 32.5 kR.

The germination percentage of the M_1 plants had a 20 and 29% reduction in 30 kR and 35 kR respectively when compared to the control. Kumar and Mallick (1986) also reported a reduction of germination when four rice varieties and their crosses were irradiated with 30 and 40 kR Gamma rays. In the nursery 20 and 25 completely white (Albino) plants were identified in 30 kR and 35 kR seedlings respectively. In the field green and white streaks (*viridis*) mutants were also observed. The Albino plants died after about 10 to 14 days. Sharma (1985) has reported a wide spectrum of chlorophyll mutations in irradiated rice.

Plant height and tiller number of 500 randomly selected plants showed a reduction of both characters in treated plants throughout the growing period. This shows that irradiation had a reducing effect on both characters. Thai-Cong-Tung *et al.*,(1971) also observed decreases in tiller number, root and shoot growth of 3 rice varieties irradiated with thermal neutrons.

An important observation made in the M_1 generation was the high percentage of sterility in the irradiated populations. The average mean value for sterility in 30 kR was 74.8 and 35 kR was 55.3 whereas the control was 20.8 (Table 1).

The increase in variances of the treated populations were high (Table 1). Results showed that irradiation had caused high sterility. Mutation breeders have observed that higher M_1 sterility give rise to better mutants in the M_2 generation (Wu and Zhang, 1983).

From a total of 5000 plants in the M_1 generation only 2041 in 30 kR and 1204 in 35 kR survived until harvest. The percentage survival is 40.8 and 24.0 in 30 and 35 kR, respectively. It shows that more than 50% of the plants had died in the first generation after irradiation. The validity of the LD_{50} value which caused 50% lethality is confirmed by these results.

The agronomic study revealed that the average mean values of the characters were adversely affected by irradiation (Table 1). Gamma irradiation also increased the variability of all the traits (Table 1). Similar behaviour was observed by Sharma (1985), where irradiation had caused higher variability in rice. This is an important aspect in plant breeding, since larger genetic variation provided a wide range for selection of useful mutants (Anonymous, 1977).

Table 1. Statistical Estimates of the M_1 Generation.

Character	Mean	Range	Variance	% increase in Variance	Skewness
1. Percentage shattering					
Control	30.0	13 - 66	115.13	-	0.66 **
30 kR	32.6	4 - 76	226.80	96.99	0.47 **
35 kR	31.3	6 - 72	256.30	122.60	0.41 **
2. Percentage Strility					
Control	20.8	11 - 41	44.22	-	0.24 NS
30 kR	74.8	12 - 99	320.70	625.23	-0.71 **
35 kR	55.3	13 - 99	605.10	1268.50	0.81 **
3. Culm Height (cm)					
Control	94.5	75 - 101	81.00	-	0.14 NS
30 kR	78.7	62 - 105	102.60	26.00	0.24 NS
35 kR	77.4	61 - 106	103.07	27.25	0.13 NS
4. Panicle Number					
Control	8	4 - 17	7.72	-	0.81 **
30 kR	7	2 - 18	8.93	15.80	0.44 **
35 kR	7	2 - 15	8.11	5.18	0.64 **
5. Thousand Grain weight (g)					
Control	21.4	18 - 25	1.66	-	0.29 NS
30 kR	20.9	18 - 28	4.24	155.40	1.21 **
35 kR	20.5	19 - 26	1.90	14.45	0.01 NS

** Significant at 1% level
NS = Not Significant

The M_2 generation planted at Pussellawa showed poor germination of the lines in the nursery. Out of 2041, 30 kR lines, 836 germinated and out of 1204, 35 kR lines, 884 germinated. It may be due to the high percentage of sterility shown in the M_1 generation. Altogether 50043, 30 kR plants and 50160, 35 kR plants were planted in the field. Five and ten chlorophyll mutants (*Viridis*) in 30 and 35 kR plants were also observed in the M_2 generation.

Flowering occurred during the months of August, September and October where the average air temperature was 18.9°C, 18.6°C and 18.5°C respectively. Since these plants were subjected to critical low temperatures, below 20°C at the young microscopic stage of flowering, (Satake, 1976) screening for cold temperature was carried out. The number of early flowering (90 to 102 days) plants in 30 kR and 35 kR were 218 and 166 respectively.

Selection for shattering was done at maturity. Altogether 20 plants in 30 kR and 101 plants in 35 kR which had less than 10% shattering, were selected. Below ten percent is the acceptable rate for cultivated rice varieties in Sri Lanka (Anonymous, 1983).

The details of the 121 categorized mutants are given in Table 2. All the 121 selected mutants had less than 10% shattering. This is a vast improvement from variety Pd 85 - 3 which had 32% shattering. Among these, 2 mutants had less than 1%, 49 had between 1 - 5% and the other 70 had 6 - 10% shattering (Table 2).

Fourteen mutants matured between 130 to 135 days which were 20 to 25 days earlier than the control. Six of these mutants had less than 25% sterility, which shows their tolerance to cold. Therefore, the main objective of inducing and selecting early maturing and less shattering mutants in variety Pd 85 - 3, while retaining its cold tolerant ability was achieved. Similar results were obtained by Yang *et al.*, (1986) where mutant M112 was identified. It was less shattering, cold tolerant and provided 10% increase in yield. Sreedharan (1979) also obtained a mutant 6 - 106 which was cold tolerant and less shattering. Ramlie *et al.*, (1990) isolated a semi dwarf mutant which was early maturing and less shattering.

Table 2. Categorized Numbers of the 121 Selected Mutants in $2M_2$ Generation.

Character	Scale	Number of Plants 30 kR	35kR	Total
1. Percentage Shattering				
Less than 1%	very low	1	1	2
1 - 5%	low	4	45	49
6 - 25%	moderate	15	55	70
2. Percentage Sterility				
1 - 5%	low	-	3	3
6 - 25%	moderate	17	59	76
25 - 50%	moderately high	3	39	42
3. Maturity Period				
121 - 135 days		4	2	6
136 - 150		9	66	75
150 - 185		7	33	40
4. Plant Height				
Less than 80 cm	Dwarf	5	14	19
81 - 110	Semi dwarf	10	83	93
110 - 130	Intermediate	15	4	9
5. Total no. of seeds				
1001 - 1500		3	6	9
501 - 1000		6	28	34
1 - 500		11	67	78

Of these mutants 79 had less than 25% sterility, which shows their cold tolerance. The other 24 were more susceptible to cold. Therefore, further selection should be carried out in future generations.

Variation in plant height such as 9 intermediate, 93 semi dwarf and 19 dwarfs were observed. The dwarf and semi dwarf mutants will be more responsive to nitrogen and resistant to lodging thus, giving a higher yield (Ganeshan and Whittington, 1983).

Nine of the mutants had more than 1000 seeds while 345 mutants had seeds between 501 to 1000. The short stature of the plants and high total number of seeds observed in some mutants can be a positive indication for yield improvement in future generations. All these mutants are to be further selected in the next generations.

The earliness of mutants will help the crop to escape the cold and may enable the farmers to cultivate two rice crops annually. The low shattering of mutants will considerably reduce losses during harvesting and handling of paddy. Therefore, these 121 mutants will provide a suitable base for breeding new high yielding varieties for farmers in cold affected areas of Sri Lanka.

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