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RADIOSENSITIVITY OF RICE GENOTYPES TO ION BEAM IRRADIATION BASED ON SEEDLING TRAITS AND PHYSIOLOGICAL INDICES

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Abstract

Three Malaysian modern rice genotypes viz., MR84, MR219 and MR284 were examined for varietal differences in radiosensitivity to ion beam irradiations. Dry healthy seeds were exposed to variable doses of ion beam radiations at 10-100 Gy with 10 Gy intervals. The result in this study demonstrated that LD_{50} for MR84, MR219 and MR284 were 70.9331 Gy, 69.3927 Gy and 52.78Gy, respectively. Shoulder dose for MR84, MR219, MR284 were ranged between 40Gy- 60Gy. This study found highly significant differences among the genotypes ($p<0.05$) for all traits studied allowing the distinction of three genotype categories. The differences among radiation treatments were highly significant ($p<0.05$) for germination percentage, shoot and root length, chlorophyll contents (a, b), plant height and panicle fertility. Furthermore, Duncan Multiple Range Test was applied to compare the mean values of all genotypes and doses. MR84 and MR219 showed no significant differences with respect to root length. Increasing in ion beam irradiation doses caused a significant reduction in shoot length and chlorophyll A content. The genotype × dose interaction for all tested traits exhibited significant differences indicating the effect of different radiation levels in performance for characters. In general, genotypes displayed variable response towards ion beam irradiations.

INTRODUCTION

Rice (*Oryza sativa* L) is the most important food crop of the developing world and the staple food of more than half of the world's population. Global consumption of rice has shown a slight increase over the last several years. In the 2018/2019 crop year, about 490.27 million metric tons of rice were consumed worldwide, which increased from 437.18 million metric tons in the 2008/2009 crop year [1]. Population growth has exceeded rice yield growth and the gap has been growing steadily larger, creating a significant imbalance between supply and demand. Rice requires specific growing conditions to be successfully cultivated [2]. Thus, identification and creation of genetic variation is of utmost importance for the genetic improvement of crops. The presence of genetic variability in crops is essential for its further improvement by providing options for the breeders to develop new varieties and hybrids. This can be achieved through mutation breeding approaches.

Induced mutation is widely used for developing improved crop varieties. This method is considered as one of the traditional but still relevant, highly effective, economical, and recognizable methods [3]. The applications of mutation techniques such as gamma-rays, ion beams, and other physical and chemical mutagens have generated a vast amount of genetic variability and played a significant role in plant breeding and genetic studies [4]. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical [5], physiological, and morphogenetic changes in cells and tissues [6]. Recently, ion beams have been used as a novel mutagen, and a total of 700 mutant varieties have been generated and registered in the Joint FAO/IAEA mutant variety database.

Energetic heavy-ion beams have been recently used to generate mutants in higher plants because they induce mutations with high frequency at a relatively low dose (i.e., at which virtually all plants survive), inducing a broad spectrum of phenotypes without affecting other plant characteristics [7]. Using this technique, unique varieties of some flowers and trees have been commercialized such as chrysanthemum (*Chrysanthemum spp*.), rose *(Rosa spp*.), and carnation (*Dianthus caryophyllus*) [8]. Because mutants produced by ion-beam radiation are not transgenic plants, they are likely to be accepted by consumers and thus represent a practical choice for rice production worldwide. During the last two decades, ion beam has evolved as a new mutation method for inducing mutation in rice and has been extensively used in many local varieties including Nipponbare [9] and Hitomebore [8] in Japan, Torjan Indonesia [10] and Indian rice, Indira Barani Dhan-1 [11]. In comparison with other countries, research on the application of ion beam in rice breeding in Malaysia is still limited [12].

The present study was carried out to determine the responses of rice genotypes to mutagenic effects of ion beam irradiation for different seedling growth, physiology, and fertility traits. These variations will be used in further selection studies for high yield production and resistance against bacterial leaf blight varieties.

MATERIALS AND METHODS

Materials

Seeds of Malaysian modern rice varieties (high yield however susceptible to bacterial leaf blight) namely MR84, MR284 and MR219 were obtained from Rice GeneBank of Malaysian Agriculture, Research and Development Institute (MARDI).

Plant Material

A total of 100 dry rice seeds were placed embryo-upward on Petri dishes and covered with 7.5 µm of kapton polymide film. A vertical beamline of the AVF-cyclotron (JAERI, Takashi), connected with Irradiation Apparatus for seed, was used for the 320 MeV carbon-ion irradiation. The carbon-ion irradiation at the doses of 10, 20, 40, 60, 80, and 100 Gy was performed under atmospheric pressure within 3 min. Control seeds were treated as unirradiated seeds [11].

Laboratory Experiment

After radiation, 30 treated and untreated (control) seeds were surface sterilized by using fungicide. The seeds were incubated overnight in the dark at room temperature followed by rinsing in distilled water. The germination rate was measured at seven days after sown in a growth chamber. Germination was considered when the emergence of coleoptiles and radical is more than 1 mm long [13]. The following formula was used to calculate the germination percentage:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated in 7 days}}{\text{Total number of seeds}} \times 100$$

For seedling growth studies, ten 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, the data for shoot length, root length were recorded. Chlorophyll content was determined by following the method of [14]. Fresh leaves were cut into small pieces and kept in 80% acetone at −10 °C for 24 hours. The extract was centrifuged, and the absorbance of the supernatant was measured at 663 nm and 645 nm. Chlorophyll a and chlorophyll b were calculated using the following formulas:

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

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

where V is the volume of the extracted sample and W is the weight of the sample.

A completely randomized design with three replications was used for all laboratory parameters.

Field Experiment

The treated and untreated seeds (control) were sown immediately in the field at Greenhouse Agency Nuclear Malaysia, Selangor with a spacing of 30 × 15 cm in a two factorial (7 treatments x 3 cultivars) randomized complete block design (RCBD) with three replications to raise the M_1 generation for evaluating the genetic effects of mutagenic treatment. At 120 days after germination, data were recorded for plant height and spikelet fertility of ten competitively guarded plants randomly selected from each entry. Spikelet fertility was calculated following the method [15].

Spikelet fertility (%) = number of fertile grains × 100/total number of florets = (number of completely filled grains + number of partially filled grains) × 100/total number of florets

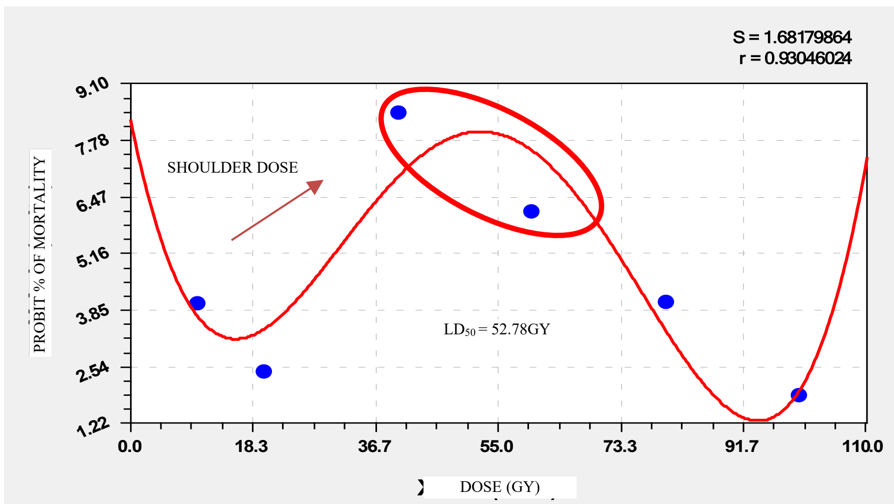
Statistical Analysis

Duncan's Multiple Range Test (DMRT) was applied to compare the mean values of all genotypes and treatments. All the data were subjected to statistical analysis using IBM SPSS Statistics 23.

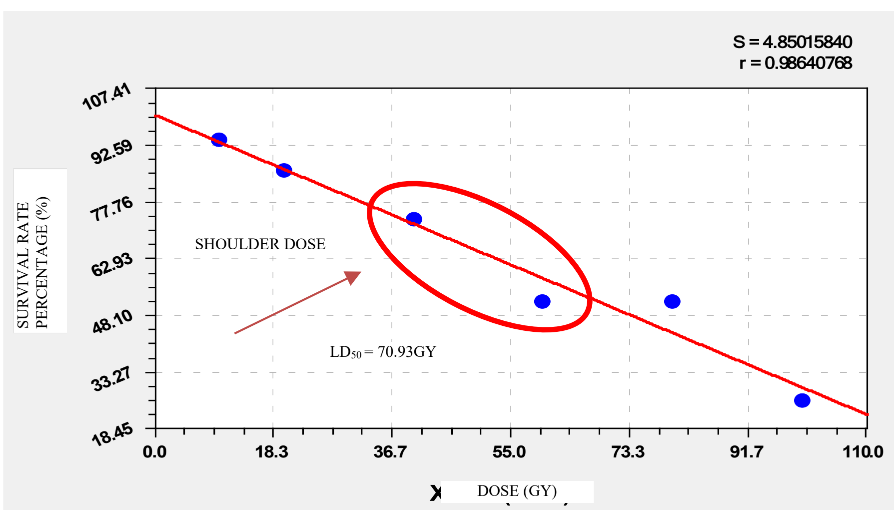
RESULTS AND DISCUSSION

Ion beam irradiation has attained importance to the mutation breeders as it produces rare mutant(s). In view of this, rice seeds of MR284, MR84, and MR219 were irradiated with different doses of ion beams. The determination of the right concentration of mutagens and doses is vital to the existence of the mutation. It is only true when the LD_{50} of the mutagen used has been identified. LD_{50} is the dose that causes half of the total irradiated seed to die. It is known to be the optimal dose that does not harm radiation in a plant with a high occurrence of beneficial mutations. As stated by [16], the value of LD_{50} is genotype-dependent and determined by the design of treatment, biological materials and subsequent environmental conditions.

(a)



(b)



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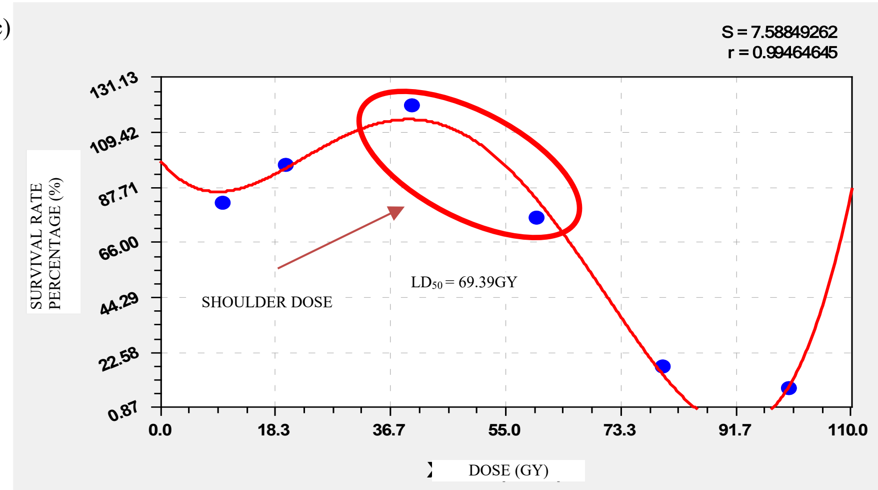


Figure 1. LD_{50} determination from a plot between % of survival rate and dose (Gy) for ion beam irradiation of (a) MR284 variety (b) MR84 (c) MR219 variety

It is therefore important to formulate the LD_{50} value for each genotype to be mutagenized. In this study, LD_{50} for MR84, MR219 and MR284 were 70.9331 Gy, 69.3927 Gy and 52.78Gy, respectively (Figure 1). Shoulder dose for MR84, MR219, MR284 were ranged between 40Gy- 60Gy.

Duncan's Multiple Range Test (DMRT) showed that there is a significant difference ($p < 0.05$) in sensitivity against ion beam irradiation of three genotypes of rice (MR284, MR219, and MR84) for germination percentage, shoot length, Chlorophyll a and b, plant height and spikelet fertility. MR284 is more sensitive than MR84 and MR219 in terms of traits studied and this difference is derived from DNA repair ability by photolyase [6]. Sensitivity against ion beam irradiation differs with the status of plants such as genotypes, target organ, developmental stage [16]. It was also found that the rice genotypes experienced a significant reduction in shoot length and Chlorophyll A due to the increasing doses in ion beam irradiation. Significant interactions also occurred between the rice genotypes and the doses of ion beam irradiation on the tested parameters. It is indicated that different genotypes are expected to result in different mutation induction effects with an increase in doses of ion beam irradiation on tested parameters.

The mean germination percentage was significantly different among genotypes; values were in the range of 58.75% to 65.76% with maximum germination by MR219 (Table 1). The rice genotype MR84 showed slight sensitivity towards radiation doses by producing lower germination percentage. The germination percentage of the seeds appeared very low (<70%) for all three genotypes which is probably due to soft embryo tissue damage caused by ion beam or seed dormancy or both. This phenomenon tends to increase with the radiation dose which is the cause of reduced germination. Radiation may damage compounds related to plant metabolism such as auxins, ascorbic acid, chlorophyll, and proteins that potentially inhibit the growth of seedlings [17]. Generally, the germination percentage showed responses towards increasing radiation doses with the lowest germination percentage (25.56%) was recorded at 100 Gy dose and the highest germination of 76.17% was recorded at 0 Gy (control) (Table 2). The decrease in germination was directly proportional to the increase of dosage and showed a definite pattern in all three varieties. The results agree with those of several authors who found that the germination of various rice plants tends to diminish with increasing radiation doses [10, 18,19]. Seeds with high radiation doses cannot germinate, or their seedlings cannot survive beyond a few days due to the changes of protein expression that influence the functioning of cells, potentially affecting the development of the embryo and preventing germination. However, the reduction of germination percentage was found not significantly differences between 10, 20, 40, 60 and 80 Gy (Table 2). Figure 2 showed the significant interaction between genotypes and doses of gamma rays for germination percentage. The values recorded for interactions ranged from 13% to 80%, 30% to 75.50%, and 33.30% to 86.60% for MR284, MR84, and MR219 respectively (Figure 2).

Table 1. Mean values of genotypes for different seedling and physiological traits

Traits	Cultivars		
	MR284	MR84	MR219
Germination (%)	58.75[a]	48.36[b]	65.76[c]
Shoot length (cm)	5.467[a]	9.89[b]	11.69[c]
Root length (cm)	5.82[a]	12.89[b]	12.95[b]
Chloro a (mg)	43.62[a]	26.69[b]	22.09[c]
Chloro b (mg)	31.42[a]	14.52[b]	9.51[c]
Plant height (cm)	104.86[a]	107.23[b]	109.12[c]
Spikelet fertility (%)	73.33 [a]	65.77 [b]	58.48 [c]

Different letter indicates significantly (p<0.05) different within column

Table 2. Treatment means for different seedling growth and physiological parameters.

Traits	Treatments						
	0 Gy	10 Gy	20 Gy	40 Gy	60 Gy	80 Gy	100 Gy
Germination (%)	76.17[a]	66.11[b]	66.53[b]	62.06[b]	56.94[b]	50.00[b]	25.56[a]
Shoot length (cm)	14.18[a]	9.07[b]	7.78[c]	11.98[d]	10.82[e]	5.18[f]	4.09[g]
Root length (cm)	17.96[a]	12.44[b]	9.82[c]	13.27[b]	10.61[c]	6.04[d]	3.73[e]
Chloro a (mg)	50.18[a]	40.09[b]	27.33[c]	32.73[d]	24.66[e]	22.97[f]	17.65[g]
Chloro b (mg)	35.44[a]	33.95[b]	17.10[c]	17.35[c]	9.891[d]	7.52[e]	8.15[f]
Plant height (cm)	111.06[a]	107.31[b]	106.61[b]	109.33[c]	106.33[b]	106.39[b]	102.44[d]
Spikelet fertility (%)	79.88 [a]	65.88 [b]	70.77 [c]	71.11 [c]	64.22[b]	55.66 [d]	53.44 [d]

**Means in column followed with different letter (s) are significantly different*

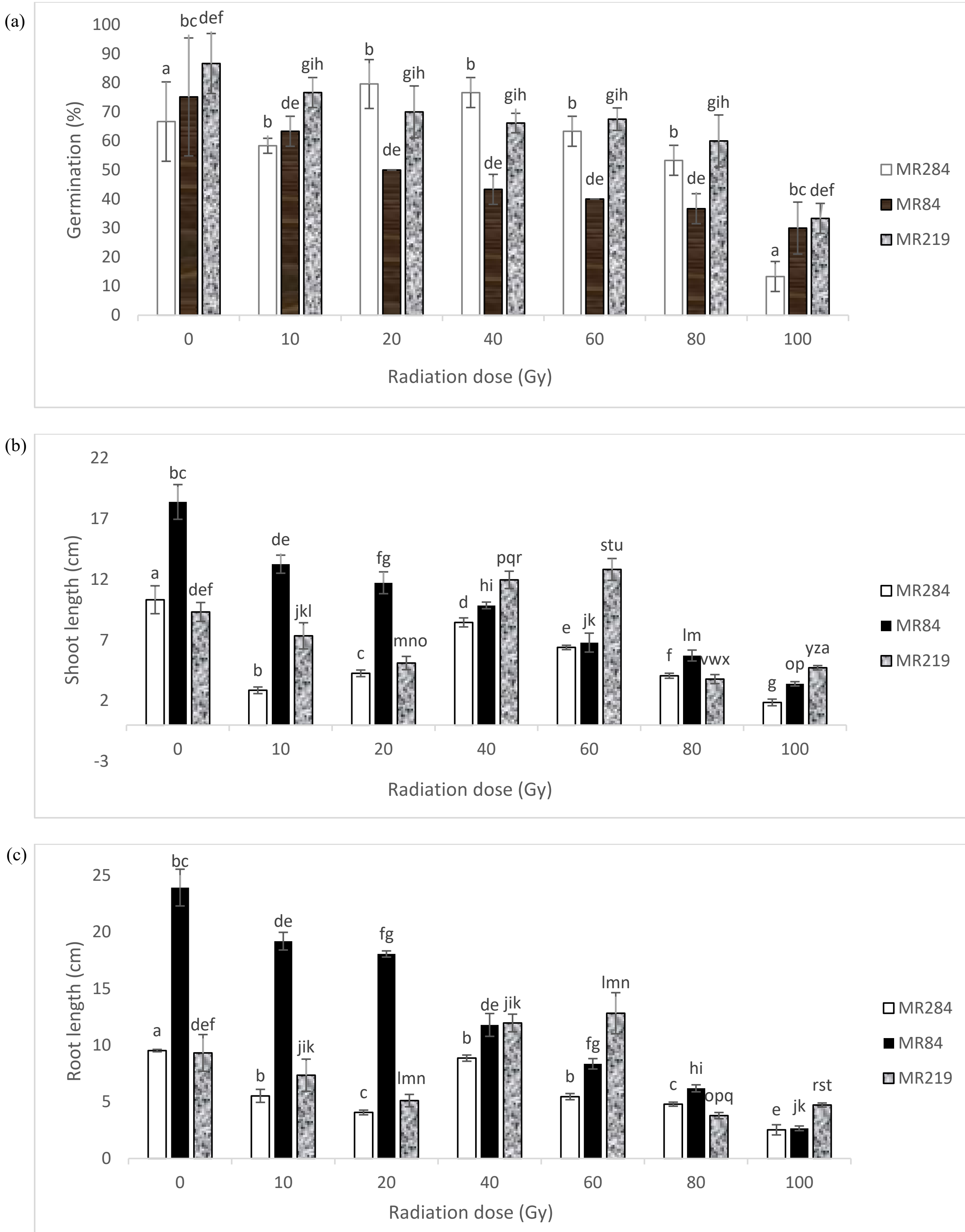


Figure 2. Bars followed with the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following ANOVA and represent mean (± SD). (a) Effects of germination percentage of MR284, MR84 and MR219 at different doses of ion beams irradiation (b) Effects of shoot length MR284, MR84 and MR219 at different doses of ion beams irradiation (c) Effect of root length of MR284, MR84 and MR219 at different doses of ion beams irradiation

Seedling height is commonly used as an index to assess the biological effects of various physical and chemical mutagens in M_1[20]. In the first generation of mutation (M_1), the effect of the ion beam on the early stage of germination was determined by shoot and root length growth on the 14th day of germination. The reduction of shoot length due to irradiation of ion beam varied from 9.067 (10 Gy) to 7.778 (20 Gy) to 11.978 (40 Gy) to 10.822 (60 Gy) to 5.178 (80 Gy) and 4.089 (100 Gy) respectively. Similarly, the reduction in root length ranged from 12.44 (10 Gy) to 9.82 (20 Gy) to 13.26 (40 Gy) to 10.61 (60 Gy) to 6.04 (80Gy) and 3.73 (100 Gy) (Table 1). Among the traits, shoot length was recorded as more sensitive and showed significant differences for ion beam treated three rice genotypes when compared to root length. In connection to this, [21] reported that the seedling height in rice decreases with increasing irradiation doses, but the decrease is not proportional to the increase in dosage. Reduced mitotic activity in meristematic tissues and reduction of moisture content in seeds after irradiation with a higher dose of gamma irradiation may be responsible for shoot and root length reduction [22]. Ionizing radiation usually affects plant metabolism and cell division, inhibiting or delaying the plant's growth [23]. In this regard, [24] found that gamma radiation can diminish the amount of proteins and chlorophyll compounds, affecting the plant metabolism. [25] also found that gamma radiation may inhibit plant growth because plants have a signal transduction mechanism that monitors cellular damage. This mechanism stops cell division whenever the cell structure is damaged. Genotypes MR219 and MR84 showed no significant differences in root length. Both shoot and root lengths showed a significant interaction for genotype x doses (Figure 2). Similarly, [26] reported highly significant interactions for these traits. Furthermore, a linear dependence of seedling height on the dosage of physical and chemical mutagens was reported by [27].

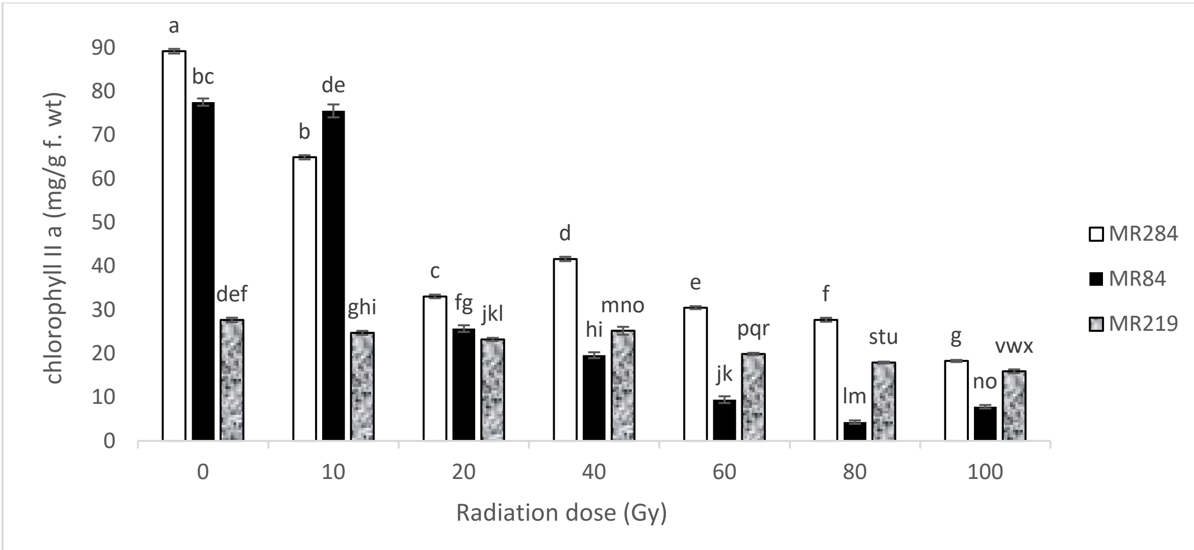
Plant height is important in rice plant breeding because it is closely related to the effective civilization of assimilation to improve plant products. Genotypes with high production are characterized by short stems and thus, the division of assimilation will be very effective in which reduction of stature increases lodging resistance [27]. The mean values for plant height ranged from 104.86 cm to 109.12 cm (Table 1). The genotype MR219 was the tallest among all the cultivars, while MR284 had relatively lesser height. Significant differences were found in control and 100 Gy plants. Maximum plant height was observed at 0 Gy while minimum at 100 Gy (Figure 3). Significant interactions were recorded for plant height (Table 3). According to Duncan's analysis ($p < 0.05$), there was no significant difference in terms of height between 10, 20, 60, and 80 Gy irradiation doses. However, all the rice seedlings of the irradiated seeds showed a slight reduction in their height as compared to the control samples. Similar results were reported by [16] who noticed a reduction in plant height as a result of irradiation on MR219 rice seeds by carbon ions. The negative impact of radiation on plants may be indirectly mediated via metabolic changes through free radical formation, as well as DNA damage to the dividing cells [28]. According to [29], cell division is the most sensitive parameter to irradiation, which might account for the growth inhibition observed in irradiated plantlets. High doses of irradiation may cause cell cycle arrest at the G_2/M phase during somatic cell division or genome damage, leading to growth inhibition [26].

Table 3. Analysis of variance for seedling and physiological traits in rice cultivars

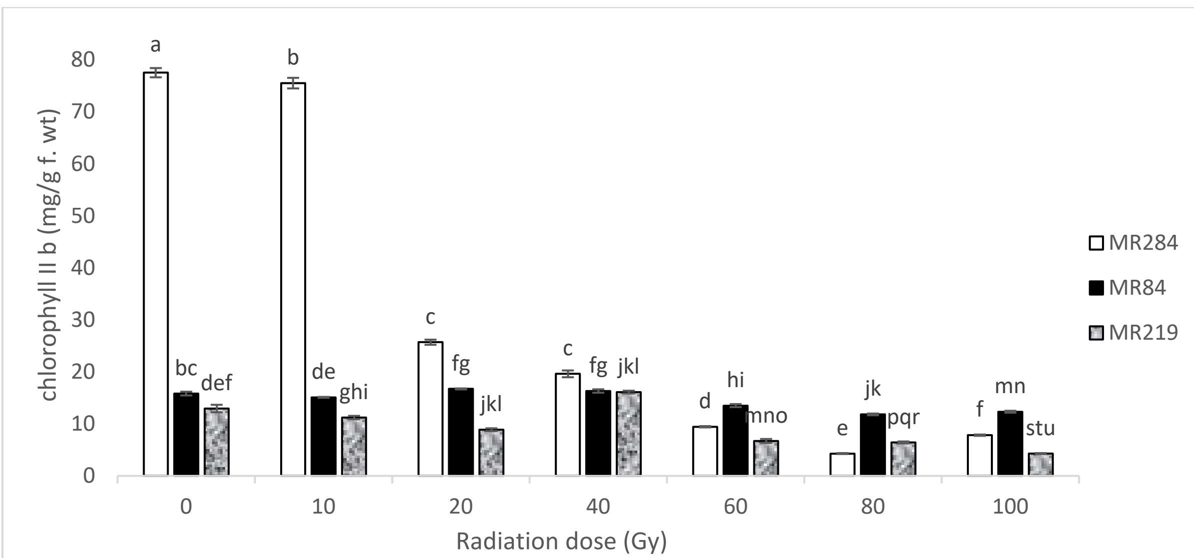
Sov	Df	Germination (%)	Shoot length (cm)	Root length (cm)	Chloro a (mg)	Chloro b (mg)	Plant height (cm)	Spikelet fertility (%)
Genotype	2	1610.420**	215.036**	353.127**	2700.099**	2767.820**	95.765**	1159**
Doses	6	2402.773**	118.834**	199.668**	1124.395**	1246.290**	65.518**	763.952**
Genotype*doses	12	325.600**	37.202**	37.975**	435.979**	913.531**	23.878**	28.111**
Error	42	76.733**	0.575**	0.853**	0.464**	0.232**	1.893**	3.968**

**Mean of three replications. Means in a column followed by asterisk are significantly different at p<0.05*

(a)


chlorophyll II a (mg/g f. wt)
90
80
70
60
50
40
30
20
10
0
0
10
20
40
60
80
100
Radiation dose (Gy)
MR284
MR84
MR219
a
bc
def
b
de
ghi
c
fg
jkl
d
hi
mno
e
jk
pqr
f
lm
stu
g
no
vwx

(b)


chlorophyll II b (mg/g f. wt)
80
70
60
50
40
30
20
10
0
0
10
20
40
60
80
100
Radiation dose (Gy)
MR284
MR84
MR219
a
bc
def
b
de
ghi
c
fg
jkl
c
fg
jkl
d
hi
mno
e
jk
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mn
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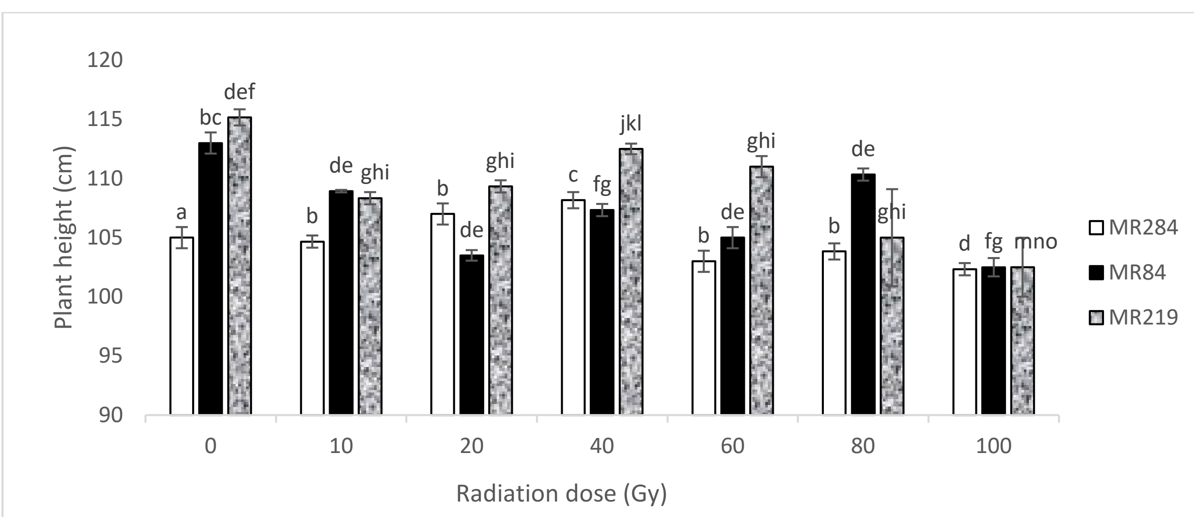
(c)


Plant height (cm)
120
115
110
105
100
95
90
0
10
20
40
60
80
100
Radiation dose (Gy)
MR284
MR84
MR219
a
bc
def
b
de
ghi
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ghi
c
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d
fg
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(d)

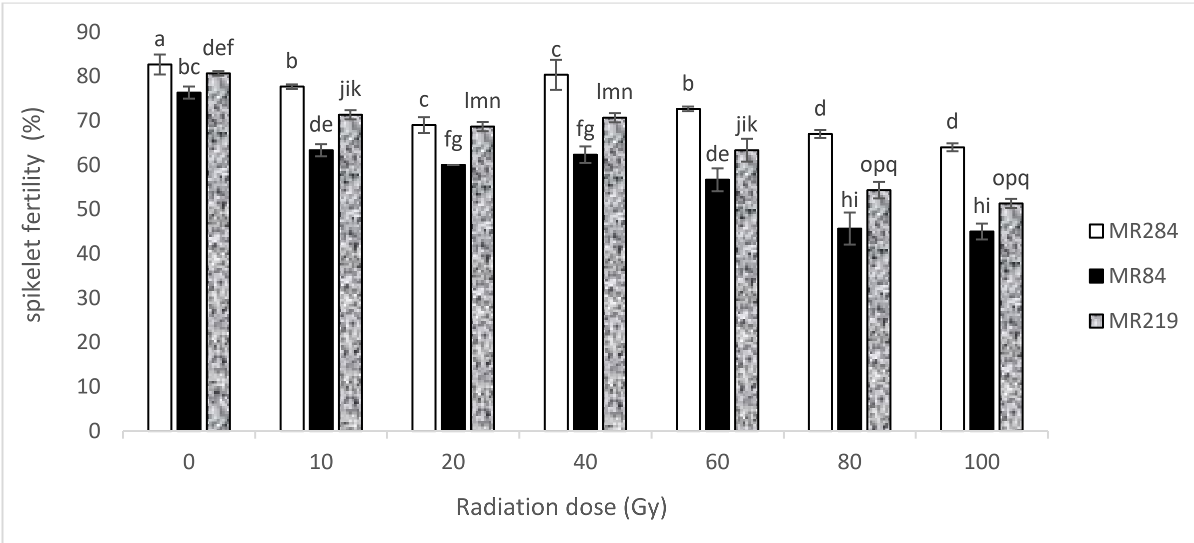

Figure 3. Bars followed with the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following ANOVA and represent mean (± SD). (a) Effects of chlorophyll a of MR284, MR84 and MR219 at different doses of ion beams irradiation (b) Effects of chlorophyll b of MR284, MR84 and MR219 at different doses of ion beams irradiation (c) Effect of plant height of MR284, MR84 and MR219 at different doses of ion beams irradiation (d) Effect of spikelet fertility of MR284, MR84 and MR219 at different doses of ion beams irradiation

Photosynthetic pigments that mainly constitute chlorophyll a and b are of vital importance in photosynthesis changes. Significant differences were observed in all genotypes (Table 1). The mean values of genotypes chlorophyll content ranged from 22.10 to 43.62 and 9.51 to 31.42 for chlorophyll a and b, respectively. Maximum values were found in MR284 while MR219 produced the lowest chlorophyll contents (a, b) (Figure 3). High values for chlorophyll a and b were found in control seeds followed by 10 Gy treatment. For chlorophyll a, there were significant differences among doses while for chlorophyll b, no significant differences were observed between 20 and 40 Gy (Table 2). Interactions between genotype x dose remained significant for chlorophyll a and b (Table 3). The present results are in accordance with the findings made by [16]. Irradiation was found to affect chloroplast development, presumably via the disruption of plastid expression [30] while photosynthetic pigments may be destroyed at high irradiation doses, leading to the loss of photosynthetic capacity [31]. Chlorophyll deficient mutants have been frequently observed in studies involving ion beam irradiation of plant materials. [32] discovered chlorophyll deficient mutants with albino, pale-green, yellow, or stripped leave phenotypes in second generation of mutation (M_2) progeny of rice as a result of carbon ion irradiation. However, [9] reported no differences in the spectrum of chlorophyll mutations between ion beams and γ rays, whereby, both high linear energy transfer ion beam and low linear energy transfer γ rays were found to induce albina, xantha, and other mutants such as striata (longitudinal white or yellow strips) and maculate (green or yellow spots distributed over the leaf) chlorophyll deficient mutants.

Genotypes differed significantly from each other for spikelet fertility. The genotype MR284 was found to be the most fertile. The decreasing trend in fertility percentage was observed but it was not proportional to the decrease in radiation level. At 100 Gy, almost 50% of plants were sterile (Figure 3). Dose × genotype interactions were significant. Generally, reproductive ability is reduced by mutagens which results in an increasing number of sterile florets compared to environmental effects. This is due to chromosomal aberrations [33]. M_1 sterility is caused by physiological damage and consequently not transferred to M_2. However, there is evidence that the radiation-induced M_1 sterility is partly transferred to later generations [34]. In the present study, the decreasing trend was observed for panicle fertility but that decrease was not proportional to the increasing radiation level. Among the cultivars, MR219 was found to be the most sterile. However, at 100 Gy, almost 50% of plants were sterile among all the genotypes tested. The results are aligned with a study by [35].

CONCLUSION

Different genotypes have different responses against ion beam irradiation for different attributes. The present study has suggested that germination percentage, shoot and root lengths, chlorophyll a, b and spikelet fertility are found to be dependent upon dose and genotype concerned. Shoulder dose for MR284, MR84 and MR219 were ranged between 40 Gy- 60Gy. Irradiation at the highest dosage (100 Gy) in this study caused some reduction and thus, this dose is not favourable. Minimum physiological and significant genetic effects in mutagenic treatment could be considered to initiate a successful mutation breeding program.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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