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Original Research Article

Effect of Gamma Rays Treatments on Percentage Germination, Morphological Variation and Chlorophyll Mutation in Musk Okra (*Abelmoschus moschatus* L.)

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Keywords

Gamma rays, Chlorophyll mutant, Germination percentage, Mutation breeding

A B S T R A C T

Abelmoschus moschatus medik. L. is an important medicinal herb of family Malvaceae. Ambrette oil is used in various pharmaceutical industries due to its high medicinal properties. Seeds of musk okra (*Abelmoschus moschatus*) were exposed to different gamma rays ^{60}codoses in 100, 200, 300, 400, 500 and 600Gy, untreated seeds used as control. Variations in the percent germination, shoot length, root length, number of leaves, phenols and chlorophyll mutants were recorded during this experiment under pot condition. Effect of mutagenic treatments was resulted in decreasing percentage germination and gamma rays treatments were produced variations in phenol content and chlorophyll mutants.

Introduction

Mutation is a sudden heritable change in an organism. Which may be structural or functional? But generally structural changes occur. It is produced by changes in base sequence of gene and it can be spontaneously or artificially both in seed and vegetative propagated crops. In plant cells; the nucleus is considered the principal site of damage by ionizing radiation. The process of leading to radiation damage may be summarized as: the initial physical, which lasts only a minute fraction of a second; the physico-chemical stage lasting about 10-16s; the chemical stage lasting a few seconds and the biological stage in which the time scale from tens of minutes to tens of years depending on the particular symptoms. Sparrow *et al.,* 3 reported that there was a direct relationship between the radiosentivity of an herbaceous plant and average volume occupied by a chromosome in the cell nucleus. Larger the chromosome volume, more sensitive is the plant and hence, smaller the radiation dose required to kill the plant or to cause a certain degree of damage. Improved quality and quantity of crops have been achieved through radiation-induced mutation.

However, the harmful effects of deleterious mutation include death in the embryonic state, inability to reproduce, increased Susceptibility to disease and decrease in life expectancy of a few months.

The seed of this plant are known as Ambrette seed, which contain moisture 11.14 %, protein 2.3 %, starch 13.35 % and fatty oil 14.5 %.Seeds yield a volatile oil known as musk seed oil or Ambrette seed oil, which is in high demand at national, international and pharmaceutical industries level due to its anti-cancerous, aphrodisiac and anti-depression properties documented by Verma *et al.,* (2011). To increase the production of this crop there is a need to have a better understanding of its genetic background.

However, there is a lack of information about varieties of *Abelmoschus moschatus*, which require variability because of their pollination status. The present study was therefore undertaken to fill the gap in knowledge of the genetic background of the crop and assess the effect of the two mutagens on the plant. The present investigation was undertaken to study the effect of physical mutagen in different concentration and dose treatments on germination and chlorophyll mutation. Such a study is needful to unveil any desirable features for quantitative traits, agronomic, Phytochemical and pharmaceutical benefits.

Materials and Methods

Healthy seeds were selected and treated with different doses of gamma rays e. g. 100, 200, 300, 400, 500 and 600 Gy untreated seeds considered as control. The various observations were recorded during the course of investigation such as germination (%), shoot length, root length, number of leaves, chlorophyll and phenol content.

Germination percentage

Twenty five healthy seeds from each treatment were sown in pots, after 7 days, number of seeds germinated was counted and the germination percentage was calculated by using below given formula

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown for germination}} \times 100$$

Estimation of phenols and chlorophyll content was done in seedlings after 30 days of sowing.

Phenols (mg/100g)

The phenol was estimated based on the method developed by Singleton *et al.*, (1965). One gram of sample from three randomly selected seedlings was homogenized with 20 ml of methanol (80 %) in a mortar and pestle for 2-3 times. The extracts were pooled and the volume was made up to 50 ml. An aliquot of 0.5 ml of the above extract was taken in test tubes and 0.2 ml of Folin-Ciocalteau’s phenol reagent was added followed by 3.3 ml distilled water duly mixing well. After 2 min, 1 ml of sodium carbonate (20 %) solution was added, mixed and allowed to stand at room temperature for 30 minutes. Then, the blue color developed was read in spectrophotometer at 700 nm. A standard curve for phenols using gallic acid (GA) as standard was prepared and total phenol content measured was expressed as mg gallic acid equivalents/100g.

Chlorophyll (mg/g)

Dimethyl sulphonic oxide method was adopted (Lichthenthales and Burchmanne, 1987) for estimation of chlorophyll. One gram of leaf sample from three randomly selected seedlings was taken in a test tube containing 10 ml of dimethyl sulphonic oxide and kept in hot air oven at 80^{0}c for 2 hours. Later, 1 ml of supernatant was decanted in another test tube and volume was made up to 5ml with dimethyl sulphonic oxide. The chlorophyll extract was transferred to cuvette and absorbance was read at 645nm and 663nm. Chlorophyll a, b and total chlorophyll can be calculated by using following formula

$$\text{Chlorophyll a} = \frac{(12.7 \times \text{O.D}_{663}) - (2.69 \times \text{O.D}_{645}) \times \text{Value} \times \text{dilution}}{\text{Weight of the sample (g)}} \times 100$$

$$\text{Chlorophyll b} = \frac{(22.7 \times \text{O.D}_{645}) - (4.68 \times \text{O.D}_{663}) \times \text{Value} \times \text{dilution}}{\text{Weight of the sample (g)}} \times 100$$

$$\text{Total chlorophyll} = \frac{(20.2 \times \text{O.D}_{645}) + (8.02 \times \text{O.D}_{663}) \times \text{Value} \times \text{dilution}}{\text{Weight of the sample (g)}} \times 100$$

Results and Discussion

Germination percentage

Significantly highest germination percentage was recorded in control (98.33 %) followed by 100 Gy (90.33 %) and lowest percentage was recorded in 600 Gy (T6) treatment (20.00 %). High levels of gamma irradiation had deleterious effects on seed germination. These results are in agreement with the results presented by Ashish *et al.,* (2011) in musk okra, Akshatha *et al.,* (2016) in *Terminalia arjuna* and Singh *et al.,* (2000), Rahman *et al.,* (1999), Jagajanantham *et al.,* (2012), Puspharajan *et al.,* (2014), Maryam and Kasimu (2016) and Elangovan and Pavadai (2015) in okra and Dodiya and Khatik (2013) in isabgol.

Low levels of gamma rays might have induced the growth stimulation signals that triggered the biosynthesis of antioxidants and hormones (Kim *et al.,* 2004). Further, proportionate reduction in germination of muskdana on gamma rays treatment was also noticed (Table and Fig 1). It is presumed that interaction effect of irradiation with other cellular molecules, esp. water generates free radicals that could combine and form some toxic substances (H_2O_2), which contribute to the destruction of cells and cause plant death (Dehgahi and Joniyasa, 2017). Reduction in germination percentage in mutants may also occur due to cytogenetic damage and physiological disturbance and disturbance in balance between inhibitors of growth regulators and promoters (Aslam *et al.,* 2016).

Shoot length (cm)

Highest shoot length was noticed at 100Gy treatment (7.42 cm) which are on par with all the treatments viz., 200Gy (7.33 cm), 500Gy (7.08 cm), 600Gy (6.87 cm), 300Gy (6.85 cm) and 400Gy (6.77 cm) except control (6.28 cm) (Table and Figure 2). Similar results were outlined by El-sharnouby *et al.,* (2007) in sweet basil and Lal and Sharma (2002) in isabgol.

In general, ionizing radiation influenced growth and development of plants. Increased shoot length might be attributed to biochemical and physiological changes induced by gamma irradiation (Al-Bachir, 2014).

Root length (cm)

Significantly superior root length was recorded at 400Gy-T_4 treatment (5.72 cm), while decreased root length was observed with 200Gy-T_2 treatment (2.75 cm) depicted in Tab and Fig. 2. Similar results were reported by previous research workers in okra (Maryam and Kasimu, 2016, Jagajanantham *et al.,* 2012 and Elangovan and Pavadai, 2015**)**.

Table.1 Effect of gamma irradiation on germination percentage at 30 DAS in muskdana under pot condition

Treatments	Germination percentage
T_1 (100 Gy)	90.33 (71.89)
T_2 (200 Gy)	72.00 (58.05)
T_3 (300 Gy)	64.00 (53.11)
T_4 (400 Gy)	68.00 (55.53)
T_5 (500 Gy)	52.00 (46.13)
T_6 (600 Gy)	20.00 (26.53)
T_7 (Control)	98.33 (83.94)
S.Em±	1.30
C.D. (P=0.05)	3.99

* DAS - Days after sowing

*Parenthesis values are Arc sign transformed values.

Table.2 Effect of gamma irradiation on seedling height, root length and number of leaves at 30 DAS in muskdana under pot condition

Treatments	Seedling height (cm)	Root length (cm)	Number of leaves
T_1 (100 Gy)	7.42	3.37	4.25
T_2 (200 Gy)	7.33	2.75	3.00
T_3 (300 Gy)	6.85	4.65	3.25
T_4 (400 Gy)	6.77	5.72	3.00
T_5 (500 Gy)	7.08	3.55	3.50
T_6 (600 Gy)	6.87	3.55	3.00
T_7 (Control)	6.28	3.67	3.25
S.Em±	0.21	0.32	0.26
C.D. (P=0.05)	0.65	1.00	0.80

Table.3 Effect of gamma irradiation on biochemical parameters (phenols and chlorophylls) of muskdana at 30DAS under pot condition

Treatments	Phenols (mg/100g)	Chlorophylls (mg/g)		
		Chlorophyll-a	Chlorophyll-b	Total chlorophyll
T_1 (100 Gy)	403.04	0.32	0.11	0.43
T_2 (200 Gy)	623.74	0.28	0.12	0.4
T_3 (300 Gy)	606.81	0.37	0.14	0.51
T_4 (400 Gy)	713.54	0.26	0.09	0.35
T_5 (500 Gy)	700.12	0.31	0.10	0.41
T_6 (600 Gy)	806.10	0.28	0.09	0.37
T_7 (Control)	568.73	0.36	0.08	0.44
S.Em±	64.51	0.02	0.01	0.03
C.D. (P=0.05)	198.77	0.06	0.03	0.09

Fig.1 Germination percentage of Muskdana at 30 DAS

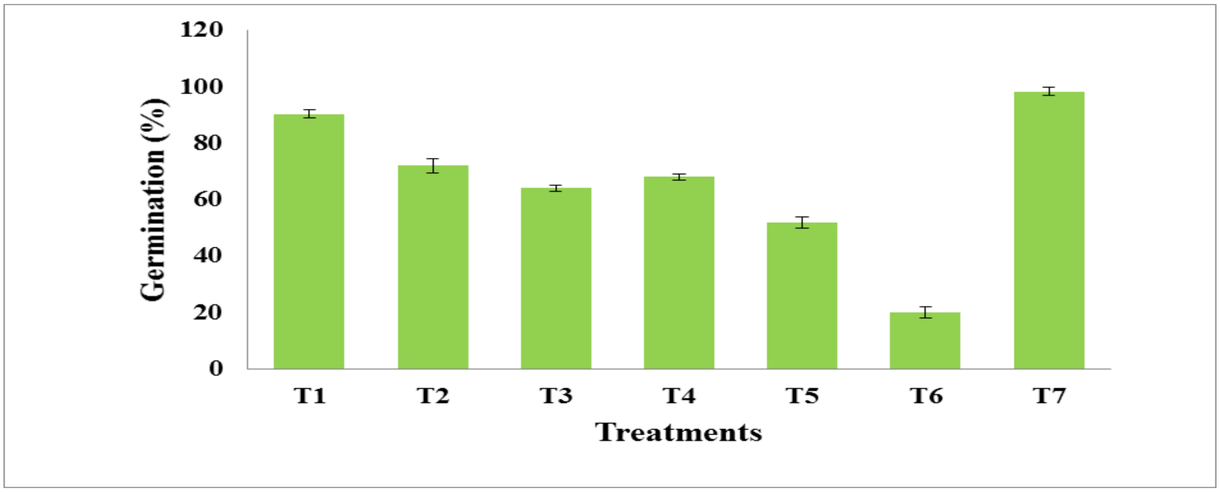


Fig.2 Seedling height, root length and number of leaves at 30 DAS

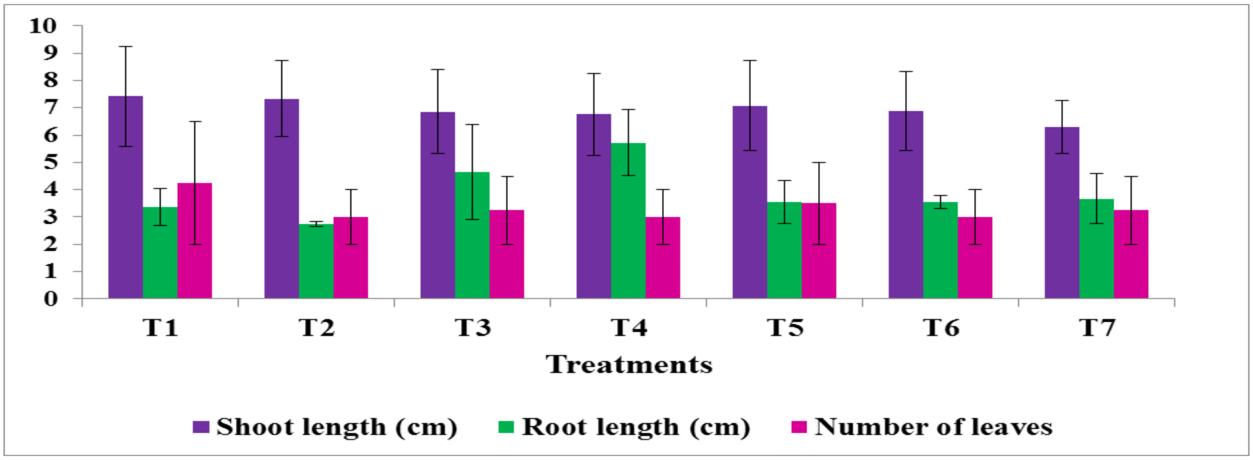


Fig.3 Phenol content at 30 DAS

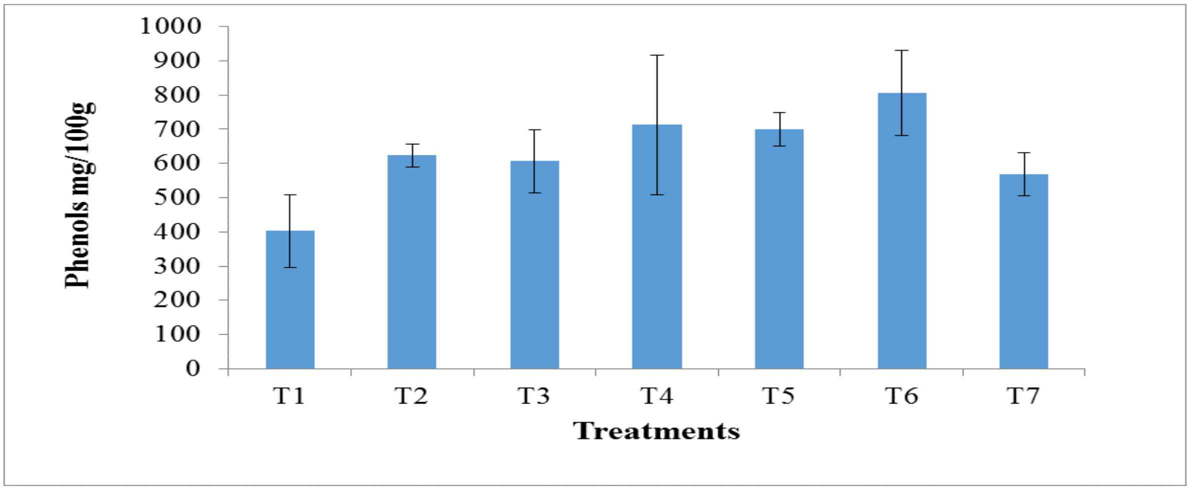


Fig.4 Effect of gamma irradiation on A) phenols and B) chlorophyll content of muskdana

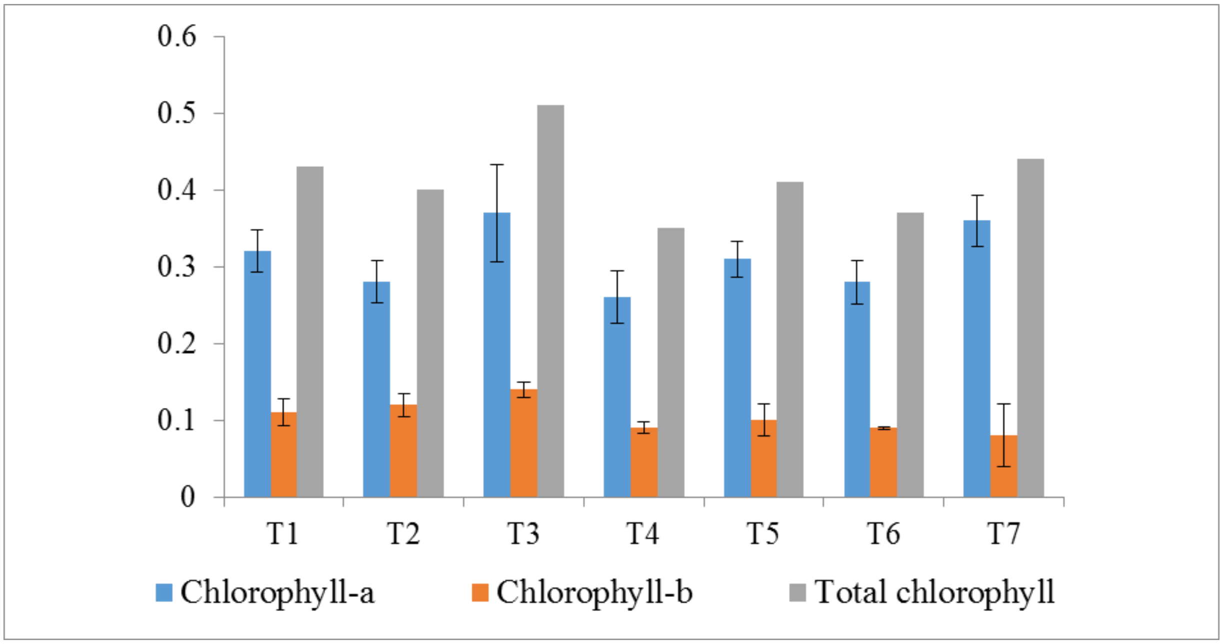


Plate.1 Effect of gamma irradiation on germination percentage of muskdana in different doses of gamma rays

T_1: 100Gy

T_2: 200Gy

T_3: 300Gy

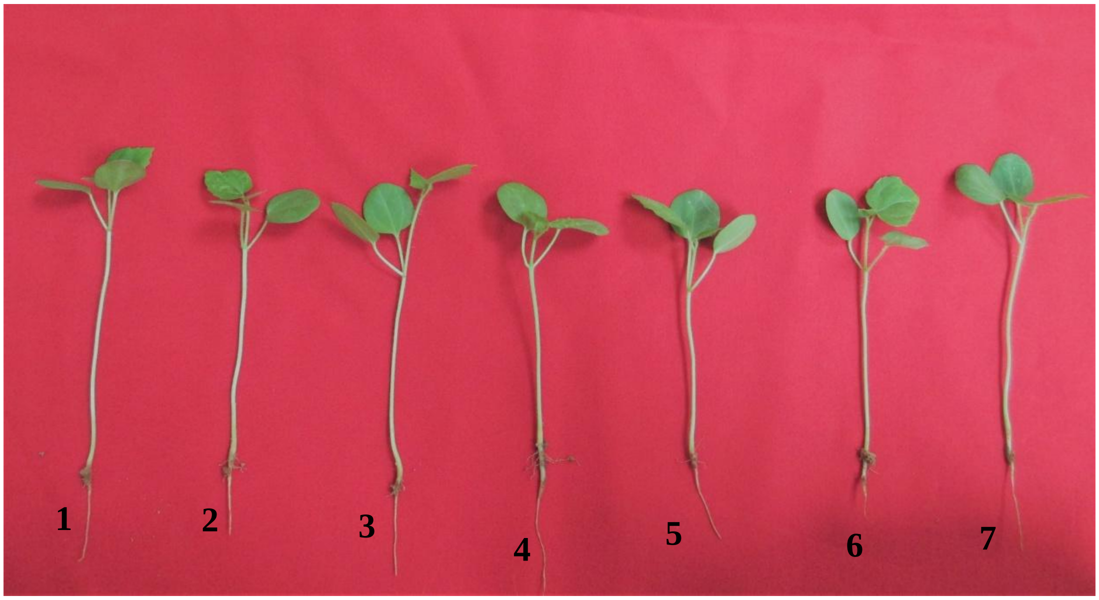
T_4: 400Gy

T_5: 500Gy

T_6: 600Gy

T_7: 0Gy (control)

Plate.2 Effect of irradiation on seedling length, root length and number of leaves of muskdana seedlings (lane 1, 2, 3, 4,5, 6 and 7 indicates control, 100, 200, 300, 400, 500 and 600 Gy irradiation doses respectively)



It is speculated that optimum dosage of irradiation was essential to activate various metabolic changes at cellular level. However, increased dosage of irradiation reduced mitotic activity of meristematic tissues and caused root development to be postponed giving poor root size and less number of rootlets per plant (Khalil *et al.,* 1986).

Number of leaves

Highest number of leaves were recorded in plants exposed to 100Gy (4.25) and lowest number of leaves was recorded in 600Gy (3.00) treatments (Tab and Fig. 2).

These results are coincided with the findings of Jagajanantham *et al.,* (2012) in okra. Gamma irradiation caused changes in plant morphology and function, as affected by various dosage and duration of exposure time (Piri *et al.,* 2011). It is also predicted that enzymes essential for leaf initiation at higher intensities of gamma irradiation might have stopped and blocked the cell division by decreasing the rate of physiological process (Akhtar, 2014).

Phenols (mg/100g)

On increasing the dose of gamma irradiation there was an increase in amount of phenols. The highest phenol content was recorded with 600Gy (806.10 mg/100g), which was on par with 400Gy (713.54 mg/100g), 500Gy (700.12 mg/100g) and 200Gy (623.74 mg/100g) and the lowest phenol content was recorded in 100Gy (403.04 mg/100g) obtained results depicted in Table 3 and illustrated in Fig 3. The results obtained are in confirmity with the findings of Oufedjikh *et al.,* (2000) in *Pterocarpus santalinus* and Akshatha *et al.,* (2016) in *Terminalia arjuna*.

Phenolic compounds are major component of the plant secondary metabolites in medicinal plants. Gamma irradiation is known to increase the activity of phenylalanine ammonia lyase (PAL), which is precursor for the synthesis of polyphenolic compounds that are capable of preventing DNA damage by reacting with free radicals induced by gamma rays (Jimenez *et al.,* 2011 and Akshatha *et al.,* 2016).

Chlorophyll content (mg/g)

Irradiation treatment T_3 (300Gy) exhibited highest chlorophyll-a, chlorophyll-b and total chlorophyll concentration of 0.37mg/g FW, 0.14mg/g FW and 0.51mg/g FW, respectively. The said treatment was on par with control (T_7) (0.36, 0.44 mg/g FW) and 100Gy (T_1) (0.32, 0.43 mg/g FW) with respect to chlorophyll-a and total chlorophyll content while it was on par with 200Gy (T_2) (0.12 mg/g FW) and 100Gy (T_1) (0.11 mg/g FW) for Chlorophyll-b content. However, minimum chlorophyll-a was, content recorded with 400Gy (T_4) 0.26mg/g FW, chlorophyll-b in control (T_7) 0.08mg/g FW and total chlorophyll content in 400Gy (T_4) 0.35mg/g FW results depicted in Table 3 and illustrated in Fig 4. It was also found that chlorophyll-a was higher than chlorophyll-b in both irradiated as well as non-irradiated plants. The concomitant results were presented by Alikamanoglu *et al.,* (2007) in *Paulownia tomentosa* and Kim *et al.,* (2004) in red pepper, who claimed that mutant phenotypes recorded highest Chlorophyll-a, Chlorophyll-b and total chlorophyll content than wild type plants.

Higher dosage of gamma irradiation had recorded low chlorophyll content. Effect of gamma irradiation intensities on chlorophyll was assessed in terms of disturbed hormonal balance, leaf gas-exchange, water exchange and enzyme activity (Stoeva, 2002). Wi *et al., (*2006) reported changes in the plant cellular structure and metabolism such as dilation of thylakoid membranes, the site of photosynthesis on exposure to gamma rays. Correspondingly, the chlorophyll content found stimulated at lower level of gamma irradiation as the integrity of thylakoid membrane remains unaffected at lower levels. Further, the thylakoid membrane contains a number of integral membrane proteins to which are bound several important prosthetic groups and light absorbing pigments, most notably chlorophylls (Pribil *et al.,* 2014).

From the data on germination studies, it is concluded that increased dose of gamma rays treatment decreased the germination percentage and *vice-versa*. The maximum shoot (7.42 cm) and root length (5.72 cm) were recorded with 100 Gy and 400 Gy respectively; significantly higher number of leaves was recorded with 100 Gy at 30 DAS. Increased dose of gamma rays treatment increased phenol content was observed and *vice-versa*. Mutations are direct or indirectly used for crop improvement breeding programme.

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