Impact of gamma (γ) irradiation on morphology, biochemical and antioxidant activity of green gram (Vigna radiata (L.) R. Wilczek)

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Research Article

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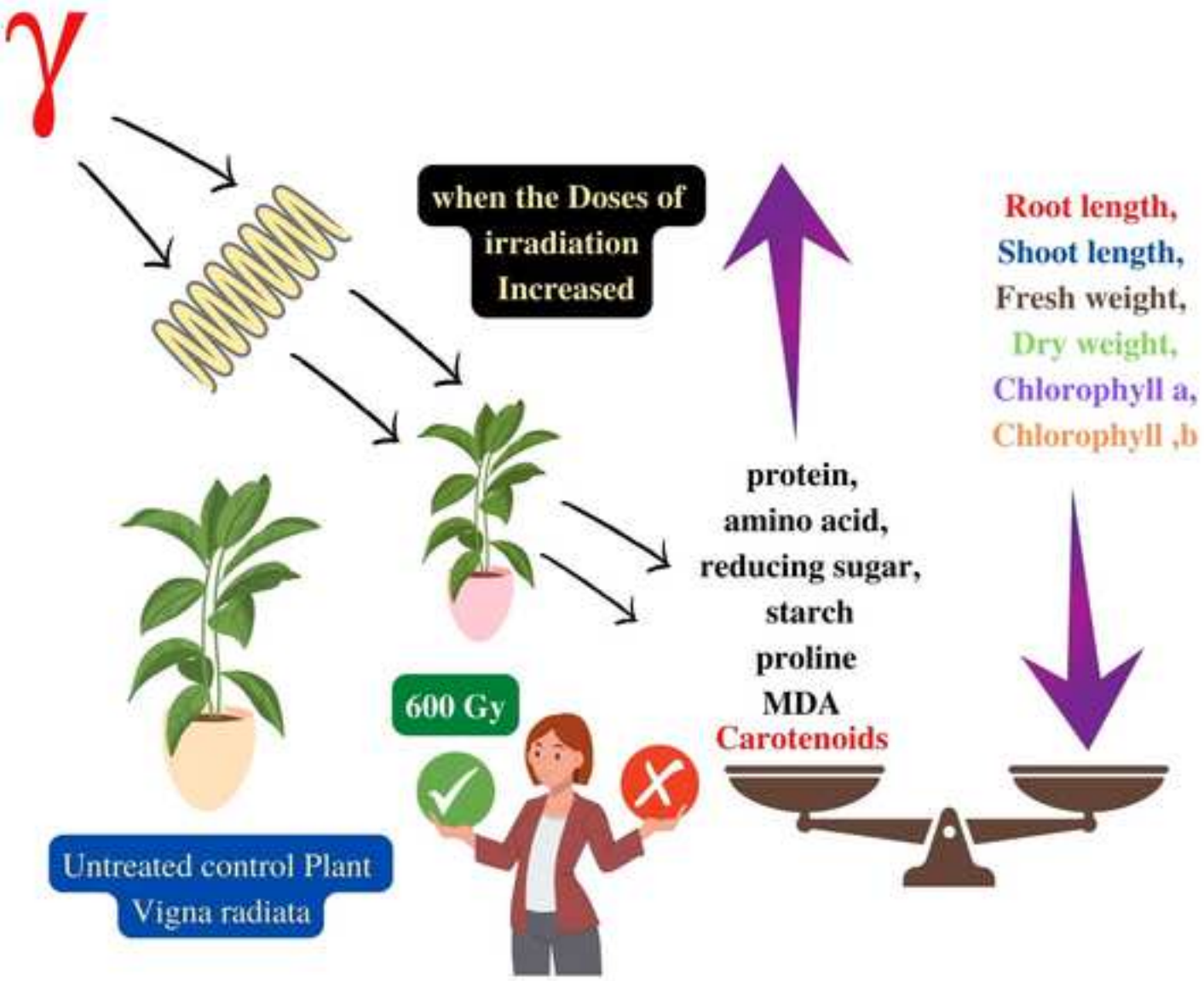
Highlights

- Irradiating flowering plants with Gamma (γ) rays produces mutants that are tolerant to abiotic stress.
- Several doses of γ radiation (100-800 Gy) were applied to green gram seeds in the ^{60}Co γ chamber.
- Changes in morphology with decreased levels were observed under ESR Spectroscopy.
- Seedlings exposed to γ radiation had fewer photosynthetic pigments (chlorophyll a & b) and increased amounts of carotenoids.
- Antioxidant enzymes were increased in irradiated seedlings which act as free radical scavengers.


γ
when the Doses of irradiation Increased
Root length,
Shoot length,
Fresh weight,
Dry weight,
Chlorophyll a,
Chlorophyll ,b
protein,
amino acid,
reducing sugar,
starch
proline
MDA
Carotenoids
600 Gy
Untreated control Plant
Vigna radiata

Impact of gamma (γ) irradiation on morphology, biochemical and antioxidant activity of green gram (*Vigna radiata* (L.) R. Wilczek)

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Abstract

One of the key sources of genetic variability that is ultimately used in the plant development program is mutations. In order to improve crops, mutagens are used, and mutants have been obtained from induced mutations. Gamma irradiation is one of the mutagens that helps to quantify the frequency and patterns of alterations in particular plants. In this present study, the gamma irradiation effect was investigated by germination study and physiological characteristics of green gram. Seeds were exposed to a gamma source (CO^{60}) at doses ranging from 100, 200, 300, 400, 500, 600, 700, and 800, with a non-irradiated sample as a control. Our results show that the germination percentages as well as morphological parameters of seedlings (root length, shoot length, fresh weight, and dry weight) decreased with increasing irradiation doses. These findings were confirmed by ESR spectroscopy with the g-factor at 2.000 ± 0.005. The photosynthetic pigments such as chlorophyll a and b were decreased in irradiated seedlings compared to control, where carotenoid content was increased. Biochemical content such as protein, amino acid, reducing sugar, starch and proline content was increased with increasing doses and it was concerned with various stretching bands analyzed by FTIR analysis. MDA content was increased with increasing doses by the production of free radicals. Antioxidant enzymes such as catalase and peroxidase were increased in irradiated seedlings, which act as free radical scavengers. These results suggested that there had been up to 600 Gy significant changes in morphology, photosynthetic pigment, biochemistry, and antioxidant analyses.

Keywords: Antioxidant; Biochemical Characteristics; ESR**;** FTIR; Gamma rays; Photosynthetic Pigments

1. Introduction

Plant breeders can improve desirable features by using various mutagenic agents and molecular tools in traditional breeding by mutation breeding, which is a sensible and alluring alternative source [1]. Radiation or chemical mutagens are used to increase crop species variability [2] by changing a small number of genes to preserve the genetic background [3]. Out of 3402 mutants, 2581 were generated using physical mutagens, whereas 1646 were produced using gamma rays, and 39 green gram varieties were released (https://nucleus.iaea.org/sites/mvd/SitePages/Search.aspx). Induced mutagenesis by gamma-irradiation is a fast and generally minimal expense way to deal with lead novel varieties with expected qualities, in contrast with traditional reproducing rehearses in designated crops. It is one of the most widely utilized physical mutagens and is effectively used in numerous crops, including sugarcane [4], barley [5], common bean [6] and fenugreek [7]. Gamma irradiation is a form of electromagnetic wave that has a high penetration capacity into molecules and can achieve ionization of the seed material by eliminating its electrons [8]. Gamma ray emitters are generated by humans using Co^{60} cells, which are often designed for industrial uses and considered an artificial source of radiation [8]. Exposure of seeds to gamma irradiation leads to mutagenic changes and enters the cells and tissues with high penetration before undergoing drastic changes. These changes can be strengthened as a direct physical result of gamma irradiation on DNA or as a result of reactive O^2 species being produced. It can interact with DNA, cellular components and biomolecules, which causes ionization, changes in the way that proteins and enzymes work, and other metabolic alterations [1,9]. Radiation-induced DNA changes in plant seeds can be a potential source of variation in offspring.

Irradiated seeds are reliant on DNA repair and gene rearrangement, which can cause aberrations in germination, morphology, and growth as well as produce mutant progeny, and these mutant progenies disrupt the enzymatic process and the metabolic pathway of the plant [10]. It is well known that gamma irradiation at lower doses induces and enhances plant development characteristics [11]. Germination and growth responses of plants to gamma irradiation typically rely on the radiation dosage quantity, exposure duration, and plant [12]. It has an impact on biomolecules, increasing the number of antioxidant enzymes and cellular membrane permeability potentials [13]. It can also be used to alter the physiological and biochemical characteristics [14], to produce free radicals [15], and to alter the seed yield [16]. The influence of gamma irradiation can modulate the photosynthetic process to increase growth [11, 17].

According to research, gamma radiation causes oxidative stress by producing excessive levels of reactive oxygen species (ROS), including superoxide radicals (O^{2-}), hydroxyl radicals (OH^{-}), and hydrogen peroxide (H_2O_2) [18], which disrupts the cellular metabolism. Antioxidants are assumed to be a substance that can slow down, stop, or prevent the oxidation process, scavenge oxygen, and add hydrogen atoms to the structure of free radicals [19]. Gamma rays can be employed for basic research in functional genomics and gene discovery as well as for creating new mutant types employing local germplasm augmentation. These novel varieties are easily adaptable in a short amount of time to various agro-climatic and growing circumstances as well as environments with scarce resources [20]. Gamma radiation is recognized to have an impact on plant development as well as improvements in cytology, biochemistry, and morphological alterations in cells and tissues.

Green gram (*Vigna radiata* (L.) R. Wilczek) is a significant protein supplement crop in subtropical regions of the world. It is also a common short duration crop on the Indian subcontinent and comprises 26 % of protein, 51 % of carbohydrates, and 7 % of other elements [21]. In order to enhance and differentiate crops, it is imperative to provide a broad spectrum of variants through hybridization, recombination, mutation, and selection [22, 23]. Induced mutation is the best choice for the process of hybridization. Recombinant mutation and selection may achieve the most important requirements for effective crop development to give the spectrum of variants and to regenerate and restore the genetic variety in green gram [24]. Thus, to break the yield plateau in greengram, a suitable variety must be created in today's intended environment.

2. Materials and methods

2.1. Seed samples and irradiation treatments

The genuine seeds of the vamban-2 variety of green gram (*Vigna radiata* (L.) R. Wilczek) were obtained from the National Pulses Research Centre in Vamban, Pudukkottai, Tamil Nadu, India. In the current investigation, gamma rays were used to treat green gram seeds. Irradiation from the ^{60}Co Source fixed in the gamma cell 5000 installed at the Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, was used in the present work. Green gram Var. Vamban-2 seeds that were healthy, dry, and uniform were subjected to treatments of 100, 200, 300, 400, 500, 600, 700, and 800 Gy. As a control, untreated seeds were used. 500 seeds were utilized for each treatment.

2.2.Morphological parameters

The shoot and root length will be measured in ten randomly selected seedlings from each dose along with control on the 7th day. The point of the root-stem transition region was taken as the total root length. The length between the stem tip and the point of the root stem transition region was taken as the stem length. The root length and the stem length were expressed in centimeters (cm) per plant. The seventh-day seedlings were separated into shoots and roots. The fresh weight of plants was determined by using an electronic balance, and the value was expressed in grams. They were kept in a hot air oven at 80 °C for 24 hours. After 24 hours, the dry weight of the shoot and root were weighed and recorded.

2.3.Photosynthetic pigment analysis

In gamma irradiated seedlings, the chlorophyll parameters were analyzed by using the acetone method [25] and carotenoid content [26]. The green, healthy, and young leaves were taken after 7 days of planting in the Petri dish with regular watering. Every sample was weighed out at approximately 0.2 g, and it was grounded with 10 ml of 80% acetone in a mortar and pestle. All the chlorophyll extracts were put into the centrifuge tube and centrifuged at 800 rpm for 10 minutes in the cooling centrifuge. The homogenate was re- extracted with 80% acetone until the green color disappeared in the residue. The clear supernatant after centrifugation was used for analysis of the content of chlorophyll a, chlorophyll b, and carotenoids. 2 ml of extract was transferred into a cuvette and the absorbance/ optical density (OD) was read at 663, 645 and 480 nm for chlorophyll a, chlorophyll b and carotenoids in a spectrophotometer against 80 % acetone as a blank. The outcomes were interpreted as milligrams per gram of fresh leaf weight.

2.4.Biochemical analysis

130 2.4.1. Reducing sugar

131 Reducing sugar was estimated according to the method [27]. Two hundred mg of fresh
132 shoot, root and leaf tissue were ground with 10 ml of 80 % ethanol and the homogenate was
133 spun at 800 rpm for 15 mts in centrifuge at 20℃. The supernatant was saved and the residue
134 was re-extracted with boiling 80 % ethanol and the supernatant was pooled. The ethanol was
135 evaporated from the supernatant and an aliquot of 20 ml was made up with distilled water. The
136 extract was used for the estimation of sugar. All the reagents without extract were used as
137 blanks. A volume of 1 ml of fresh copper reagent and 1 ml of extract (prepared by mixing
138 copper tartrate solution and copper sulfate solution (25:1 v/v) were added. The mixture was
139 heated in a marble covered test tube in a boiling water bath for 20 minutes, then 1 ml of
140 arsenomolybdate reagent was added after the mixture had been cooled. The final volume is
141 made up of 20ml of distilled water. The green color complex was read at 520 nm in a
142 spectrophotometer against the blank (reagent without extract). The sugar content of the sugar
143 was calculated from the standard graph using glucose. It was expressed in mg/g fresh weight
144 basic.

145 2.4.2. Starch

146 Starch content was estimated using the earlier described method [28]. Soluble sugar
147 extraction was taken. To the residue, 5 ml of distilled water was added and 6.5 ml of 52 % of
148 perchloric acetic acid was also added, stirred well and heated at 80 °C in a water bath for 30
149 minutes. Then 20 ml of distilled water was added and centrifuged at 800 rpm for 15 minutes,
150 and the supernatant was saved. The residue was re-extracted and the supernatant was pooled
151 and made up to 10 mL with distilled water. The extract was filtered through Whatman No.1

filter paper and used for the estimation of starch. Ten ml of cold anthrone reagent was added with 1ml of perchloric acid (PCA) extract and it was diluted with 5ml of deionised water. The test tube was heated for 10 minutes at 100 °C in a boiling water bath. The test tube was cooled rapidly, and absorbance was read at 630 nm in a spectrophotometer. All the reagents without extract were used as blanks. Starch content was calculated by multiplying glucose equivalents with the conversion factor 0.9. It was expressed in mg/g fresh weight basic.

2.4.3. Protein

The protein was estimated according to the standard method [29]. Five hundred milligrams of fresh plant tissue were ground with 20ml of 20 % trichloroacetic acid (TCA) in a pestle and mortar. The homogenate was spun at 800 rpm for 15 minutes in a centrifuge. After centrifugation, the supernatant was discarded and the pellet was saved. 5ml of 0.1N sodium hydroxide (NaOH) was added to the pellet to solubilize the protein, and the aliquot was spun again at 800rpm for 15 min. Finally, the supernatant was made up to 10ml with 0.1N NaOH and used for the estimation of protein content. 0.5 ml of extract was taken in a clean test tube, in which 5 ml of reagent-D and 1 ml of folin phenol were added and kept at room temperature for 10 minutes. The blue color complex was read against the blank (reagent without extract) at 640 nm in a spectrophotometer (systronics). Bovine serum albumin was used as a standard graph to calculate protein content. It was expressed in mg/g fresh weight basic.

2.4.4. Amino acid

The free amino acid was estimated according to the earlier method [30]. 500 mg of plant tissue was taken and homogenized with 10ml of 80 % boiling ethanol. The extract was centrifuged at 800 rpm for 15 min and the supernatant was made up to 10 ml with 80 % ethanol

used for the estimation of free amino acids. One ml of ethanol extract was taken in a 25ml test tube and neutralized with 0.1N NaOH using methyl red. To this, 1 mL of Ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes, and then 5ml of diluting reagent was added, cooled and made up to 25ml with distilled water. The absorbance was read at 570 nm in a spectrophotometer. It was expressed in mg/g fresh weight basic.

2.5. Antioxidant enzyme activity

Total CAT activity was measured [31]. The technique described by [32] was used to carry out POD activity. Plant material was collected and frozen at -80^{o} C. The extraction buffer consists of a 50mM phosphate buffer (pH 7.0). Plant material was homogenized in 5 ml of extraction buffer and centrifuged at 4000 rpm for 20 min. The supernatant was used for enzyme assay, and 2.6 ml of 50 mM potassium phosphate buffer and 0.4 ml of 15 mM hydrogen peroxide (H_2O_2) were added to it. The absorbance of crude extract was measured with UV-Spectrophotometer. Units/mg protein unit was used to express the enzyme activity as units/mg of protein.

2.6. Proline content

Proline was extracted and estimated according to the earlier reported protocol [33]. Five hundred mg of frozen plant material was ground with 10 ml of 3 % aqueous sulphosalicylic acid in a pestle and mortar. The homogenate was filtered through Whatman No.1 filter paper. The residue was re-extracted and pooled, and the aliquot was made up to 20 ml with aqueous sulphosalicylic acid. The extract was used for the estimation of proline. Two ml of acid ninhydrin and two ml of glacial acetic acid were allowed to react with two ml of proline extract. The mixture was incubated for 1 hour at 100°C in a boiling water bath. Then the test tube was

196 transferred to an ice bath to terminate the reaction. After that, 4 ml of toluene was added and
197 mixed for 30 seconds, and the toluene containing chromophore was separated from the aqueous
198 phase by using a funnel. The absorbance was read at 520 nm in the spectrophotometer without
199 using reagent as a blank. The proline content was determined from the standard graph with a
200 series of prolines and expressed in mg per gm of fresh weight.

201 2.7. Lipid peroxidation

202 Lipid peroxidation can be estimated by the standard method [34] by detecting the
203 malondialdehyde (MDA) concentration at 532 nm. In the extraction buffer of 0.1 M potassium
204 phosphate, samples were homogenized. A tube holding 1 ml of 20 % TCA and 0.5 % TBA
205 received 2 ml of enzyme solution and it was centrifuged for 10 minutes at 14000 rpm after 30
206 minutes of heating at 95 °C. Utilizing an extinction value of 155 mM-1 cm^{-1}, it was calculated.

207 2.8. Electronic spin Resonance (ESR) analysis

208 Gamma-irradiated green gram seed samples were placed in ESR quartz tubes in order
209 to register paramagnetic species. ESR spectra were recorded at room temperature with the
210 Bruker Biospin EMX spectrometer operating at X-band (9.1 GHz). The ESR parameters were
211 set at 100-KHz modulation frequency, microwave power of 5 mW, modulation amplitude of
212 2.5 G, sweep time of 4 min, and receiver gain of 2 x 10^4. The variations in the steady state of
213 the relative concentration of the paramagnetic species generated at different absorbed doses
214 were obtained. The signal's intensity was calculated as the peak and reported as arbitrary units
215 per kilogram of sample weight (AU/mg).

216 2.9. Fourier transform infrared spectroscopic (FTIR) analysis

Using gamma-irradiated samples of mung bean seed powder, Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify the 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 spectra were collected between 4000-400 cm^{-1} at room temperature.

2.10. Data analysis

Analysis of the sample was done seven days after germination. Results are given as the mean ± standard error. To discover differences in the average of all parameters between the irradiated samples, 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.

3. Results

3.1. Morphological analysis of gamma irradiated seedlings

Seedlings grown from irradiated seeds exhibit a significant reduction in length. Gamma ray doses of 100, 200, 300, 400, and 500 Gy were used to measure the increased seedling length; dosages of 600, 700, and 800 Gy caused a dramatic drop, as seen in Fig. 1 and 2. Gamma radiation exposure caused the maximum reduction of seedling length (4.86) in 800 Gy as compared to control (16.26). Studying the fresh and dried weight (g) of shoots, roots, and leaves reveals that weight decreases with increasing doses as compared to control. When compared to control seedlings (shoot: 0.094; root: 0.032; leaf: 0.067), 800 Gy showed a decrease in fresh weight (shoot: 0.063; root: 0.006; leaf: 0.011). The dry weight was also measured in the

238 seedlings and 800Gy showed the decreased value (shoot: 0.005, root: 0.002, and leaf: 0.002)
239 as compared to control (shoot: 0.017; root: 0.008; leaf: 0.013) and it was shown in Fig.2.

240 3.2. Photosynthetic parameter analysis

241 Gamma irradiation showed a significantly lower amount of the photosynthetic pigments
242 such as chl a and b content and an increase in the carotenoid content as compared to control,
243 which makes chlorophyll estimation one of the key criteria in determining production capacity.
244 Chlorophyll "a" content is reduced at 800 Gy (0.216) when compared to control (0.978).
245 Chlorophyll "b" content was increased when compared to chlorophyll "a" and decreased when
246 compared to the control. The greatest reduction known as the maximum reduction was in 800
247 Gy (0.249) as compared to control (0.562). Gamma radiation treatment improves carotenoid
248 content. It was observed in 800 Gy (1.689) an increased content as compared to control (1.229)
249 and this was shown in Fig.3.

250 3.3. Biochemical analysis

251 Depending on the dosages of gamma radiation, the biochemical composition of the
252 seedlings displayed some variations with increased doses of irradiation up to 600 Gy.

253 When compared to control, the effect of gamma radiation on the decreasing sugar,
254 starch, protein, and amino acid content was increased. Increased irradiation dose resulted in
255 greater sugar content reduction. When compared to the control (shoot: 0.821; root: 0.464; leaf:
256 0.542), it demonstrates the increased content at 600Gy (shoot: 1.084; root: 1.080; leaf: 1.008).
257 The average starch content performance was in 600 Gy (shoot: 2.422; root: 1.458; leaf: 1.087),
258 which was higher than in control (shoot: 1.829; root: 1.398; leaf: 0.478). The protein content
259 showed the biggest difference, and it was boosted by 600 Gy (shoot: 3.594; root: 2.999; leaf:

3.721) as compared with control (shoot: 2.488; root: 2.396; leaf: 2.079). An increase in gamma irradiation dose led to an increase in amino acid content. In comparison to control (shoot: 0.743; root: 0.705; root: 0.456), it displays the maximum value in 600Gy (shoot: 0.829; root: 0.826; leaf: 0.792). Proline displays more content in 600 Gy (shoot: 1.572, root: 1.398, leaf: 1.387) than the control (shoot: 1.518, root: 1.324, leaf: 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 control leaf (0.243) and denoted in Fig.4.

3.4. Enzymatic antioxidant

Under the growth conditions of this experiment, CAT and POD activity were increased with increased doses (800Gy). CAT activity in seedlings treated with 800 Gy gamma irradiation was increased in shoot: 2.740; root: 2.721; leaf: 2.809 versus shoot: 2.571; root: 2.345; leaf: 2.166 in control plants. POD activity showed an important increase in response to gamma irradiation. In 800 Gy of gamma irradiation, there was more inflation in shoot: 7.103; root: 6.829 and in leaf: 6.567 than that observed in control plants (shoot: 3.156; root: 3.062; leaf: 3.177) and it was represented in Fig. 5.

3.5. Correlation coefficient analysis

Through correlation, characteristics related to morphology, photosynthetic processes, antioxidants, and metabolic pathways are clearly shown. 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

283 "a," has a strong positive association. Since antioxidant enzymes like catalase and peroxidase
284 function as ROS scavengers to shield seedlings from harm, their activity is highly negatively
285 connected with morphological traits. The biochemical components have a highly substantial
286 and positive association with one another, according to the Pearson correlation coefficient. The
287 significant information was displayed in Tables 1 & 2 for morphology and biochemical content,
288 respectively.

289 3.6. ESR analysis of gamma irradiated samples

290 A single signal is observed in the ESR spectra of all irradiated and non-irradiated green
291 gram samples. The g-value was set at 2.000 ± 0.005 for an irradiated plant sample. In the case
292 of irradiated plant samples, the intensity of signals was increased with increased doses. Gamma
293 irradiated green gram powder provides the typical spectrum of a central signal with a g factor
294 of 2.005. The spectrum of irradiated seed powder was exemplified in Fig.6.

295 3.7. FTIR spectroscopy analysis of gamma irradiated samples

296 The FTIR spectrum of gamma irradiated presents a number of peaks between 4000-
297 400 $cm^{-1}$are due to various stretching bands of biomolecules such as proteins, amino acids,
298 lipids, carbohydrates, and various fingerprint regions. The FTIR spectrum shows a broad
299 spectrum in both irradiated and non-irradiated (control) green gram samples. The peak comes
300 in the range between 3712-2839 cm^{-1} such as 3305 cm^{-1}, 2928 cm^{-1} in control and 3458 cm^{-1},
301 3366 $cm^{-1,}$ 3343 cm^{-1} and 3308 cm^{-1} in irradiated samples consigned to hydroxyl compounds.
302 Peaks obtained in 2922 cm^{-1}, 2926 cm^{-1} 2928 cm^{-1} 2929 cm^{-1} and 2931 cm-1 mainly
303 characterize C-H extending vibration by lipids. The peaks at 1650 cm^{-1}, 1644 cm^{-1}, 1546 cm^{-1},
304 1545 cm^{-1}, 1542 cm^{-1} and 1540 cm^{-1} in irradiated samples as amino acids. The bands that
305 appeared between 1500-1100 cm^{-1} were of the fingerprint region, and the peaks obtained

306 between 1200-900 cm^{-1}, 922-770 cm^{-1} and 1300-600 cm^{-1} attributed to the presence of
307 carbohydrates. Absorption bands were shown between 400-560 cm^{-1} and peaks such as 530 cm^{-1}, 529 cm^{-1}, 440 cm^{-1} and 439 cm^{-1} were specified with the presence of starch molecules and
309 the transmittance percentage of these biochemical characteristics was represented in Fig.7.

310 **4. Discussion**

311 Gamma irradiated green gram seeds such as 100, 200, 300, 400, 500, 600, 700 & 800
312 Gy doses were sown and morphology parameters such as seedling length, fresh weight and dry
313 weight were observed on the 7^{th} day. The results of the study demonstrated that gamma
314 radiation is sufficient to reduce the root percentage while not exceeding in length, and at higher
315 doses of gamma irradiation, shoot and root length were decreased. It resulted in deteriorated
316 mitotic activity in meristematic tissues and decreased the moisture content of seeds [35]. Rising
317 doses resulted in a decrease in seedling length, which was demonstrated in green gram [36], in
318 chickpea [37], in groundnut [38] and in paddy [39] (Fig.1). A reduction in fresh weight and dry
319 weight of seedlings was also noted in this study, and the similarity was seen in crops such as
320 rice [40], in *Lepidium sativum* [41], and in *Vigna unguiculata [42]*. Reduction of water content
321 as a result of gamma radiation can cause a decrease in biomass production [43] [41] (Fig. 2).
322 This was confirmed by ESR Spectroscopy, as shown in Fig 6. The release of chlorophyll from
323 its protein complex through de-phytolization can gradually reduce the chlorophyll content after
324 gamma irradiation treatment. For gamma-irradiated and control seedlings, a dose-dependent
325 significant variation in chlorophyll a and b content was discovered, and chlorophyll b was less
326 abundant than chlorophyll a. [44] Gamma rays are known to destroy chlorophyll molecules
327 and limit the rate of photosynthetic activity [45,46]. According to [14], the destruction of
328 chlorophyll b rather than chlorophyll a is due to its biosynthesis or degradation of its precursors.

Inhibiting gamma irradiation on seedlings increased chlorophyllase activity, promoted chlorophyll deprivation, and ultimately decreased photosynthetic activity [47]. In this study, carotenoid concentrations were increased by 800 Gy (Fig.3). According to [48], carotenoid levels increased at the same level of irradiation, whereas chlorophyll a and b are essentially insensitive to it. Carotenoids are crucial for protecting chlorophyll from oxidative damage and scavenging free radicals in light [49]. Studies of correlation can be used to identify features and to 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, as was shown in Table 1.

The biochemical investigation showed the beneficial effect on seedlings of gamma irradiation treatment, which produces free radicals [50]. Plant cells evolve a defense mechanism against gamma radiation [20] [51]. Increases in biochemical traits including sugar and starch content were seen in 600 Gy, and a similar effect was shown in lupine [52]. 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 [53].

High irradiation doses provide high chemical extractability by creating a disulfide bond between polypeptide chains, which has an impact on the accumulation and conformation of low molecular weight proteins [54]. The same outcome was attained with soybean seeds [55]. Gamma radiation-induced changes in amino acid content may be caused by the production of free radicals. The findings of irradiated soy flour [56], sesame [57], and mung bean [58] were in agreement with this conclusion (Fig.4). 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 [59]. The outcome displays increased proline content in wheat

[60], *Allium sativum* [61], and *Terminalia arjuna* [62], all of which have considerably favorable correlations with one another and are depicted in Table 2.

Different doses of gamma radiation encourage the synthesis of antioxidant enzymes, enhance the production of ROS, and can also alter several environmental stresses [63] [64]. Catalase, peroxidase, and lipid peroxidation activities show the highest production at 800 Gy. Catalase activity was controlled by the radiation exposures during the developmental phase [65], and it reduced the damage caused by irradiation [66]. It was stimulated by irradiation at 5k Gy in *Vicia faba* L. [67] and also seen in two rice cultivar seeds at irradiation of 200 Gy [68]. Cellular function depends on peroxidase activity, which might change in response to gamma radiation. Peroxidase is more effective than catalase due to its protective action in removing H_2O_2, peroxides, and particularly lipid hydrogen peroxides [69]. Similar results were observed in *Phaseolus vulgaris* [70] and *Triticum aestivum* [71]. Gamma radiation may cause an accumulation of free radicals like O_2 and H_2O_2, which would distress the system for removing them and cause lipid peroxidation. Different MDA concentrations were used as a marker for the oxidation of cell membranes brought on by stress [72] (Fig.4). 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 [51]. A study found that the enhanced content was present in chickpea, in *Zizania latifolia* [73], in soybean [74], and in rice [75].

The FTIR spectroscopic analysis showed the broad spectrum of irradiated and non - irradiated (Control) green gram samples. Coca seeds [76] and rice seedlings [77], the peak ranged between 3712-2839 cm^{-1} such as 3305 cm^{-1}, 2928 cm^{-1} in control, and 3458, 3366, 3343, and 3308 cm^{-1} in irradiated samples, were assigned to hydroxyl compounds. Souza et al. [78]

declares that the vibration caused by lipids comes under the range between 3000-2800 cm^{-1} (2922, 2926, 2928, 2929, and 2931 cm^{-1}).

The maximum intensity was obtained at 1650, 1644, 1546, 1545, 1542, and 1540 cm^{-1} in irradiated samples and it comprises amino acids and protein absorption bands, including amide I and amide II bands that are mainly traced between 1660-1600 cm^{-1}, 1585-1481 cm^{-1} and 1551-1460 cm^{-1} [79]. Fingerprint regions appeared between 1500-1100 cm^{-1} and the bands such as 1404, 1403, 1402, 1245, 1241, 1154 and 1153 cm^{-1} exhibited its presence. 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} ([80]), and the presence of starch molecules was shown between 400-560 cm^{-1} such as 530, 529, 440 and 439 cm^{-1} [81]. Thus, FTIR analysis is a technique used to explore the biochemical compounds and functional groups that were obtained in irradiated samples. From the above findings, it could be concluded that λ-irradiation has a significant effect on morphology and biochemical and antioxidant activities. In the doses, 600 Gy shows the incredible changes, and the bargain was obtained at 800 Gy, as shown in Figure 7. From this, up to 600 Gy, the plants show restorative effects on green gram plants.

Conclusion

In summary, the above finding suggests that there is a significant change in germination percentage and morphological characteristics such as plant height, shoot and root length, and fresh and dry mass, which suggest decreased content. Photosynthetic pigments like chlorophyll a and b show decreased value due to chloroplast damage, but carotenoids show increased content because they act as antioxidants. Biochemical characteristics such as reducing sugar, starch, protein, amino acids, and proline content were increased with increased doses. This was

confirmed by ESR and FTIR techniques, which show the changes occurred due to gamma irradiation.

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Author Contributions

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

Conflict of Interest

The authors declare no conflict of interest.

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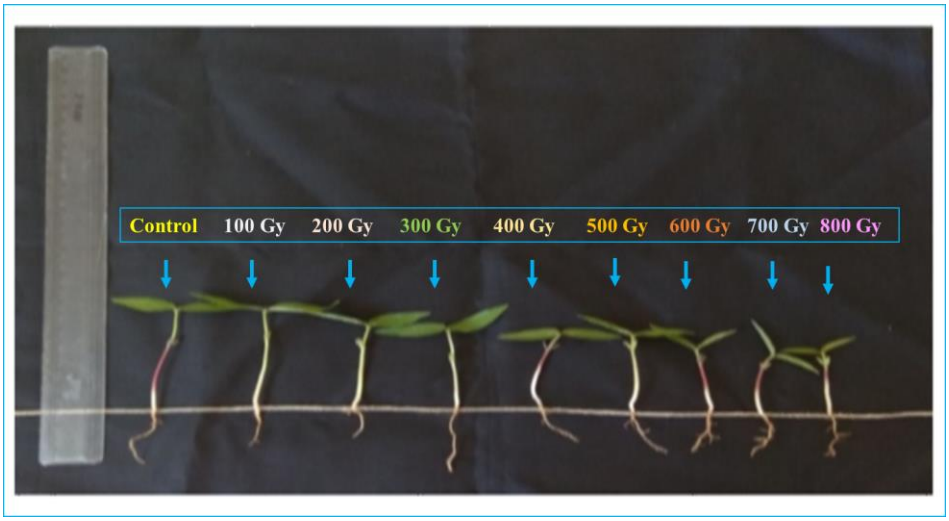

Figure 1. Effect of gamma irradiation on seedling at 7th day

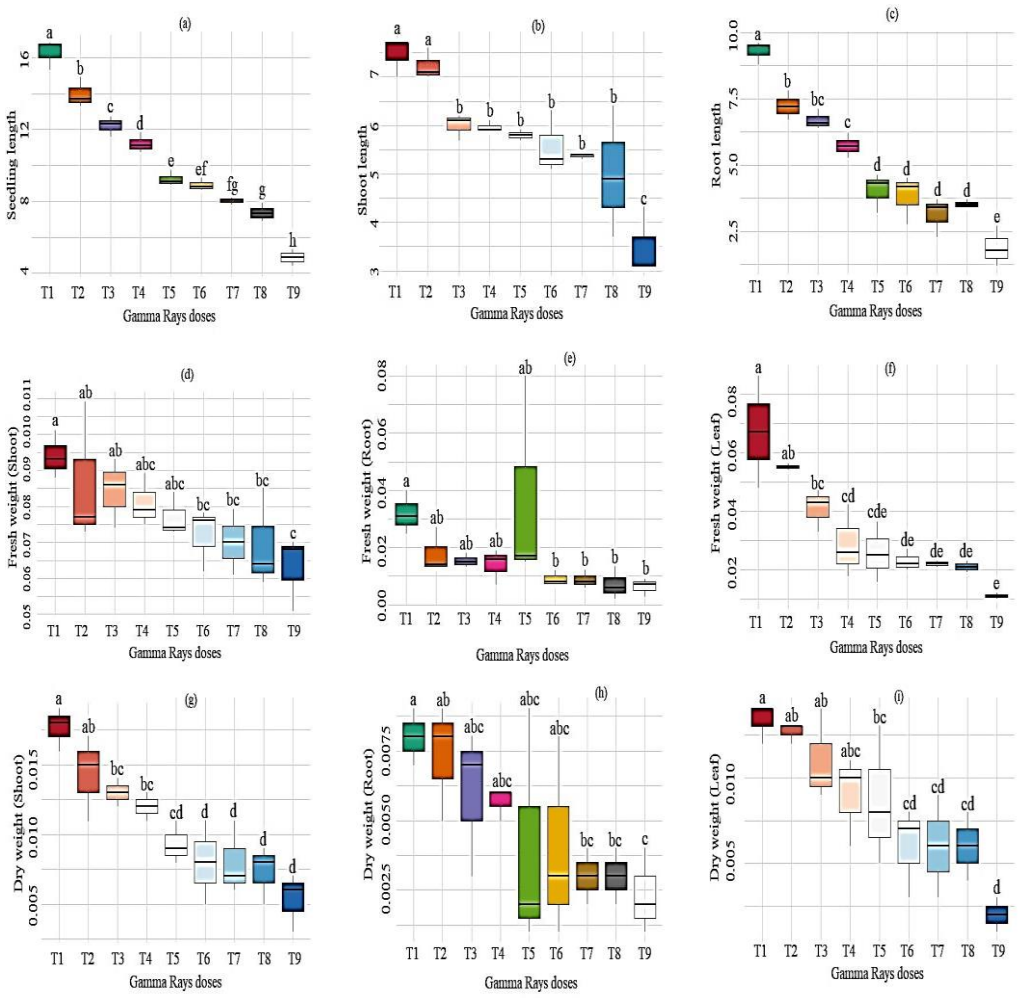

Figure 2 Morphological analysis of gamma irradiated seedlings

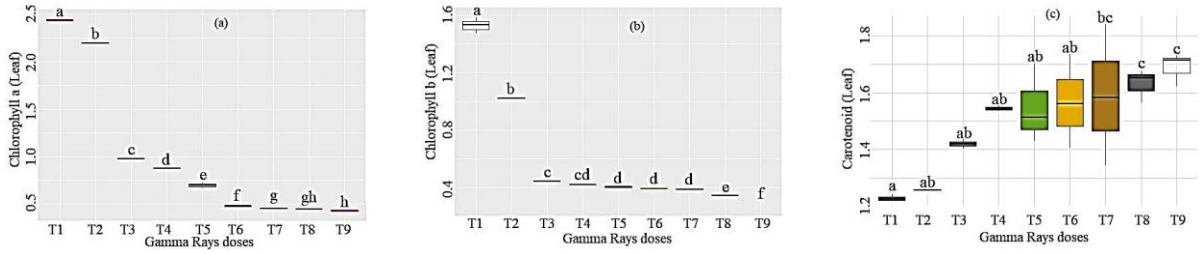

Figure 3. Photosynthetic parameter analysis

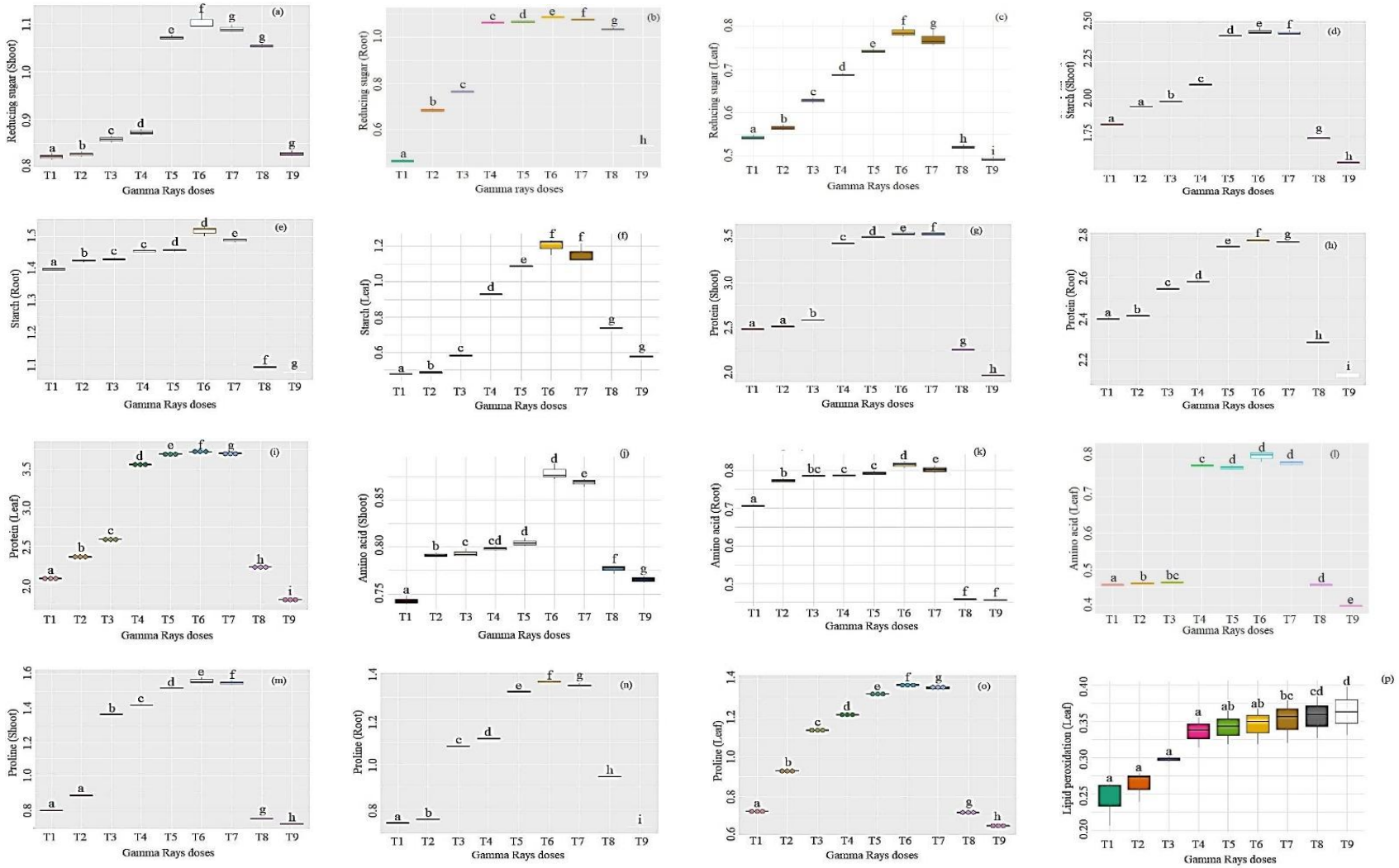

Figure 4. Biochemical parameter analysis in shoot, root & leaf: Reducing sugar (a-c), Starch (d-f), Protein (g-i), Aminoacid (j-l), Proline (m-o) and Lipid peroxidation (p)

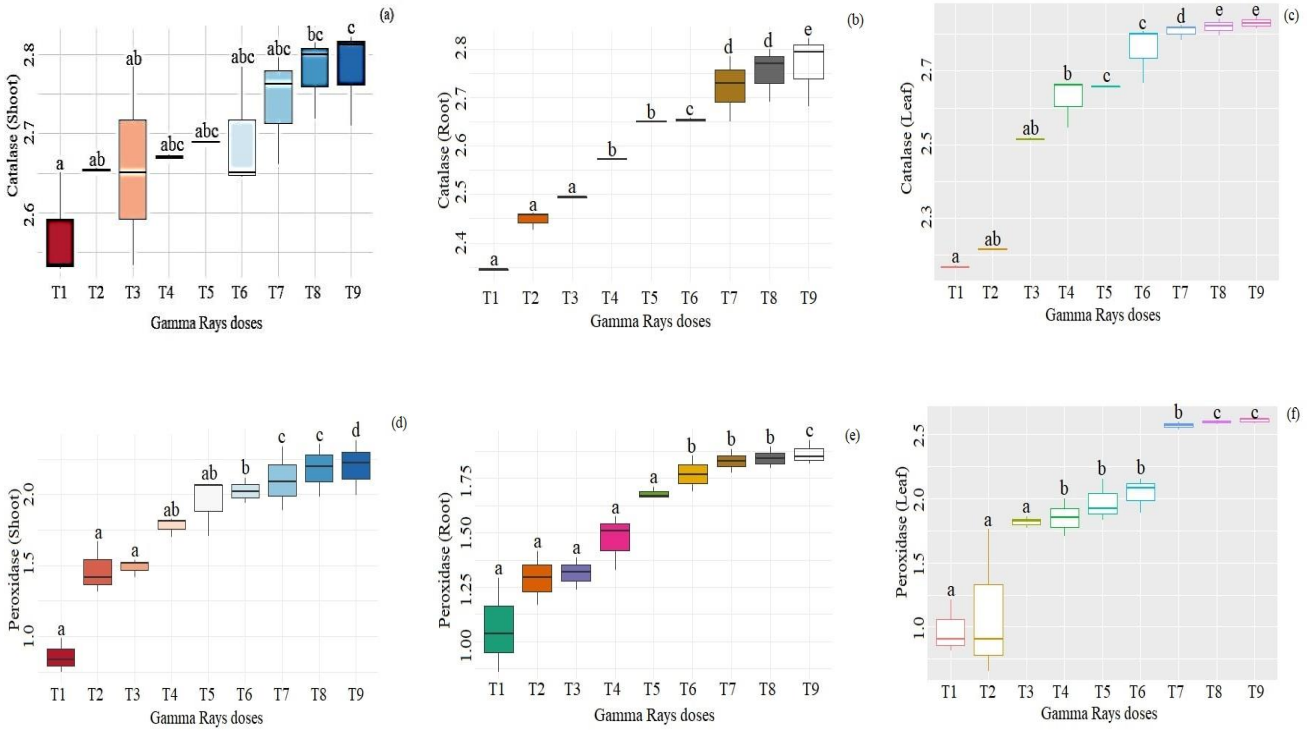

Figure 5. Enzymatic antioxidant activity: Catalase (a-c) and Peroxidase (d-f)

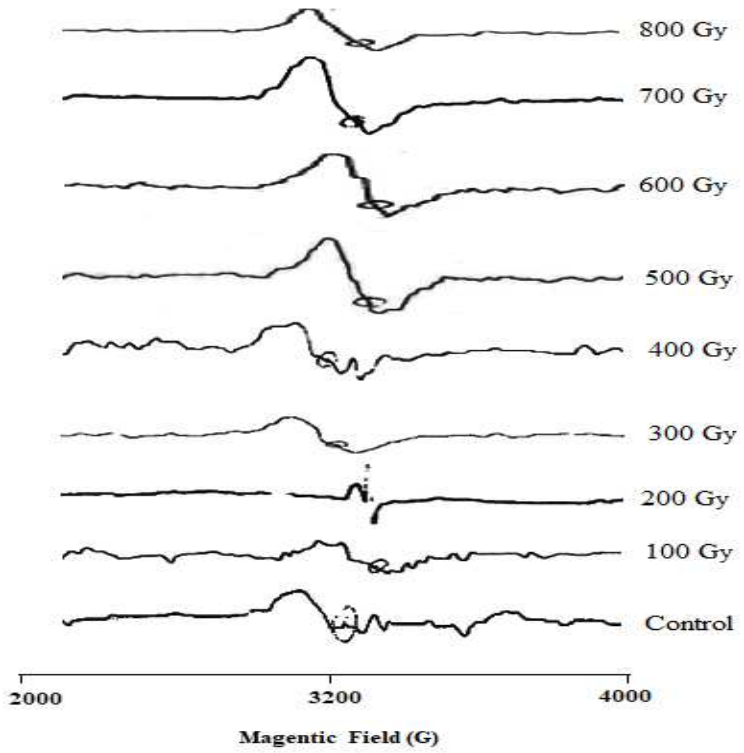

Figure 6. ESR spectroscopy analysis of gamma irradiated and control of green gram seed sample X-band ESR spectrum of control and different dose of gamma irradiated green gram seeds using 100-KHz modulation frequency, microwave power 5 mW. Circles are indicating ESR spectrum of peaks with g-value 2.005

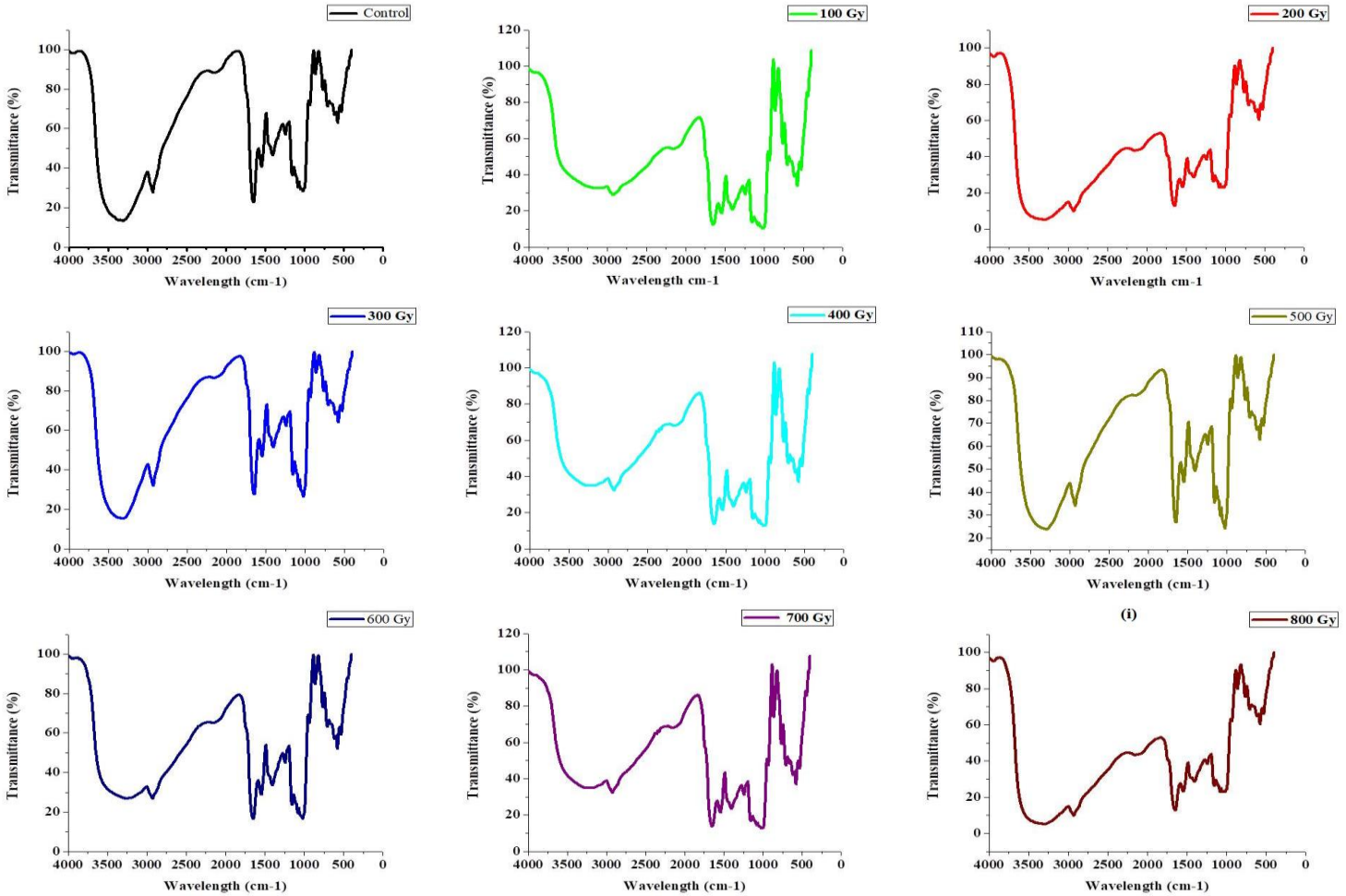

Figure 7. FTIR analysis of biochemical component of gamma irradiated green gram seed powder: a) Control; b) 100 Gy; c) 200 Gy; d) 300 Gy; e) 400 Gy; f) 500 Gy; g) 600Gy; h) 700 Gy; i) 800 Gy. *Spectral range between 4000 – 400 cm^{-1} for different doses of gamma irradiation

List of Tables

	SEL	SL	RL	FWS	FWR	FWL	DWS	DWR	DWL	CHLa	CHLb	CARO	CATS	CATR	CATL	PODS	PODR	PODL	MDA
SEL	1	.896**	.967**	.726**	.392*	.861**	.896**	.680**	.827**	.897**	.854**	-.824**	-.725**	-.940**	-.928**	-.882**	-.899**	-.878**	-.835**
SL		1	.799**	.653**	.401*	.741**	.820**	.514**	.732**	.781**	.749**	-.806**	-.606**	-.767**	-.816**	-.720**	-.746**	-.786**	-.782**
RL			1	.667**	.393*	.862**	.893**	.717**	.801**	.885**	.842**	-.736**	-.679**	-.929**	-.897**	-.869**	-.893**	-.838**	-.829**
FWS				1	.290	.610**	.722**	.424*	.565**	.634**	.591**	-.712**	-.625**	-.652**	-.684**	-.658**	-.697**	-.690**	-.589**
FWR					1	.413*	.448*	.561**	.524**	.383*	.385*	-.170	-.347	-.381*	-.394*	-.345	-.387*	-.379	-.254
FWL						1	.820**	.723**	.773**	.891**	.875**	-.758**	-.608**	-.871**	-.907**	-.836**	-.839**	-.818**	-.748**
DWS							1	.611**	.737**	.829**	.785**	-.766**	-.703**	-.860**	-.852**	-.827**	-.856**	-.766**	-.821**
DWR								1	.660**	.656**	.601**	-.423*	-.336	-.697**	-.657**	-.575**	-.620**	-.553**	-.450*
DWL									1	.747**	.684**	-.560**	-.637**	-.782**	-.805**	-.717**	-.757**	-.738**	-.630**
CHLa										1	.957**	-.819**	-.629**	-.894**	-.976**	-.872**	-.860**	-.891**	-.863**
CHLb											1	-.775**	-.620**	-.840**	-.908**	-.862**	-.791**	-.829**	-.816**
CARO												1	.687**	.837**	.861**	.834**	.798**	.810**	.851**
CATS													1	.782**	.690**	.776**	.749**	.708**	.641**
CATR														1	.936**	.956**	.939**	.901**	.851**
CATL															1	.906**	.915**	.926**	.873**
PODS																1	.944**	.885**	.868**
PODR																	1	.907**	.874**
PODL																		1	.849**
MDA																			1

Table 1. Pearson's correlation coefficient analysis of morphology. LENG: seedling length, SFW: Shoot fresh weight, RFW: Root fresh weight, LFW: Leaf fresh weight, SDW: Shoot dry weight RDW: Root Dry weight, LDW: Leaf dry weight, CHLA: Chlorophyll a, CHLB: Chlorophyll b, CARO: Carotenoid, Measurement was taken as mg/g fresh weight. CAT: Catalase, POD:Peroxidase, as Unit/mg protein MDA: Malondialdehyde of gamma irradiated green gram seedling as mM/g frsh weight. Correlation was significant at *P< 0.05, **P<0.01.

	SUGAR S	SUGAR R	SUGAR L	STARCH S	STARCH R	STARCH L	PROT S	PROT R	PROT L	AMINO S	AMINO R	AMINO L	PROL S	PROL R	PROL L
SUGAR S	1	.826**	.675**	.697**	.205	.857**	.618**	.636**	.674**	.727**	.160	.666**	.558**	.812**	.581**
SUGAR R		1	.750**	.722**	.347	.868**	.763**	.690**	.834**	.717**	.338	.805**	.720**	.861**	.741**
SUGAR L			1	.976**	.790**	.892**	.968**	.984**	.972**	.861**	.770**	.939**	.962**	.946**	.974**
STARCH S				1	.817**	.860**	.952**	.988**	.947**	.839**	.797**	.907**	.918**	.915**	.952**
STARCH R					1	.464*	.800**	.853**	.735**	.573**	.992**	.677**	.783**	.603**	.817**
STARCH L						1	.879**	.830**	.916**	.843**	.427*	.934**	.816**	.931**	.821**
PROT S							1	.953**	.988**	.768**	.776**	.979**	.921**	.892**	.939**
PROT R								1	.945**	.813**	.836**	.897**	.956**	.921**	.970**
PROT L									1	.805**	.720**	.981**	.941**	.935**	.957**
AMINO S										1	.550**	.777**	.789**	.842**	.829**
AMINO R											1	.647**	.787**	.588**	.822**
AMINO L												1	.881**	.889**	.893**
PROL S													1	.933**	.986**
PROL R														1	.924**
PROL L															1

Table 2 Correlation coefficient analyses of biochemical characteristics. Sugar: Reducing sugar, PROT: Protein, AMNIO: Aminoacid, PROLI: Proline and the content were measured as mg/g freshweight. S: Shoot, R: Root L: Leaf Correlation was significant at *P< 0.05, **P<0.01

Conflict of interest

The authors of the present study stated no conflicts entertained.

Supplementary Files

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- ImpactofgammaFigurespage0005.jpg