PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 10(2): 289–301
https://doi.org/10.14719/pst.2141

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Evaluation of morphophysiological, biochemical and antioxidant activity of green gram (*Vigna radiata* (L.) R.Wilczek) in responses to gamma irradiation

Soundarya Vasudevan[1], Arulbalachandran Dhanarajan[1*], Yasmin Kasim[2], Vanmathi Sankar[1], Govindasamy Balasubramani[3], Selvakumar Gurunathan[4] & Aamir Raina[5]

[1]Division of Crop Mutation and Molecular Breeding, Department of Botany, Periyar University, Salem 636 011, Tamil Nadu, India
[2]Department of Botany, Shri Sakthikailash Women's college (Arts and Science) Military road, Salem 636 003, Tamil Nadu, India
[3]Division of Research and Innovation, Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha Nagar, Thandalam, Chennai 602 105, Tamil Nadu, India
[4]Department of Genetics and Plant Breeding, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Chengalpattu, Chennai 603 201, Tamil Nadu, India
[5]Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

*Email: arul78bot@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 23 September 2022
Accepted: 03 January 2023

Available online
Version 1.0 : 19 March 2023
Version 2.0 : 01 April 2023

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

CITE THIS ARTICLE

Vasudevan S, Dhanarajan A, Kasim Y, Sankar V, Balasubramani G, Gurunathan S, Raina A. Evaluation of morphophysiological, biochemical and antioxidant activity of green gram (*Vigna radiata* (L.) R.Wilczek) in responses to gamma irradiation. Plant Science Today. 2023; 10(2): 289–301. https://doi.org/10.14719/pst.2141

Abstract

Mutation breeding plays a vital role as a source of genetic diversity to improve plant growth and development. Green gram (*Vigna radiata* (L.) R. Wilczek) "Vamban 2" variety was selected for this investigation. The doses applied to the healthy seeds of green gram were 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy and 800 Gy and non-treated seeds were kept as control throughout this study. A lethal dose (LD50) was observed at 500 Gy, whereas seedling length, fresh and dry weight decreased as the applied doses increased while compared to the control. Irradiated seedlings showed a decreased content of chlorophyll a compared to Chlorophyll b and increased carotenoid content compared to the control. Biochemical characteristics such as reducing sugar, starch, protein, amino acid and proline content were increased and noted maximum at 800 Gy. Antioxidants and lipid peroxidation (MDA) increased gradually along with increasing doses. FTIR analysis exhibited maximum functional groups at 600 Gy and ESR data showed ample hyperfine range of structure at 500 Gy, 600 Gy and 700 Gy. This investigation found considerable alterations in morphology, photosynthetic pigment, biochemical characteristics and antioxidant analyses, which suggest an idea to select an appropriate dose of gamma irradiation in green gram for successive breeding programme.

Keywords

Green gram, gamma rays, antioxidant, biochemical characteristics, photosynthetic pigments

Introduction

Green gram (*Vigna radiate* (L.) R. Wilczek), commonly known as mung bean is one of the major pulse crops containing 26 % of protein, 51 % of carbohydrates and 7 % of other elements (1). It is India's third most crucial pulse crop after the chickpea and pigeon pea (2). It is a self-pollinating legume crop with a short life span and provides lysine rich seed protein compared to many cereals (3). Since it is a highly self-pollinated crop, the natural crossing face limitations in producing new genetic combinations spontaneously. Also, it is cultivated mainly under

rain-fed and poor soils conditions (4) that led to genetic erosion and reduction in genetic variation over the years (5). The available natural variation in green gram is insufficient to achieve the required improvement to get appropriate yield potential. Reports and information on natural mutations in mung bean are scanty (6), and it requires induced mutagenesis to achieve variations in the development of novel traits. Generation of variations in crop varieties is the vital step in the plant breeding programmes to select the best performing genotypes by employing different screening methods. Gamma irradiation is a proven mutagen to generate genetic variation at the genome level to confer the genotypic and phenotypic variations in crop plants which is the base for crop improvement programmes (7). Gamma rays penetrate the cells and tissues and produce ROS species, consequently inducing mutations in seeds and other plant materials (8). Gamma irradiation can modulate the photosynthetic process and biochemical characteristics by producing these free radicals, resulting in increased growth and seed yield. Of 3402 mutants, 2581 were generated using physical mutagens, 1646 were produced using gamma rays and 39 green gram varieties were released (https://nucleus.iaea.org/sites/mvd/SitePages/Search.aspx). These novel varieties adapt quickly to agro-climatic and environmental circumstances (9). Thus, the present study was focused on evaluating the effect of gamma irradiation in 7th day seedlings of green gram on morphophysiological characteristics, biochemical and antioxidant potential for optimizing a desirable dose for developing novel variations in green gram.

Materials and Methods

Seed samples and mutagenic treatments

Green gram variety “Vamban 2” seeds were selected and obtained from National Pulses Research Centre Vamban, Pudukkottai, Tamil Nadu. Co^{60} as a source of gamma irradiation was utilized from Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. Healthy, dry and well-matured seeds covered with blotting paper were treated with various doses of gamma rays (Gray - Gy) at 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy and 800 Gy and untreated seeds were used as a control for this investigation. The uncertainty range of about 1-3 Gy/sec was observed as an average measurement of radiation. The gamma chamber and Fricke dosimetry system were calibrated for applied doses in line adhering to the guidelines of the International Atomic Energy Agency. To obtain 7^{th} day seedlings of green gram, irradiated seeds along with control were grown in Petri plates (15 seeds /petri plate) under laboratory conditions.

Germination %

Irradiated seeds along with control were kept for germination in petri plates with 2 layers of absorbent cotton covered with filter paper. The germination % was recorded on 5^{th} day.

$$\text{Seed germination (\%)} = \frac{\text{No of seeds germinated}}{\text{Total no of seeds kept for germination}} \text{X } 100$$

Lethal dose 50% (LD_{50}) was calculated based on germination %.

Morphological parameter analysis

Ten randomly selected seedlings were used to measure the shoot and root length from each dose along with control on 7^{th} day. Then, seedlings were separated into shoots and roots to weigh the fresh weight after initial measurement. Dry weight was measured after being stored in a hot air oven (Scientec - India) over a period of 24 hrs at 80 ^{0}C.

Photosynthetic pigment analysis

In gamma irradiated seedlings, the chlorophyll parameters were analyzed using the acetone method (10) and carotenoid content (11). Fresh young leaves of about 200 mg were grounded with 10 ml of 80% acetone and centrifuge (Remi R 24) at 800 rpm for 10 min. After centrifugation, a clear supernatant was obtained for analysis of photosynthetic pigments. The absorbance/ optical density (OD) was read at 663, 645 and 480 nm in UV spectrophotometer (SL159, Elico, Hyderabad, India) against 80 % acetone as a blank. The outcomes were interpreted as mg/g fresh weight of leaf. The following formula was used for the estimation.

Chlorophyll ‘a’ = (12.7*A663) - (2.69*A645)

Chlorophyll ‘b’ = (22.9*A645) - (4.68*A663)

Carotenoids = A480 + (0.114* A663) – (0.638 * A645)

*A663, A645 and A480 are absorbance at 663,645 and 480 nm in the UV spectrophotometer respectively.

Biochemical analysis

Reducing sugar

A 200 mg of fresh shoot, root and leaf samples were macerated with 10 mL of 80% ethanol and centrifuged (Remi R 24 - India) at 800 rpm for 15 mins. The supernatant was saved by re-extraction of the residues and utilized for the measurements. For estimation, 1 mL of the extract was mixed with 1 mL of reagent C (prepare by mixing copper sulphate and copper tartrate solution (25:1v/v) and incubated for 20 mins at 100 ^{0}C in a boiling water bath (Sunbim (India)). Then, 1mL of arsenomolybdate reagent was added after the mixture had been cooled. A final amount of 20 mL was made up by adding distilled water. The green colour complex was detected and it was read at 520 nm in the UV spectrophotometer (SL159, Elico, Hyderabad, India). The sugar content of the sample was calculated from the standard graph using glucose and it was expressed in mg/g fresh weight (12).

Starch

Starch content was estimated using the Clegg (1965) method (13). A soluble sugar extract was prepared by mixing it with 6.5 mL of 52% perchloric acid (PCA) solution, 5 mL of distilled water, and heated at 80^{o}C for 30 min in a water bath (Sunbim-India). The homogenate was centrifuged (Remi R 24- India) at 800 rpm for 15 mins. The supernatant was kept and utilized for the estimation of starch. From the supernatant, 1 mL of the extract was taken and mixed with 10 mL of the anthrone reagent and it was diluted with

5 mL of deionized water. The sample was heated in a boiling water bath at 100°C for 10 min. Absorbance was read at 630 nm in a spectrophotometer and the content was expressed in mg/g fresh weight.

Protein

The protein content was estimated according to standard procedure (14). A 500 mg of the fresh shoot, root and leaf tissue were ground with 20 mL of 20 % Trichloroacetic acid (TCA) in a pestle and mortar. The homogenate was spun at 800 rpm for 15 min in a centrifuge (Remi R 24- India). The supernatant was made up to 10 mL with 0.1N NaOH and used for the estimation of protein content. The blue colour complex was read against the blank (reagent without extract) at 640 nm in a UV spectrophotometer (SL159, Elico, Hyderabad, India). Bovine serum albumin was used as a standard graph to calculate protein content. It was expressed in mg/g fresh weight.

Amino acid

The free amino acid was estimated according to standard procedure (15). A 500 mg of plant tissue was taken and homogenized with 10 mL of 80 % boiling ethanol. The extract was centrifuged (Remi R 24 - India) at 800 rpm for 15 min and the supernatant was made up to 10 mL with 80 % ethanol. For estimation, 1 mL of extract was taken and neutralized with 0.1N NaOH and methyl red indicator and then 1 mL of Ninhydrin reagent was added and it was incubated at 65 ^{0}C for 20 mins in boiling water bath (Sunbim-India). When cooled, the extract was transferred into a 2 mL cuvette and the absorbance was read at 570 nm in a UV Spectrophotometer (SL159, Elico, Hyderabad, India). The free amino acid was expressed in mg/g fresh weight.

Proline

Proline was extracted and estimated (16). A 500 mg of frozen plant material was ground with 10 mL of 3 % aqueous sulphosalicylic acid in a pestle and mortar. The homogenate was spun at 800 rpm for 10 mins in a centrifuge (Remi R 24). The extract was used for the estimation of proline. The estimation was done by mixing the 2 mL of an extract with 2 mL of ninhydrin and 2 mL of glacial acetic acid. It was incubated at 100°C for 1 hr in a heating mantle (Sunbeam- India) and it was cooled by keeping it in an ice bath. Finally, 4 ml of toluene was added and the absorbance was read at 520 nm in the UV spectrophotometer (SL159, Elico, Hyderabad, India) using the reagent as a blank. The proline content was determined from the standard graph and expressed in mg/g fresh weight.

Lipid peroxidation

Lipid peroxidation can be estimated (19) by detecting the malondialdehyde (MDA) concentration at 532 nm. Utilizing an extinction value of 155 mM^{-1} cm^{-1}, it was calculated. For extraction 0.1g of leaf tissues were homogenized with 0.5 mL of trichloroacetic acid (TCA) (W/V) and it was centrifuged (Remi R 24) at 15000 rpm for 10 mins. The supernatant was collected and mixed with 1.5 mL of 0.5% of Thiobarbituric acid (TBA) diluted in 20 % TCA. It was incubated in a water bath at 95 ^{0}C for 25 min and then cooled by an ice bath. The absorbance was measured at 532 and 600 nm in the UV Spectrophotometer (SL159, Elico, Hyderabad, India). MDA concentration was calculated using Lambert beer law with an extinction coefficient of 155 $mM^{-1}cm^{-1}$. Results are presented as MDA g/FW.

Enzymatic antioxidant activity

Catalase activity was measured (17) and Peroxidase by Machly and Chance (18). The extraction buffer consists of a 50 mM phosphate buffer (pH 7.0). Plant material was homogenized in 5 mL of extraction buffer and centrifuge (Remi R 24) at 4000 rpm for 20 min. For estimation, 2.6 mL of 50 mM potassium phosphate buffer, 0.4 mL of 15 mM H_2O_2 and 0.04 mL of enzyme extract made up the assay combination for catalase. Monitoring the disappearance of H_2O_2 at 240 nm allowed for the determination. The peroxidase assay combination for estimation consisted of 2 mL of 0.1 N phosphate buffer, 1 mL of 0.01 M pyrogallol, 1 mL of 0.005 M H_2O_2 and 0.5 mL of enzyme extract. The absorbance of crude extract was measured at 420 nm in a UV-Spectrophotometer (SL159, Elico, Hyderabad, India). Units/mg protein was used to express the enzyme activity.

Electronic spin resonance (ESR) analysis

In order to find the ROS species, gamma-irradiated green gram seed samples were placed in ESR quartz tubes along with control. The ESR spectra were recorded at room temperature with the Bruker Biospin EMX spectrometer operating at X-band (9.1 GHz). ESR spectra were observed with microwave power of 5mW, amplitude of 2.5 G and receive gain of $2x10^4$ at modulation frequency of 9.1 GHz. The variations in the steady state of the relative concentration of the paramagnetic species generated at different absorbed doses were obtained. The signal's intensity was calculated as the peak and reported as arbitrary units per kg of sample weight (AU/mg) was analyzed at Indian Institute of Technology, Mumbai, India.

Fourier transforms infrared spectroscopic (FTIR) analysis

Using gamma-irradiated samples of mung bean seed powder, Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify the functional groups characteristic peaks of biological components. Using the potassium bromide (kBr) pellet method, the spectra of samples that had and had not been exposed to radiation were obtained. The peaks were found between 4000–400 cm^{-1} at room temperature. FTIR analysis was done at Archbishop Casimir Instrumentation Center (ACIC), St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Data analysis

Analysis of the sample was done seven days after germination of seedlings. Results are given as the mean ± standard error. Experimental data were statistically assessed using one-way analysis of variance. The correlation was tested with Dennett's test at a 5% level of probability ($P < 0.05$), and the correlation was examined using IBM SPSS Statistics 21 software.

Results

Germination %

Gamma irradiated green gram seeds showed highest reduction of seed germination in 800 Gy (27%). Based on the seed germination % on the 7th day the LD50 values were fixed at 500 Gy and it means that 50 % of plants would be dead after the exposure of seeds to gamma irradiation (Fig. 1a)

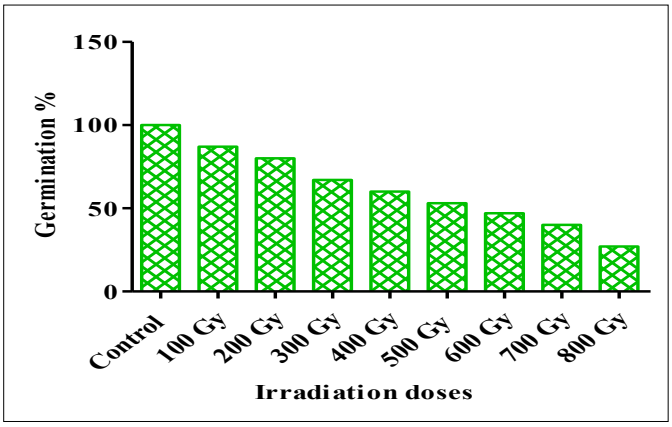


Fig. 1a. Effect of gamma irradiation on seed germination**.**

Morphological parameter analysis of gamma irradiated seedlings

Among the applied doses of gamma rays, seedlings exhibited a significant reduction in length as the dose increases when compared with the control. Gamma ray doses of 100, 200, 300, 400 and 500 Gy showed increased seedling length; dosages of 600, 700 and 800 Gy caused a dramatic drop (Fig. 1b & 2). Gamma radiation exposure in 800 Gy

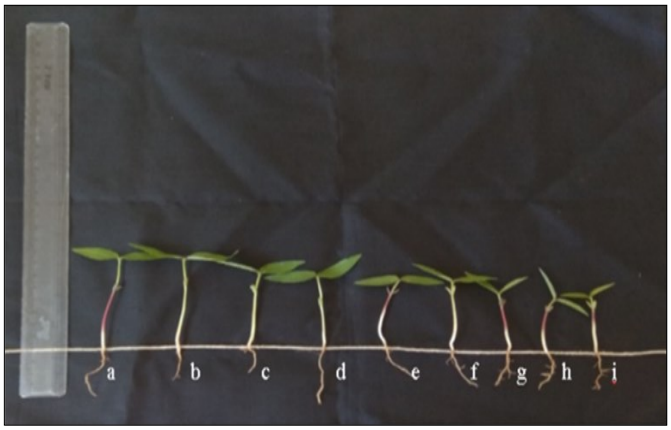
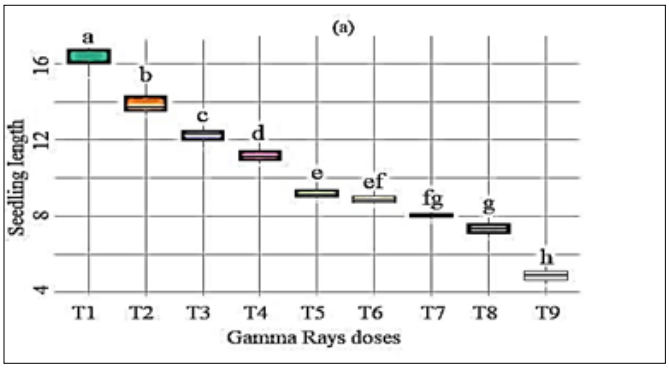
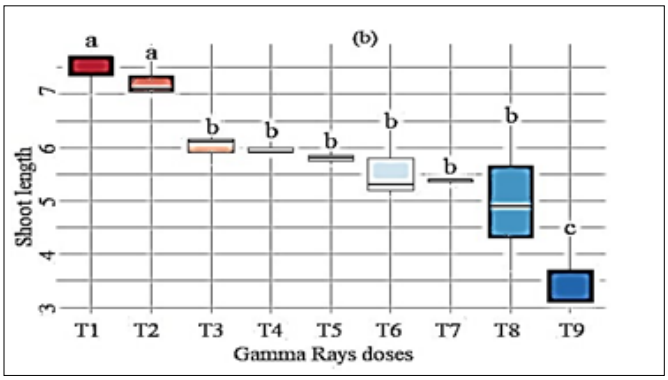
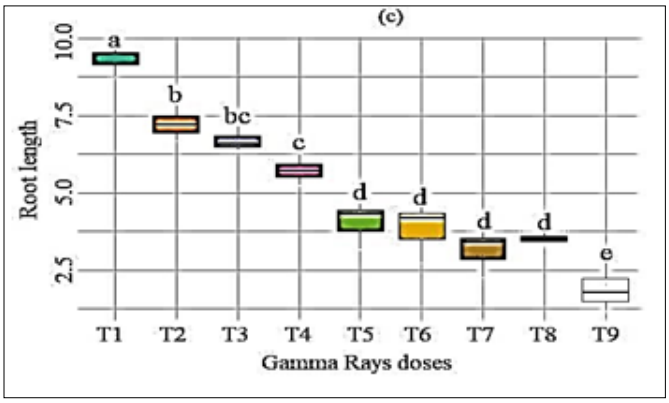


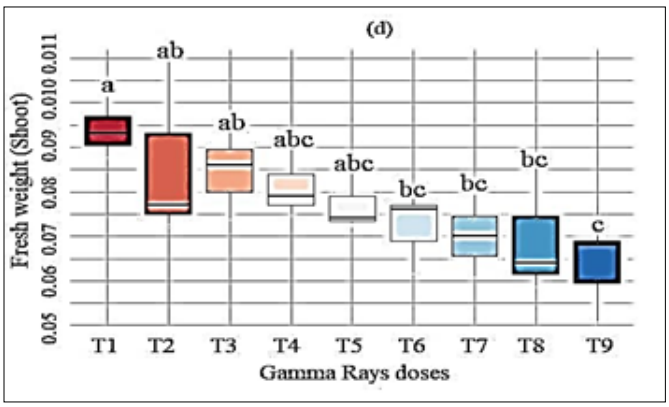
Fig. 1b. Gamma irradiation impact on seedling length at 7th day. **a**) Control **b**) 100 Gy **c**) 200 Gy **d**) 300 Gy **e**) 400 Gy **f**) 500 Gy **g**) 600Gy **h**) 700 Gy **i**) 800 Gy.

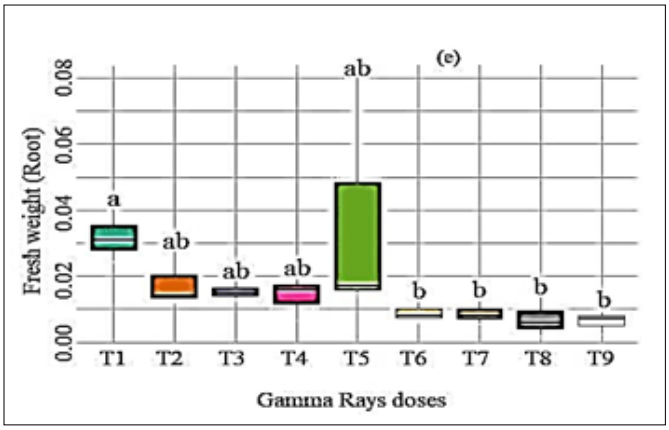
caused the maximum reduction of seedling length (4.86) as compared to the control (16.26). Studying the fresh and dry weight (g) of shoots (S), roots (R) and leaves (L) reveals that weight decreased with increasing doses as compared to the control. When compared to the control seedlings (S: 0.094; R: 0.032; L: 0.067), 800 Gy showed a decrease in fresh weight (S: 0.063; R: 0.006; L: 0.011). The dry weight was also measured in the seedlings and 800Gy showed the decreased value (shoot: 0.005, root: 0.002 and leaf: 0.002) as compared to the control (S: 0.017; R: 0.008; L: 0.013) (Fig. 2).











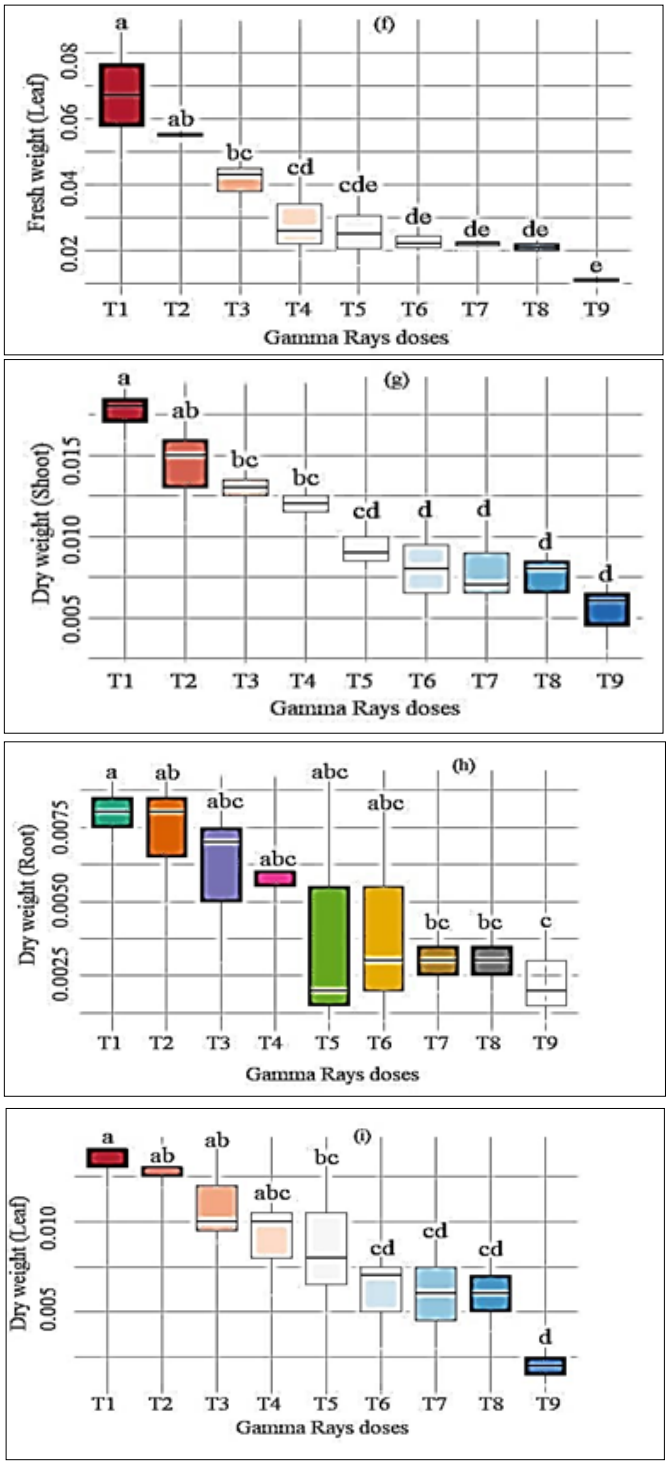


Fig. 2. Morphological parameter analysis of gamma irradiated seedlings. **a-** Seedling length **b-** Shoot length **c-** Root length **d-f –** Fresh weight of shoot, root and leaf **g-i –** Dry weight of shoot, root and Leaf. Boxplot followed by the same letter is not different at the 1% level of significance based on Dun-can's Multiple Range Test. **T1**- Control, **T2-T9** – Doses from 100 Gy – 800 Gy.

Photosynthetic pigment analysis

Chlorophyll estimation is one of the key criteria in determining yield capacity. Irradiated seedlings showed a significantly lower amount of the photosynthetic pigments and a steady increase in carotenoid content as compared to the control. Chlorophyll "a" was reduced maximum at 800 Gy (0.216) when compared to control (0.978). Chlorophyll "b" was more when compared to chlorophyll "a" and less when compared to the control. The greatest reduction was recorded at 800 Gy (0.249) as compared to the control (0.562). Gamma radiation treatment improved carotenoid content. It was observed that 800 Gy (1.689) increased carotenoid content as compared to the control (1.229) (Fig. 3).

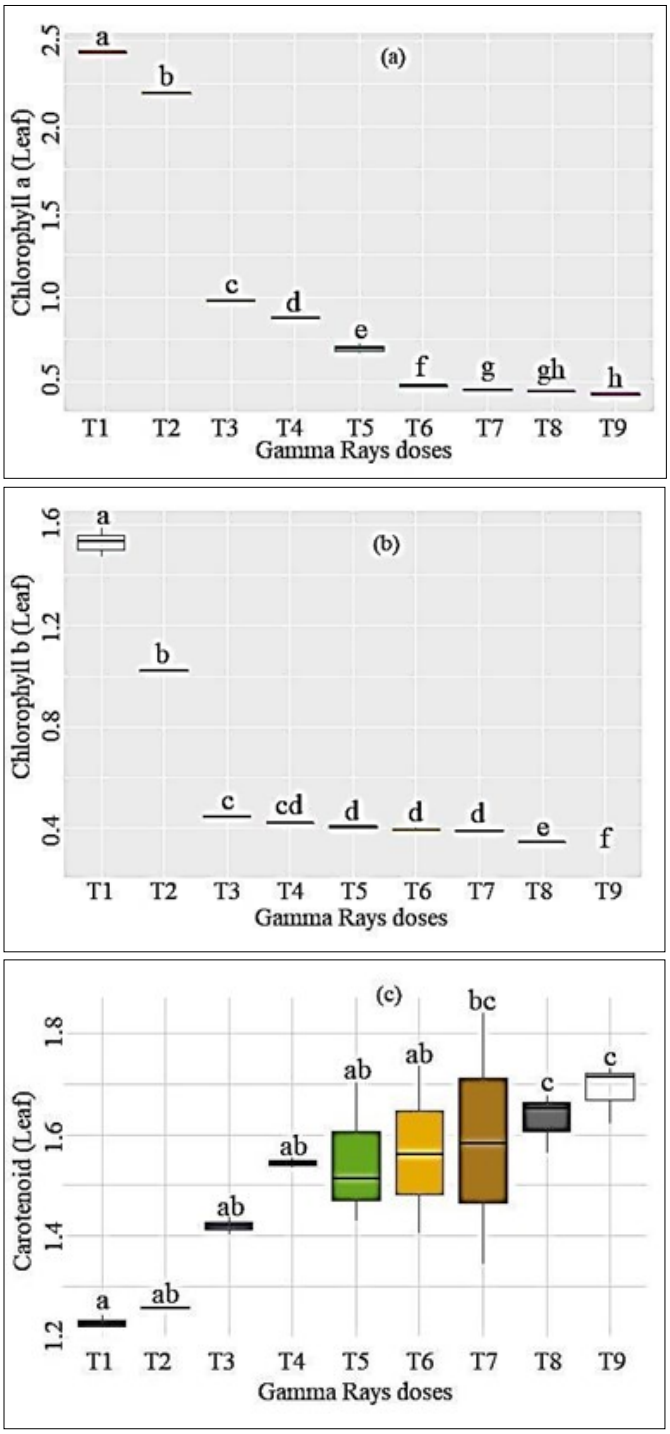


Fig. 3. Photosynthetic parameter analysis. **a)** Chlorophyll a **b)** Chlorophyll b **c)** Carotenoid Boxplot followed by the same letter is not different at the 1% level of significance based on Dun-can's Multiple Range Test. **T1-** Control**, T2-T9 –** Doses from 100 Gy – 800 Gy**.**

Biochemical analysis

Depending on the dosages of gamma irradiation, the biochemical composition of the seedlings exhibited some variations with increased doses of irradiation up to 600 Gy. When compared to the control, the effect of gamma irradiation on the reducing sugar, starch, protein, and amino acid content was increased. Increased irradiation dose resulted in increased sugar content when compared to control (S: 0.821; R: 0.464; L: 0.542), it exhibited maximum content at 600Gy (S: 1.084; R: 1.080; L: 1.008). The average

starch content was recorded in 600 Gy (S: 2.422; R: 1.458; L: 1.087), which was higher than in the control (S: 1.829; R: 1.398; L: 0.478). The protein content was boosted in 600 Gy (S: 3.594; R: 2.999;L: 3.721) as compared with control (S: 2.488; R: 2.396; L: 2.079)(Fig. 4a). An increase in gamma irradiation dose led to an increase in amino acid content in comparison to control (S: 0.743; R: 0.705; L: 0.456), it displayed the maximum value in 600Gy (S: 0.829; R: 0.826; L: 0.792). Proline exhibited more content in 600 Gy (S: 1.572, R: 1.398, L: 1.387) than in control (S: 1.518, R: 1.324, L: 1.324). MDA content in leaf tissues subjected to gamma irradiation caused a linear increase and reached the highest level at 800 Gy (0.638) as compared to the control leaf (0.243) (Fig. 4b).

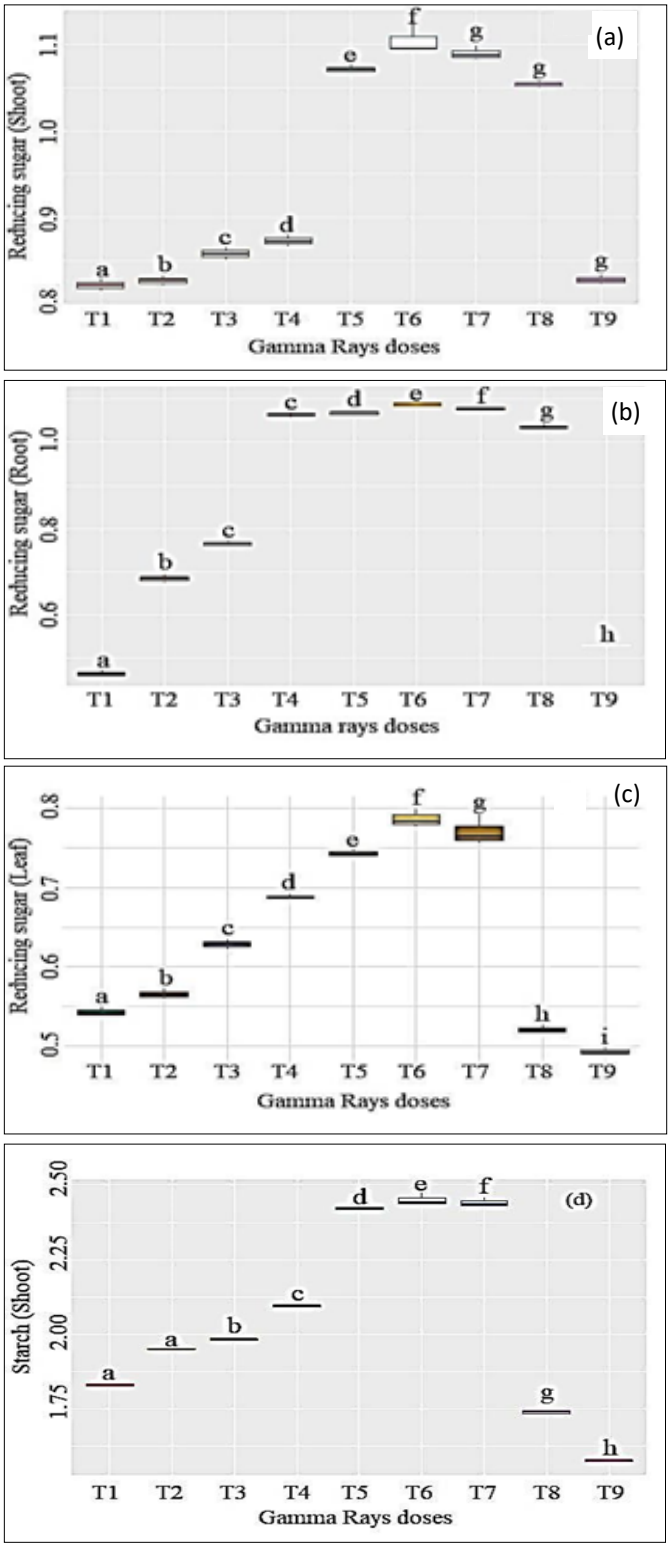
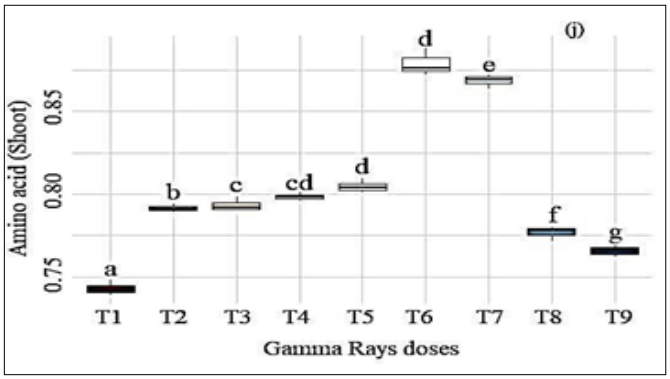
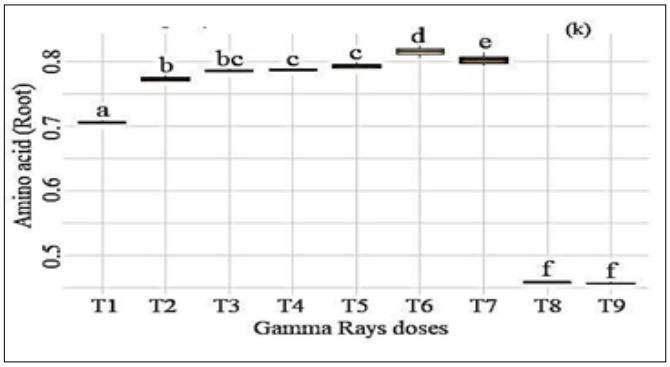
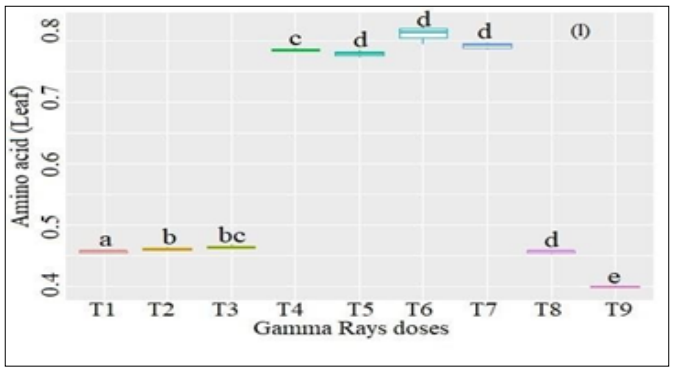
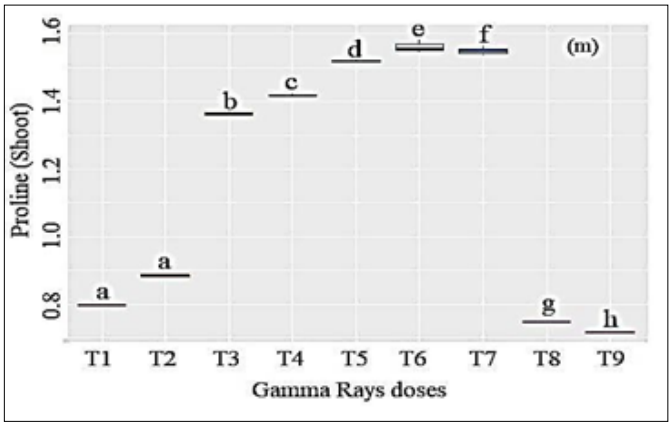


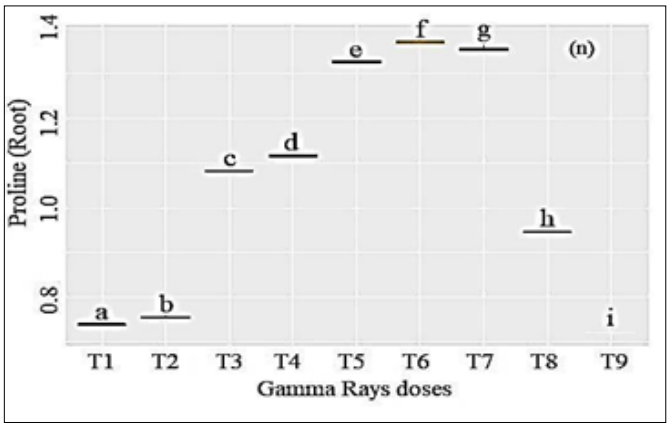
Fig. 4a. Biochemical parameter analysis in shoot, root and leaf**. a-c -** Reducing sugar **, d-f -** Starch **, g-i -** Protein Boxplot followed by the same letter is not different at the 1% level of significance based on Dun-can's Multiple Range Test. T1- Control, T2-T9 – Doses from 100 Gy – 800 Gy.

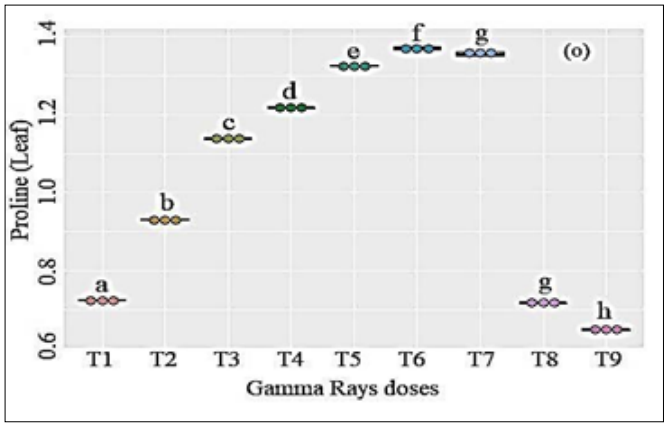












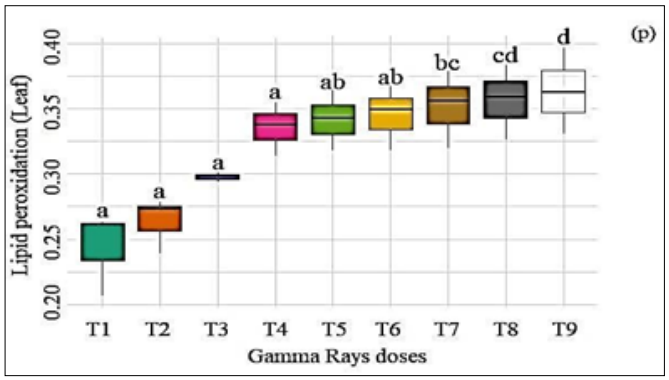
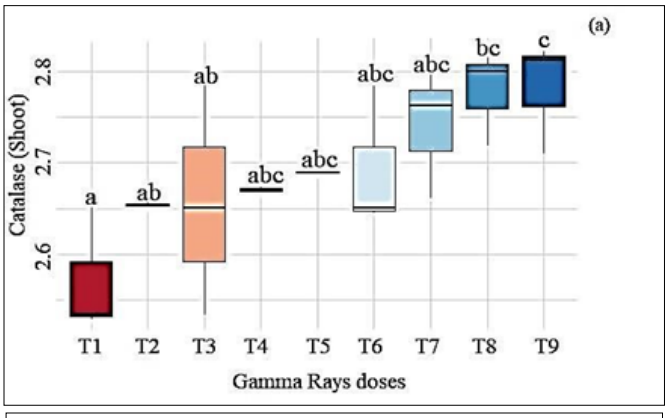
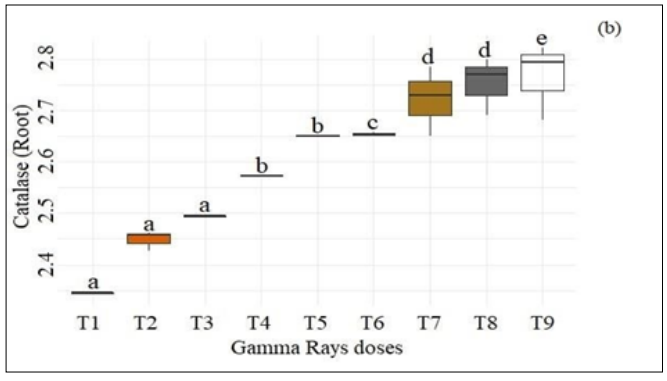


Fig. 4b. Biochemical parameter analysis in shoot, root and leaf. **j-l –** Amino-acid, **m-o -** Proline **, p -** Lipid peroxidation. Boxplot followed by the same letter is not different at the 1% level of significance based on Dun-can's Multiple Range Test. **T1**-Control**, T2-T9–**Doses from 100 Gy – 800 Gy.

Enzymatic antioxidant activity

Under the growth conditions of this experiment, CAT and POD activity were increased with increased doses. CAT activity recorded the maximum level in 800 Gy (S: 2.740; R: 2.721; L: 2.809) compared to control (S: 2.571; R: 2.345; L: 2.166). POD activity showed a significant increase in 800 Gy (S: 7.103; R: 6.829; L: 6.567) than control seedlings (S: 3.156; R: 3.062; L: 3.177) (Fig. 5.)





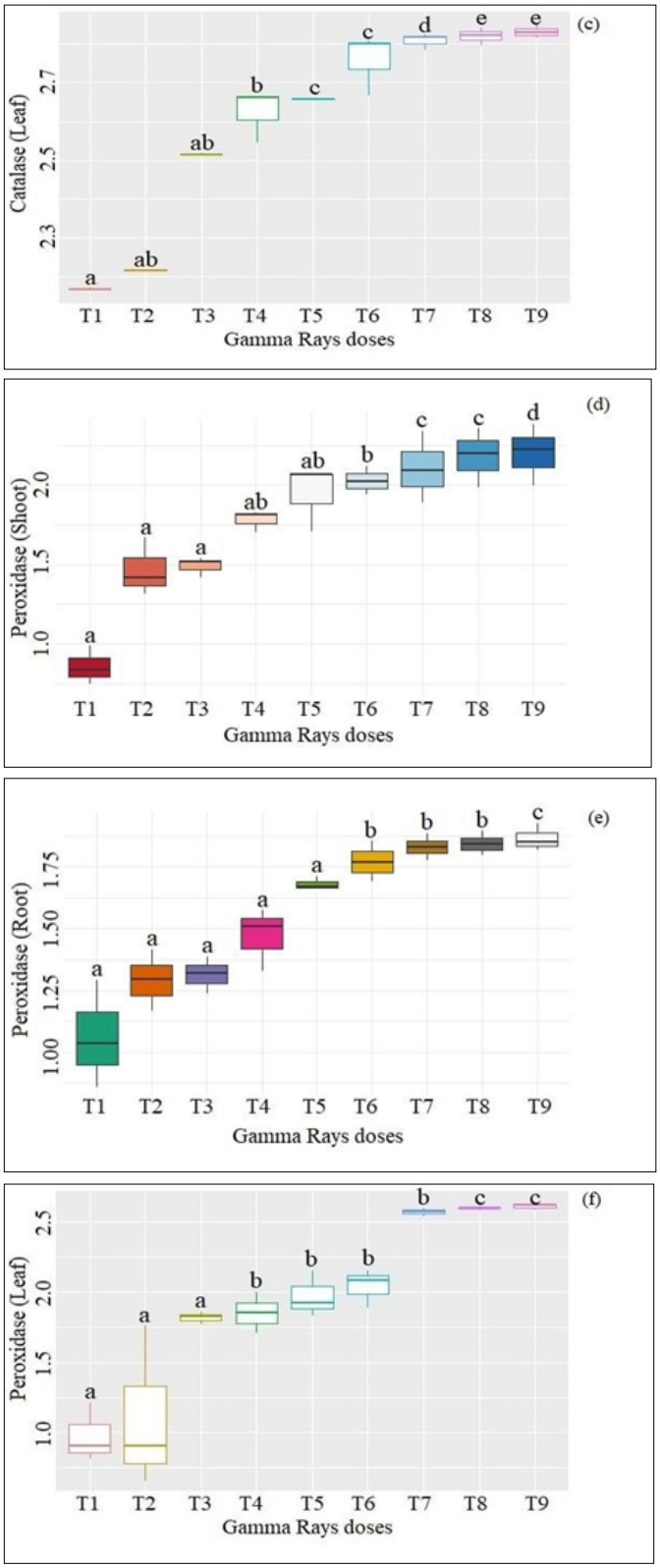


Fig. 5. Enzymatic antioxidant activity. **a-c –** Catalase, **d-f -** Peroxidase Boxplot followed by the same letter is not different at the 1% level of significance based on Dun-can's Multiple Range Test. **T1-** Control**, T2-T9 –** Doses from 100 Gy – 800 Gy.

Correlation coefficient analysis

Through correlation, characteristics such as morphology, photosynthetic pigments and antioxidants are clearly analyzed. Positive character correlation is thought to be advantageous in this analysis, while negative character correlation is thought to cause delays in the recovery of these combinations. Gamma radiation and various seedling characteristics exhibited a strong association with each other. Except for carotenoid concentration and antioxidant enzymes like catalase and peroxidase activity, almost all of the characters had positive correlations and the chlorophyll parameter, which denotes chlorophyll "a," had a strong positive association. Since antioxidant enzymes like catalase and peroxidase function as ROS scavengers to shield seedlings from harm, their activity was highly negatively connected with morphological traits. The biochemical components had a highly substantial and positive association with one another, according to the Pearson correlation coefficient. The significant information was depicted in Supplementary Tables 1 and 2 for morphology and biochemical content respectively.

ESR analysis of gamma irradiated samples

A single signal was observed in the ESR spectra of all irradiated and non-irradiated green gram samples. The g-value was set at 2.000 ± 0.005 for an irradiated plant sample. In the case of irradiated plant samples, the intensity of signals was increased with increased doses. The peak intensity was higher in 800 Gy as compared to the control. Gamma irradiated green gram powder provided the typical spectrum of a central signal with a g factor of 2.005. The spectrum of irradiated seed powder is exemplified in Fig. 6.

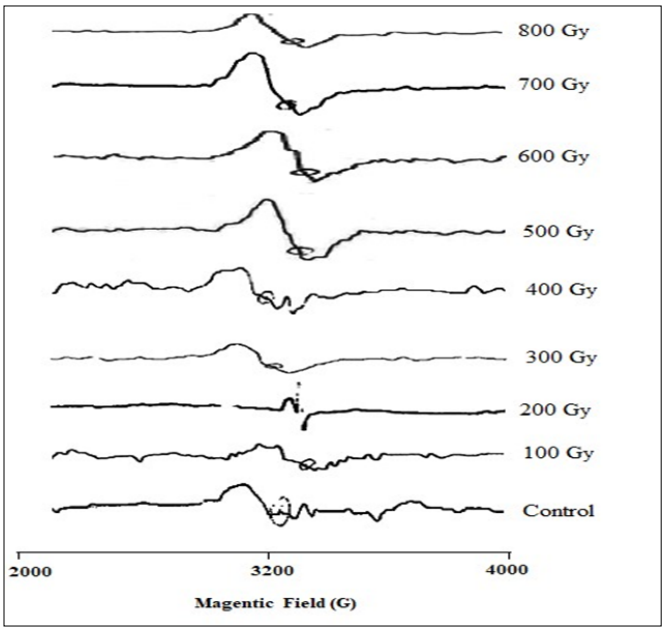


Fig. 6. ESR spectroscopy analysis of gamma irradiated and control of green gram seed sample. X-band ESR spectrum of control and different dose of gamma irradiated green gram seeds using 100-KHz modulation frequency, microwave power 5 mW. Circles are indicating ESR spectrum of peaks with g-value 2.00.

FTIR spectroscopy analysis of gamma irradiated samples

The FTIR spectrum of gamma irradiated presented a number of peaks between 4000- 400 cm^{-1} due to various stretching bands of biomolecules such as proteins, amino acids, lipids, carbohydrates and various fingerprint regions. The FTIR spectrum showed a broad spectrum in both irradiated and non-irradiated (control) green gram samples. The peak came in the range between 3712-2839 cm^{-1}, such as 3305 cm^{-1}, 2928 cm^{-1} in the control and 3458 cm^{-1}, 3366 $cm^{-1,}$ 3343 cm^{-1} and 3308 cm^{-1} in irradiated samples consigned to hydroxyl compounds.

Peaks obtained in 2922 cm^{-1}, 2926 cm^{-1} 2928 cm^{-1} 2929 cm^{-1} and 2931 cm^{-1} mainly characterized C-H extending vibration by lipids. The peaks at 1650 cm^{-1}, 1644 cm^{-1}, 1546 cm^{-1}, 1545 cm^{-1}, 1542 cm^{-1} and 1540 cm^{-1} in irradiated samples as amino acids and also indicated the presence of the aromatic compounds.

The bands that appeared between 1500-1100 cm^{-1} were found to be the presence of the carbonyl group, and the peaks obtained between 1200-900 cm^{-1}, 922-770 cm^{-1} and 1300-600 cm^{-1} attributed to the presence of polysaccharides which represents the carbohydrates. Absorption bands between 400-560 cm^{-1} and peaks at 530 cm^{-1}, 529 cm^{-1}, 440 cm^{-1} and 439 cm^{-1} were found to be the presence of halogen compounds, which also specified the presence of starch molecules. From FTIR analysis, significant changes have been recorded in the functional groups when green gram seeds were subjected to gamma irradiation. The occurrence of these compounds revealed the presences of protein, amino acid, carbohydrates and starch. Lipids and transmittance % of these characteristics was represented in Fig. 7.

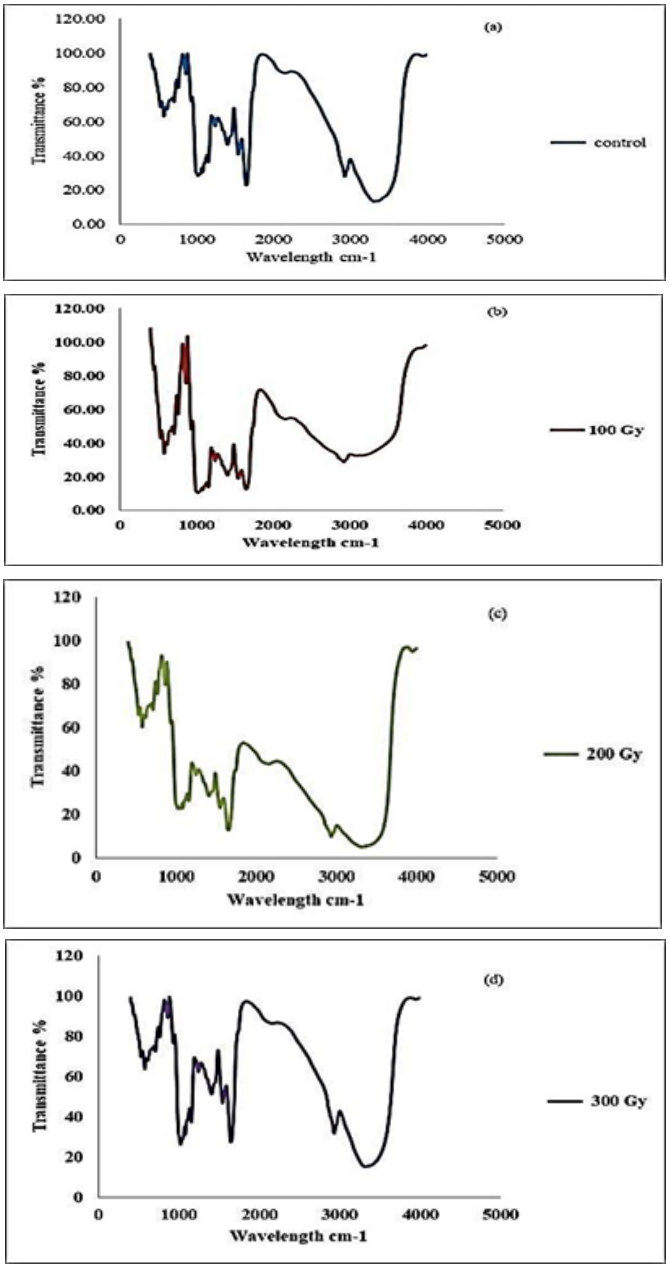


Fig. 7. FTIR analysis of biochemical component of gamma irradiated green gram seed powder. **a)** Control, **b)** 100 Gy, **c)** 200 Gy, **d)** 300 Gy , **e)** 400 Gy, **f)** 500 Gy , **g)** 600Gy,**,h)** 700 Gy, **i)** 800 Gy, *Spectral range between 4000 – 400 cm^{-1} for different doses of gamma irradiation.

Discussion

In this study gamma irradiated green gram seeds of 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy and

800 Gy doses were grown in petri plates and morphology parameters were observed on 7th day. Reduction in seed germination was observed due to the disturbance at the cellular level caused in meristematic tissue of the radical/plumule or by chromosomal damage (20). Similar inhibitory effects were reported in *Vigna mungo* (21) and *Pisum sativum* (22). The study findings also showed that a higher dose of gamma radiation was sufficient to reduce the root percentage while not exceeding in length. It resulted in deteriorated cell division in the meristem and decreased the moisture content of seeds (23). Rising doses resulted in a decrease in seedling length, which was revealed in green gram (24), chickpea (25) groundnut (26) and paddy (27). A reduction in the weight of seedlings was also noted in this study, and the similarity was seen in crops such as rice (28), *Lepidium sativum* (29) and *Vigna sesquipedalis* (30). Chlorophyll separation from its protein complex through de-phytolization can gradually reduce the chlorophyll content after gamma irradiation treatment. A dose-dependent significant variation in chlorophyll a and b content was discovered, and chlorophyll b was less abundant than chlorophyll a (31). The limitation of photosynthetic activity can be done by destroying the chlorophyll molecules (32, 33). Biosynthesis or degradation of chlorophyll b's precursors causes its breakdown rather than chlorophyll a inhibiting gamma irradiation on seedlings increased chlorophyllase activity, promoted chlorophyll deprivation and decreased photosynthetic activity (34). In this study, carotenoid concentrations were increased by 800 Gy. According to one report (35), carotenoid levels increased at the same level of irradiation, whereas chlorophyll a and b were essentially insensitive to it. Carotenoids are crucial for protecting chlorophyll from oxidative damage and scavenging free radicals in light (36). Studies of correlation can be used to identify features and highlight the scope and constraints of choosing desirable traits. It assesses the interrelationships between the characteristics, and all of them had positive correlations with one another, except carotenoids.

The biochemical investigation showed the beneficial effect on seedlings of gamma irradiation treatment, which produces free radicals (37). Plant cells evolve a defense mechanism against gamma radiation (38). Increases in biochemical traits, including sugar and starch content were observed in 600 Gy, and a similar effect was shown in lupine (39). At 800 Gy, the protein content was enhanced. Gamma-irradiation responses to protein synthesis can result in the breakdown of protein molecules into free amino acids (40).

High irradiation doses provide high chemical extractability by creating a disulfide bond between polypeptide chains, which has an impact on accumulation and conforms to the presence of low molecular weight proteins (41). The same outcome was attained with soybean seeds (42). The generation of free radicals may contribute to the alteration in amino acid by gamma irradiation. The findings of irradiated soy flour (43), sesame (44) and mung bean (45) were in agreement with findings made in this study. Proline functions as an osmoregulatory system to protect enzyme structure and activity against stress. It lessened the *in vitro* enzyme denaturation brought on by different stresses (46). The outcome displays increased proline content in wheat (47), *Allium sativum* (48) and *Terminalia arjuna* (49), all of which have considerably favorable correlations with one another.

Gamma radiation promotes the synthesis of antioxidant enzymes, enhances the production of ROS and can also alter several environmental stresses (50, 51). Catalase, peroxidase and lipid peroxidation activities showed the highest production at 800 Gy. Catalase activity was controlled by the radiation exposures during the developmental phase (52) and it reduced the damage caused by irradiation (53). It was stimulated by irradiation at 5 kGy in *Vicia faba* L. (54) and also seen in two rice cultivar seeds at irradiation of 200 Gy (55). Peroxidase is more effective than catalase due to its protective action in removing H_2O_2 (56). Comparable results were observed in *Phaseolus vulgaris* (57) and *Triticum aestivum* (58). Free radicals like O_2 and H_2O_2 accumulation by gamma radiation distressed the system for removing them and caused lipid peroxidation. Different MDA concentrations were used as a marker for the oxidation of cell membranes brought on by stress. When plant cells were damaged and free radicals were produced, the MDA content increased. The MDA concentration was increased when the plant cells were injured by the production of free radicals (59). A study found that the enhanced content was present in chickpeas, *Zizania latifolia* (60), soybean (61) and rice (62). The intensity of band produced by ESR signals was gradually increased in higher doses of gamma irradiation. It reacted rapidly with almost all the structural and functional molecules. ROS species produced by radiation may acted as stress signal and triggered the production of antioxidants.

FTIR analysis is a technique used to explore the biochemical compounds and functional groups that were obtained in irradiated samples. In Coca seeds (63) and in rice seedlings (64), the peak ranged between 3712-2839 cm^{-1}, such as 3305 cm^{-1} and 2928 cm^{-1} in control and 3458, 3366, 3343 and 3308 cm^{-1} in irradiated samples were consigned to hydroxyl compounds. It was declared that the vibration caused by lipids comes under the range between 3000-2800 cm^{-1} (2922, 2926, 2928, 2929, and 2931 cm^{-1}) (65). According to one report the maximum intensity obtained at 1650, 1644, 1546, 1545, 1542 and 1540 cm^{-1} in irradiated samples were comprised of amino acids and amide I and amide II bands of protein absorption (66). The metabolites such as carbohydrates were screened in the peaks obtained between 1200-900 cm^{-1}, 922-770 cm^{-1} and 1300-600 cm^{-1} (67) and the presence of starch molecules was shown between 400-560 cm^{-1} such as 530, 529, 440 and 439 cm^{-1} (68). Thus, from the above findings, it could be concluded that gamma irradiation had a significant effect on morphology and biochemical and antioxidant activities. Among the doses, 600 Gy showed incredible changes and the bargain was obtained at 800 Gy.

Conclusion

In this study, gamma irradiated green gram seeds revealed an increased values of morphological characteristics such as germination %, plant height, fresh and dry mass at upto 500 Gy and gradually decreased with higher doses. Chlorophyll a and b showed decreased value due to chloroplast damage, but carotenoids which served as antioxidants were increased based on the free radical production detected by ESR spectroscopy. Biochemical characteristics such as reducing sugar, starch, protein, amino acids and proline content were increased with increased doses and their frequencies were identified by FTIR. As a result, this study, suggests that lower doses of gamma irradiation could be employed to create novel genotype with desired features for crop development.

Acknowledgements

The authors are thankful to the Department of Science and Technology for their financial assistance for this work. We are thankful to Dr. B.K.D. as, SO/G, Shankar Bhujbal, SO/C, NA and BTD, BARC, Trombay, Mumbai, India for their kind help in utilizing gamma irradiation. We also thank our Department of Botany at Periyar University in Salem, Tamil Nadu, India, for providing laboratory space for doing the research.

Mrs. V. Soundarya is thankful to the Department of Science and Technology (DST), New Delhi, India for selecting her as an INSPIRE Fellow (IF160742) and providing financial assistance for this study.

Authors contributions

Laboratory experiment, analysis of data, interpretation and statistical analysis (DAB and VS); Fieldwork and data collection (VS and SV); composing a manuscript (DAB, VS, BG, SG, AR and KY).

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None.

Supplementary data

Table 1. Pearson's correlation coefficient analysis of morphology

Table 2. Pearson's correlation coefficient analysis of Biochemical parameters

References

1. Afzal MA, Murshad AN, Bakar MA, Hamid A, Salahuddin ABM. Mungbean Cultivationin Bangladesh, Pulse Research Station, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. 2008.
2. Mohan S, Sheeba A, Kalaimagal T. Genetic diversity and association studies in greengram (*Vigna radiata* (L.) Wilczek). Legu Res-An Int J. 2021;44(7):779-84.
3. Baskaran L, Sundaramoorthy P, Chidambaram A, Ganesh KS. Growth and physiological activity of greengram (*Vigna radiata* L.) under effluent stress. Int J Bot. 2009;2(2):107-14.
4. Govardhan G, Vinoth R, Praveen K, Selvakumar G, SM. A need of heterosis in mungbean (*Vigna radiata* L.) for the pulses crop improvement-special review. J Inno Agri. 2015;2(1):1-6.
5. Rukesh AG, Rahuman MA, Latitia SC, Packiaraj D. Impact of gamma irradiation induced mutation on morphological and yield contributing traits of two genotypes of Green gram (*Vigna radiata* L.). J pharmacogn phytochem. 2017;6(6):1229-34.
6. Singh AK. Induced genetic variability in M3 generation of mungbean. J food legu. 2009;22(3):162-65.
7. Tah PR. Studies on Gamma Ray Induced Mutations in Mungbean [*Vigna radiata* (L.) Wilezek]. Asian J Plant Sci. 2006;5(1):61-70. https://doi.org/10.3923/ajps.2006.61.70
8. Zaka R, Chenal C, Misset MT. Effects of low doses of short-term gamma irradiation on growth and development through two generations of *Pisum sativum*. Sci Total Env. 2004;320(2):121-29. https://doi.org/10.1016/j.scitotenv.2003.08.010
9. Jan S, Parween T, Siddiqi TO, Zafar M. Effect of gamma radiation on morphological, biochemical and physiological aspects of plants and plant products. Environ Rev. 2012;20:17-39. https://doi.org/10.1139/a11-021
10. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris* L. Plant Physiol.1949;24:1-15. https://doi.org/10.1104/pp.24.1.1
11. Kirk JTO, Allen RL. Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione. Biochem. Biophysics Res J. Canada 1965;27:523-30. https://doi.org/10.1016/0006-291X(65)90516-4
12. Nelson N. A photometric adaptation of the somogyi method for the determination of glucose. J Biol Chem. 1944;153:375-80. https://doi.org/10.1016/S0021-9258(18)71980-7
13. Clegg KM. The application of the anthrone reagent to the estimation of starch in cereals. J Sci Food Agric. 1965;7:40-44. https://doi.org/10.1002/jsfa.2740070108
14. Lowry OH, Rosebrough NZ, Randall RJ. Protein measurements with the Foline-phenol reagent. J Biol Chem. 1960;193:265-75. https://doi.org/10.1016/S0021-9258(19)52451-6
15. Moore S, Stein W. Photometric Ninhydrin methods for use in the chromatograph of amino acids. J Biochem.1948;176:367-88. https://doi.org/10.1016/S0021-9258(18)51034-6
16. Bates LS, Waldern Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973;39:205-07. https://doi.org/10.1007/BF00018060
17. Chandlee JM, Scandalios JG. Analysis of variants affecting the catalase development program in Maize Scutellum Theo Appl Genet. 1984;69:71-77. https://doi.org/10.1007/BF00262543
18. Machly AC, Chance B. Methods of biochemical analysis. In D.Glick (ed). In Sci pub Inc. New York. 1967;357-824.
19. Dhindsa RS, Matowe W. Drought tolerance in two mosses correlated with enzymatic defense against lipid peroxidation. J Exp Bot. 1981;32:79-91. https://doi.org/10.1093/jxb/32.1.79
20. Deepika Minakshi, Pal Pahuja SK. Morphologicalvariations induced by Ethyl Methane Sulphonate in Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). Forage Research. 2016;41(4):218-21.
21. Ramya R, Nallathambi G, Ganeshram S. Genetic variability, heritability and genetic advance in induced mutagenesis black gram (*Vigna mungo* (L.) Hepper). Plant Archives. 2014;14(1):139-41.
22. Dhulgande GS, Ghogare DS, Dhale DA. Mutagenic effect on seed germination, seedling growth and seedling survival of pea (*Pisum sativum* L.). International Journal of Current Research. Bioscience Plant Biology. 2015;2(4):59-64.

23. Choudhary KS. A simple and reliable method to detect gamma irradiated lentil (*Lens culinaris* Medic.) seeds by germination efficiency and seedling growth test. Rad Phy Chem. 2002;64:131-36. https://doi.org/10.1016/S0969-806X(01)00467-4

24. Khan S, Wani MR, Parveen K. Induced genetic variability for quantitative traits in *Vigna radiata* (L.)Wilczek. Pak J Bot. 2004;36:845-50.

25. Raut VK, Patil JV, GAwande VL. Correlation and path analysis for quantitative traits in chickpea. Indian J Pulses Res. 2004;17-82-2.

26. Lukanda L, Funny-Biola C, Tshiyoyi-Mpunga A, Mudibu J, Ngoie-Lubwika M, MuhendiTshibingu R, KalonjiMbuyi A. Radiosensitivity of some groundnut (*Arachis hypogaea* L.) British Biotechnol J. 2012;2(3):169-78. https://doi.org/10.9734/BBJ/2012/1459

27. Talebi AB, Talebi AB, Shahrokhifar B. Ethyl Methane Sulphonate (EMS) induced mutagenesis in Malaysian rice (cv MR219) for lethal dose determination. Ame J Plant Sci. 2012;4:2186-92. http://dx.doi.org/10.4236/ajps.2012.312202

28. Tabasum A, Cheema AA, Hameed A, Rashid M, Ashraf M. Radio sensitivity of rice genotype to gamma radiations based on seedling traits and physiological indices. Pak J Bot. 2011;43:1211-22.

29. Abdul, Khan, Habib, Zahir. Gamma irradiation effects on some growth parameters of *Lepidium sativum* L. J Agric Food Ind Organ. 2010; https://www.cabdirect.org/cabdirect/abstract/20103304184

30. Kon E, Ahmed OH, Saamin S, Majid NM. Gamma radio sensitivity on long bean (*Vigna sesquipedalis*). Am J Appl Sci. 2007;1090-93. https://doi.org/10.3844/ajassp.2007.1090.1093

31. Ling APK, Chia JY, Hussein S. Physiological responses of *Citrus sinensis* to gamma irradiation. World Appl Sci J. 2008;5:12-19.

32. Byun MW, Jo C, Lee KH, Kim KS. Chlorophyll breakdown by gamma irradiation in a model system containing Linoleic acid. JAOCS. 2002;79(2):145-50. https://doi.org/10.1007/s11746-002-0449-y

33. Stoeva N, Bineva TZ. Physiological response of beans (*Phaseolus vulgaris* L.) to gamma-irradiation treatment. I. Growth, photosynthesis rate and contents of plastid pigments. J Environ Protec Ecol. 2001;2:299-303.

34. Chowdhury MR, Johri JK. Seasonal variations in chlorophyll content and chlorophyllase activity in Bangla and Mitha varieties of betelvine (*Piper betle* L.) grown in different soil treatments. Natl Bot Res Inst., Lucknow. 2003.

35. Kim JH, Chung BY, Kim JS. Effects of in Planta gamma-irradiation on growth, photosynthesis and antioxidative capacity of red pepper (*Capsicum annuum* L.) plants. J Plant Biol. 2005; 48:47-56. https://doi.org/10.1007/BF03030564

36. Kopsell DA, Kopsell DE, Lefsrud MG. Pre-harvest cultural growing conditions can influence carotenoid phytochemical concentrations in vegetable crops. Acta Hort. 2007; 841:283-93. https://doi.org/10.17660/ActaHortic.2009.841.34

37. Variyar PS, Limaye A and Sharma A. Radiation-Induced Enhancement of Antioxidant Contents of Soybean. J Agri Food Chem. 2004;52:3385-88. https://doi.org/10.1021/jf030793j

38. Qui X, Wang F, Wang X, Zhou G, Li Z. Effect of combined treatment of 60Co γ- ray and EMS on antioxidase activity and ODAP content in *Lathyrus sativus*. Chin J Appl Ecol. 2000;11:957-58.

39. Khodary SE, Moussa AH. Influence of gamma radiation and/or salinity stress on some physiological characteristics of lupine plants. Egyp J Biotechnol. 2003;13:29⊠36.

40. Hieng B, Ugrinovic K, Sustar-Vozlic J, Kidric M. Different classes of proteases are involved in the response to drought of *Phaseolus vulgaris* L. cultivars differing in sensitivity. J Plant Physiol. 2004;161:519-30. https://doi.org/10.1078/0176-1617-00956

41. Singh PK, Singh D. Effect of γ radiation on seed storage proteins of Chickpea using SDS-PAGE Sch. Acad J Biosci. 2015;3(1B):104-10.

42. Stajner D, Milosevic M, Popovic BM. Irradiation effects on phenolic content, lipid and protein oxidation and scavenger ability of soybean seeds. J Mol Sci. 2007;8:618-27. https://doi.org/10.3390/i8070618

43. Abd-ELkalik K, El-Demerdash HM, Mekkawy SH. Evaluation of chemical changes in some soybean mutants induced by gamma irradiation. J Rad Res Appl Sci. 2010;3:149-63.

44. Swailam MH. Quality characteristics of irradiated sesame seeds during storage. J Rad Sci Appl. 2009;22:323-42.

45. Hussein. Effect of ascorbic acid and/or sodium chloride on irradiated mungbean seeds, Arab J Nucl Sci Appl. 2010.

46. Ashraf M , Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007;59:206-16. . https://doi.org/10.1016/j.envexpbot.2005.12.006

47. Borzouei A, Kafi M, Khazaei H, Naseriyan B, Majdabad A. Effects of γ radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. Pak J Bot. 2010;42:2281-90.

48. Rashad Kebeish, Hanan E Deef, Nagwa El-Bialy. Effect of gamma radiation on growth, oxidative stress, antioxidant system and alliin producing gene transcripts in *Allium sativum*. Int J Res Stu Bio sci. 2015;3:161-74.

49. Akshatha Chandrashekar KR, Somashekarappa HM, Souframanien J. Effect of gamma irradiation on germination, growth and biochemical parameters of *Terminalia arjuna* Roxb. Rad Protect Environ. 2013;36:38-44. https://doi.org/10.4103/0972-0464.121826

50. El-Beltagi HS, Mohamed AA, Rashed MM. Response of antioxidative enzymes to cadmium stress in leaves and roots of Radish. Not Sci Biol. 2010;2:76-82. https://doi.org/10.15835/nsb245395

51. El-Beltagi HS, Ahmed OK, El-Desouky W. Effect of low doses γ-irradiation on oxidative stress and secondary metabolites production of Rosemary (*Rosmarinus officinalis* L.) callus culture. Rad Phy Chem. 2011;80:968-76. https://doi.org/10.1016/j.radphyschem.2011.05.002

52. Kim DS, Kim JB, Goh E J, Kim WJ, Kim SH, Seo YW, Jang CS, Kang SY. Antioxidant response of *Arabidopsis* plants to gamma irradiation: Genome-Wide expression profiling of the ROS scavenging and signal transduction pathways. J Plant Physiol. 2011;68:1960-71. https://doi.org/10.1016/j.jplph.2011.05.008

53. De Freitas RB, Augusti PR, De Andrade ER, Rother FC, Rovani BT, Quatrin A, Alves NM, Emanuelli T, Bauermann LF. Black grape juice protects spleen from lipid oxidation induced by gamma radiation in rats. J Food Biochem. 2012;18(10):12154-67.

54. Aly AA, El-Beltagi HES. Influence of ionizing irradiation on the antioxidant enzymes of *Vicia faba* L. Grasas Aceites. 2011;288-94. https://doi.org/10.3989/gya.111509

55. Silva AS, Danielowwski R, Braga Eb, Deuner S, Magalhães Junior AM, Peters JA. Development of rice seedlings grown from pre-hydrated seeds and irradiated with gamma rays. Cienc E Agrotec. 2011;35:1093-1100. https://doi.org/10.1590/S1413-70542011000600008

56. Croute F, Delaporte E, Bonnefoy JY Fertin C, Thivolet J, Nicolas JF. Interleukin-1 beta stimulates fibroblast elastase activity. Braz J Dermatol. 1991;124:538-41. https://doi.org/10.1111/j.1365-2133.1991.tb04946.x

57. Stoeva N. Physiological effects of the synthetic growth regulator Thidiazurol (drop) on gamma irradiated stress in peas plants (*Pissum sativum*). J Cent Euro Agri. 2002;6:349-58.

58. Zhang, Mao. Radiation-induced changes in enzymes of wheat

during seed germination and seedling growth, He Nong Xue Bao. 1993;7:93-97.

59. Beyaz R, Kaya G, Cocu S, Sancak C. Response of seeds and pollen of *Onobrychis viciifolia* and *Onobrychis oxyodonta* var. *armena* to NaCl stress. Scientia Agricola. 2011; 68(4):477-81. https://doi.org/10.1590/S0103-90162011000400013

60. Fan J, Shi M, Huang JZ, Xu J, Wang ZD, Guo DP. Regulation of photosynthetic performance and antioxidant capacity by 60Co γ -irradiation in *Zizania latifolia* plants. J Environ Radioact. 2014;129:33-42. https://doi.org/10.1016/j.jenvrad.2013.11.013

61. Stajner D, Popovic BM, Taski K. Effects of γ-irradiation on antioxidant activity in soybean seeds. Cent Eur J Biol. 2009;4:381-86. https://doi.org/10.2478/s11535-009-0019-z

62. Kim SH, Song M, Lee KJ, Hwang SG, Jang CS, Kim JB. Genome-wide transcriptome profiling of ROS scavenging and signal transduction pathways in rice (*Oryza sativa* L.) in response to different types of ionizing radiation. Mol Biol Rep. 2012;39:11231 -248. https://doi.org/10.1007/s11033-012-2034-9

63. Hu Y, Pan ZJ, Liao W, Li J, Gruget P, Kitts DD, Lu X . Determination of antioxidant capacity and phenolic content of chocolate by attenuated total reflectance- Fourier transformed - infrared spectroscopy. Food Chem. 2016;202:254-61. https://doi.org/10.1016/j.foodchem.2016.01.130

64. Silva SD, Feliciano RP, Boas LV, Bronze MR. Application of FTIR-ATR to Moscatel dessert wines for prediction of total phenolic and flavonoid contents and antioxidant capacity. Food Chem. 2014;150:489-93. https://doi.org/10.1016/j.foodchem.2013.11.028

65. Souza LD, Devi P, Divya Shridhar MP, Naik CG. “Use of Fourier Transform Infrared (FTIR) spectroscopy to study cadmium-induced changes in *Padina tetrastromatica* (Hauck). Analy Chem Insights. 2008;3:135-43. https://doi.org/10.4137/117739010800300001

66. Ji Y, Yang X, Ji Z, Zhu L, Ma N, Chen D, Jia X, Tang J, Cao Y . DFT-Calculated IR Spectrum Amide I, II, and III Band Contributions of N-Methylacetamide Fine Components, ACS Omega. 2020;5:8572 -78. https://doi.org/10.1021%2Facsomega.9b04421

67. Vodnar DC, Pop OL, Socaciu C. Monitoring Lactic Acid Fermentation in Media Containing Dandelion (*Taraxacum officinale*) by FTIR Spectroscopy. Not Bot Hort Agrobot Cluj-Napoca. 2012;40:65-68. https://doi.org/10.15835/nbha4016653

68. Kizil R, Irudayara J, Seetharaman K. Characterization of irradiated starches by using FT Raman and FTIR Spectroscopy. J Agri Food Chem. 2002;50:3912-18. https://doi.org/10.1021/jf011652p