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DEVELOPING HIGH YIELDING, LODGING TOLERANT BIROI TYPE RICE LINES THROUGH MUTATION BREEDING TECHNIQUE

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Abstract

Red rice is increasing its popularity for its functionality and healthy food value hence raised the market demand now-a-days. But the yield of this group of rice is very poor. Local rice Biroi having red pericarp, low yielding and lodging susceptible variety was irradiated with five (100, 150, 200, 250 & 300 Gy) doses of gamma ray. A total of 6 M_3 plants were first selected from irradiated 1805 M_2 plants. Two years replicated yield trial experiments were conducted in different locations of Bangladesh. Among them two mutants were selected for higher yield and moderate lodging tolerance. The selected mutants Biroi-250-2-2 and Biroi-250-2-3 showed 11% to 13% higher yield than their original parent which gave red pericarp. These two mutants might be a good breeding material for red rice, lodging resistance variety development program.

Key words: Red pericarp, lodging tolerance, high yield, biroi

Introduction

Rice is the main food for more than half of the world's population and is the staple food for the people of Bangladesh, constituting over 91% of the food grain production, and providing 62% of the calorie with 46% of the protein intake in the average daily diet (HIES, 2010). The rice production area in Bangladesh is approximately 15.4 million hectares (ha) producing 63.64 million tons of rice annually (BBS, 2019).

There are around 40,000 variants of rice in the world. Red colored rice is a variety of rice that contains anthocyanin. During the milling process, only the husk is removed from the rice grains but retain all nutrients, vitamins, and minerals intact in the bran layer and in the germ. Red rice is enriched with antioxidants and magnesium compared to polished rice. It is used in breads, nutty, colored pasta, vinegar, alcoholic beverage, drugs, and cosmetics (Patindol *et al*., 2006). It has antioxidant activity with procyanidins (Oko *et al*., 2012) and exists great gene diversity which make red rice important to cultivate as resistance to drought, flood, submergence, alkalinity, salinity, and resistance to pests and diseases (Chaudhary and Tran, 2001). But traditional red rice varieties also typically have weak stems, low tillering ability, and long droopy leaves, turn yellow during grain development, and become logged at maturity. However, farmers still plant them widely because they can be grown under low inputs and produce a reasonable yield under the seasonal environmental

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conditions to which modern varieties are not adapted. Nonetheless, this limited yield makes farmers less interested in cultivating red rice despite its high medicinal value and use as a functional food (BRRI, 2016).

Biroi is a traditional local rice variety of Bangladesh containing red pericarp in grain and considered as red rice. Higher plant height, longer duration and lodging susceptibility are the key characteristics of Biroi cultivar (BINA, 2017). Stem lodging hindrances the photosynthetic effectiveness of the canopy that affects the grain filling (Weng *et al*., 2017). Hence the rice grain yield and quality reduced by 60-80% as photosynthesis is directly associated with lodging (Jency *et al*., 2020).

Modern breeding techniques including mutation could improve the lodging (Jency *et al*., 2020) and yield of red rice to overcome this problem. Successful breeding for crop improvement, however, depends on genetic variation in the parents (BRRI, 2016) which limit breeding progress and/or yield and quality crop improvements (Corneous and Sneller, 2012).

Mutation can play a vital role in improving desired characters of red rice. The technique has been successfully utilized by Bangladesh Institute of Nuclear Agriculture (BINA) and many other research institutes on different crops (Miah and Bhatti, 1968; Azad *et al*., 2012).

As red rice is a very good source of human health concern, we designed an experiment to improve this rice through mutation breeding. We had developed some lines of Biroi those possess earliness, high yielding and moderate lodging tolerant comparing to parent. The objective of this research is to develop a lodging tolerant premium quality red pericarp rice variety that will maintain the nutritional balance as well as the food demand in the world prospective.

Material & Methods

The local popular germplasm Biroi was used as experimental material and collected from different area of Gafargaon and Fulpur Upazila, Mymensingh were irradiated through physical mutagen (gamma rays) from Electronics Section, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh. About 500 mature and viable seeds (moisture 12%) of the Biroi variety were packed in butter paper covers and supplied for each dose of irradiation at 150, 200, 250, 300 and 350Gy with 60_{Co} (Cobalt-60) irradiator (Model: IAEA-TECDOC-539, IAEA, 1990) at BINA irradiation chamber, Mymensingh. Germination and survival test were done from irradiated seeds. Seeds were grown dose wise with dense spacing in the raised nursery beds established at the plant breeding division, BINA. After a month, the seedlings were transplanted in a non-replicated design with 10 m^2 area with the spacing of 15 cm in plant and 20 cm in row (Kato *et al.*, 2020). Recommended doses of nitrogen, phosphorus, potassium, sulphur and zinc were applied in the form of Urea, TSP, MoP, Gypsum and Zinc Sulphate @ 195, 50, 70, 55, and 5.6 kg ha^{-1} respectively. Cultural and intercultural practices were followed as and when necessitated. Data on plant height (cm), effective tillers hill^{-1}, panicle length (cm), filled and unfilled grains panicle^{-1} were recorded from five randomly selected competitive hills at maturity. Maturity time was assessed plot basis. Grain yield was recorded from an area of 1.0 m^2 which later converted to t ha^{-1}.

Results & Discussions

Determination of Lethal Dose (LD_{50}) of Mutagens

As the relative effectiveness of the mutagens is essential to determine the correct dose/concentration of the mutagens (Zhao *et al*., 2016) LD_{50} was calculated (Fig. 1). Probit analysis (Finney, 1978) was carried out using seed germination values (Poornima *et al.*, 2017) for gamma rays to determine the LD_{50}. The expected LD_{50} value for the seeds was 250 Gy (Fig. 1) and LD_{50} was found 300 for the hard coated seeds of rice (Huang *et al.*, 2009). To comprehend, higher doses caused injury to the cell, which may be vital, and inhibited many cellular activities, eventually causing death of the cells. It had been noticed that, due to these chaos of the mutagens, seeds treated at high doses 350 Gy most of the seeds did not germinate, or their seedlings could not survive beyond a few days (Liu *et al*., 2012). The social acceptance of gamma-ray irradiation is quite high and numerous useful practical mutant varieties have been developed in the past 60 years (Nakagawa and Kato, 2017). This method has been proven to be useful in generating higher yield mutants, which hence contribute to the discovery of novel genes for higher yield.

Growing of M_1 generation of Biroi rice in T. Aman season

Germination% was decreased with the increase of the irradiation doses (Fig. 1). The result was agreed with Liu *et al*., 2012. The plant height was also decreased gradually with the increase of gamma rays doses. Zanzibar and Sudrajat (2016) was reported the similar result. Finally, the M_1 seeds from the survived plants were bulked dose wise and kept for growing as M_2 generation in the next growing season.

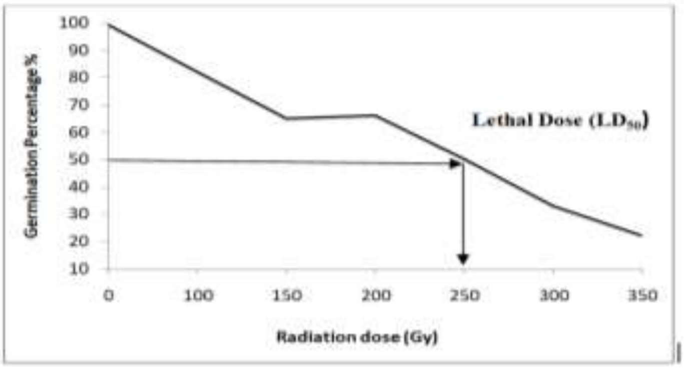

Fig. 1. Determination of LD_{50} based on germination percentage of irradiated seed

Screening of the candidate mutant of M_2 plants and M_3 lines

A wide range of variations was noticed among the M_2 generations for novel altered phenotypes in the traits *viz*., plant height, effective tiller hill^{-1}, panicle length, filled grain panicle^{-1}, unfilled grain panicle^{-1}, yield plant^{-1}. Five seeds of each panicle were bulked together from each dose (150, 200, 250, 300 and 350 Gy) of irradiation. The total number of 1805 M_2 plants was collected from irradiated Biroi variety according to the homogeneity of the panicles. Then M_3 population was grown in the field for selection. In our selection, we considered their positional advantage over other plants and their position in the field so that the effect of non-uniform soil fertility could be eliminated. In M_3 generations several mutant has been selected from different doses. Two mutants 2-1 & 2-2 were selected from 300 Gy and eight mutants were selected from 200 Gy of irradiation. The progeny were recorded as Biroi-300-2-1, Biroi-300-2-2, Biroi-200-2-1, Biroi-200-2-2, Biroi-200-2-3, Biroi-200-2-4, Biroi-200-2-5, Biroi-200-2-6, Biroi-200-2-7 and Biroi-200-2-8 respectively (Table 1). Likewise, six M_3 mutants were selected from 250 Gy of irradiation and named as Biroi-250-2-1 to Biroi-250-2-6. Total 16 mutants from M_3 generation were planted along with biroi variety as a check. Five plants from each line excluding border plants were harvested for yield evaluation. All the panicles of each plant were cut at the neck and collected into individual paper bags. The panicle weight of each plant was measured as the individual plant yield. To fulfill the research objectives the mutants having white pericarp and lodging susceptible was discarded even they are short duration and moderately high yielding mutant (Table 1). Only six mutants from 250 Gy dose having red pericarp and moderately tolerant to lodging were selected and planted for M_4 generation.

Table 1. Grain color, lodging status and yield of some M_3 mutants of Biroi during 2016-17

Variety/mutant	Duration (days)	Pericarp color	Lodging condition	Yield (tha^{-1})
Biroi-300-2-1	136	White	Susceptible	4.30
Biroi-300-2-2	138	White	Susceptible	4.46
Biroi-200-2-1	141	White	Susceptible	5.20
Biroi-200-2-2	133	White	Susceptible	5.33
Biroi-200-2-3	145	White	Susceptible	5.80
Biroi-200-2-4	146	White	Susceptible	5.65
Biroi-200-2-5	142	White	Susceptible	4.98
Biroi-200-2-6	137	White	Susceptible	4.50
Biroi-200-2-7	128	White	Susceptible	4.25
Biroi-200-2-8	135	White	Susceptible	4.32
Biroi-250-2-1	140	Red	Moderately tolerant	5.30
Biroi-250-2-2	139	Red	Moderately tolerant	6.50
Biroi-250-2-3	145	Red	Moderately tolerant	6.00
Biroi-250-2-4	143	Red	Moderately tolerant	6.66
Biroi-250-2-5	137	Red	Moderately tolerant	5.10
Biroi-250-2-6	142	Red	Moderately tolerant	5.56
Biroi (Parent)	148	Red	Susceptible	4.30

Performance of selected mutants in M_4 and M_5 generation

In M_4 generation, maturity date among the mutants ranged from 123-141 days (Table 2) and was shorter than parent (145 days). Among the mutants three had relatively longer days (133-141) to mature and produced higher yield. The long maturity duration allows the plants to attain higher yield and biomass (Dixit *et al*., 2014). Plant height ranged between 85.60 to 136.8 cm with Biroi-250-2-3 being the shortest and Biroi-250-2-1 the tallest. Number of effective tillers ranged between 7.0 to 10.6 with the parent being the highest and the mutants Biroi-250-2-2 and Biroi-250-2-6 the lowest. Panicle length ranged between 14.87 to 26.8 cm with the mutant Biroi-250-2-6 being the longest and Biroi-250-2-5 the shortest (Table 2). Filled grains panicle^{-1} ranged between 122.0 to 233.6 with the parent being the lowest and the mutant Biroi-250-2-6 the highest. Unfilled grains panicle^{-1} ranged 23 to 57.6. Grain yield ranged between 3.9 to 6.86 t ha^{-1} with the mutant Biroi-250-2-5 being the lowest and Biroi-250-2-3 the highest (Table 2). Three mutants (2-2, 2-3 & 2-4) were red colored similar maturity period (133-141 days), intermediate plant height (85-96 cm), 7-8 no. effective tiller hill^{-1}, average no. of filled grain panicle^{-1} was 166-194 and thus the yield (>6 ton/ha) which was higher than the parent and within the mutants as well. The mutants having red grain color with medium plant height possess moderate lodging resistance (Mackill *et al*., 1996). In addition, lower positioning of panicles in the plant's canopy is known to be associated with increased tolerance of lodging (Setter *et al*., 1997). Two dominant genes with complementary gene action (ISASaT, 2017), one increases the pigment content and other accumulates the pigments (Nagao *et al*., 1957) might be responsible for red pericarp. Therefore, the mutants with red decorated grain are needed to further confirmation by molecular analysis.

Table 2. Grain yield and yield components of some M_4 mutants of Biroi during 2018-19

Variety/ mutant	Days to maturity	Plant height (cm)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Unfilled grains panicle^{-1}	Grain yield (t ha^{-1})
Biroi-250-2-1	127	136.8±2.63	8.0±0.55	25.0±0.95	205.8±6.26	44.0±9.33	4.0±0.02
Biroi-250-2-2	141	92.8±1.94	7.0±0.45	24.8±0.49	166.6±9.93	57.6±7.11	6.18±0.08
Biroi-250-2-3	133	85.60±1.70	7.4±0.81	24.8±0.49	194.4±14.96	55.6±9.37	6.86±0.05
Biroi-250-2-4	133	96.2±1.98	8.0±0.71	23.8±1.02	166.2±18.79	23.00±6.24	6.43±0.04
Biroi-250-2-5	127	134.2±2.03	7.6±0.60	14.87±0.37	180.0±6.61	24.6±1.63	3.9±0.03
Biroi-250-2-6	123	130.4±1.21	7.0±0.63	26.8±1.30	233.6±16.63	35.2±6.98	5.34±0.09
Biroi (Parent)	143	125.8±1.85	10.6±0.68	24.2±0.58	122.0±5.15	24.2±1.91	4.05±0.02

In Table 3, results showed significant variation among the mutants and check for most of the characters in combined over locations and individual locations from combined analysis, it was observed that plant height of all the genotypes ranged from 101.29-146.74 cm., effective tillers was found lowest (8.93) in mutant Biroi-250-2-3 and highest (19.08) in Biroi-250-2-6. Significant variation was found in panicle length, filled grain panicle^{-1} and unfilled grain panicle^{-1}. Some mutants showed lower effective tillers hill^{-1} than the parent but produced longer panicle length, higher filled grains panicle^{-1} and lower unfilled grains panicle^{-1} which contributed to the higher yield. In Rice, higher Leaf Area Index (LAI), more filled grains panicle^{-1} and significant panicle length are the pre-requisite for maximizing yield (Wu *et al*., 2005).

Table 3. Grain yield and yield components of some M_5 mutants of Biroi at different locations during 2019-20

Location	Mutants/Variety	Plant height (cm)	Effective tillers hill^{-1}	Panicle length (cm)	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Yield (t ha^{-1})
Mymensingh	Biroi	141.73 b	10.93 b	21.86 c	126.93 d	44.33 ab	3.91 d
	Biroi-250-2-1	140.47 b	9.66 bc	25.20 a	207.33 a	39.06 ab	4.01 d
	Biroi-250-2-2	92.73 d	10.20 bc	24.73 ab	177.07 abc	39.06 ab	6.10 a
	Biroi-250-2-3	87.67 d	9.20 cd	25.13 a	200.73 a	39.80 ab	6.23 a
	Biroi-250-2-4	98.33 c	8.20 d	23.53 b	161.93 bc	30.60 b	5.56 b
	Biroi-250-2-5	154.00 a	14.40 a	26.06 a	191.00 ab	47.26 a	3.95 d
	Biroi-250-2-6	142.59 b	13.39 a	24.86 ab	156.95 cd	41.95 ab	4.59 c
Nalitabari	Biroi	120.93 a	10.40 b	24.36 a	91.60 a	32.73 ab	3.96 c
	Biroi-250-2-1	153.00 a	9.93 b	24.80 a	106.73 a	34.00 ab	4.13 bc
	Biroi-250-2-2	130.70 a	10.46 b	25.43 a	96.67 a	16.66 b	6.03 a
	Biroi-250-2-3	114.67 a	8.86 b	22.93 a	89.93 a	23.40 ab	6.06 a
	Biroi-250-2-4	116.47 a	10.60 b	23.00 a	98.60 a	19.26 ab	5.66 a
	Biroi-250-2-5	133.73 a	9.93 b	24.30 a	105.73 a	29.20 ab	4.10 bc
	Biroi-250-2-6	137.40 a	25.03 a	21.26 b	109.47 a	37.06 a	4.56 b
Combined mean over location	Biroi	131.33abc	10.66 ab	23.12 b	109.27 b	38.53 a	3.94 d
	Biroi-250-2-1	146.74 a	9.33 b	25.00 a	157.03 a	36.53 ab	4.07 d
	Biroi-250-2-2	109.49 abc	10.15 b	25.36 a	138.63 ab	26.13 ab	6.02 a
	Biroi-250-2-3	101.29 c	8.93 b	23.96 b	145.47 ab	31.70 ab	6.03 a
	Biroi-250-2-4	107.40 bc	9.40 b	23.26 b	130.26 ab	24.93 b	5.62 b
	Biroi-250-2-5	143.87 ab	12.16 ab	25.18 a	148.37 ab	38.23 a	4.02 d
	Biroi-250-2-6	139.57 ab	19.08 a	17.06 c	135.40 ab	39.03 a	4.54 c

Table 4. Grain yield and yield components of some M_6 mutants of Biroi during 2020-21

Mutants/Variety	Plant height (cm)	Effective tillers hill^{-1}	Panicle length (cm)	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Yield (t ha^{-1})	BLB severity (%)
Biroi	141.73 b	10.93 a	21.86 c	126.93 d	44.33 d	3.91 d	-
Biroi-250-2-1	143.73 b	7.13 d	25.27 b	159.80 a	45.67 cd	4.00 bc	30
Biroi-250-2-2	92.93 d	8.32 cd	22.04 d	146.33 bc	79.20 ab	6.00a	-
Biroi-250-2-3	87.93 d	9.87 b	24.80 bc	141.00 bc	85.80 a	5.97 a	-
Biroi-250-2-4	98.53 c	8.50 cd	23.87 cd	130.87 cd	60.00 cd	5.41 bc	25
Biroi-250-2-5	151.80 a	9.20 bc	26.60 a	153.47 b	82.93 ab	4.03 ab	20
Biroi-250-2-6	142.27 b	9.73 bc	23.53 cd	131.93 cd	58.67 bc	3.95c	15

During aman season 2020-21, considering the yield performance and lodging tolerant ability of the mutant further trial was done for M_6 generation and bacterial leaf blight (BLB) severity test also done (Table 4). Among six mutants, Biroi-250-2-1, Biroi-250-2-4, Biroi-250-2-5 and Biroi-20-2-6 were BLB susceptible. Mutant Biroi-250-2-2 & Biroi-250-2-3 was BLB tolerant and produced higher yield than parent and other mutants. These two mutants also showed higher yield in Nalitabri substation, and Mymensingh Headquaters of BINA in M_5 generation (Table 3) and M_4 generation (Table 2). Although mutant Biroi-250-2-4 had good performance regarding yield and lodging tolerant ability in different generations we discarded this mutant because of BLB susceptibility (Table 4). Finally two mutants were selected based on moderate plant height, lodging tolerance, red colored pericarp and BLB tolerance which will need further trail in next season.

Conclusions

The higher yields of the selected mutants were likely to have been caused by the increase of positive allele frequency in mutants through mutagenesis. Biroi-250-2-2 and Biroi-250-2-3 mutants can be evaluated at the next trail to develop a high yielding moderate lodging tolerant reddish grain with having BLB tolerant and semi dwarf biroi type rice variety.

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