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Effect of gamma irradiation on the chlorophyll content of tree tomato (*Solanum betaceum* Cav.) in M_1 generation

Akoijam Merina Chanu, Rocky Thokchom, Th. Bhaigyabati, Ningthoujam Sandhyarani Devi and Thokchom Ronald Meitei

Abstract
Gamma irradiation stimulates a broad range of change in physiology and biochemical alterations in plants by modulating specific defensive and metabolic pathways. Pre-sowing seed irradiation is deliberated as an effective method of increasing production, yield and chemical compositions in plants. In the current study, *Solanum betaceum* Cav. (Tree tomato) is induced to gamma irradiations with absorbed doses 0 Gy, 10 Gy, 25 Gy, 50 Gy, 75 Gy and 100 Gy and 0 Gy as a control dose. When they were exposed to the variable doses of gamma radiations, they show consistent changes in the growth and development of the plants. The current study experiments on the chlorophyll content of the irradiated and non-irradiated plants in M1 generation. The result of the study shows that the effect of gamma irradiations on chlorophyll content is significant in M1 generation of the tree tomato. From the data it shows that the chlorophyll content decreases significantly after gamma irradiation is used on first generation when compared with the control dose. The highest chlorophyll content (chl a: 16 µg/ml, chl b: 3.43µg/ml, total chlorophyll: 19.43µg/ml) is recorded in the non-irradiated plants. Among the gamma irradiated plants, chlorophyll content of 10 Gy (8.32µg/ml) is found highest while, lowest (2.61µg/ml) is observed at higher irradiation i.e.,100 Gy at M1 generation. This shows that chlorophyll content decreases with the increase in gamma doses. The above biochemical analysis confirms that photosynthetic components are very responsive to gamma irradiation and are good index of persistence which provides sufficient evidences to the effect that γ-irradiation. Thus, gamma rays demonstrate to be a prime tool to enhance the efficiency of breeding and frequency of regeneration.

Keywords: *Solanum betaceum*, gamma ray, chlorophyll content, solvent

Introduction
Chlorophyll is a green photosynthetic pigment usually found in green parts of plant. It is an antioxidant compound stored in the chloroplast of green leaf plants and mainly present in the area of green leaves, stems, flower and roots (Srichaikul *et al*., 2011; Mirza *et al*., 2013; Kamble *et al*., 2015) [13, 9, 5]. The main pigments of the plant photosystems are chlorophyll a and chlorophyll b (Kamble *et al*., 2015; Richardson *et al*., 2002) [5, 12]. Concentration of leaf chlorophyll can be obtained from the physiological condition of a leaf or plant (Kamble *et al*., 2015) [5]. It helps in indicating the content of chloroplast, photosynthetic mechanism and plant metabolism). The concentration of chlorophyll a is 2-3 times higher than that of chlorophyll b (Srichaikul *et al*., 2011) [13]. Plants are sensitive to radiations. Under heavy irradiations, the growths of plant are found to be retarded to the extent of becoming lethal. The physiological changes or disturbances under irradiations detect the correlation between the content of chlorophyll and gamma irradiation. The current study attempts to show the biochemical analysis of the chlorophyll content in tree tomato (Solanum betaceum Cav.). Tree tomato (Solanum betaceum Cav.), is an edible fruit belonging to the Solanaceae family with high nutritional value and the potential for becoming premium products in local and export markets (Enciso-Rodríguez *et al*., 2010) [1]. The use of ionizing radiation is the elementary tool of nuclear technology in terms of enhancing the yield in plants which results in induced mutations in plants. The usages of the technology are seen in extensive assortments of characters. The technology has been used to enhance induced mutation breeding which include plant architecture, yield, blossoming and adulthood duration, superiority and tolerance to biotic and abiotic pressures. According to Kharkwal (2000) [6], about 89% of mutant variations have been established using physical mutagens such as X-rays, gamma rays, thermal and fast neutrons whereas with gamma rays alone accounting for the development of 60% of the mutant varieties.

Thus, the mutation might have valuable and sophisticated efficient values. There are numerous practices of nuclear techniques in agriculture. Gamma rays have been used for developing biotic- abiotic stresses tolerance and plant characteristics in the plants grown from the seed (Jain, 2010) [3]. With this view, variable doses of gamma rays were used as induced mutagen on chlorophyll content of leaves of Tree tomato (*Solanum betaceum* Cav.).

Materials and Methods

The present investigation entitled "Effect of gamma radiation on the chlorophyll content of tree tomato (Solanum betaceum Cav.) in M1 generation" was carried out at the experimental field of Department of Agriculture, School of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences (PDDUIAS), Utlou, Manipur during rabi season, 2018-2019. A poly house experiment was carried out in Horticulture Research Farm, School of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences during 2018- 2019. Seed mutagenesis was performed at the Department of Radiology and Cancer Centre, Regional Institute of Medical Sciences (RIMS), Imphal.

Field trial

The seeds were collected from Krishi Vigyan Kendra (KVK), Utlou, Bishnupur district. The healthy seeds were irradiated by gamma-rays with different doses and non-irradiated seeds were served as control and arranged in a completely randomized block design in three replicates. After mutagenesis, the seeds were soaked for 24 hours in distilled water at a temperature of around 25ºC. Three replicates of 30 seeds of each mutagen dose were germinated in poly trays containing sand, coco-peat and compost and kept under laboratory conditions supplied with water every day in order to maintain moisture. The field experiment lies in 24.71˚N latitudes and 93.84˚E longitudes. It is situated at an altitude of 820 m above mean sea level. Irradiated seeds of each dose were transplanted at 4 weeks on August, 2018 in poly bags; all pots were equally spaced with equal soil contents (coco-peat, sand and compost) at the ratio of 1:1:1 in each pot.

Extraction of Chlorophyll

0.5g fresh plant leaf sample of each of the irradiated and non-irradiated were measured precisely and taken. Each sample was homogenized in mortar and pestle with 10 ml of 80% acetone separately. The homogenized sample mixtures were centrifuged at 10,000 rpm for 15 minutes at 4℃. The supernatants which measures at 0.5 ml were taken and mixed with 4.5 ml of 80% acetone, making up each sample supernatant to 5ml. The solution mixtures were analyzed for chlorophyll a (Chl a) and chlorophyll b (Chl b) by observing the absorbance at different wavelengths and for the respective solvents using spectrophotometer (Thermo Fisher UV 2700) as per the equation given below (Porra 1989, Lichtenthaler 1987 and Lichtenthaler and Wellburn 1983) [14, 7-8].

Solvent	Equations
80%	Acetone
Chl a =	12.7(A663) - 2.69 (A645)
Chl b =	22.9 (A645) – 4.68 (A663)

Result

The chlorophyll content of non-irradiated and irradiated plants is shown in Table 1 and Figure 1. The results showed that the effect of gamma radiations on chlorophyll content was significant in M1 germination of tree tomato. From the data that the chlorophyll content decreased significantly after gamma radiation was used in first generation when compared with the control dose. The highest chlorophyll content (chl a: 16 µg/ml, chl b: 3.43µg/ml, total chlorophyll: 19.43 µg/ml) was recorded in the non-irradiated plant. Among the gamma irradiated plants, chlorophyll content of 10 Gy (8.32µg/ml) was found highest, while lowest (2.61µg/ml) was observed at higher irradiation i.e.,100 Gy at M1 generation. This shows that chlorophyll content decreases with the increase in gamma dose. In addition, it was also observed that the concentration of chlorophyll a was relatively higher than chlorophyll b in non-irradiated and irradiated plants. Gamma irradiation resulted in greater reduction in the amount of chlorophyll b as opposed to chlorophyll a (Fukuzawa *et al*., 1998) [2]. The reduction in chlorophyll b is due to a more selective destruction of chlorophyll b biosynthesis or degradation of chlorophyll b precursors as reported by (Mishra *et al*.,2007; Neelam *et al*.,2014) [10, 11].

Table 1: Effects of different gamma doses on chlorophyll content in M1 plants.

	Chlorophyll content		
Doses	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll content**
0Gy	15.99 ± 5.04	3.43 ± 0.40	12.94 ± 8.41
10 Gy	4.88 ± 0.76	1.85 ± 0.13	4.48 ± 2.46
25 Gy	4.28 ± 0.82	1.51 ± 0.39	3.86 ± 2.17
50 Gy	3.49 ± 0.96	1.39 ± 0.24	3.25 ± 1.75
75 Gy	3.22 ± 0.96	1.27 ± 0.31	2.99 ± 1.62
100 Gy	1.91 ± 0.29	0.70 ± 0.33	1.74 ± 0.96
CD (5%)	3.82	0.56	6.82
CV %	0.38	0.18	0.78

Assays were performed in triplicates. Values are expressed as means ± SD.

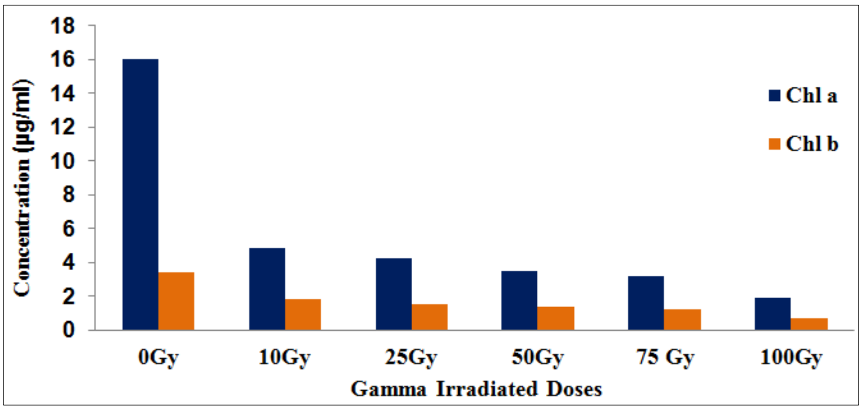

Fig 1: The effects of different gamma doses on chlorophyll content in M1 plants

Conclusion

The increase in doses of gamma rays decreases the chlorophyll content in the tree tomato. The concentration of chlorophyll a and chlorophyll b decreases with an increase in the dose of gamma rays. The present research clearly proves that induced mutation can be successfully employed to create genetic variability when it is preferred to improve definite traits in plants. Therefore, physical mutagenic treatments at lower frequency may be employed in inducing superior genotypes with significant alterations in growth and metabolism of the plant. However, further study is required to check the results in next generations of the present studied tree tomato plant.

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