1. Bangladesh

High Yielding mutants with shorter life cycle selected in rice irradiated with carbon ion beam

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1.1 Summary

To investigate the possibility of induced fixed and stable mutants in M_1 generation of rice in which, one hundred and fifty seeds of the highly photoperiod sensitive and tall *indica* type rice cv. Ashfal and popular variety BRRI dhan29 were irradiated with different doses of carbon ion beams from the Radiation Applied Biology Division, Quantum Beam Science Division, Japan Atomic Energy Agency, Japan. In case of BRRI dhan29 and Mutant of BRRI dhan29, it was grown during Boro season, 2012 and in the case of Ashfal M_1 was grown during long day length at Boro (December-May) season and M_2 during short day length at Aman season (July–December) in 2009 whilst M_3 once again grown during Boro season in 2010. Nine M_1 hills in which, 2 from each of 40, 120 and 160Gy, and 1 from each of 60, 80 and 200 Gy were headed even under long day in Boro season despite none of the parent plants of Ashfal headed during Boro season. Of the heading of M_1 plants that had the plant height as the parent that could not produce any seed. Interestingly only the shorter plant height that capable to produced seeds. The M_2 progenies took 85 days and 110 days for heading and maturity respectively, during short days in Aman season. In contrast, the parent Ashfal headed after 7 November and matured at 152 days. In M_3 generation, the progenies bred true as in M_2 for heading, maturity and plant height. It was further confirmed that the Ashfal parent and the mutants had similar 1000-grain weight. Interestingly in case of mutant of BRRI dhan29 showed higher yield and early maturity compared to the parent of BRRI dhan29. Finally, it could be concluded that it is possible to induce stable mutant(s) with photoperiod insensitive and shorter plant height and also higher yield and early maturity traits in rice treated by 40 to 200Gy dose range of carbon ion beams in M_1 of *indiac* rice cv. Ashfal and popular rice variety in Bangladesh BRRI dhan29.

Key words: rice, indica type, ion beams, mutation, photoperiod sensitivity, fixed M_1 mutant.

1.2 Introduction

Rice (*Oryza sativa* L.) is a short day plant (Vergara and Chang, 1985). Almost all rice cultivars matured in a shorter time when they were grown under a short photoperiod (about 10 h) than under a long photoperiod (about 14 h). The degree of photoperiod sensitivity greatly varies among cultivars. In Bangladesh, rice is being grown in three seasons in a year; Aman season (July to December), Boro season (December to May) and Aus season (March to June). Ashfal is a local T.aman (July to December) cultivar being cultivated in the coastal south western part of Bangladesh from time immemorial. It is highly photoperiod sensitive with long duration and tall height cultivar. It is prone to lodging but had some degree of tolerant to salinity and submergence. Its grains are medium bold and contains 10.3% protein and 28.6% amylose (Kamruzzaman and Asaduzzaman, 2004). Ashfal cannot be grown in Boro (long day) season as it is highly photosensitive. It often becomes lodged even in Aman season due to its tall culm which cause yield losses to a considerable extent.

Recently, ion beam irradiation has been appeared to utilized an effective means of inducing mutations. The biological effects of ion beams have also been investigated and observed to be shown a high relative biological effectiveness (RBE) in lethality, mutation and also to transfers high energy to the target compared to low linear energy transfer (LET) radiation such as Gamma-rays, X-rays and electrons (Blakely,1992). It has been also demonstrated that ion beams induce mutations at high frequency and induce novel mutants in *Arabidopsis* (Hase *et al*. 2000, Shikazono *et al*. 2003, Tanaka *et al*. 1997, 2002). The use of ion beams for inducing mutations in rice (*Oryza satva* L.) breeding has also been attempted (Hayashi et al. 2007; Hidema *et al*. 2003; Rakwal *et al*. 2008). It is generally and widely accepted that in mutation breeding induced traits become fixed during M_2-M_4 generations (Azad *et al*., 2010; Hamid *et al*., 2006; Shamsuzz*aman et al*., 1998; Azam and Uddin, 1999). But it has been reported that it is possible to isolate fixed mutants even in M_1 generation of heavy ion irradiated sweet pepper (Honda *et al*., 2006).

In this study, we aimed to describe the possibility to get fixed M_1 mutant(s) with photoperiod insensitive, short day-length and shorter plant height from local T.aman Ashfal and also to get higher yield and early maturity from BRRI dhan29 in rice by ion beam irradiation.

1.3 Materials and Methods

Dehusked seeds of Ashfal rice and BRRI dhan29 were exposed to 26.7 MeV/n carbon ions with different doses of 0, 10, 20, 40, 60, 80, 100, 120, 160 and 200 Gy in January 2009 at Japan Atomic Energy Agency (Takasaki, Gunma, Japan). One hundred and fifty seeds were used for each irradiation dose. The irradiated seeds were allowed to germinate in petri dishes. Seedlings were transplanted on February 11, 2009. Single seedlings were transplanted into each hill. Spacings were maintained between rows and hills are of 20 cm and 15 cm respectively. All plants were grown under natural environment at the Bangladesh Institute of Nuclear Agriculture (BINA) head quarters' farm Mymensingh, Bangladesh. The day length at the time of heading in Boro (December to May) season is between 12 - 13 h whilst that in Aman season (July- December) is 10 - 11 h. Fertilizers were applied at a rate of N-90 Kg, P-18 Kg, K-63 Kg, S-10 Kg and Zn-1.60 Kg/ha in the forms of urea, tripple super phosphate, muriate of potash, gypsum and zinc sulphate equally in all plots. All fertilizers were applied during final land preparation excluding urea. Urea was applied at three equal installments at 7, 30 and 55 days after transplanting. Three hand weeding were made and the plots were kept saturated with irrigation till maturity. Heights were measured randomly from ten M_1 plants of each irradiated dose at 3 weeks after transplanting. M_2 seeds were harvested individually from three fertile M_1 plants at 5 months after transplanting. The M_2 seeds were spread on a seed bed on July 16, 2009. Survived seedlings were transplanted on August 17, 2009 and they were grown during Aman season. Number of days to heading was recorded as the number of days required from sowing time when 50% of plants of each line headed. Days of maturity was recorded as the number of days required from sowing time when 90% of plants of each line appeared with yellowish grains. These two data were recorded through visual observation by visiting the plots every alternate day. Plant heights, number of effective tillers per plant and panicle length were measured at the time of maturity with randomly selected 5 competitive plants. M_3 seeds were harvested separately from each M_2 plant.

M_3 plants were grown as plant progeny during next Boro (long day) season (December 2009 to May 2010). Data were recorded as the same as described in M_2. Grain characteristics such as length (L) and width (W) of unhusked and husked grain were recorded in October 2012. The mutants derived from 140 Gy dose were excluded previously that are not included.

1.4 Results

Seeds of Ashfal irradiated with carbon ions were grown during Boro (long day) season in 2009. Survival rate and plant height was determined to examine the effect of carbon ion irradiation over the plant growth cycle. Survival rate of the irradiated seeds were significantly decreased at 60Gy and remains gradually at higher doses (Table 1). Seedling height also gradually decreased with the application of increased rate of irradiation dose. The height of the seedlings were reduced almost half compared to unirradiated seeds and it was started at 80Gy and gradually goes to at higher doses. Since the cultivar Ashfal is a highly photoperiod sensitive in which, none of the control plants headed during the Boro season. However, we have found nine M_1 plants headed under the same growing conditions (Table 1).

Table 1. Effects of carbon ion irradiation on survival rate, seedling height and heading in highly photosensitive rice cultivar Ashfal grown in Boro season

Dose (Gy)	Number of seeds sown	Number of survived plants	Survival rate (%)	Seedling height* (cm)	Number of headed plants	Number of fertile plants
0	150	147	98.0	23.5 ± 0.4	0	–
10	150	122	81.3	23.0 ± 0.7	0	–
20	150	141	94.0	21.7 ± 0.4	0	–
40	150	121	80.7	19.2 ± 0.8	2	1
60	150	94	66.0	15.5 ± 0.2	1	0
80	150	15	10.0	13.5 ± 1.5	1	0
100	150	9	6.0	12.5 ± 0.5	0	–
120	150	6	4.0	11.6 ± 1.3	2	0
160	150	11	7.3	12.9 ± 1.1	2	1
200	150	3	2.0	12.3 ± 2.0	1	1

* Mean ± SE of seedling heights at 3 weeks after transplanting were shown.

It was confirmed that photoperiod sensitivity was genetically altered in the fertile M_1 plants in which, M_2 seeds were harvested from them and grown during next Aman (short day) season in 2009. Seedlings were raised in seed bed with 301, 650 and 246 M_2 seeds which were derived from the 40, 160 and 200Gy treated with carbon ion beam respectively. Germination rate was appeared very low which probably due to damage of soft embryo tissue caused by ion beam or the effect of seed dormancy occurred or the effect of both. Of which 9, 126 and 18 M_2 seedlings were transplanted to the field. The parent Ashfal headed within 122 days and matured within 152 days after sowing in the field. In contrast, M_2 progenies derived from 40 and 200Gy doses of carbon ion beams and 200Gy has taken almost 85 and 110 days to heading and maturity respectively. The number of effective tillers per plant was observed 13 in Ashfal whilst it ranged from 4 to 13 in M_2 progenies (Table 2). The panicle length was found similar to Ashfal in all M_2 progenies studied. These facts suggests that the photoperiod insensitive phenotype of the three fertile M_1 plants was inherited to all M_2 progenies without any segregations.

Table 2. Characteristics of M_2 progenies grown in Aman season and M_3 progenies grown in Boro season

Mutant line	**M_2 generation grown in *aman* season, 2009**			**M_3 generation grown in *boro* season, 2010**		
	Plant height (cm)	**Number of effective tillers/plant**	**Panicle length (cm)**	**Plant Height* (cm)**	**Number of effective tillers/plant***	**Panicle length* (cm)**
RM(1)-40(C)-1-1	75	10	21	75.6 ± 1.0	10.0 ± 0.6	22.6 ± 0.8
RM(1)-40(C)-1-2	73	11	23	77.8 ± 1.7	8.2 ± 0.7	22.8 ± 0.5
RM(1)-40(C)-1-3	77	5	23	79.0 ± 0.7	9.0 ± 0.6	22.4 ± 0.5
RM(1)-40(C)-1-4	73	10	19	77.0 ± 1.8	8.4 ± 1.0	22.8 ± 0.6
RM(1)-40(C)-1-5	76	7	24	76.8 ± 2.0	9.2 ± 0.7	22.8 ± 0.2
RM(1)-40(C)-1-6	72	7	21	74.0 ± 1.3	9.0 ± 0.8	22.8 ± 0.5
RM(1)-40(C)-1-7	74	7	21	81.0 ± 1.7	11.8 ± 0.6	23.0 ± 1.7
RM(1)-40(C)-1-8	81	6	22	–	–	–
RM(1)-40(C)-1-9	91	13	28	83.4 ± 5.1	11.6 ± 1.4	26.2 ± 1.6
RM(1)-200(C)-1-1	68	13	26	70.6 ± 2.0	9.2 ± 1.1	23.0 ± 0.4
RM(1)-200(C)-1-2	64	10	22	65.9 ± 0.7	6.0± 0.7	21.6 ± 0.7
RM(1)-200(C)-1-3	68	6	23	67.4 ± 2.4	7.8 ± 0.9	23.2 ± 0.7
RM(1)-200(C)-1-4	67	8	22	69.4 ± 4.8	8.6 ± 0.9	24.6 ± 1.4
RM(1)-200(C)-1-5	66	5	22	66.0 ± 1.4	6.2 ± 0.5	22.6 ± 0.9
RM(1)-200(C)-1-6	69	8	21	67.8 ± 1.2	7.8 ± 0.6	22.4 ± 0.7
RM(1)-200(C)-1-7	61	6	20	69.8 ± 1.6	6.6 ± 0.7	22.4 ± 0.5
RM(1)-200(C)-1-8	62	9	22	67.6 ± 2.0	8.6 ± 0.5	22.6 ± 0.4
RM(1)-200(C)-1-9	71	7	22	70.2 ± 0.7	7.6 ± 0.4	22.0 ± 0.4
RM(1)-200(C)-1-10	56	5	19	69.4 ± 1.6	9.8 ± 0.6	22.6 ± 0.5
RM(1)-200(C)-1-11	58	7	22	69.4 ± 1.1	7.4 ± 0.2	22.2 ± 0.5
RM(1)-200(C)-1-12	64	6	21	69.2 ± 1.2	9.6 ± 1.7	21.8 ± 0.7
RM(1)-200(C)-1-13	75	6	23	69.4 ± 1.0	11.0 ± 0.9	22.6 ± 0.2
RM(1)-200(C)-1-14	66	9	21	69.6 ± 1.0	8.8 ± 0.5	23.2 ± 0.9
RM(1)-200(C)-1-15	63	7	21	67.0 ± 0.8	8.4 ± 0.9	22.6 ± 0.8
RM(1)-200(C)-1-16	56	4	16	70.6 ± 1.9	10.6 ± 1.0	22.6 ± 0.7
RM(1)-200(C)-1-17	61	8	21	65.0 ± 1.5	10.2 ± 0.8	22.8 ± 0.5
RM(1)-200(C)-1-18	48	4	20	70.6 ± 2.0	9.2 ± 1.1	23.0 ± 0.4
Ashfal (parent)	148	13	24	–	–	–

*Mean ± SE of 5 plants, RM indicates Rice Mutant; 40(C) and 200 (C) indicate irradiation with 40

For further confirmation of photoperiod insensitivity of mutant lines, M_3 plants derived from the each M_2 plants were grown during next Boro (long day) season in 2010. Interestingly none of the parental Ashfal was found to be headed in this season whilst all mutant lines headed almost 90 days after sowing and matured within 119 days after sowing. Days to heading and maturity were found slightly longer than in the case of M_2 generation where that were grown during Aman season. It was observed that in M_2 generation, the plant height of mutant lines were markedly shorten than that of Ashfal (Figure 1).The mutant lines from 40Gy were slightly shorten in plant height than that of mutant lines from 200Gy and it was found consistent with the plant height of each mutant line observed in M_2 generation.

Length (L) and width (W) of unhusked and husked grain and their ratio altered significantly in mutants over the parent Ashfal (Table 3). Length of unhusked and husked grain increased significantly in all the mutants except the line RM(1)-160(C)-1. In RM(1)-160(C)-1, it was found to decreased significantly. In contrast, width of unhusked and husked grain decreased significantly in all mutants whilst ratios of length and width (L/W) of unhusked and husked grain increased significantly. Finally, 1000-grain weight remained unchanged in most of the mutants except 3.

Table 3 .Grain characteristics of eight mutants along with parent Ashfal

Mutant/variety	**Grain length, L (mm)**	**Grain breadth (mm)**	**L: W**	**1000-Grain weight (g)**
RM(1)-200 (C)-1-1	9.4 (7.0)	2.4 (2.0)	3.92 (3.50)	24.0
RM(1)-200 (C)-1-3	8.9 (7.0)	2.4 (2.0)	3.71(3.50)	23.9
RM(1)-200 (C)-1-9	9.1 (7.0)	2.3 (2.2)	3.96 (3.18)	22.5
RM(1)-200 (C)-1-10	9.4 (7.2)	2.4 (2.0)	3.92 (3.60)	24.0
RM(1)-200 (C)-1-13	8.5 (6.8)	2.3 (2.0)	3.70 (3.40)	23.8
RM(1)-200 (C)-1-17	9.3 (7.0)	2.4 (2.0)	3.88 (3.50)	24.2
RM(1)-200 (C)-1-18	9.4 (7.0)	2.4 (2.0)	3.92 (3.50)	22.6
RM(1)-160 (C)-1	7.5 (5.8)	2.8 (2.2)	2.68 (2.64)	20.5
Ashfal	7.9 (6.2)	3.0 (2.6)	2.63 (2.38)	24.2
SE	0.2 (0.2)	0.1 (0.1)	0.18 (0.15)	0.4

Figures in parenthesis indicate grain characteristics from husked grain.

Preliminary yield trial with M_6 high yielding mutant lines of boro rice

Seedlings of 10 mutant lines derived from BRRI dhan29 by irradiated its seeds with carbon ion beams in Boro season along with unirradiated BRRI dhan29 were transplanted on 22 January 2012 at 41 days after seed sowing. The experiment followed RCB design with 3 replications. Seedlings were transplanted at 15cm distances within rows of 20cm apart. A unit plot size was 3.0m × 2.0m. Recommended doses of fertilizers, cultural and intercultural operations were also followed as and when necessitated. Data on plant height, days to maturity; number of effective tiller, panicle length, filled and unfilled grains panicle^{-1} and yield plot^{-1} were recorded. Maturity was recorded plot basis while plant height, effective tiller number, panicle length, filled and unfilled grains/panicle was recorded from hills per plot at harvesting time. Grain yield was recorded from 1m^2 areas which was latter converted to yield/ha at 14% moisture condition of grain. Moisture data was recorded with a grain moisture meter. Finally, the recorded data were subjected to proper statistical analyses following the design used and are presented in Table 4.

It appears that the mutants and the check variety differed significantly for yield and yield attributing traits except panicle length (Table 4). The mutants took 147 to 157 days to mature whilst their parent BRRI dhan29 took 159 days. The mutant RM (2)-40 (C) -3-1-7 took the shortest period only 147 days for maturity. Filled grains was significantly the highest in the mutant RM(2)-50 (C) -2-1-1 and it also had the highest grain yield at 14% moisture content of 8.58t/ha, which was 1.35 t/h more than BRRI dhan29. This higher yield of this mutant was attributed to its considerably longer panicle length and significantly higher number of filled grains/panicle (Table 4).This mutant together with those with higher filled grains/panicle, longer panicle length, statistically as par yield and shorter maturity period would be put into advance yield trial in the next Boro season.

Table 4. Yield and some yield attributing traits of 10 mutant lines derived from BRRI dhan29 with carbon ion beam irradiation along with unirradiated BRRI dhan29 at BINA farm, Mymensingh

Mutant/variety	**Days to maturity**	**Plant height (cm)**	**Effective tiller (no.)**	**Panicle length (cm)**	**Filled grains panicle^{-1} (no.)**	**Unfilled grains panicle^{-1} (no.)**	**Grain yield/ha (t)**
RM(2)-40 (C) -1-1-1	151	112.27	6.93	26.53	139.13	27.00	7.02
RM(2)-40 (C) -1-1-10	149	96.30	8.20	27.00	119.33	23.87	6.93
RM(2)-40 (C) -1-1-7	149	95.53	8.60	25.87	113.60	26.13	5.80
RM(2)-40 (C) -4-2-5	149	91.67	11.40	25.40	109.13	10.87	6.90
RM(2)-40 (C) -4-2-7	159	115.00	8.80	25.73	94.33	18.07	6.53
RM(2)-40 (C) -4-2-8	157	117.60	6.47	28.20	140.60	30.80	6.34
RM(2)-40 (C) -1-1-5	149	112.87	7.93	25.33	136.67	21.07	7.30
RM(2)-40 (C) -3-1-7	147	108.80	7.33	25.80	116.47	21.40	7.10
RM(2)-40 (C) -4-2-2	157	103.07	9.33	25.20	103.73	24.73	6.88
RM(2)-50 (C) -2-1-1	155	115.40	7.80	27.00	169.00	22.87	8.58
BRRI dhan29	159	97.50	10.40	24.57	127.75	22.65	7.23
LSD(0.05)		9.47	1.53	NS	31.42	6.76	0.72

NS- not significant

1.5 Discussion

Heavy ion irradiation has been attaining an important to the mutation breeder as it produced rare mutant(s). In view of this, seeds of Ashfal rice and BRRI dhan29 were irradiated with different doses of carbon ion beams and was grown in Boro season. Three of nine headed plants were found fertile and the rest six were exhibited sterile in case of Ashfal. Since the Ashfal is a highly photoperiod sensitive cultivar and none of the control plants headed during Boro season. These results suggest that photoperiod sensitivity was altered by carbon ion irradiation in a few M_1 plants. Plant heights of M_2 progenies were found significantly shorten than Ashfal and this is also observed consistent with the fact that the fertile M_1 plants had shorter plant height than Ashfal. This might be due to the fact that the fertile mutant lines shifted from vegetative phase to reproductive phase earlier than parental Ashfal and it is caused by ion beam irradiation. It has been reported that one dominant and one recessive genes are responsible for photoperiod sensitivity in rice (Yu and Yao, 1968; Yokoo and Fujimaki, 1971; Yokoo *et al*.,1980). It could be happened that carbon ion beams irradiation inactivated or down regulated the activity of the dominant gene in the irradiated seeds which makes the M_1 plants homozygous monogenic recessive for photoperiod insensitivity. These facts leaded M_1 plants to bred true in M_2 and M_3 generations. The M_2 progenies from 160Gy were omitted from these evaluations because they were prone to lodging for weak culm although they had shorten plants. Moreover, these progenies had shorter panicle with smaller grains and lower number of seed grains. The mutants derived from 40Gy dose also did not continue after M_3 generation because of their inferior performance. The grain characteristics such as size and shape although most of the mutants altered but grain weight mostly remained unchanged as the parent. This also confirmed that the M_1 plants that mutated as photoperiod insensitive with shorten plant height were not contaminated with other variety. If it was contaminated then 1000-grain weight must differed significantly with Ashfal and the contaminants. The mutant of BRRI dhan29 showed higher yield and early maturity compared to parent of BRRI dhan29 which could be due to the useful effect of carbon ion beam.

Hence, it could be concluded that it is possible to induced fixed and stable mutant(s) in M_1 generation of *indica* type rice Ashfal and BRRI dhan29 through 40-200Gy doses of carbon ion beams irradiation. This result is also in agreement with the results of Honda *et al*. (2006) who reported genetically fixed mutants in M_1 generation of sweet pepper for dwarf height and yellow pericarp. Therefore, all of these suggest heavy ion beams irradiation has unique properties that induce fixed mutation even in M_1 generation. In other mutational studies showed that mutants are usually detected in M_2 and M_3 generations and thus need to handle numerous plants to screen. If mutants can be screened in M_1 generation, the number of plants that must be handle is dramatically decreased. Therefore, further studies are needed to investigate whether other crop species could induce fixed mutant(s) in M_1 generation.

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