Pak. J. Bot., 43(2): 1211-1222, 2011.

RADIO SENSITIVITY OF RICE GENOTYPES TO GAMMA RADIATIONS BASED ON SEEDLING TRAITS AND PHYSIOLOGICAL INDICES

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Abstract

Three Basmati rice genotypes viz., 00515, 99417 and Super Basmati were examined for varietal differences in radio sensitivity to gamma radiations. Dry healthy seeds were exposed to variable doses of gamma radiations i.e., 150- 400 Gy with 50 Gy intervals. Highly significant differences among the genotypes ($p<0.01$) for all traits were observed. The differences among radiation treatments were highly significant ($p<0.01$) for shoot and root length, shoot and root fresh weight, water uptake, chlorophyll contents (a, b), plant height and panicle fertility while non significant differences were observed for germination percentage only. The genotype × dose interactions were non significant for germination percentage, shoot length, root length, shoot fresh weight and plant height indicating stability of performance for characters across different radiation levels. In contrast, chlorophyll (a, b), root fresh weight, water uptake and panicle fertility exhibited significant differences for interactions. Mutagenic treatments shifted mean values towards negative direction for almost all traits but not in a definite pattern. However, water uptake of seeds increased with increasing gamma radiation doses. In general, genotypes displayed variable response towards gamma radiations.

Introduction

Rice is a staple food for large part of the world's human population and is ranked the second most consumed cereal grain. Asia produces 92% of the world's rice. In the last 30 years growth in rice output has been achieved primarily by increasing yield but despite of enormous accomplishments yield gains appear to be flattening. Thus identification and creation of genetic variation is of utmost importance for genetic improvement of crops. Induced mutation which increases genetic variability is one of the traditional but still relevant, highly effective, economic and recognized methods for enhancing natural and genetic resources and developing improved cultivars of cereals, fruits and other crops (Lee *et al*., 2002). The application of mutation techniques i.e., gamma rays and other physical and chemical mutagens has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies (Hajos., 2009). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical (Hameed *et al*., 2008), physiological and morphogenetic changes in cells and tissue (Gunckel & Sparrow, 1961).

Gamma radiation treatment of the rice cultivar ‘Calrose’ was used to select the semi dwarf rice cultivar ‘Calrose 76’ (Rutger *et al*., 1977). Many mutant varieties became the national leading variety e.g., the rice variety Yuanfengzao in the 1970’s and early 1980’s, and Zhefu 802 in the late 1980’s and early 1990’s in China for the early season rice production (Ahloowalia *et al*., 2004; Liu *et al*., 2004). Highly resistant rice starch mutants are useful for the dietary management of metabolic disorders such as diabetes and hyperlipidemia (Chen *et al*., 2006). A great majority of mutant plays an important nutritional role in developing countries where crop varieties (64%) were developed by the use of gamma rays (Ahloowalia *et al.*, 2004). Currently, rice mutant resources include collections that were generated by insertional, radiation or chemical mutagenesis (Hirochika *et al.*, 2004; Kurata *et al.*, 2005), but only irradiated or chemically induced rice mutants have been proved to be very useful in plant breeding programs, providing excellent varieties in a direct or indirect way. During the last seven decades, more than 2252 mutant varieties have been officially released in the world (Maluszynski *et al.*, 2000). But in Pakistan only six Basmati rice cultivars viz, Basmati 370, Basmati Pak, Basmati 385, Super Basmati, Shaheen Basmati and Basmati 2000 have been approved for general cultivation.

The present study was carried out with the objective to determine the response of rice genotypes to mutagenic effects of gamma radiation for different seedling growth, physiological and fertility traits in order to induce genetic variability in Basmati rice for production of high yielding varieties. These variations will be used in further selection studies for producing high yielding and good quality basmati varieties.

Materials and Methods

a. Plant material: The seed material comprised of two advance lines/genotypes of rice viz., 00515, 99417 and one commercial cultivar Super Basmati. Pure and healthy dry seeds were irradiated by six different doses of gamma rays i.e., 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy and 400 Gy at room temperature (22-25°C) in a Cobalt 60 gamma cell-220 (Atomic Energy of Canada Ltd.) of 381.43 curie strength delivering 290 Gy/ hr at the time of irradiation at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The moisture content of the seeds at the time of mutagenic treatments was maintained up to 12 to 13% by keeping them in a desiccator containing calcium chloride.

b. Laboratory experiment: Germination percentage was accessed by sowing 50 seeds of treated as well as untreated (control) in Petri dishes on moist filter papers. Daily count was taken and after a week final germination percentage was calculated. For seedling growth studies, 20 seeds were placed between two wet blotters vertically arranged between slots in PVC racks placed in plastic trays containing enough water to immerse the lower edge of the filter papers. After two weeks data for shoot length, root length, shoot fresh weight and root fresh weight were recorded.

Chlorophyll content was determined by following the method of Arnon (1949). Fresh leaves were cut into small pieces and kept in 80% acetone at -10°C for 24 hours. The extract was centrifuged and absorbance of supernatant was measured at 663 nm and 645 nm. Chlorophyll a and chlorophyll b were calculated by following formula

$$\text{mg Chl a} = [12.7\ (\text{OD } 663) - 2.69(\text{OD}645)] \times V/1000 \times W$$

$$\text{mg Chl b} = [22.9\ (\text{OD } 645) - 4.68(\text{OD}663)] \times V/1000 \times W$$

Where V = Volume of extracted sample and W = Weight of the sample

For water uptake studies, 30 seeds were weighed and then placed in Petri dishes on moist filter papers. According to plant requirement an equal volume watering was done and after 72 hours, seeds were surface dried with tissue paper and weighed on electric balance. Water uptake for each entry was calculated on weight basis by subtracting the initial weight from final weight. Completely randomized design with three replications was used for all laboratory parameters.

c. Field experiment: The treated seeds along with control (untreated seeds) were sown immediately in the field with a spacing of 30 × 15 cm in a two factorial randomized complete block design (RCBD) with 3 replications to raise the M_1 generation for evaluating the genetic effects of mutagenic treatments. At maturity data were recorded for plant height and panicle fertility on 10 competitive guarded plants randomly selected from each entry.

d. Statistical analysis: Duncan's Multiple Range Test (DMRT) was applied to compare the mean values of all genotypes and treatments and all the data were subjected to statistical analysis using MSTAT-C.

Results

Analysis of variance revealed highly significant differences among the genotypes ($p<0.01$) for all traits (Table 1). The differences among treatments were also highly significant ($p<0.01$) for shoot and root length, shoot and root fresh weights, water uptake, chlorophyll contents (a, b), plant height and panicle fertility while non significant differences were observed for germination %. The genotype × dose interactions were significant for root weight, chlorophyll contents (a, b), water uptake and panicle fertility while non significant for rest of the traits.

Germination percentage: The mean germination percentage was significantly different among genotypes and values were in the range of 86.66% to 99.81% with maximum germination by super basmati (Table 2). This trait showed very little response towards increasing radiation doses and thus giving statistically non significant differences for all treatments. However the lowest germination percentage (93.33) was recorded at 150 Gy dose and the highest germination of 96.44 was recorded at 200 Gy (Table 3). Effect of interaction between genotypes and doses of gamma rays for germination percentage was non-significant. The values recorded for interaction ranged from 80 to 90, 97.33 to 98.67, 100 to 100 for 00515, 99417 and super basmati respectively (Fig. 1). The rice genotype "00515" showed slight sensitivity toward radiation doses by producing differences at 150 Gy.

Shoot and root lengths (cm): Genotypic differences in mean values for shoot and root lengths were significant and were in the range of 11.6 cm to 15.09 cm and 11.6 to 12.6 (cm) respectively. Maximum shoot length was recorded for 99417. Highest dose of gamma radiation 400 Gy produced significant differences for shoot length in genotype "00515" and super basmati while genotype "99417" did not respond to radiations in spite of producing maximum shoot length. Genotype "00515" got superiority in root length and produced significant differences at 250 Gy while in other genotypes differences appeared at 200 Gy showing more radio sensitivity for this trait. Shoot length did not increase or decrease in definite pattern in response to different doses while with increasing radiation doses decline in root length values was observed. The mean values for gamma doses indicated lowest values at highest radiation level and maximum values in control for both traits. Interactions were non significant (Fig. 1).

Table 1. Analysis of variance for seedling and physiological traits in rice genotypes.

Sov	Df	Germination percentage	Shoot length (cm)	Root length (cm)	Fresh shoot weight (mg)	Fresh root weight (mg)	Chloro a (mg)	Chloro b (mg)	Water uptake	Plant height (cm)	Panicle fertility (%)
Factor A	2	1098.92**	84.14**	5.09**	5753.71**	684.84**	2777.63**	1262.29**	2104.73**	2184.09**	295.15**
Factor B	6	9.31NS	50.97**	62.79**	5664.36**	1294.03**	945.00**	305.77**	1832.13**	273.85**	1596.67**
AB	12	15.66NS	5.86NS	1.79NS	713.39	451.65**	214.38**	246.62**	874.26*	70.69NS	214.80**
Error	42	15.75	3.23	1.38	805.079	142.16	22.09	45.33	419.62	49.87	60.54

**=Highly significant NS=non significant

Table 2. Means values of genotypes for different seedling and physiological traits.

Traits	Genotypes		
	00515	99417	Super Basmati
Germination (%)	86.67 b	98.48 a	99.81 a
Shoot length (cm)	15.06 a	15.09 a	11.61 b
Root length (cm)	12.65 a	11.92 b	11.71 b
Shoot fresh weight (mg)	184.8 a	189.1 a	158.5 b
Root fresh weight (mg)	78.14 a	73.05 ab	66.48 b
Chlorophyll a (mg)	54.85 b	61.25 a	38.91 c
Chlorophyll b (mg)	22.19 b	30.44 a	14.94 c
Water uptake	148.6 a	137.5 ab	128.8 b
Plant height (cm)	99.41 b	110.6 a	90.12 c
Panicle fertility (%)	63.43 a	56.08 b	61.07 ab

Table 3. Treatment means for different seedling growth and physiological parameters.

Traits	Treatments						
	0 GY	150 Gy	200 Gy	250 Gy	300 Gy	350 Gy	400 Gy
Germination (%)	95.56 a	93.33 a	96.44 a	95.11 a	94.22 a	95.56 a	94.67 a
Shoot length (cm)	16.59 a	16.16 a	15.28 ab	13.77 bc	12.75 c	13.21 bc	9.669 d
Root length (cm)	15.93 a	14.90 a	12.57 b	11.57 bc	10.80 c	10.66 c	8.206 d
Shoot fresh weight (mg)	208.1 a	203.8 ab	184.9 ab	173.1 ab	167.8 bc	171.2 ab	133.4 c
Root fresh weight (mg)	86.78 a	83.78 ab	84.67 a	67.33 bc	60.89 c	70.00 abc	54.44 c
Chlorophyll a (mg)	65.42 a	65.48 a	46.26 bc	49.19 bc	52.42 b	43.16 cd	39.77 d
Chlorophyll b (mg)	32.13 a	27.73 ab	19.64 bc	19.73 bc	23.16 bc	20.80 bc	14.47 c
Water uptake	125.6 b	136.8 ab	145.8 ab	144.8 ab	161.2 a	136.7 ab	117.2 b
Plant height(cm)	103.4 ab	105.3 a	104.5 a	100.7 abc	100.3 abc	93.95 bc	92.09 c
Panicle fertility (%)	83.97 a	70.58 b	60.00 c	57.40 c	51.12 cd	54.48 c	43.81 d

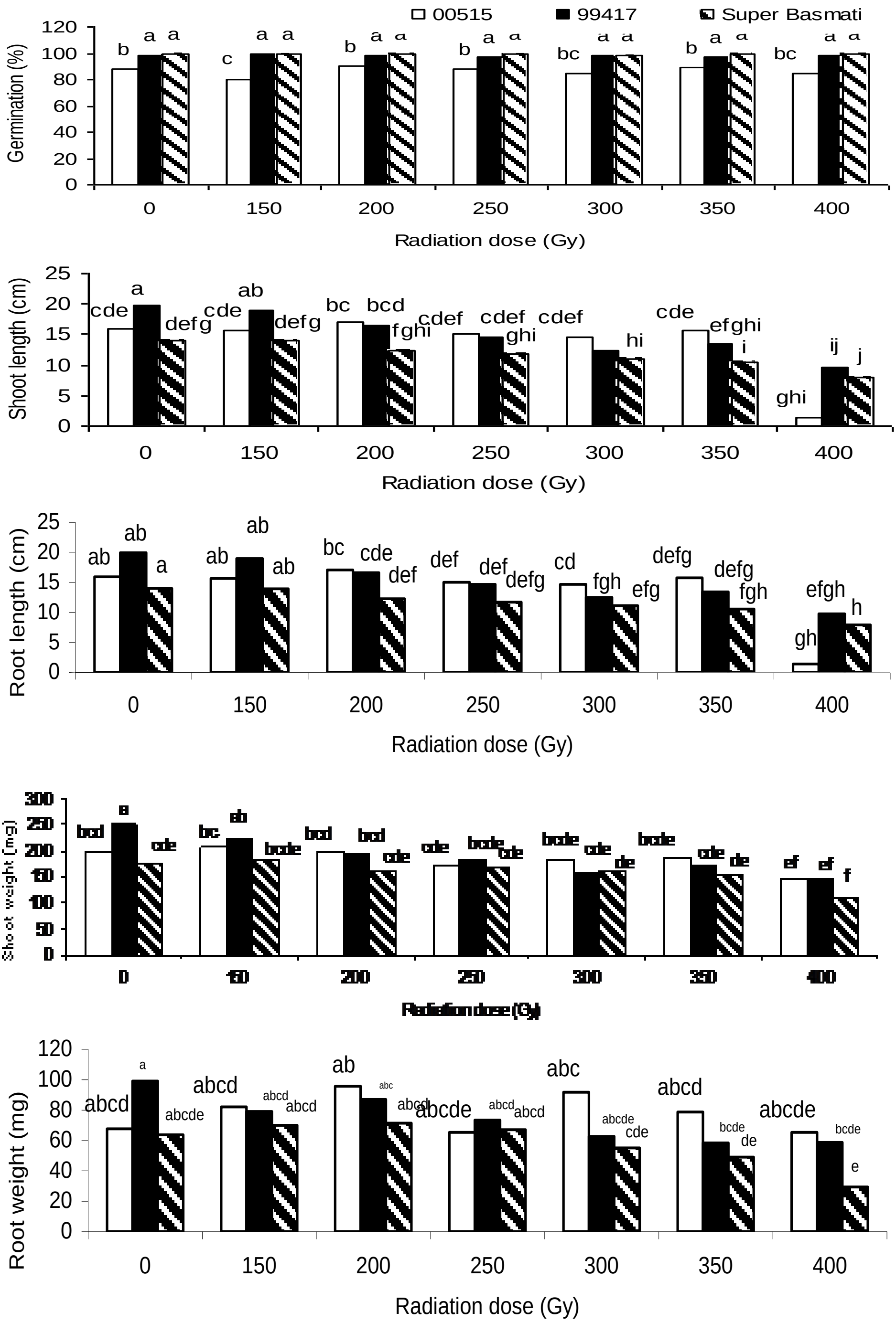

Fig. 1. Effect of gamma radiations on germination and other seedling traits in different rice genotypes.

Shoot and Root fresh weight (mg): At highest radiation level 400 Gy, minimum values were recorded for both traits in comparison with control. The radiation treatment reduced shoot and root weight but that reduction was not proportional with decrease in dose level. Genotype “99417” produced maximum shoot weight (189.1g) while maximum root weight was observed for 00515. At 400 Gy super basmati produced significant difference by giving minimum values for above traits. However at all radiation doses non significant differences were detected in genotypes “00515” and “99417” for shoot and root weight (Fig. 1). Maximum of seedling weight was observed in control and at 100 Gy while minimum weight was observed at 300 Gy treatments.

Chlorophyll content (mg): The mean values of genotypes ranged from 38.91 to 61.25 and 14.94 to 30.44 for chlorophyll a and b, respectively. Maximum values were found in “99417” while super basmati produced lowest chlorophyll contents (a, b). Gamma radiation induced differences were also significant. As compared to control high values for chlorophyll a were recorded at 150 Gy. In case of chlorophyll b highest value for treatments was found in control followed by 150 Gy treatments. Interactions remained significant for chlorophyll a and b (Fig. 2). Differences started appearing at 200 Gy in all the genotypes for chlorophyll a while for b significant differences were started at 150 Gy in 00515 and 99417 while super basmati did not respond to any dose.

Water uptake: In general, significant differences were detected among genotypes with mean values ranging from 128.8 to 148.6. Genotype 00515 absorbed maximum water uptake. Radiation dose 300 Gy leads other doses by giving maximum value for this trait**.** Increase in water uptake value was not exactly proportional to increase in radiation level; however maximum reduction occurs at highest dose as compared to control. Dose × genotypic interaction was significant for this trait. Significant differences were observed for 00515 and 99417 at 250 Gy and 150 respectively Super basmati showed least sensitivity to gamma radiation by producing significant difference at highest dose 400 Gy (Fig. 2).

Plant height (cm): The mean values for plant height ranged from 90.12 to 110.6. The genotype 99417 was tallest among all the genotypes, while super basmati had relatively lesser height. Maximum plant height was observed at 150 Gy while minimum at 400 Gy. Non significant interactions were recorded for plant height (Fig. 3).

Panicle fertility (%): Genotypes differed significantly from each other for panicle fertility. The genotype 00515 was found to be more fertile one. Decreasing trend in fertility % was observed but that was not proportional to decrease in radiation level. At 400 Gy almost 50% plants were sterile. Dose × genotype interactions were significant**.** 00515 was affected at 400 Gy while other genotypes showed no radio sensitivity (Fig. 3).

Discussion

With perusal of results it is evident that the genotypes and doses under study varied significantly for most of the traits showing presence of sufficient genetic variability. The non significant genotype × dose interactions for germination percentage, shoot and root length, shoot weight, plant height indicated a consistency in performance of each genotype across different irradiation treatments except for root weight, chlorophyll (a, b) contents, water uptake and panicle fertility for which these genotypes did not perform uniformly at all doses.

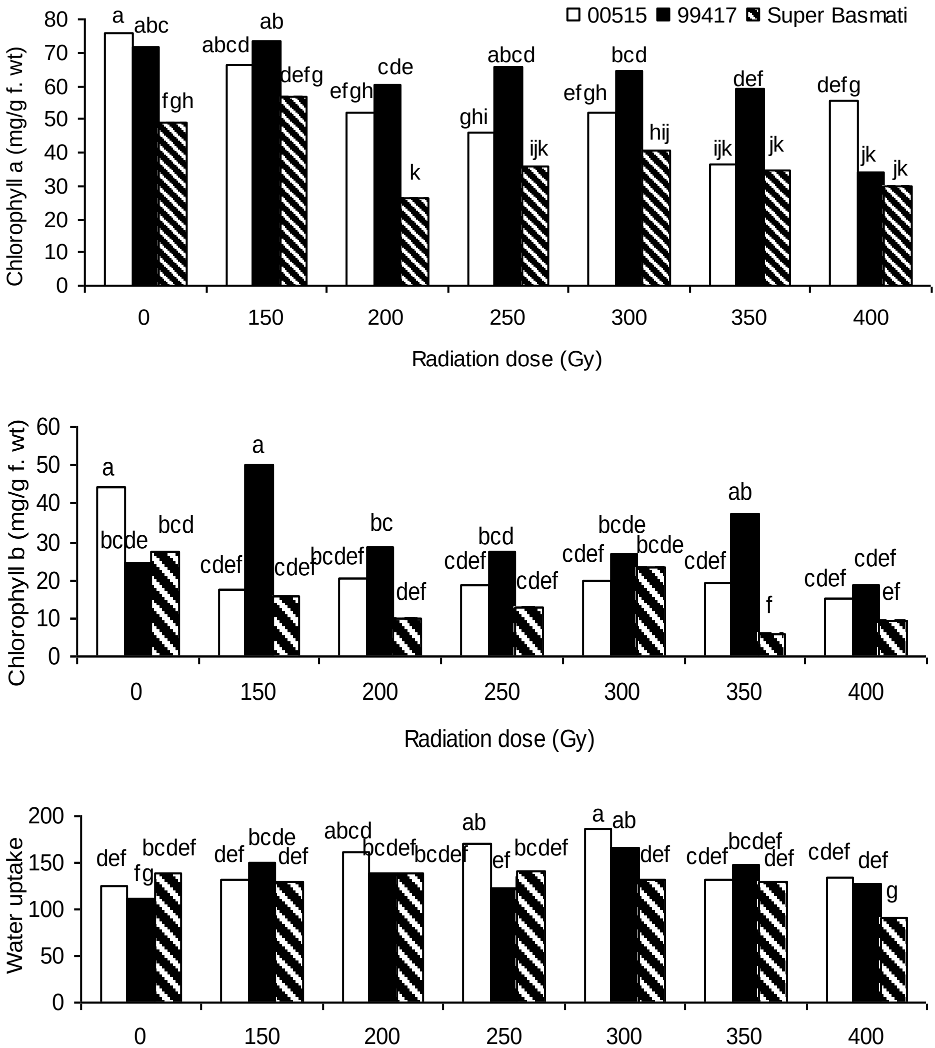

Fig. 2. Effects of gamma radiations on some physiological traits in different rice genotypes.

The germination percentage remained almost unaffected by increasing gamma radiation doses although the highest germination of 96.44% was recorded at 200 Gy. Effect of interaction between genotypes and doses of gamma rays for germination percentage was also non-significant. These findings match with a previous report by Soriano (1961) depicting that germination percentage did not reduce greatly on exposure to 200, 250 and 300 Gy gamma radiations. Such little or no effect of gamma radiation on germination of genotypes under study depicts that these genotypes may be successfully used for the creation of genetic variability through induced mutations in further breeding experiments. However, reduced germination rate with increasing radiation dose has also been reported (Cheema & Atta 2003; Wootton., 1988).

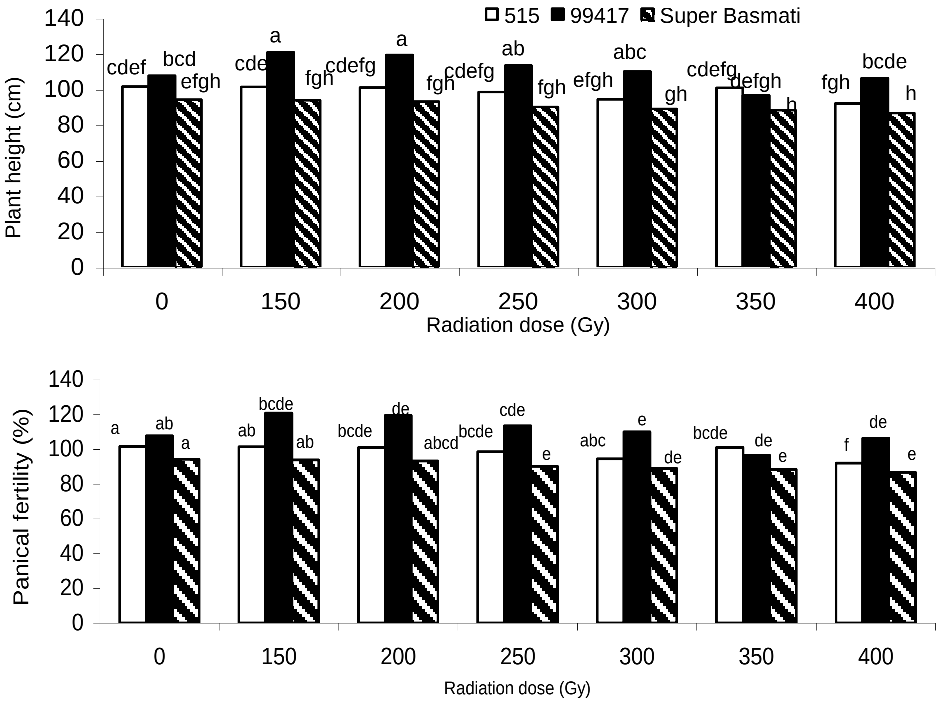

Fig. 3. Effects of gamma radiations on plant height and panicle fertility in different rice genotypes.

Seedling height is widely used as an index in determining the biological effects of various physical and chemical mutagens in M_1 (Konzak *et al.*, 1972). In present study, variable response of genotypes was observed for shoot and root lengths. However shoot length did not increase or decrease in definite pattern in response to different doses while root length decreased with increasing radiation doses. In this connection, Cheema & Atta (2003) reported that the seedling height in rice decreased with the increasing irradiation doses, but the decrease was not proportional to the increase in dosage. They also reported a reduction in root length with each corresponding increase in gamma ray dose that is inline with present findings. Non significant interactions between genotypes x radiation doses for these traits showed same response of varieties towards radiation. Similarly, Cheema & Atta (2003) also pointed out that the tested rice varieties in their study did not differ in radio sensitivity with respect to seedling height at 300 Gy. However in contrast to our findings, highly significant interactions for these traits have also been reported by Ashraf *et al.*, (2003). Moreover, a linear dependency of seedling height on the dosage of physical and chemical mutagens has also been reported by Wang *et al.*, (1995). In our study, maximum values were found in control for both root and shoot lengths while at highest radiation level (400 Gy) minimum values were recorded. This is inline with a previous report in which maximum of stem length seedling was observed in control while minimum of stem length was observed in gamma 200 Gy treatment Moradi *et al.*, (2009) similarly gamma irradiation dose (300 and 400 Gy) induced reduction in rice seedling height has also been reported by Soriano (1961). However, present findings differed form those reported by Moradi *et al.*, (2009) where maximum root length at 200 Gy and minimum at 300 Gy gamma treatments was observed as compared to control.

Previously, maximum seedling weight was observed in control and at 100 Gy while minimum was observed in at 300 Gy that was also highest in the experiment (Moradi *et al.,* 2009). Our findings are in line with this report as maximum root and shoot weights were also observed in controls and minimum at highest radiation dose of 400 Gy. However in the present study, reduction in these traits was not proportional with increasing dose. In contrast to above mentioned findings, a slight stimulation in shoot fresh weights of rice mutants at 150 and 200 Gy radiation dose has also been reported (Sheeren *et al.,* 2009).

Gamma irradiation can significantly curtail the synthesis of chlorophyll content (Dale *et al.,* 1997). In the present study, with exception of 150 Gy, chlorophyll a and b reduced significantly at all other gamma radiation doses as compared to the control. Moreover, genotypic and gamma radiation induced differences were significant with significant interactions for chlorophyll a and b. In a previous report, there was a significant, though relatively small interaction between potato cultivars and irradiation level, which was attributable to a few cultivars showing a slightly higher reduction in chlorophyll synthesis with increased levels of gamma irradiation (Dale *et al.,* 1997). Moreover it has also been reported in citrus that non-irradiated plantlets demonstrated the highest amount of chlorophyll content as compared to irradiated (10-50 Gy) plantlets (Ling *et al.,* 2008).

The physical process of water uptake leads to the activation of metabolic processes as the dormancy of the seed is broken following hydration (Katembe *et al.,* 1998). Significant differences were detected among genotypes for water uptake on gamma irradiation treatments. Although increase in water uptake was not exactly proportional to increase in radiation level but overall seed water uptake increased with only reduction at highest dose (400 Gy) as compared to control. Water absorbed by seeds immediately after irradiation could reduce the damaging effects of ionizing radiation (Ajvi & Larson 1999).

Plant height is a fundamental trait from breeding point of view. In rice, higher yields are usually obtained from shorter crops because the reduction of stature increases lodging resistance. Among the whole range of rice cultivars with different height, semi-dwarf ones are the highest yielding since a severe reduction in height tends to decrease production and to hinder development during early stages of growth and also at harvesting (Domingo *et al.,* 2007). In current study, 99417 was the tallest genotype, while Super Basmati had lesser height. Minimum value was recorded at 400 Gy. The reduction of plant height in M_1 generation could be due to the inhibition of DNA synthesis or other physiological damage after mutagenic treatment. Similar results have also been reported in rice by other workers (Sarawgi & Soni 1993; Cheema & Atta, 2003).

Mutagens generally reduce the reproductive ability of the plant and increase the number of sterile florets much more than the environmental effects. The decrease in fertility of rice after irradiation is considered to be due to chromosomal aberrations (Matsuo & Onozawa, 1961). There is evidence that the radiation-induced M_1 sterility is partly transferred into later generations (Anon., 1977). A large part of M_1 sterility is caused by physiological damage and consequently not transferred to M_2. In present study, decreasing trend was observed for panicle fertility but that decrease was not proportional to increasing radiation level. Among the genotypes only 00515 was affected at 400 Gy while other genotypes showed no radio sensitivity. In this connection, Cheema & Atta, (2003) reported that seed fertility decreased with increase in gamma radiation dose in an approximately linear fashion in all tested rice varieties. The induction of M_1 sterility in rice by gamma radiation has been reported by various workers (Sarawgi & Soni, 1993;

Sanjeev *et al.*, 1998). In conclusion, the genotypes had differential sensitivity for different attributes. The characters like chlorophyll (a, b), root weight, water uptake and panicle fertility were found to be dependent upon dose and genotype concerned. The use of highest dosage (400 Gy) in this study caused some reduction in physiological processes thus this dose is not favorable. The ultimate aim of a mutagenic treatment should be to induce mutations leading to genetic improvement of a specific trait. The mutagenic treatments with low physiological and strong genetic effects may be utilized for launching a successful mutation breeding program.

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(Received for publication 21 June 2010)