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RESEARCH ARTICLE

Exploring the role of gamma irradiation in modulating physiological and biochemical traits of dragon fruit (*Selenicereus undatus*)

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Abstract

Dragon fruit (*Selenicereus* spp.), known as pitaya or pitahaya, a tropical climbing fruit from the family Cactaceae, is rich in antioxidants, vitamins and fiber. Commercial propagation through stem cuttings limits genetic diversity, hindering cultivar development. However, white-fleshed varieties perform poorly in acidic soils, which restricted the cultivation that necessitated the need of genetic improvement. To exploit the potentiality of gamma irradiation research trial was carried out to study the influence of gamma irradiation at doses of 0, 100, 200, 300, 400 and 500Gy on the germinability, growth, biochemical content and enzyme activity of white-fleshed dragon fruit seedlings. The study reported that untreated seeds demonstrated the highest germinability, mean daily germination, survival percentage and growth rate. Lower irradiation doses (≤ 200 Gy) had less influence on physiological and growth parameters, while levels ≥ 400 Gy significantly reduced germination and development; however, irradiation at 302 Gy was identified as the lethal dose (LD_{50}). The maximum total chlorophyll, protein and nutrient attributes were observed in untreated seedlings and moderately increased to 200 Gy, but the concentration declined sharply at higher doses. The study revealed that biochemical markers associated with abiotic stress tolerance traits likes, proline, phenol, flavonoid and antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase) were significantly higher at the 200 Gy irradiation level. The findings suggested that gamma irradiation at 200 Gy dose may be ideal for inducing mutagenesis in dragon fruit, promoting abiotic stress tolerance with minimal adverse impact on seedling growth traits and development of abiotic stress tolerant mutant lines.

Keywords: biochemical; dragon fruit; enzyme activity; gamma rays; germination; growth; mutation; nutrient content

Introduction

Dragon fruit (*Selenicereus spp.*) is an herbaceous perennial climbing tropical fruit belonging to the family Cactaceae, native to southern Mexico (1). In recent years, this fruit has gained significant attention from both growers and consumers owing to its rich nutritional profile, including vitamins, minerals, pigments, dietary fibres and antioxidants and its promising economic value (2, 3). The distinctive red colour of the fruit pulp is attributed to the presence of vitamins, which exhibit potent antioxidant properties, regulate diabetes, cholesterol and high blood sugar levels and even have cancer-preventive potential (4, 5). Despite its benefits, dragon fruit is commonly propagated through stem cuttings, resulting in a narrow genetic base and low genetic variation. This lack of diversity challenges the development of new cultivars through traditional clone selection methods (6). Additionally, white-fleshed dragon fruit has been observed to perform poorly in acidic soils of the eastern tropical regions, further emphasizing the need for genetic improvement.

Mutation breeding is a promising approach for overcoming these limitations. It induces heritable changes,

creating genetic variability essential for crop improvement (7). The spontaneous mutations induce at a very low frequency (10^{-4} to 10^{-6}) and require a long time to manifest. Induced mutations accelerate this process, enhancing genetic diversity and improving crop traits within a shorter period (8, 9). Physical mutagens agents such as gamma rays, X-rays and UV rays, as well as chemical mutagens like EMS (ethyl methanesulfonate), MMS (methyl methanesulfonate) and DES (diethyl sulfate), have been widely used in fruit crop improvement (10). Among these, gamma rays have significantly affected plant morphology and physiology by interacting with cellular molecules to produce free radicals (11, 12). However, highest doses of gamma irradiation caused cellular damage, leading to stress tolerance and inhibition of physiological traits (13, 14).

Earlier studies have reported the efficacy of both chemical and physical mutagens in improving dragon fruit traits (15, 16). Specifically, investigations (15) revealed a higher frequency of variation in dragon fruit treated with EMS (3.71 %-3.95 %) and gamma irradiation (38.5-42.4 Gy). These treatments induced physiological and biochemical changes, with lower EMS concentrations exhibiting stimulatory effects, while LD_{50} doses showed inhibitory impacts on physiochemical traits (16). However, limited studies have explored the effects of gamma irradiation on dragon fruit in the Indian subcontinent. The present study aims to evaluate gamma irradiation's influence on the physiological, biochemical and enzyme activity traits of dragon fruit in the M_1 generation. This research seeks to provide insights into the potential of gamma rays as a mutagenic tool for improving dragon fruit cultivation in diverse agroclimatic conditions.

Material and Methods

The study was conducted at the Central Horticulture Experiment Station, ICAR-IIHR, Bhubaneswar, India, at an elevation of 45 m above mean sea level, with geographical coordinates of 20°27' N latitude and 85°40′ E longitude. The research was conducted during 2022 and 2023. The site falls within the eastern coastal region of India, which experiences a tropical climate. In this study, seeds of white-fleshed dragon fruit (*Selenicereus undatus*) underwent gamma irradiation using a Gamma Chamber 5000 at the Quality Control Laboratory of Professor Jai Shankar Telangana State Agricultural University, Hyderabad, India.

Six levels of gamma irradiation were applied: T_1: Control (0 Gy), T_2: 100 Gy, T_3: 200 Gy, T_4: 300 Gy, T_5: 400 Gy and T_6: 500 Gy. Each treatment was replicated three times. A total of 300 dried irradiated seeds per replication were placed in Petri dishes on wet filter paper and kept in a seed germinator at 25 °C and 60 % relative humidity.

Germination Characteristics

Seed germination was recorded at 24 hr intervals for 15 days. The germination percentages were calculated using the equation: (NS/TS) X 100. Where, NS= Number of germinated seeds. Mean Germination Time (MGT) is used in seed germination studies to assess the average time it takes for a group of seeds to germinate under specific conditions. The formula calculated the mean germination time: (n1 x d1 + n2 x d2 + n3 x d3 -----n)/ Total number of days. Where, n= number of germinated seeds, d = number of days (17, 18). The mean daily germination (days) measure provides information about the germination speed and can be useful for understanding the dynamics of seed germination over time (19). The survival rate measures the percentage of plants that remain alive after a particular period or under specific conditions. The survival percentage was recorded after 30 days of germination of the seedlings by dividing the number of surviving plants by the total number of plants at the beginning.

Growth characteristics

The growth rate represents the rate at which a specific trait or measurement increases over time, typically expressed in milligrams per day. This concept is widely used in mutagenized dragon fruit seedlings to quantify changes in particular characteristics over a given period. It is calculated using the formula: $(M_2-M_1) / (T2-T1)$, where M_1 and M_2 denote the initial and final seedling mass, respectively and T_1 and T_2 represent the corresponding time points. Additionally, the shoot-root ratio in plants refers to the proportion of biomass (fresh weight) allocated to the shoot compared to the root in seedlings

Biochemical characteristics

The acetone extraction method determined the chlorophyll and carotenoid content (mg g^{-1}) in newly growing seedlings (20, 21). For this analysis, 0.1 g of the shoot sample was homogenized in 10 mL of 80 % acetone, followed by centrifugation at 5000 rpm for 15-20 min until the pellet became colourless, ensuring all pigments were extracted into the supernatant. The optical density (OD) of the supernatant was measured using a spectrophotometer (Eppendorf, Bio Spectrophotometer Fluorescence) at specific wavelengths: OD_{663} for chlorophyll a, OD_{645} for chlorophyll b and OD_{470} for carotenoids. The total chlorophyll and carotenoid content were calculated using the Equation 1-2 formulas (20, 21):

$$\text{Total chlorophyll (mg/g)} = (8.05\ OD_{663} + 20.29 \times OD_{645}) \times \frac{V}{1000 \times W} \quad \text{(Eqn. 1)}$$

$$\text{Carotenoid} = [(1000 \times OD_{470}) - (3.27 \times \text{Chla}) - 104 \times \text{Chl b})] \times \frac{V}{229 \times 1000\ W} \quad \text{(Eqn. 2)}$$

Where V represents the volume of acetone (mL) and W denotes the sample weight (g).

The sample's total protein content (g per 100 g) was estimated using the Bradford assay (22). For this analysis, 0.1 mL of clarified shoot juice was transferred into a clean test tube, followed by adding 5 mL of Bradford reagent. The mixture was then incubated in the dark at room temperature for 20 min. The absorbance was recorded at 595 nm using a spectrophotometer, with a calibration curve prepared using Bovine Serum Albumin (BSA) as the standard. The total flavonoid content was determined

following the method (23). For this, 5 g of seedling shoot sample was finely ground and homogenized using 80 % methanol in a mortar and pestle. A reagent blank was prepared using distilled water. For flavonoid estimation, 1.0 mL of extract was transferred into each test tube, followed by the sequential addition of 0.3 mL of 5 % $NaNO_2$, 10 % $AlCl_3$ and 3.4 mL of NaOH. The mixture was allowed to stand at room temperature for approximately 10 min. Absorbance was recorded at 510 nm against the blank and expressed in mg quercetin equivalent (QE) 100 g^{-1} fresh weight.

Phenol content analysis was performed following the modified method (24). Fresh dragon fruit shoot samples (5 g) were finely ground and homogenized using 10 times volume of 80 % methanol) in a mortar and pestle. A reagent blank was prepared with distilled water. 0.2 mL of Folin-Ciocalteu phenol reagent was added and shaken to the homogenized mixture. After 2 min, 1 mL of 20 % Na_2CO_3 solution was added to each tube and heated to dryness on a water bath for 30 min. The absorbance was measured at 700 nm using a UV-Vis spectrophotometer against the reagent blank. The total phenol content was expressed as mg gallic acid equivalents (GAE).

Proline content (µg g^1 FW) was estimated using a rapid colorimetric method (25). A fresh shoot sample (0.5 g) was homogenized in 5 mL of 3 % sulphosalicylic acid and centrifuged at 7826 x g for 10 min at 4 °C. The reaction mixture consisted of 1 mL of the supernatant, 5 mL of acid ninhydrin reagent and 5 mL of glacial acetic acid. The mixture was then heated at 100 °C for 1 hr. Following incubation, 4 mL of toluene was added and vortexed and the absorbance of the chromophore-containing toluene layer was measured at 520 nm using a spectrophotometer.

Nutrient content in seedlings

Di-Acid digestion

Weighed 0.5 g of samples. The sample was allowed for pre-digestion by adding 5 mL concentrated HNO_3 to it and kept overnight. The pre- digested samples were taken for digestion by adding 5 mL of di-acid mixture HNO3:HClO4 =3:2 and kept it on hot plate at and the contents were converted to a gel-like substances. Few mL of double distilled water was added to it. The contents were then filtered with Whatman No. 42 filter paper and transferred to a 50 mL volumetric flask, the volume of which was made up to 50 mL with double distilled water. The aliquot was used for analysis of total content of different elements as P, K, Ca and Mg by standard methods (26).

The nitrogen content in dragon fruit seedlings was analyzed using modified micro-Kjeldahl method (26). For this procedure, 0.5 g of dried seedling sample was digested in micro-Kjeldahl flask using salt mixture (1:10 ratio of K_2SO_4:CuSO4.5H_2O with metallic selenium) and 10 mL of concentrated sulfuric acid. The digested sample was then distilled in micro-Kjeldahl distillation apparatus, where 35 mL of 40 % NaOH solution was added and the mixture was distilled into 2 % boric acid containing a mixed indicator. The ammonia in distillate was absorbed in boric acid and titrated with standard 0.1 N H_2SO_4 to calculate nitrogen percentage. Phosphorus content was determined using the di-acid digestion method, followed by molybdate phosphoric acid yellow colour method. The absorbance was measured at 440 nm using a spectrophotometer, as outlined (26) and the results were expressed as a percentage of phosphorus. Potassium content in plant samples was estimated using the flame emission photometry method (26). Before analysis, the samples were digested in a di-acid digestion solution to ensure proper extraction of potassium.

Calcium content was analyzed using an atomic absorption spectrophotometer with a nitrous oxide acetylene flame. The diacid-digested sample was appropriately diluted and calcium concentration was measured at a wavelength of 422 nm. The calcium content was then calculated on dry weight basis by following the method (26). Magnesium content was determined using an atomic absorption spectrophotometer with an oxidizing air-acetylene flame. The diacid-digested sample was suitably diluted and the concentration was measured at 202.6 nm. The final magnesium percentage was calculated on dry weight after applying the appropriate dilution factor, as outlined (26). Zinc and iron content in plant samples were analyzed using an atomic absorption spectrophotometer (27). The results were expressed in parts per million (ppm).

Enzymatic activity

Preparation of enzyme extract

Preparation of enzyme extract mutagenized dragon fruit seedling was collected fresh from each treatment in ice box to prevent the proteolytic activity. One gram of cleaned sample was homogenized in pre-chilled mortar and pestle at 4 °C in 5 mL of chilled phosphate buffer (50 mM; pH 7.0). The homogenate was centrifuged at 14000 × g for 20 min at 4 °C. The supernatant so obtained was filtrated through two layers of muslin cloth and stored at -20 °C to be used as an extract to estimate the following antioxidant enzymes.

The activity of superoxide dismutase (SOD) was assessed based on its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) following the method (28). The reaction mixture (3 mL) contained 0.2 mL of 0.2 M Methionine, 0.1 mL of 3 mM ethylenediamine tetraacetic acid (EDTA), 0.1 mL of 1.5 M Na_2CO_3, 0.1 mL of 2.2 mM NBT, 0.1 mL of 2 µM riboflavin, 0.1 mL of enzyme extract and 1.5 mL of 100 mM phosphate buffer (pH 7.8). Riboflavin was added last and the tubes were thoroughly mixed before being placed 30 cm beneath a light bank with two 15 W fluorescent lamps for 15 min. The absorbance of the reaction mixture was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme extract required to reduce the absorbance by 50 % compared to tubes without enzyme.

Ascorbate peroxidase (APX) activity was determined by spectrophotometer (29). The assay was conducted in 1 mL cuvette containing 250 µM ascorbate, 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1.5 mM H_2O2 and 100 µL of enzyme extract. The decline in absorbance at 290 nm, caused by ascorbate oxidation, was recorded and enzyme activity was expressed as µmol of ascorbate consumed per minute per gram of protein.

Catalase (CAT) activity was measured based on the reduction in absorbance of H_2O_2 at 240 nm, following the method (30). The 3 mL reaction prepared by mixture 1.5 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.5 mL of 75 mM H_2O_2, 5 µL of enzyme extract and water to adjust the final volume. Absorbance was recorded for 1 minute and CAT activity was expressed as µmol of H_2O_2 decomposed per minute per gram of fresh weight.

Statistical analysis

The experiment design was in the form of a completely randomized design with three replications. The statistical analysis system significant of the differences between the mean values of various traits determined using of statistical XLSTATE software and OPSTAT whereas means were compared using Tukeys' HSD test ($P<0.05$). Pearson correlation coefficients and principal component analysis (PCA) were calculated variance of germination growth, biochemical attributes and increase the interpretability of the relationship between the variables and the observations.

Results and Discussion

Morphological parameters

Germination, mean daily germination (MDG), mean germination time (MGT) and survival rate

The germination percentage, mean daily germination (% / day) and Mean germination time (days) were significantly influenced by different doses of g- irradiation. The maximum germination percentage and MDG were exhibited at T_1- Control (86.28 and 16.63) and the minimum MGT (3.13) in mutagenized dragon fruit seedlings. The lower MGT value indicates faster and more uniform germination, while a higher MGT value suggests slower and less uniform germination. The survival percentage collinearly decreases dose-dependently and the maximum survival rate is recorded at T_1- Control (85.83) followed by T_2- 100 Gy (81.53). All treated seedlings with control, the minimum mean value of survival rate was observed T_5-500 Gy. Similarly, results were recorded by various scientists and researchers in Cowpea, dianthus and pea (31-33). The inhibition of germination at higher doses of mutagen due to damage to chromosomes and subsequent mitotic retardation and similarly found in carrot tissue and Ashwagandha (33, 34).

Determination of LD_{50} of γ - irradiation in dragon fruit seedlings

This study primarily focused on determining the optimum dose of g- radiation to induce mutations in dragon fruit. The LD_{50} for gamma irradiation was estimated using a linear regression equation model based on radiation doses: Y=89.78x-0.1319xR^2 = 0.9726 (Fig. 1). The results indicated that the lethal dose (LD_{50}) for dragon fruit seed germination was 302 Gy (Fig. 1). A similar result was observed in research in which increasing gamma-ray doses induced lethal doses (LD_{50}) in papaya at 120 Gy. The LD_{50} dose of gamma irradiation has also been predicted in various fruit crops, including grapes, papaya and strawberries (37- 40).

Growth rate

Growth rate slowly increased in a mutagen-dose-dependent manner. The highest plant biomass and growth rate was exhibited at control (17.54 mm/week). However, the minimum value was found to be T_6-500 Gy (5.15 mm/week), which significantly reduced higher doses of mutagen. Similarly, a result was reported by many scientists when lower doses of mutagen increased the plant biomass or growth rate due to changing the hormonal signalling network in plant tissue and increasing the antioxidant capacity in cells, reducing stress and increasing growth in papaya (11, 12). However, higher doses of gamma irradiation significantly reduced plant biomass or growth rate due to cellular damage, thus leading to auxin degradation and a similar result was reported in lemon (41, 42).

Shoot &root percent and shoot and root ratio

The effect of different doses of gamma irradiation on shoot %, root % and shoot/root ratio was significantly influenced by various doses of gamma irradiation. The shoot % and

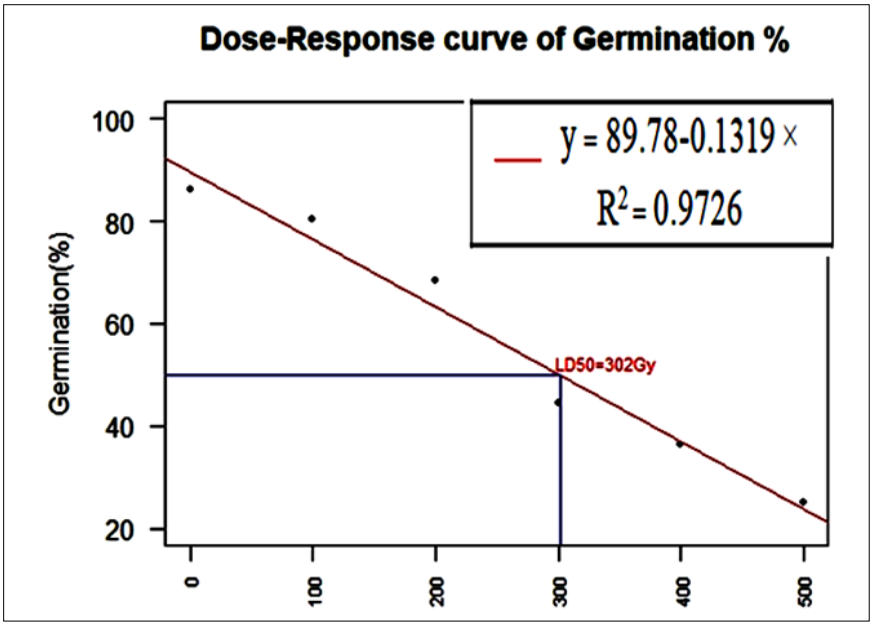

Fig. 1. Determination of LD_{50} of γ - irradiation in germination dragon fruit.

shoot/root ratio gradually increased according to doses up to 200 Gy gamma irradiation. However, the maximum shoot (93.43 %) and shoot/root ratio (15.85) were recorded at T_3:100 Gy and 200 Gy (T_3), while the maximum root (17.61 %) was found at 600 Gy. The results were indicated when higher doses of 500 Gy of gamma irradiation caused apparent depression of shoot % and shoot/root ratio, further increasing root percent, respectively, compared to the control. The results showed lower doses of gamma rays in untreated wheat with no significant variation; however, similar results were found in plants (12). Similarly, in higher radiation doses, shoot/root and shoot percentages were reduced, while in lower doses, i.e., 0.1 k Gy, they were not significantly different from the control in lentils (43).

Bio-chemical content

Total chlorophyll and carotenoids content

Different doses of gamma irradiation significantly influenced the total chlorophyll and carotenoid contents. The total chlorophyll and carotenoids decreased with increasing doses of gamma rays. Higher doses of gamma irradiation reduced the content of photosynthetic pigments. The maximum content of total chlorophyll (0.549 mg/g) and carotenoids (0.065 mg/g) was observed in the control treatment (T_1), while the minimum contents were found at T_6 (500 Gy), with values of 0.317 mg/g and 0.033 mg/g, respectively. The chlorophyll content is a key indicator of photosynthetic pigments in plants. Research reported increased chlorophyll in red pepper while higher photosynthetic activity in gamma-irradiated maize (44). Research indicates that higher mutagen doses reduce chlorophyll by damaging pigments and chloroplasts, as seen in sweet orange. Carotenoids, essential for chlorophyll-binding proteins, are vital for photosynthesis. Research indicates that non-irradiated citrus plantlets had the highest chlorophyll content compared to irradiated ones (45).

Total protein

Biochemical analysis of total protein content revealed the highest value in the control (T_1) (1.71 g/100g) and the lowest (0.49 g/100g) in T_6 (500 Gy). Protein content decreased with increasing mutagen concentration, with higher doses disrupting protein synthesis (46). Similar trends were reported in gamma-irradiated *Vigna sinensis* seedlings, where lower doses led to production in protein due to increased amino acid production, while higher doses disrupted synthesis and affect cellular functions such as gas exchange and enzyme activities; comparable findings were observed in *Citrus sinensis* (45, 47).

Flavonoid content and phenol content

Flavonoid, a class of secondary metabolites, plays a critical role as an antioxidant and chelating agent in plant cells. Their antioxidant activity depends on the structure and hydroxyl group substitution patterns. Gamma irradiation significantly increased flavonoid and phenol contents, with maximum flavonoid (40.78 mg QE/100g) at T_4(300 Gy) and phenols (90.69 mg GAE/100g) at T_3 (200 Gy). The total phenol and flavonoid content correlated plant to resistance under stress conditions. However, the lowest values were recorded at the highest dose. Similar results were reported in *Gerbera jamesonii* (48). Research indicates a negative correlation between total phenol content and apricot plant growth (49) while increasing endogenous antioxidants in tamarind juice (45). These findings suggest that higher phenolic content enhances antioxidant properties, positively correlates with gamma irradiation and contributes to plant stress resistance mechanisms.

Proline

Different doses of gamma radiation increased the proline content of dragon fruit seedlings, in Table 1. The seeds treated with varying doses of gamma radiation (100, 200 300, 400 and 500 Gy) caused a significant increase in the total proline contents compared to untreated control plants. Proline content was significantly enhanced with increased doses of gamma irradiation and the maximum value was recorded in T_3:300Gy (18.21µg/g). Where the minimum was noted at T_1: Control (11.08). Proline is indicated to act as a compatible osmolyte and its increased production confirms osmo-tolerance in plants (50, 51). Similarly, a result found proline content significantly increased with doses of gamma irradiation (52).

Enzymatic activity

Table 2 illustrates the effect of gamma irradiation on the superoxide dismutase, Ascorbate peroxidase and catalase activities of dragon fruit seedlings. Superoxide dismutase, ascorbate peroxidase and catalase enzyme activity increased with increasing gamma radiation doses up to 200 Gy. The maximum superoxide dismutase (57.21 units/mg protein/ min), Ascorbate peroxidase (0.46 µmol/min/g) and catalase enzymes (4.22 µmol H_2O_2/min/g) increase was observed in 200 Gy of seedlings of dragon fruit, respectively. Beyond 200 Gy, enzyme activity declined. Higher activity of SOD, POX and GR was also reported in irradiated shoots of ber (53). The increased activity of enzymatic antioxidants is likely a result of the up regulation of genes triggered by irradiation stress. Oxygen radicals generated during plant metabolism must be scavenged for normal growth. Superoxide radicals are

Table 1. Influences of γ- irradiation on germinability and growth traits of dragon fruit

γ- irradiation	Germination (%)	Mean daily germination (%/day)	Mean germination time (days)	Survival rate (%)	Growth rate (mm/day)	Shoot percentage	Root percentage	Shoot/root ratio
T_1:Control	86.28^a	16.63^a	3.13^a	85.83^a	17.54^a	91.58^b	6.57^d	13.94^b
T_2:100 Gy	80.28^b	14.15^b	3.38^b	81.53^b	14.38^b	93.43^a	6.96^d	13.42^c
T_3:200 Gy	68.4^c	11.62^c	3.70^c	79.66^b	11.65^c	93.36^a	5.89^e	15.85^a
T_4:300 Gy	44.5^d	8.06^d	4.21^d	58.91^c	9.53^d	88.65c	10.35c	8.57^d
T_5:400 Gy	36.5^e	6.12^e	4.75^e	76.65^d	6.43^e	83.59^d	16.41^b	5.09^e
T_6:500 Gy	25.17^f	5.41$_f$	5.29$_f$	34.54^e	5.15^f	82.39^e	17.61^a	4.68^d

Table 2. Influences of γ -irradiation on biochemical attributes of dragon fruit seedlings

γ-irradiation	Total chlorophyll (mg/g)	Carotenoid (mg/g)	Protein (g 100 g^{-1})	Flavonoid (mg QE/100g)	Phenol (mg GAE/100g)	Proline (µg/g)	Superoxide dismutase (units/mg protein/min)	Ascorbate peroxidase (µmol/ min/g)	Catalase (µmol H_2O_2/ min/g)
T_1:Contro	0.549^{a}	0.065^{a}	1.71^{a}	25.68^{d}	66.41^{d}	11.08^{c}	31.83^{e}	0.20^{c}	2.29^{d}
T_2:100 Gy	0.406^{bc}	0.051^{cd}	1.65^{b}	31.64^{b}	73.44^{c}	16.54^{a}	52.93^{b}	0.42^{a}	4.33^{b}
T_3:200 Gy	0.418^{c}	0.058^{ab}	1.48^{b}	35.13^{a}	90.69^{a}	18.21^{a}	57.21^{a}	0.46^{a}	4.42^{a}
T_4:300 Gy	0.398^{cd}	0.055^{bc}	1.06^{c}	40.78a	76.48^{b}	12.31^{b}	45.86^{c}	0.35^{b}	3.87^{c}
T_5:400 Gy	0.391^{d}	0.043^{d}	0.85^{d}	38.54^{b}	70.99^{c}	11.92^{b}	39.72^{c}	0.23^{c}	3.51^{c}
T_6:500 Gy	0.317^{e}	0.033^{e}	0.49^{e}	24.86 ^{b}	67.45^{d}	11.58^{b}	36.98^{d}	0.15^{d}	3.34^{c}

neutralized by SOD, producing H_2O_2, which is further broken down primarily by ascorbate peroxidase (APX) and catalase (CAT) (54). Among antioxidant enzymes, peroxidase is crucial in detoxifying H_2O_2, protecting cellular components like proteins and lipids from oxidative damage (55). The application of ionizing radiation has been reported to increase the activity of POD, CAT and SOD in various plant species (11).

Nutrient content

Nutrient content in mutagenized dragon fruit seedlings was significant influence by irradiation levels (Table 3). The highest nutrient levels were observed under control conditions, with a slight reduction at low irradiation levels. N, P, K, Ca, Mg, Fe, Zn and Mn concentrations were relatively higher at 100 Gy and 200 Gy. However, a pronounced decline in nutrient content was observed at higher irradiation levels, with the lowest levels recorded at 500 Gy. At this dose, shoot nutrient content was approximately 50 % lower compared to 100 Gy, indicating the adverse effects of high irradiation levels on plant physiological activity. Similarly, low-dose irradiation has been reported to enhance P and K levels in potato plant (56). The observed decline in N, P and K content at higher irradiation doses may result from the suppression of essential enzymes such as phenylalanine ammonia-lyase (PAL) and polyphenol oxidase, which are vital for nitrogen assimilation and the production of secondary metabolites. As noted, this enzymatic disruption impairs plant growth and nitrogen uptake (57). Comparable patterns have been reported in other crops, including red radish (*Raphanus sativus*L.) (58), eggplant (*Solanum-melongena*) (59), wheat (*Triticum-aestivum*), chickpea (*Cicer arietinum*) and tomato (*Solanum-lycopersicum*) (60-62).

The result clearly indicated that the micronutrient content of dragon fruit seedlings such as calcium (0.79 %), magnesium (0.51 %), iron (30.45 ppm), zinc (18.02 ppm) and manganese (35.43ppm) content were estimated maximum value in untreated seedlings and the minimum content was observed in the seedlings treated with 500 Gy irradiation (Table 3). The study's finding aligns with the finding (57), where higher mutagen doses inhibited enzymatic activity and disrupted mineral assimilation. Increased calcium uptake in wheat at lower doses suggesting enhanced metabolic efficiency (60). Similarly, Enhanced magnesium content in basil at lower doses of mutagen might link to improved enzymatic functions (63). Similarly increased micronutrient uptake, including iron at moderate mutagen doses in irradiated plants attributing to improved metabolic functions and nutrient transport (64). Similar trends in zinc accumulation, emphasizing that moderate mutagen doses promote nutrient uptake by enhancing root efficiency (65).

Analysis of traits associated for influences of gamma irradiation on germinability, growth and biochemical attributes and nutrient content of dragon fruit

Pearson correlation coefficient analysis was performed on the germinability, growth, biochemical attributes, enzyme activity and nutrient content of dragon fruit seedlings (Fig. 2). The data demonstrated that germination rate was positively correlated to mean germination time at P< 0.001 % and mean daily germination, survival rate at P< 0.01 % with potassium, Iron and manganese at P < 0.5, However root percentage, mean germination time, total phenol and proline are negatively correlated of dragon fruit seedlings. Likewise, these parameters survival rate are highly positively correlated, germination %, Mean daily germination and nutrient content at P<0.01 respectively. However, bio-chemical attributes of mutagenized dragon fruit seedlings viz, total chlorophyll content was highly correlated with carotenoids, carbohydrate, protein nitrogen, phosphorus, potassium, iron and zinc are correlated, while root %, total flavonoid, total phenol proline SOD, APX and CAT content are the negative correlated and non-significant impact of all most traits. Similarly nutrient uptake viz, Nitrogen was highly positively correlated with plant biomass, growth rate, shoot %, shoot root ratio, protein content and proline at P <(0.001) However, survival rate, total chlorophyll content, phosphorus, calcium and iron content are highly demonstrated at P < 0.01 generation) to develop a suitable mutant line.

Table 3. Influences of gamma irradiation on nutrient content of dragon fruit

γ- irradiation	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)
T_1:Control	1.68^{a}	0.49^{a}	1.45^{a}	0.79^{a}	0.51a	30.45^{a}	18.02^{a}	35.43^{a}
T_2:100 Gy	1.55^{b}	0.45^{b}	1.38^{b}	0.68^{b}	0.47^{b}	27.83^{b}	15.49^{b}	28.83^{b}
T_3:200 Gy	1.47^{b}	0.42^{b}	1.25^{c}	0.62^{c}	0.42^{b}	22.63^{c}	14.58^{c}	26.63^{c}
T_4:300 Gy	1.21^{c}	0.36^{c}	1.13^{d}	0.51^{d}	0.28^{c}	18.69^{d}	13.06^{d}	19.69^{d}
T_5:400 Gy	0.81^{d}	0.29^{d}	0.88^{e}	0.18^{e}	0.23^{d}	14.59^{e}	7.54^{e}	15.59^{e}
T_6:500 Gy	0.59^{e}	0.21^{e}	0.53^{f}	0.099^{f}	0.18^{e}	12.88^{f}	6.75^{f}	12.88^{f}

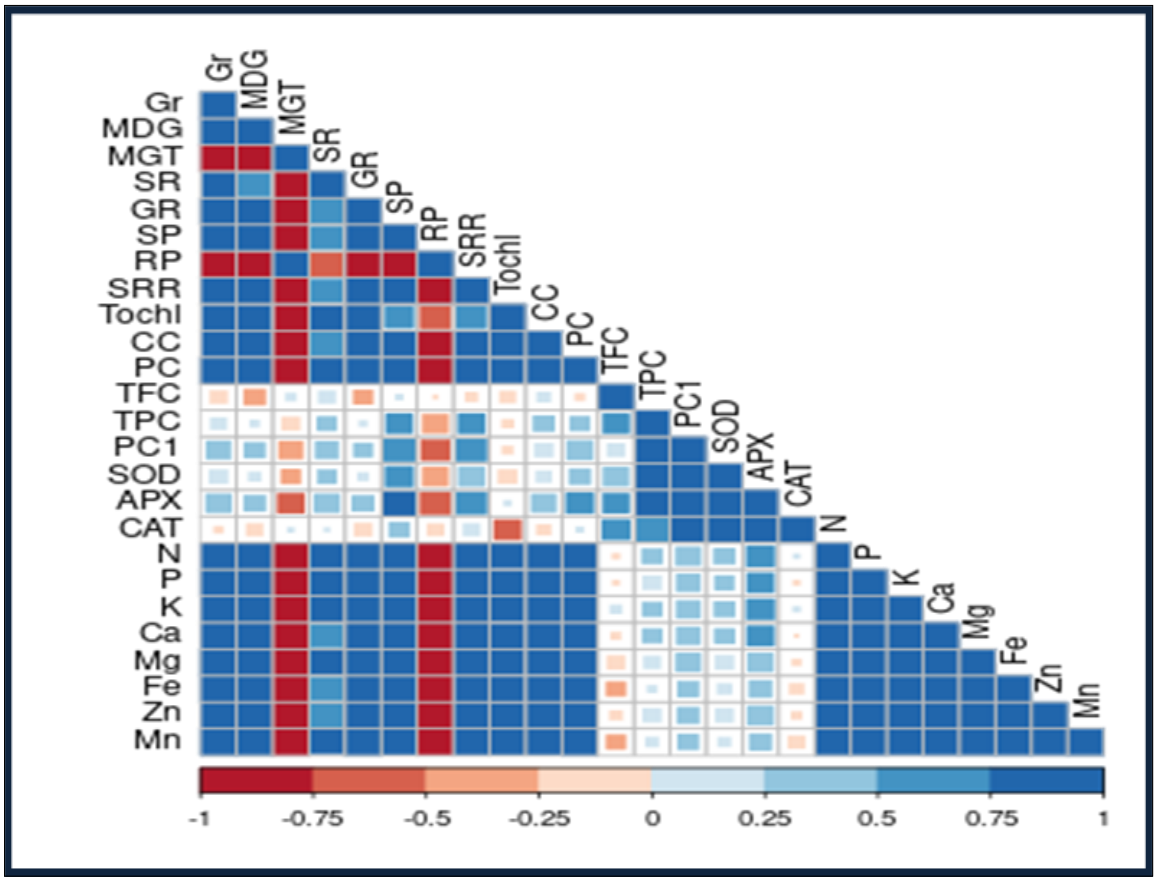

Fig. 2. Correlation matrix depicting relationship among various traits. Gr-Germination rate; MDG-Mean daily germination; MGT- Mean germination time; SR- Survival rate; GR- Growth rate; SP- Shoot percentage; RP- Root percentage; SRR- Shoot root ratio; Tochl,- total chlorophyll; CC- Carotenoid; PC- Protein content; TFC- Total flavonoid content; TPC- Total phenol content; PC1- Proline content; SOD- Superoxide dismutase; APX-Ascorbate peroxidase; CAT-Catalase N- Nitrogen; P-Phosphorus; K-Potassium; Ca-Calcium; Mg- Magnesium; Fe,- Iron; Zn,-Zinc; Mn- Manganese.

Principal component analysis (PCA) of germination, growth, biochemical attributes and enzyme activity on mutagenized seedlings of dragon fruit

Twenty-five parameters observed the variation in germination, growth rate, biochemical attributes, enzyme activity and nutrient content. From PCA biplot analysis, traits were divided into main and subgroups based on homogeneity and dissimilarity. Four sets of traits were reported which were considered in PC1 and PC2. Maximum traits were presented *viz*., mean daily germination, survival rate, growth, shoot %, shoot root ratio, carotenoids, carbohydrate, protein content, nitrogen, phosphorus, potassium, magnesium, manganese, zinc, iron were clustered under group I and total phenol content, proline, superoxide dismutase, ascorbate peroxidase and catalase under come cluster II. However total flavonoid III. Meanwhile, mean germination time and root presence were found in cluster IV.

It was observed that the PCA biplot expressed that group I and II. This significantly contributed to PC1 being highly involved with T_1 (Control) and T_3 (200Gy) wherever the traits correlated to with groups III and IV contribute more towards PC2. The length and intensity of colour of a vector in biplot expressed how well the qualities are represented and how much they share with the principal components. Group I contributed more towards PC1 followed by group II and group III. Group I, II, III and IV's parameters seem to be free from each other traits based on angles between vectors derived from the middle point of biplot (Fig. 3). That the reason Principal component analysis, it can be assumed that gamma irradiation treatment influences on the physiological and biochemical traits on dragon fruit seedling along the PC1 will be effective for enhances germination, growth rate, biochemical content and nutrient uptake as most of the factors expressed positive correlations with each other. Whereas PC2 contributes more towards mean germination time, total flavonoid and root ratio % on dragon fruit seedling.

Conclusion

Based on the findings, it can be concluded that lower doses of gamma irradiation (≤ 200 Gy) have minimal adverse effects on the germination traits, growth attributes, chlorophyll content and nutrient levels of dragon fruit seedlings. Notably, a dose of 200 Gy was effective in inducing abiotic stress tolerance by enhancing proline, phenol, flavonoid, enzyme activity and mineral content, while maintaining minimal damage to germination and growth. Conversely, higher doses of gamma irradiation (≥ 400 Gy) were harmful, significantly reducing germination, seedling growth, chlorophyll content and nutrient levels. These results highlight that 200 Gy is the optimal irradiation dose for promoting abiotic stress tolerance, creating genetic diversity and selecting stress-tolerant mutant lines for potential agricultural improvement for dragon fruit cultivation.

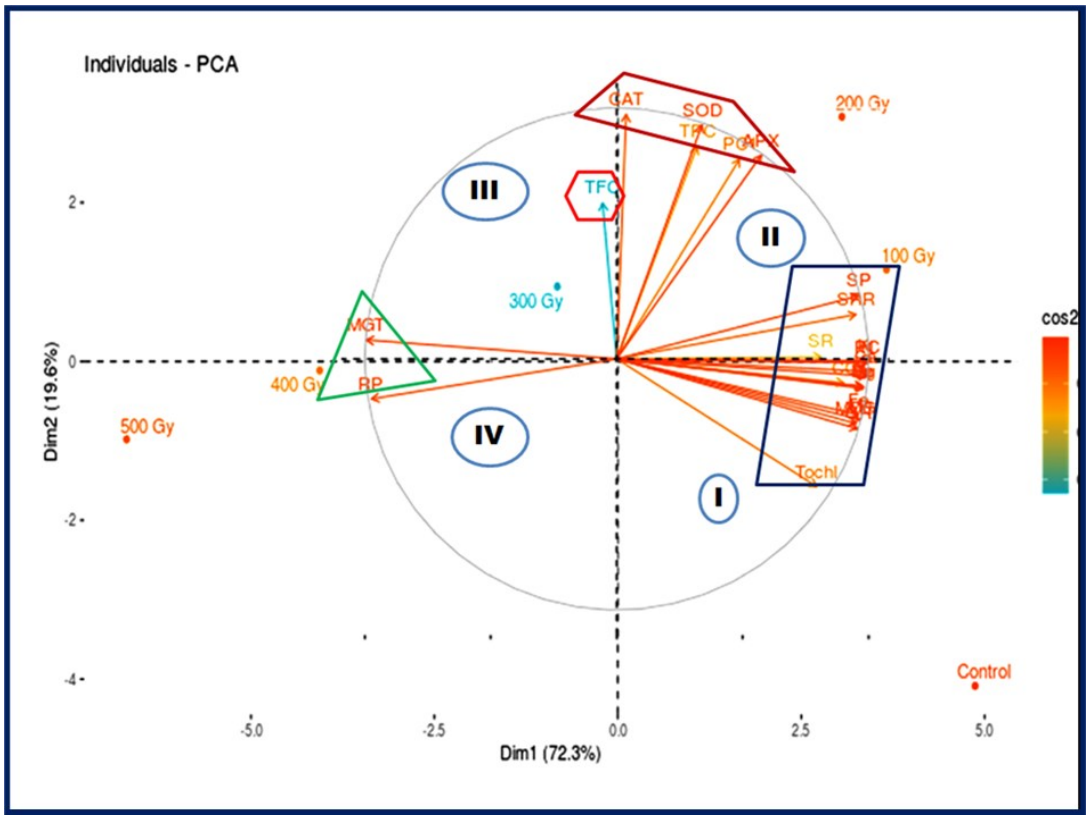

Fig. 3. Principal component analysis (PCA) of germination, growth, biochemical attributes and enzyme activity on mutagenized seedlings of dragon fruit.

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Authors' contributions

KK[1] and SCS were responsible for conceptualization, supervision and validation and participated in writing, reviewing and editing. KKS contributed to conceptualization, data curation, formal analysis, investigation, methodology, visualization, original draft, and writing review and editing. SN was involved in data curation, formal analysis, methodology, and writing - review and editing. LP contributed to data curation and methodology. CJ participated in data curation, formal analysis and investigation. PN was involved in data curation, formal analysis and writing - review and editing. DG contributed to data curation and methodology. SNS was responsible for visualization. KK[2] participated in writing, reviewing and editing. All authors read and approved the final manuscript. [KK[1] stands for Kundan Kishore and KK[2] stands for Km Kusum]

Compliance with ethical standards

Conflict of Interest: The authors declare no competing interests.

Ethical issues: None

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