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# Morphological and biochemical responses of different varieties of *Tagetes patula* L. to gamma radiations

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**Abstract**
Ionizing Radiations has emerged as a useful mutagen in the mutation breeding of ornamental plants result in the induction of novel mutations. This study investigates the morphological and biochemical responses of different varieties of *Tagetes patula* L. (French marigold) *viz*., Bonanza, Guljafri Orange, Guljafri Yellow, Nana patula Yellow and Safari Red toward different dosage of gamma irradiation. In this study, the dry seeds of different varieties of *T. patula* L. were irradiated at 0, 2.5, 10.0, 15.0, 20.0 and 25.0 kR. The study demonstrated that low dosage (2.5 kR) of gamma irradiation had a stimulating effect on the plant height, number of flowers per plant and flower diameter in all the varieties of *T. patula* L. Meanwhile, dosage higher than 10.0 kR caused reductions in all the morphological parameters studied as compared to the control samples. The highest total soluble protein content [(4.11±0.47) mg/g FW] was observed in plantlets irradiated at 2.5 Kr. The present study also revealed that higher dosage of gamma irradiation (15.0 to 25.0 kR) had stimulatory effect on the specific activity of peroxidase content of *T. patula* L. varieties while low doses (2.5 and 10.0 kR) had a inhibitory effect on the specific activity of peroxidase. Conclusion is gamma irradiation administered at low to moderate doses of 2.5 to 25.0 Kr may induce *T. patula* L. var Bonanza, Safari Red, Guljafri Yellow and Nana patula Yellow mutants with superior novel characters.

**Keywords:** gamma irradiation, mutation breeding, total soluble protein content, peroxidase activity

## 1. Introduction

Gamma irradiation has emerged as a new physical mutagen in the mutation breeding of ornamental plants, result in the induction of novel mutations. During past decades, mutation breeding has been used for generating genetic variations of new crop varieties [1]. The crop plant characteristics that have been improved by mutation breeding are those that have either not found with natural selection or were not achieved during previous plant breeding efforts [2]. The successful achievement with mutation breeding techniques for the improvement of the major crops in the world would indicate that it is no longer a controversial breeding technique, but an important way to complement the conventional breeding technology. Recently, physical mutagens have been established as effective tools of inducing mutations in many plant species due to its high frequency and broad spectrum [3]. The conventional approaches of plant breeding have exploited the available genetic variability in *T. patula*. Mutagens have remarkable possibilities of improving plants with regard to their qualitative and quantitative traits. The current study was conducted with the aim of determining the morphological and physiological responses of *T. patula* L. *viz*., Bonanza, Guljafri Orange, Guljafri Yellow, Nana patula Yellow and Safari Red toward gamma irradiation at different doses. Thus, this study attempted to further improve the quality and traits of *T. patula* L. *viz*., Bonanza, Guljafri Orange, Guljafri Yellow, Nana patula Yellow and Safari Red through ion beam irradiation.

## 2. Materials and Methods

### 2.1 Plant materials and irradiation method

The planting material (seeds) of all the varieties of *Tagetes viz*., Bonanza, Guljafri Orange, Guljafri Yellow, Nana patula Yellow and Safari Red will be treated with gamma rays (Cobalt-60) at 2.5, 10.0, 15.0, 20.0, 25.0 kR, respectively, which would be applied to the seeds just before sowing in the gamma chamber of National Botanical Research Institute, Lucknow and then immediately sown for raising nursery on raised beds and seedlings (about 30 days old) will be used as a planting material to be transplanted in experimental plot. Untreated seeds of each variety will be planted beside the treated population for the ease of comparison. Variations caused by these mutagenic treatments would be recorded by observing the difference in growth and flowering attributes between treated and untreated seed derived plants.

### 2.2 Morphological studies
Both control and irradiated plantlets of different varieties of *T. patula* L. were subjected to morphological studies, whereby, the survival percentage of the seeds was determined after 30 days of germination. Meanwhile, the height and number of branches per plant were observed and recorded at maturation of the plantlets.

### 2.3 Biochemical studies
#### 2.3.1 Protein extraction
Leaves of irradiated and non-irradiated seeds were extracted with Tris protein extraction buffer (pH 8.0) at the ratio of 1 g of sample to 10 ml of protein extraction buffer. The samples were homogenized in an ice bath with the addition of a protein extraction buffer. The crude extracts were then transferred to a 1.5 ml centrifuge tube followed by centrifugation at 15,000 rpm for 20 min at 4°C. The resulting supernatant was transferred to a new centrifuge tube and was used for the determination of total soluble protein content of different varieties of *T. patula* L. plantlets.

#### 2.3.2 Determination of total soluble protein content
The total soluble protein contents of the irradiated and non-irradiated varieties of *T. patula* plantlets were determined by using the Bradford method [4]. The total soluble protein assay was carried out by addition of 20 µl of sample extract to 80 µl of protein extraction buffer and 5 ml of protein reagent. The solution was thoroughly mixed by vortexing, followed by a determination of absorbance at 595 nm using GENESYS 20 spectrophotometer (Thermo Scientific, USA). The total soluble protein content of the samples was determined from the standard curve plotted by using bovine serum albumin (BSA; Sigma Aldrich, USA) as the standard at 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mg/ml. The total soluble protein content of the samples was then expressed in mg/g FW of the plant material.

### 2.4 Statistical Analysis
In this study, the experiments were carried out in three replicates for both morphological and biochemical studies where each set of the experiments was repeated once. The results obtained from the morphological and biochemical studies of the plant samples were subjected to one-way analysis of variance (ANOVA) as well as determine the significance differences between the mean of each parameter tested in the study at $P<0.05$.

## 3. Results and Discussion
### 3.1 Height of plantlets
The morphological study was carried out after maturation of the plantlets of all the five varieties to compare the heights of the irradiated and non-irradiated plantlets Fig 1. There was significant difference in the height between all the irradiation doses in all the varieties of *T. patula* L. However, all the plantlets of the irradiated seeds showed a slight reduction in their height as compared to the control samples in higher doses with an exception for plantlets irradiated at 10 Gy of carbon ion beam, which were 3.9% taller than the control samples.

**Plant Height (cm) at maturity**
It is evident from the data that plant height gradually decreased with increase in gamma rays dose. It reflects that in $M_1$ generation, plants which were given 25.0 Kr gamma rays treatment recorded minimum plant height at maturity (26.71 cm) while, maximum plant height was observed in 2.5 Kr gamma rays treatment (40.27 cm). In $M_2$ generation, maximum plant height (39.01 cm) was observed in lower dose (2.5 Kr) gamma rays whereas; smaller plant height (24.68 cm) was recorded at higher dose (25.0 Kr) which was at par with rest of treatments. In $M_3$ generation, maximum plant height (41.73) at maturity was observed in 2.5 Kr dose whereas, smaller plant height (25.48 cm) was recorded in 25.0 Kr gamma ray dose.
Variety differences were also significant in $M_1$, $M_2$ and $M_3$ generation. Maximum plant height was recorded in variety Guljafri yellow in $M_1$ (66.81 cm) which was at par with variety Guljafri orange (34.66 cm), in M2 and M3 the variety Guljafri yellow again exhibited maximum plant height (65.50, 66.17) cm which was at par with variety Guljafri orange (31.98, 35.24) cm, respectively.
The untreated plants of all the varieties in $M_1$, $M_2$ and $M_3$ resulted in maximum plant height except Guljafri yellow at 2.5 Kr gamma rays, which exhibited highest plant height (78.29, 77.89, 79.58) cm, respectively whereas, variety Bonanza resulted in all three generations minimum plant height (15.12, 15.37, 14.29) cm, respectively which was significantly shorter than the other interactions.
In general, there was reduction in plant height after gamma radiations in all the varieties except in Guljafri yellow where it has increased at 2.5 Kr. Reductions were more at higher doses in all the varieties. Significantly longer plant height in Guljafri yellow at 2.5 Kr might be due to the reason that it was an early variety and growth was faster as compared to other varieties. Inactivation of auxin and decrease in auxin content with increase in radiation doses is responsible for reduction of plant height.
The present study revealed that inhibited growth in terms of height was evident in *T.patula* L. plantlets with increasing doses of gamma radiation administered on the seeds. The negative impact of radiation on plants may be indirectly mediated via metabolic changes through free radical formation, as well as indirectly, by DNA damage to the dividing cells [5]. According to [6], cell division is the most sensitive parameter to irradiation, which might account for the growth inhibition observed in irradiated plantlets.
On the contrary, stimulated growth was observed on *T.patula* L. var Guljafri orange plantlets derived from seeds that were irradiated with low doses of gamma rays. The stimulation of plant height at low doses of gamma irradiation was frequently observed on plants [7, 8]. This may be due to the hormetic effects of low-dose ionizing radiation on plants that accelerated the cell proliferation, stimulated germination and growth, or even increased yield. These effects could be characterized as the modulation of photosynthesis and antioxidant machineries [7].

### 3.2 Number of flowers per plant
Fig. 2 reveals that there were significant effects of gamma radiations and varieties on number of flowers per plant. The irradiation of plants with gamma rays, irrespective of varieties caused non-significant reduction in number of flowers per plant. Maximum number of flowers per plant (17.04) was recorded in control, and was higher than the rest of the treatments. Further, increase in gamma rays dose caused

significant reduction in flower number. The higher doses of gamma rays (25.0 Kr) resulted in minimum number of flowers per plant (15.90). In $M_2$ and $M_3$ generation also, similar reduction in number of flowers per plant were recorded at higher dose whereas, control resulted in more number of flowers (16.93, 16.98, respectively) as compared to higher doses of gamma radiations. Minimum flowers per plant (15.85, 15.83) were recorded in the plants treated with 25.0 Kr treatment in $M_2$ and $M_3$ generations, respectively.

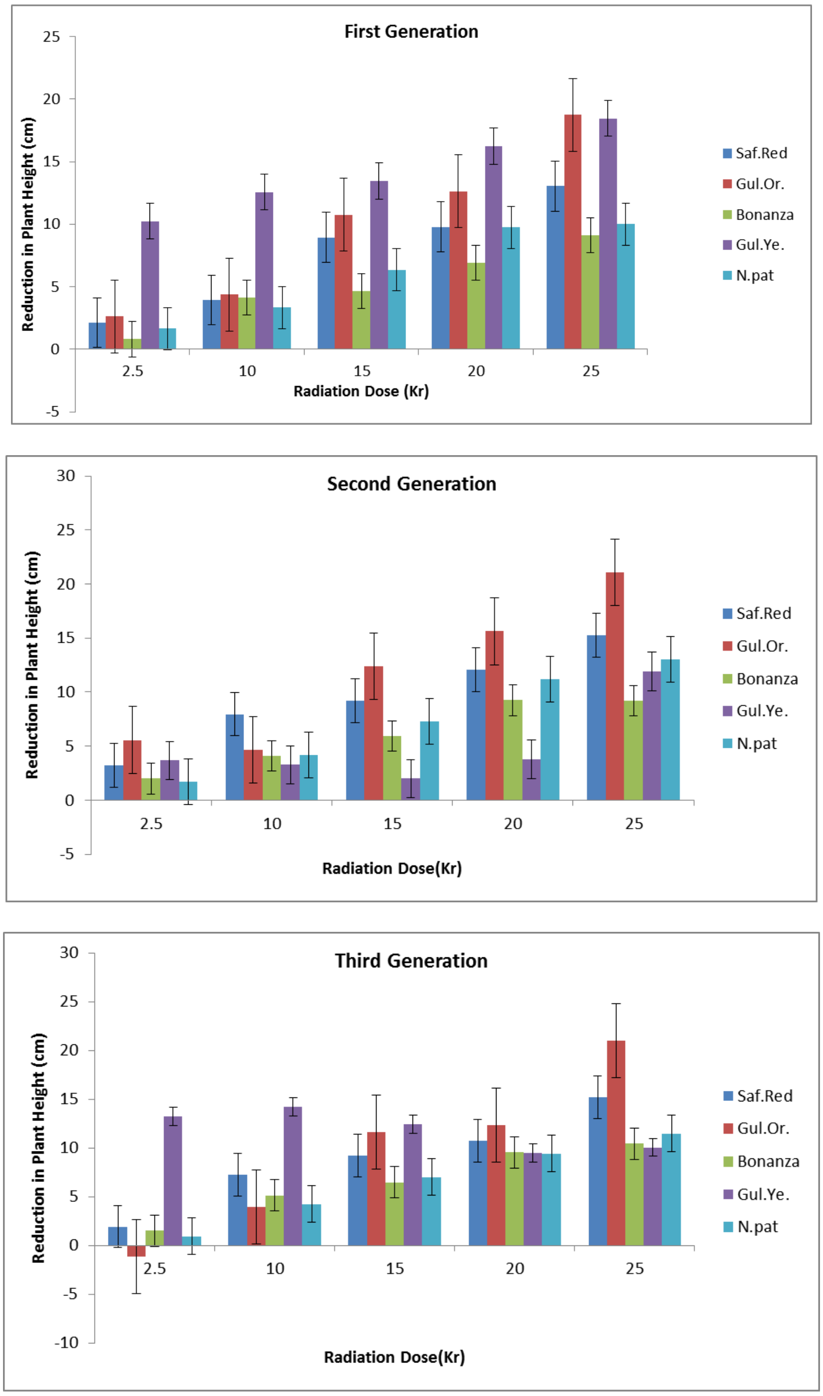



**Fig 1:** Effect of gamma irradiation on plant height at maturity on five varieties of *T. patula* L (Each *bar* represents Mean ±SD [Standard deviation] $p < 0.05$

Among the variety, Guljafri Orange exhibited maximum number of flowers per plant in $M_1$ (19.98) as well as Bonanza in $M_2$ (20.15) and $M_3$ (20.13), whereas, minimum number of flowers per plant in $M_1$, $M_2$ as well as in $M_3$ generation was observed in variety Safari red (18.13, 18.08, 18.10, respectively).

The interaction effect of varieties and gamma rays treatment was significant in $M_1$ generation but in $M_2$ and $M_3$generation was found to be non-significant. In general, interactions of all the varieties with control resulted in highest number of flowers per plant and interaction with highest dose (25.0 Kr) caused more reduction in flowers in all the varieties as compared to lower doses.

Decrease in flowers per plants with higher doses is mainly due to disturbances in plant physiological processes and reduction in vegetative growth of plant. Researchers also recorded increase in flower number at lower doses of gamma radiation in zinnia, whereas number of flower heads reduced drastically at higher doses of gamma rays [9]. These results also corroborate with the findings [10] who recorded maximum number of florets per spike in untreated plants and reduction in florets at higher doses of gamma rays in gladiolus.

### 3.3 Flower diameter after gamma rays

The data revealed to the effect of gamma rays treatment on the flower diameter have been presented in Fig 3. It is evident from the data that highly significant differences for this character were recorded in all the three generations. The perusal of the data revealed that irrespective of the varieties, lower doses (2.5 Kr) treated plants had significantly larger flower size in $M_1$ (6.32cm), $M_2$ (5.67cm) and $M_3$ (6.23cm) than un-radiated plants in all the three generations, while exposure to higher doses gamma rays reduced the flower size significantly. The 25.0 Kr gamma rays treated plants recorded lowest flower diameter (5.99 cm) followed by 20 Kr gamma rays treatment (5.70 cm) in $M_1$ generation. In $M_2$ generation, 5.92 cm in 25.0 Kr followed by 5.63 cm in 20.0 Kr and in $M_3$ generation, 5.93 cm in 25.0 Kr followed by 5.63 cm in 20.0 Kr recorded lowest flower diameter. The variety differences for flower diameter were significant in $M_1$, $M_2$ as well as in $M_3$ generation. Irrespective of gamma rays treatment, variety Bonanza exhibited significantly maximum flower diameter (8.21), whereas minimum flower diameter was recorded in variety Guljafri yellow (4.53 cm) in $M_1$ generation. In $M_2$ generation, significantly maximum flower diameter was recorded in the variety, Guljafri orange (5.73 cm) while, minimum flower diameter was exhibited by variety Bonanza (4.44 cm). In $M_3$ generation, maximum flower diameter was recorded in the variety, Bonanza (8.21 cm) while, minimum flower diameter was examined by variety Safari red (5.71 cm). A critical perusal of data revealed that in $M_1$, $M_2$ and $M_3$ generations, variety Bonanza (8.38, 8.39,8.44 cm) treated with 2.5 Kr followed by Safari red had maximum flower size (6.47,6.32, 6.44 cm), respectively in comparison to untreated plants. Minimum flower size in $M_1$, $M_2$ and $M_3$ generations was exhibited by Guljafri yellow (4.34, 4.31, 4.28 cm) with 25.0 Kr gamma radiations.

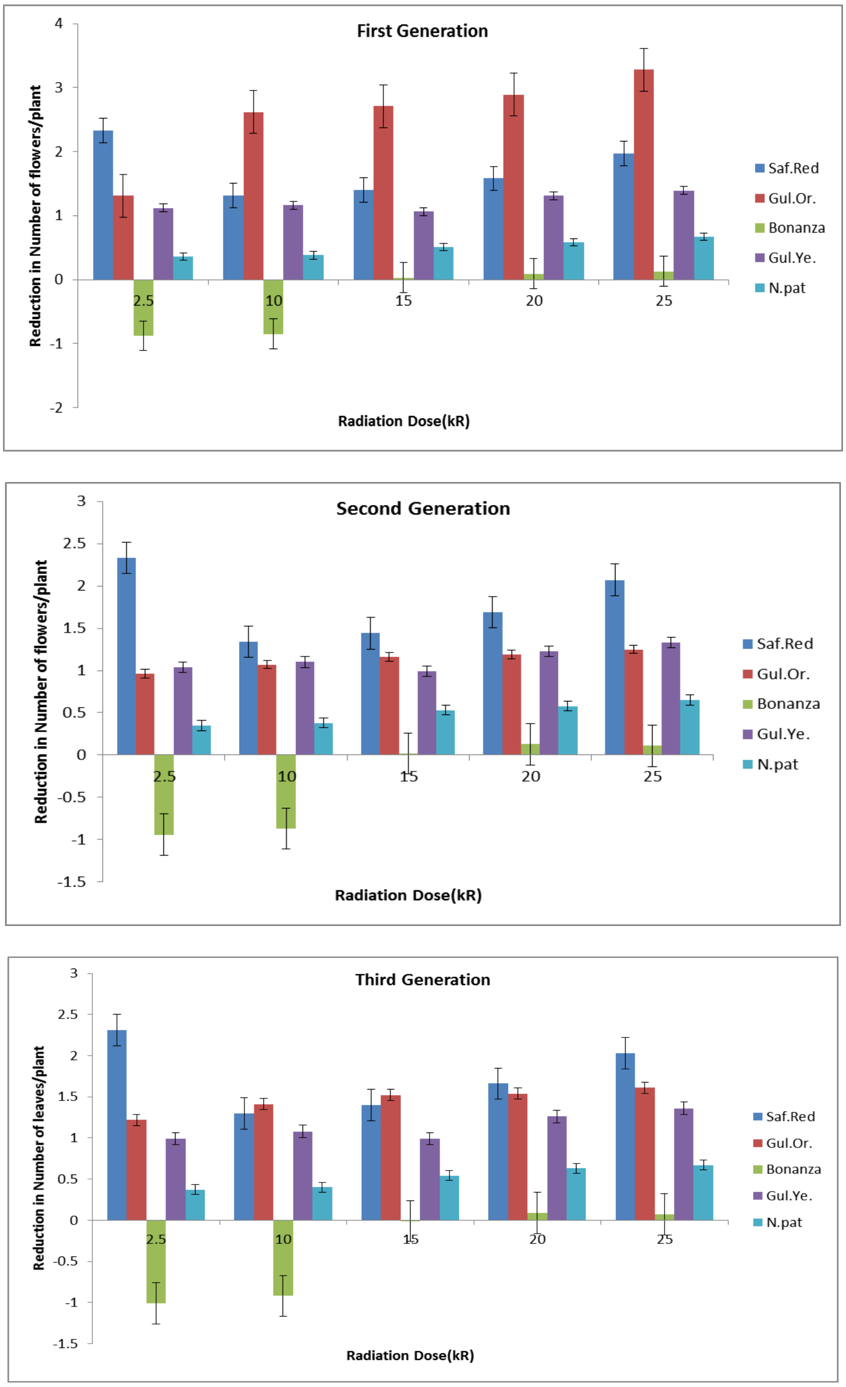



**Fig 2:** Effect of gamma irradiation on number of flowers per plant on five varieties of *T. patula* L (Each *bar* represents Mean ±SD [Standard deviation] $p < 0.05$

In general, higher doses of gamma radiation reduced flower size drastically. This might be due to reduction in vegetative growth of plants. Reduction in flower size at higher doses of gamma rays treated was also reported in chrysanthemum [11]. These results are also in lines with findings who reported reduction in flower size of dahlia cv. 'Pinki' after gamma radiations of rooted cuttings. At higher doses, effect of gamma radiations was more pronounced, which resulted in smaller flower size [12].

### 3.4 Total soluble protein content

The biochemical assay revealed that the total soluble protein content of different varieties of *T. patula* L. showed some variability with different doses of gamma irradiation. According to Fig. 4 irradiation of seeds at lower doses (2.5 to10 Kr) resulted in plantlets with relatively high total protein content in *T. patula* L. var Safari Red and *T. patula* L. var Nana patula yllow compared to the other varieties in $M_1$, $M_2$ and $M_3$ generations. However, irradiation with higher doses (15 to 25 Kr) of gamma irradiation gradually reduced the total soluble protein content of the resulting varieties of *T. patula* L.

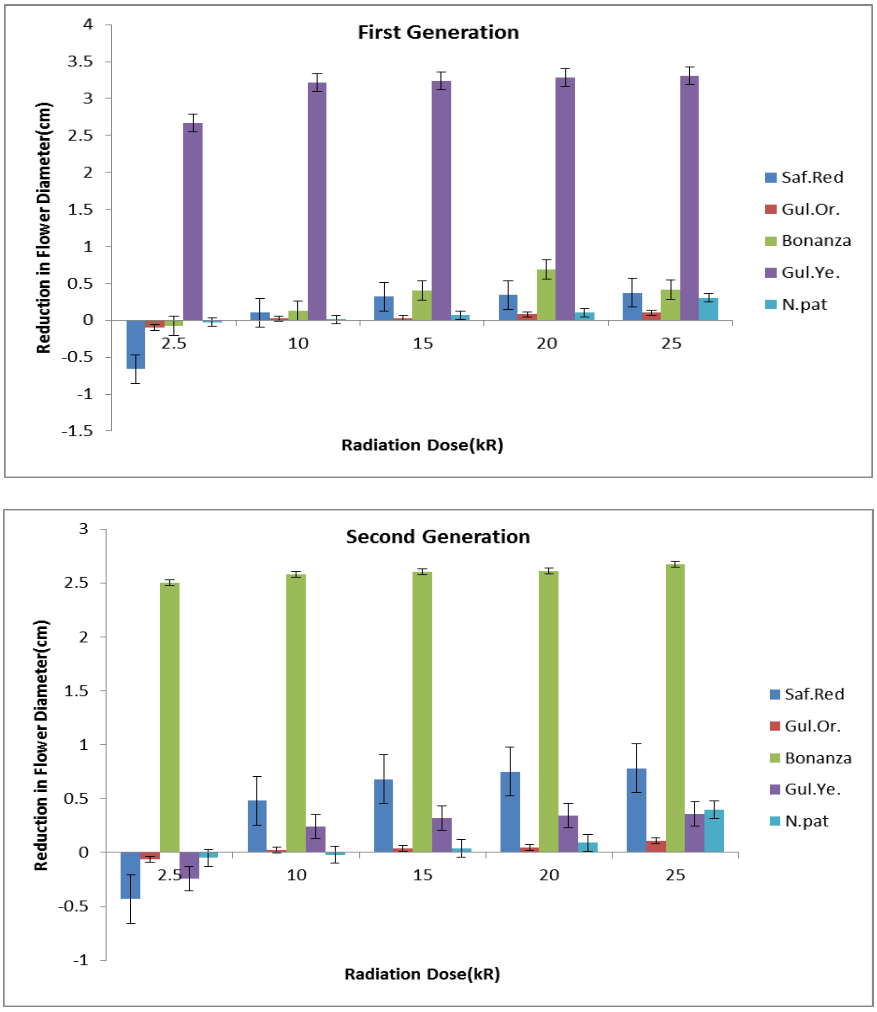



**Fig 3** Effect of gamma irradiation on flower diameter on five varieties of *T. patula* L (Each *bar* represents Mean ±SD [Standard deviation] $p< 0.05$

Gamma irradiation on the seeds of different varieties of *T. patula* L. causes a considerable stress on the plant. Plants may alter their metabolic mechanisms and gene expressions to cope with the stress effect [13]. The plants stress often results in an alteration of gene products [14], which plays an important role in the molecular changes of stress tolerance [13].

Different genes displayed different patterns of gene expression in response to irradiation in tobacco plants, thereby complex signaling mechanism is involved in the irradiation-induced defense by plants [15]. Therefore, altered gene expression under radiation stress, the qualitative and quantitative variation in protein content was obvious [16]. These proteins might play a role in signal transduction, anti-oxidative defense, metal binding and osmolyte synthesis, which are essential to a plant's function and growth [17]. This might be the possible explanation for the increase in total soluble protein content at low irradiation doses as observed in the present study.

On the other hand, radiation may disrupt hydrogen bond, destroy sulfydryl groups, or affect other reactive groups in the side chain of protein molecules [18]. This can cause irreversible change in the protein conformation, which tends to occur with high irradiation doses. In addition, fragmentation and aggregation of protein molecules might also occur in response to radiation exposure [19]. These phenomena might lead to a reduction in total soluble protein content when the seeds were irradiated at high doses of ion beam, as observed in the current study.

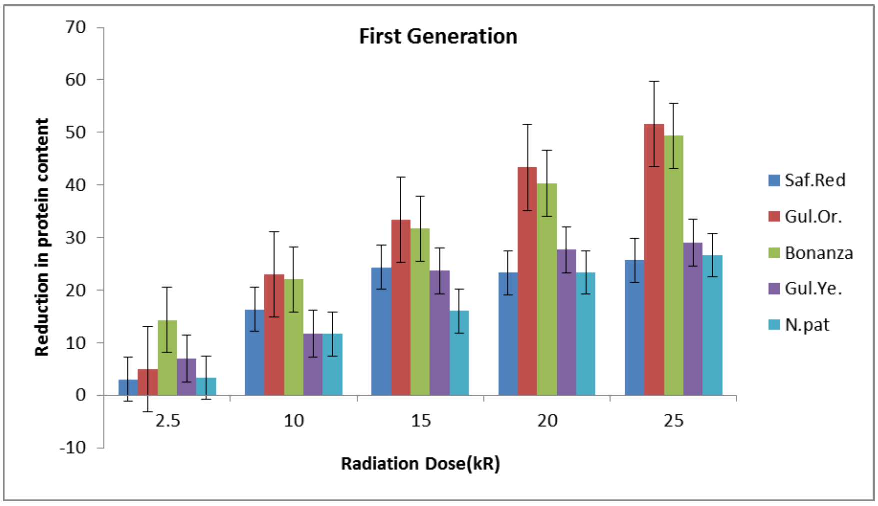
Extensive research has been carried out to investigate the effects of γ irradiation and other ionizing radiation on the total protein content of plants, while little research evidence was found regarding the effects of ion beam irradiation on the protein content in a plant model. In the study which investigated the effect of γ irradiation on edible seed protein, it was discovered that the total protein content decreased with increasing doses of γ irradiation as compared to the control samples [20, 21] revealed that there was a significant increase in protein contents of corn seeds and *Zea mays* after irradiating the seeds at 0, 17.39, 86.96, 130.40, and 173.90 Gy of γ rays. Similarly, discovered an increase in the protein content of *O. sativa* after irradiation.

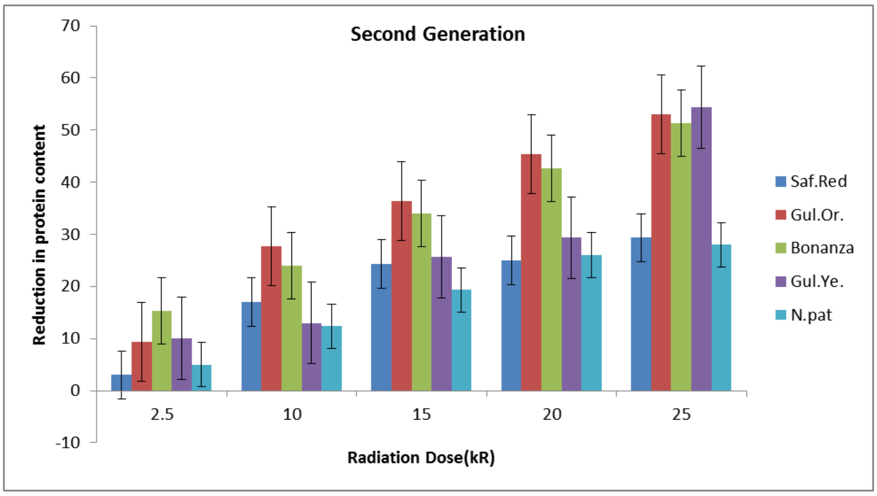
### 3.5 Specific activity of peroxidase

Specific activity of peroxidase of varieties of *T. patula* L. was determined after maturation of different varieties of *T. patula* L. As illustrated in Fig. 5 an increase in the specific activity of peroxidase was observed with increasing doses of ion beam. Non-irradiated plantlets were found to have specific activity of peroxidase of 547.67 U/mg, which was the lowest among all the treatments. On the other hand, the highest specific activity of peroxidase (1,165.42 U/mg) was recorded in plantlets irradiated with 25.0 kR of gamma irradiation, which was significantly greater than that of the control samples. Meanwhile, plantlets at 15 kR also exhibited relatively high specific activity of peroxidase, which was 748.19 U/mg. This study also revealed that plantlets irradiated at 15.0, 20.0 and 25.0 kR showed greater specific activity of peroxidase of 17.09%, 24.14%, and 27.45%, respectively, over the control samples. However, statistical analysis showed that the specific activity of peroxidase was not significantly different between all the treatments.

Gamma irradiation generates free radicals, which might be inhibitory to the biochemical processes [23]. Water radiolysis as the predominant effect of radiation in organisms which induces reactive oxygen species (ROS) formation, one can assume that plant enzymes that are involved in cell protection against oxidative stress will display similar responses under radiation stress as under other stress factors [24]. According to the research conducted by [25] heavy ion obviously enhanced ROS, which is reflected by the increased production of $O_2$ and $H_2O_2$. Consequently, plants need to develop antioxidant enzymes and antioxidative molecules to mitigate and repair the damage initiated by free radicals or ROS [23]. Therefore, peroxidases play an essential protective role against oxidative stress and they are the indicators of cellular damage [26].

Peroxidase, antioxidant enzymes play an important role in plant defense systems (Veitch, 2004). In this study, it has been demonstrated that the specific activity of peroxidase increased with increasing doses of gamma irradiation. All *T. patula* L. plantlets derived from irradiated seeds were found to have higher specific activity of peroxidase as compared to the control plantlets of different *T. patula* L. varieties.



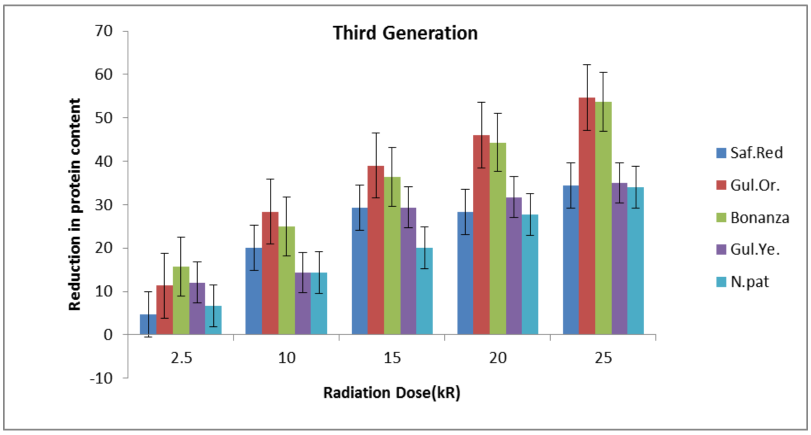



**Fig 4:** Effect of gamma irradiation on protein content of five varieties of *T. patula* L (Each *bar* represents Mean ±SD [Standard deviation] $p < 0.05$

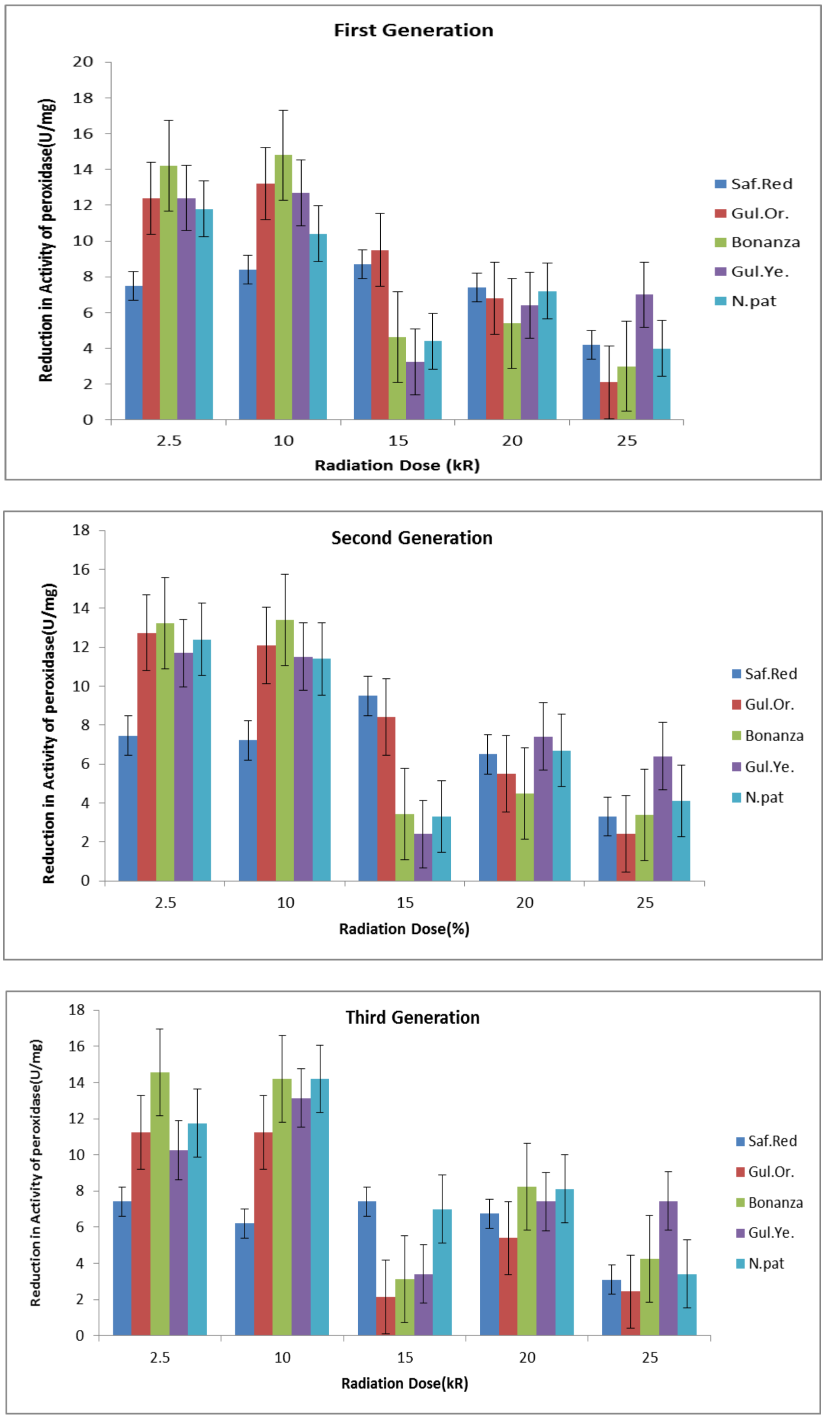



**Fig 5:** Effect of gamma irradiation on specific activity of five varieties of *T. patula* L (Each *bar* represents Mean ±SD [Standard deviation] $p < 0.05$

## Conclusion

In short, the morphological and biochemical responses of different varieties of *T. patula* L. towards high energetic gamma beam are dose-dependent. Low irradiation doses at 2.5 to 10.0 kR stimulate plant height, number of flower per plant, flower diameter as well as total soluble protein content of Safari Red followed by Nana patula Yellow, while irradiation at 15.0 to 25.0 kR resulted in an increase in peroxidase activity. Therefore, it is postulated that irradiation administered at low to moderate doses of 2.5 to 25.0 kR may induce mutants with superior characteristics of different varieties of *T. patula* L in $M_1$, $M_2$ and $M_3$ generations. Further investigations were carried out on further progeny of T. *patula* L. plantlets, by this possible mutants were induced by irradiation. Study shows that gamma irradiation on *T. patula* L. is transmittable to the succeeding generations.

## References


1. Fu HW, Li YF, Shu QY. A revisit of mutation induction by gamma rays in rice (*Oryza sativa* L.): implications of microsatellite markers for quality control. Mol. Breed. 2008; 22:281-288.
2. Sharma KD. Induced Mutagenesis in Rice. *In*: Rice Genetics: Proceedings of the International Rice GeneticsSymposium. International Rice Research Institute, Manila. 1986, 680.
3. Hayashi Y, Takehisa H, Kazama Y, Ichida H, Ryuto H, Fukunishi N, Abe T. Effects of ion beam irradiation on mutation induction in rice. Cyclotr. Their Appl. 1986; 18:237-239.
4. Bradford M. A rapid and sensitive method for the quantification of the icrogram quantities of proteins utilizing the principle of protein dye binding. Anal. Biochem. 1976; 72(1-2):248-254.
5. Jones HE, West HM, Chamberlain PM, Parekh NR, Beresford NA, Crout NMJ. Effects of gamma irradiation on *Holcus lanatus* (Yorkshire fog grass) and associated soil microorganisms. J Environ. Radioact. 1976; 74(1-3):57-71.
6. Vazquez-Tello A, Uozumi T, Hidaka M, Kobayashi Y, Wanatabe H. Effect of 12C+5 ion beam irradiation on cell viability and plant regeneration in callus, protoplasts and cell suspensions of *Lavatera thuringiaca*. Plant Cell Rep. 2005; 16(1-2):46-49.
7. Kim JH, Baek MH, Chung BY, Wi SG, Kim JS. Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annum* L.) seedlings from gamma-irradiated seeds. J Plant Biol. 2004; 47(2), 314-321.
8. Ling APK, Chia JY, Hussein S, Harun AR. Physiological responses of *Citrus sinensis* to gamma irradiation. World Appl. Sci. J. 2008; 5(1):12-19.
9. Kole PE, Meher SK. Effect of gamma rays of some quantitative and qualitative characters in zinnia *ginnia elegans.* J. of Orna. Horti. 2005; 8(4):303-305.
10. Sisodia A, Singh AK. Influence gamma radiation on morphological changes, post harvest life and mutagenesis in gladiolus. Inter. J of Agri., Envir. and Biotech. 2004; 7(3):535-545.
11. Kumari K, Dhatt KK, Kapoor M. Induced mutagenesis in Chrysanthemum morifoliumvariety' Otome pink' through gamma irradiation. Biosc. 2013; 8(4):1489-1492.
12. Dwivedi AK, Banerji BK. Effect of gamma irradiation on dahlia cv. 'Pinki' with particular reference to induction of somatic mutation. Journal of Ornamental Horti. 2008; 11(2):148-151.
13. Rao KVM. Introduction. In: Physiology and Molecular Biology of Stress Tolerance in Plants. Springer, the Netherlands, 2006, 1.
14. Humera A. Biochemical and Molecular Markers of Somaclonal Variants and Induced Mutants of Potato (*Solanum tuberosum* L.). PhD Thesis, University of Punjab Lahore, Pakistan, 2006.
15. Cho HY, Lee HS, Pai HS. Expression pattern of diverse genes in response to gamma irradiation in *Nicotiana tabacum*. J Plant Biol. 2000; 43(2):82-87.
16. Corthals G, Gygi S, Aebersold R, Patterson SD. Identification of proteins by mass spectrometry. Proteome Res. 2000; 2(1):286-290.
17. Qureshi MI, Qadir S, Zolla L. Proteomics-based dissection of stress-responsive pathways in plants. J Plant Physiol. 2007; 164(10):1239-1260.
18. Hayden GA, Friedberg F. Effects of gamma radiation on ribonuclease. Radiat. Res. 1964; 22(1):130-135.
19. Gaber MH. Effect of γ-irradiation on the molecular properties of bovine serum albumin. J Biosci. Bioeng. 2005; 100(2):203-206.
20. Maity JP, Chakraborty S, Sandeep K, Subrata P, Jean J, Samal AC *et al*. Effects of gamma irradiation on edible seed protein, amino acids and genomic DNA during sterilization. Food Chem. 2009; 114(4):1237-1244.
21. Iqbal J, Kutaček M, Jiraček V. Effects of acute gamma irradiation on the concentration of amino acids and protein-nitrogen in *Zea mays*. Radiat. Bot. 1974; 14(3):165-172.
22. Khanna VK, Maherchandani N. Effects of gamma irradiation and seedling growth of "Kabuli" and "Desi" chickpea on the activity of alpha amylase. Indian J Genet. Plant Breed. 1985; 28(2):3-10.
23. Verma S, Lakra N, Sarma A, Misha SN. Effect of Li+ Heavy Ion on Hydrogen Peroxide Decomposing Enzymes in Leaves of *Brassica juncea.* MS Thesis, Maharshi Dayanand University, Rothak, 2009.
24. Zaka R, Vandecasteele CM, Misset MT. Effects of low chronic doses of ionizing radiation on antioxidantenzymes and G6PDH activities in *Stipa capillata.* J Exp. Bot. 2002; 53(376):1979-1987.
25. Zhang L, Zhang H, Zhang X, Zhu J. Assessment of biological changes in wheat seedlings induced by 12C6+-ion irradiation. Nucl. Sci. Tech. 2008; 19(3):138-141.
26. Hameed A, Mahmud Shah T, Atta BM, Haq MA, Sayed H. Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in Desi and Kabuli chickpea. Pak. J Bot. 2008; 40(3):1033-1041.