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Morphological, Biochemical, Anatomical and Genetic Variation of *Zamioculcas zamiifolia* in Response to Gamma Irradiation

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ABSTRACT

This study evaluated the effects of gamma irradiation doses (0, 1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 Gy) on the survival, morphological, anatomical, biochemical and genetic characteristics of *Zamioculcas zamiifolia* over the two successive seasons (2023 and 2024). It was observed form the obtained results that most of the irradiated doses 5-20 Gy led to a high percentage of germination loss leaflets exposed to these previous doses failed to initiate rhizogenesis and showed complete mortality, except the low doses (1 and 2.5 Gy) whereas non-irradiated (control) exhibited 100% survival. Lower doses (1 and 2.5 Gy) influenced growth parameters variably. Although plant height, leaflet area, and rhizome number were generally reduced compared with the control,1 Gy enhanced leaves and root numbers enhanced chlorophyll and carotenoid content, while 2.5 Gy increased leaflet number, rhizome size, root length, and biomass accumulation. These findings indicate that high gamma doses are lethal at the initiation, whereas low doses may stimulate specific growth traits in a dose-dependent manner. From another point, the obtained results demonstrated a clear radiation hormesis effect; gamma irradiation induced a significant progressive accumulation of total phenolic compounds and total carbohydrates. Most notably, a simultaneous upregulation of both gibberellic acid (GA3) and abscisic acid (ABA) was observed with increasing doses. At 2.5Gy, total phenolic compounds reached 36.21 and 37.86 mg/g while total carbohydrates peaked at 69.29 and 70.55 mg/g in the first and the second season, respectively. GA3 and ABA surged significantly, showing a balanced synergetic co-existence rather than conventional hormonal antagonism. These findings suggest that sub-lethal gamma irradiation doses functions as a metabolic stress, triggering protective antioxidant pathways and osmoregulatory mechanisms while successfully preserving photosynthetic efficiency and growth plasticty in *Zamioculcas zamiifolia* .Transverse sections of *Zamia* leaflets exhibited distinct anatomical differences across the various irradiation treatments. Furthermore, ISSR marker detected limited genetic variation between control and gamma-irradiated Zamia plants, generating 32 bands with 12.5% polymorphism, indicating that gamma irradiation induced measurable DNA changes and potential breeding variability.

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Introduction

Zamioculcas zamiifolia L. (Lodd.) Engl. is a commercially important, export-oriented potted foliage plant widely used for indoor landscaping. Its high tolerance to environmental stress and pests reduces the need for intensive cultivation practices (**Seneviratne *et al.*, 2020**). The species exhibits strong export demand due to its ornamental value (**Tarran *et al.*, 2007; Wong, 200**9). Additionally, *Z. zamiifolia* L. enhancing its desirability as a houseplant (**Sriprapat and Thiravetyan, 2013**). The plant is primarily propagated through leaf cuttings, and well-drained, friable substrates are typically preferred for cultivation. There is growing interest in developing novel cultivars with desirable traits, including diverse leaf shapes, colors, sizes, as well as plant architectures (**Datta and Da Silva, 2006; De, 2017**). Among conventional breeding approaches, mutation breeding has proven effective for the development of novel cultivars. Gamma irradiation using Cobalt-60 is a prominent physical mutagen, as it interacts with cellular atoms and molecules to generate free radicals, thereby inducing modifications in plant morphology, anatomy, biochemistry, and physiology depending on the applied dose (**Wi *et al.*, 2007**). Approximately 64% of radiation-induced mutant varieties have been developed using gamma rays, compared with 22% using X-rays **(IAEA, 2015)**. Gamma rays, as a high-energy form of electromagnetic radiation, are widely utilized due to their ease of application, good tissue penetration, reproducibility, and high mutation frequency (**Chahal and Gosal, 2002**). Their short wavelength and high penetrative power allow interaction with cellular atoms and molecules to produce free radicals, making them the most effective physical mutagen for inducing mutations in crop plants (**Jan *et al.*, 2011**).

Physical mutagens, particularly gamma radiation, have been successfully applied in the improvement of ornamental plants, including Matthiola, Dimorphotheca, Dianthus (**Khattab and Hegazi, 2015)**, Philodendron (**El-Khateeb *et al.*, 2016**), Zinnia (**Pallavi *et al.*, 2017**), Borgo (**Fayed, 2023**), and *Z. zamiifolia* (**Gouda *et al.*, 2023**). This study was conducted to investigate the effects of gamma irradiation on mature leaflets, focusing on the anatomical, physiological and genetic changes in *Zamioculcas zamiifolia* L.

Materials and Methods

This study was carried out during the 2023 and 2024 seasons within the greenhouse located at the Experimental Garden of the Horticulture Research Institute, Agricultural Research Center; Giza, Egypt. The irradiation process was carried out at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt.

Plant Material

Stock *Zamioculcas zamiifolia* (ZZ plant) pots were obtained from a private indoor plant nursery. Botanical Gardens Research Department Horticultural Research Institute, Agricultural Research Center, Giza, Egypt. The selected plants exhibited uniform growth and were at comparable developmental stages, as determined by their size, age, and number of leaves.

Soil Analysis

The physical and chemical properties of the soil used in the study were determined and were as follows: Coarse sand (65%); Fine sand (24.1%); Silt (6.3%); Clay (4.6%); O.M. (95%); EC (1.66 dS.m-1); pH (5.9); HCO3- (1.93 meq.l-1); Cl- (23 meq.l-1); Ca+ (3.6 meq.l-1); Mg+ (3.2 meq.l-1); Na+ (15 meq.l-1); K+ (5.7 meq.l-1).

Irradiation process and growth conditions

The mature basal leaflets were excised from the leaves in early March, randomly mixed and divided into groups each group contained five leaflets exposed to different doses of gamma radiation (0,1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 Gy) in the 2023 seasons at dose rates of 0.66815 Gy/h and 0.58588 Gy/ h, using the Indian gamma cell that uses 60Co as a radiation source at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. Since all leaflets exposed to radiation doses of 5-20 Gy failed to survive, the experiment was repeated during the subsequent season (2024) to verify the observed results. The irradiated leaflets and the control group (non-irradiated) were planted in plastic pots and covered with a transparent plastic sheet to maintain humidity. Plants were regularly irrigated during the initial growth stage in both experimental seasons. After approximately two months, when rhizome initiation was observed, the plants were transplanted into 20 cm pots containing a mixture of peat moss and sand 2:1 v/v). Five pots were assigned to each treatment and each pot contained one leaflet. Standard horticultural practices were applied, including monthly fertilization with a commercial NPK fertilizer (Krista, 19–19–19) at a rate of 2 g per pot. After six months, the following parameters were recorded.

Vegetative growth parameters

Survival percentage, Plant height (cm), number of leaves per plant, number of leaflets per leaves, leaflet area (cm^2) of the recently fully expanded mature leaf (the fourth leaf from the plant top) of five plants from each treatment was calculated as a relation between unit area and leaf weight using the following equation of **Koller (1972).**

$$\text{Leaflet area (cm2)} = \frac{\text{Disk area} \times \text{No. Disks} \times \text{Leaflet FW}}{\text{Disk FW}}$$

Number of rhizomes per plant, rhizome diameter (mm), root length (cm), aboveground (leaves with leaflets) and

fresh and dry weights (g) underground (rhizome + roots) fresh and dry weights (g).

Chemical investigations

a. Photosynthetic pigments

Fresh leaves were extracted with dimethylformamide solution [HCON (CH3)2] and placed overnight at a cool temperature (5°C). Chlorophyll a, b and carotenoids were measured by a Shimadzu UV-120-02 spectrophotometer at wavelengths 663, 647 and 470 nm, respectively. Chlorophyll and carotenoids were calculated according to the equation described by **Moran (1982).**

Chlorophyll a =12.70 A663 – 2.79 A647

Chlorophyll b =20.76 A647 – 4.62 A663

Total Chlorophyll =17.90 A647 + 8.08 A663

Total Carotenoids = [1000 A470 – (3.72 Chla -104 Chlb)] /229

b. Total carbohydrates.

Total carbohydrate content was determined according to the official methods of **A.O.A.C. (2000).**

c. Total soluble phenols

Total soluble phenols were determined by a colourimetric method using Folin-Denis reagent according to **Swain and Hillis (1959).**

d. Phytohormones

Gibberellin and abscisic acid were determined in young leaves of Zamia plants at 60 days after planting in the seasons of 2023 and 2024. The method of extraction was essentially similar to that adopted by **Shindy and Smith (1975)** and described by **Hashem (2006)**, while the determination was according to **Vogel (1975).** HPLC Agilent 1200 series equipped with Quaternary pump, Auto sampler, column compartment test at 35 C°, multi-wavelength detector set at 330 nm, 280 nm for detection of Gibbrellin and abscisic acid.

Anti-oxidant enzymes activity 2.4

Enzymes assays

The activities of antioxidant enzymes Superoxide dismutase, Catalase and Peroxidase. were assayed in fresh leaf extracts following the standard agricultural protocols described by **DaCosta and Huang (2007)** based on the original methods of **Giannopolitis and Ries (1977)** and **Chance and Maehly(1955)**.

Anatomical study

Samples were dehydrated, infiltrated, and embedded through a tertiary butyl alcohol paraffin- wax series according to the procedure of **Johannsen (1940)**. Sections were cut 10 µm thick with a rotary microtome and stained with hematoxylin and then enclosed with Kandapalsm.

Molecular genetic identification

A. DNA extraction

DNA extraction from leaves of the three Zamia treatments (control, 1 and 2.5 Gy) was performed using DNeasy Plant Mini Kit (QIAGEN). The amplification reactions were performed in a volume of 12.5 µl, which contained 6.25 µL 2 × Green Taq Mix (Vazyme, Nanjing, China), 0.5 µL of each primer (0.4 µM), 5 µL of template genomic DNA (4 ng/µL), and 0.75 µL of distilled deionized water. PCRs were conducted in a DNA thermocycler (Multigene gradient, Labnet, NJ, USA).

B. Inter Simple Sequence Repeats (ISSR)

The PCR program consisted of an initial denaturation at 94 °C for 5 min, followed by 40 cycles according to the following procedure: denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 1 min; the last cycle was followed by a final extension for 7 min at 72 °C. PCR products were separated by electrophoresis on 1.5% agarose gel submerged in 0.5 × TBE buffer and stained with 1 µg/µl of ethidium bromide (**Soliman et al., 2017**). The DNAs were visualized under UV light using the Gel Doc system (EnduroTM GDS, Labnet). The fragment size was estimated by using a DNA marker (100 bp plus II DNA ladder, TransGen Biotech Co., Ltd). DNA bands were treated as dominant markers and were scored in terms of a binary code as presence (1) or absence (0).

Table 1. List of the ISSRs primers codes and their nucleotide sequences.

Primer no.	Primer sequences (5' ⟶ 3')
UBC 811	(GA)8 C
UBC 807	(AG)8T
17898A	(CA)6AC
HB-10	(GA)6 CC
HB-12	(CAC)3 GC

C. Data analysis

The similarity matrices were done using Gelworks ID for advanced software, the UVP-England Program. The relationships among genotypes revealed by dendrograms were done using the SPSS Windows (Version 22) program. DICE computer package **Dice, 1945** was used to calculate the pairwise difference matrix and plot the phenogram among cultivars (**Yang and Quiros, 1993**).

Experiment layout and statistical analysis:

The experiment was arranged in a completely randomized design (CRD) with ten treatments (nine irradiation doses + control), each treatment contained five replicates (one leaflet per replicate). The experiment was conducted over two successive seasons using the same experimental design and treatment structure. Following planting, seven treatments resulted in complete mortality of plant material, and consequently growth measurements could be obtained for these treatments. Therefore, growth parameters were recorded and statistically analyzed only for the three surviving treatments. The collected data from each season were analyzed separately using the appropriate analysis of variance (ANOVA) model with the MSTAT computer program (MSTAT Development Team, 1989). Treatment means were compared using the least significant difference (LSD) test, following the procedure described by **Gomez and Gomez (1984)**, to determine statistically significant differences among treatments.

Results and Discussion

1. Vegetative growth parameters:

After one month of culture, all leaflets exposed to gamma-irradiation doses of 5-20 Gy failed to initiate rhizogenesis and subsequently died. In contrast, all non-irradiated control leaflets exhibited 100% survivability.

The present study demonstrated that gamma rays influence certain vegetative growth parameters of the ZZ plant across the two seasons. Data presented in Table 2 indicate that the number of leaves during the first season was not significantly different because of gamma irradiation exposure. Plants recorded values of 3.0, 3.4, and 3.6, when exposed to 0, 1 and 2.5 Gy, respectively. However, in the second season, the highest vegetative growth parameter recorded was 6.60 at a dosage of 1Gy.this result agrees with that obtained by **Moustafa *et al.* (2018)** who found that low doses of gamma irradiation increase leaf number of gladiolus plant.

In the second season, the highest number of leaflets was recorded at a gamma irradiation dose of 2.5 Gy, reaching 25.42 leaflets. In contrast, the effect of gamma irradiation on leaflet numbers was not statistically significant during the first season. These results agree with those obtained by **Datta (1997)** on Lantana, **Gonzales (2007)** on Gladiolus, and **El-Khateeb et al. (2007)** on Melissa; they found that number of leaves was significantly decreased with increasing gamma doses.

Regarding leaflet area, the control treatment produced the highest values in both seasons (15.62 and 14.88 cm^2, respectively). Among the irradiated treatments, the 1 Gy dose resulted in larger leaflet areas (13.69 and 13.30 cm^2 in the first and the second seasons, respectively) compared with the 2.5 Gy treatment (11.00 and 11.66 cm^2, respectively). The same result was obtained by **Moustafa et al. (2018)** who found that low doses of gamma irradiation increase leaf area of gladiolus plant.

Similarly, the effects of gamma irradiation on rhizome numbers and rhizome size were not statistically significant during the first season. The recorded rhizome numbers were 3.6, 2.8, and 2.8 for the control, 1 Gy, and 2.5 Gy treatments, respectively, whereas rhizome sizes were 1.20, 1.66, and 1.16 cm, respectively. During the second season, the 2.5 Gy treatment produced the highest rhizome size (1.95 cm), while the mean rhizome number was 2.54. this result is on the same line of **Moustafa et al. (2018)** who found that number of florets/spike and corm size as well as corm number per plant increased with low doses of gamma irradiation

As for root number, data revealed that root number scored the lowest value at 2.5 Gy in both seasons.as for root length, it was observed that there was no significant difference between different treatments in the first season, whereas in the second one 2.5 Gy scored the highest value. As for aerial part fresh weight, aerial part dry weight, root fresh weight and root dry weight, data revealed that 2.5 Gy scored the highest values in both seasons.

Gamma irradiation negatively influenced plant height, Leaflet area, and the quantity of rhizomes across both seasons. The control treatment exhibited the highest values of plant height, Leaflet area, and the quantity of rhizomes across both two seasons. Similarr findings were obtained by **Datta (1997)** on Lantana depressa. **Gonzales (2007)** on Orchids found that plant height decreased with doses increasing. **Suraninpong and Wuthisuthimethavee (2013)** on Anthurium, **Patil (2014)** and **Upadhyay (2014)** on gladiolus, found that plant growth decreased with the increasing gamma doses.

Table 2. Effect of Gamma irradiation on growth parameters of Zamioculcas zamiifolia plant.

Treatments	Leaves No.	Plant height (cm)	Leaflet No.	Leaflet area (cm^2)	Rhizome Number	Rhizome diameter (cm)	Root Number	Root Length	Aerial part F.W	Aerial part D.W	Root F.W	Root D.W
						1st Season						
Control	3.0 ±1	5.10±0.65^{a}	2.86±0.49	15.62±1.11^{a}	3.6±0.55	1.20±0.19	13.4±1.67^{a}	11.34±0.9	21.82±1.19^{c}	2.02±0.14^{b}	31.74±1.41^{b}	1.54±0.14^{b}
1Gy	3.4±1.14	3.50±0.47^{b}	2.40±0.89	13.69±1.25^{b}	2.8±0.45	1.66±0.44	13.8±2.39^{a}	10.34±1.0	26.82±2.56^{b}	1.35±0.06^{c}	23.76±1.2^{c}	1.24±0.09^{c}
2.5 Gy	3.6±0.89	3.96 ±29^{b}	2.00±0	11.00±1.43^{c}	2.8±0.84	1.12±0.21	10.4±0.89^{b}	12.24±2.8	36.00±2.19^{a}	2.60±0.09^{a}	34.54±1.45^{a}	1.69±0.08^{a}
LSD	NS	0.59	NS	1.58	NS	NS	2.86	NS	1.81	0.12	1.33	0.08
						2nd Season						
Control	5.4±1.14^{b}	10.42±1.11	19.40±2.07^{c}	14.88±0.83^{a}	2.54±0.54^{a}	1.65±0.54^{b}	11.88±1.95^{b}	12.60±1.55^{c}	22.24±1.57^{c}	2.02±0.11^{b}	31.25±1.98^{b}	1.57±0.09^{b}
1Gy	6.6 ±1.14^{a}	9.8±1.13	21.52±1.58^{b}	13.30±1.5^{b}	2.14±0.44^{a}	1.45±0.41^{b}	13.60±1.3^{a}	13.44±1.02^{b}	25.4±1.86^{b}	1.33±0.09^{c}	23.62±0.0.84^{c}	1.25±0.09^{c}
2.5 Gy	5.0±1.0^{b}	10.34±2.05	25.42±2.07^{a}	11.66±1.4^{c}	1.60±0.44^{b}	1.95±0.23^{a}	11.80±1.14^{b}	14.52±1.36^{a}	36.42±1.51^{a}	2.56±0.22^{a}	33.97±1.06^{a}	1.75±0.11^{a}
LSD	0.90	NS	1.66	1.17	0.47	0.20	1.49	0.42	1.65	0.08	1.45	0.17

- Means± S.D in each column followed by the same letter(s) are not significantly different from each other at 5% level
- NS: not significant

2. Chemical constituents.

2.1. Photosynthetic pigments

Regarding the response of photosynthetic pigment to gamma irradiation, the data presented in **Table 3** clearly indicate a progressive and significant enhancement in **chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids** with increasing low doses of gamma radiation. The highest significant values for all investigated pigments were consistently achieved under the 2.5 Gy treatment across both vegetative growth seasons, followed by the 1Gy treatment, whereas the control plants exhibited the lowest pigment concentrations. Same results were obtained by **Lal et al. (2024)** who found that low doses of gamma irradiation scored the highest value of chlorophyll a, b, total chlorophyll and carotenoids of chrysanthemum. Leaf color variation may be due to its effect on inducing a beneficial mutation which led to a variation in cell structure and vital process like expansion the effect of low doses of gamma ray on increasing the concentration of photosynthetic pigments might be related to its effect on inducing a beneficial mutation which led to a variation in cell structure and vital process like expansion of thylakoids membranes and amelioration of photosynthesis process which reflect on pigment bulk that adjust the color of plant leaves (**Gaafar et al. 2016**).

Table 3. Effect of Gamma irradiation on photosynthetic pigments concentration (mg/g F.W) of Zamioculcas zamiifolia L leaves during two seasons.

Chemical characters / Treatments	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Total Carotenoid	
	2023	2024	2023	2024	2023	2024	2023	2024
Control	5.32 c	4.67 c	3.06 c	2.41 c	8.38 c	7.08 c	4.50 c	4.81 c
1Gy	6.23 b	5.17 b	3.93 b	3.35 b	10.16 b	8.52 b	5.04 b	4.97 b
2.5Gy	6.73 a	5.21 a	4.83 a	4.32 a	11.56 a	9.53 a	6.87 a	7.02 a
LSD	0.20	0.02	0.72	0.02	0.16	0.07	0.02	0.02

Total phenolic content

Respecting the effect of gamma irradiation on total phenolic compounds, data revealed in Tabel 4 that there was a progressive, significant increment in total phenolics with increasing doses of gamma radiation. The highest values of total phenolics compounds were observed under the 2.5 Gy treatment, yielding 36.21 in the first season (S1) and 37.86in the second season (S2), compared to their respective control values of 21.80 and 26.15. The same result was obtained by **El Sayed et al. (2023)** who found that the highest doses of gamma irradiation increase the total phenolic content of gardenia plants. The importance of phenolphenolic compounds performs significant physiological and ecological roles; participating in resistance to various types of stresses. Additionally, increases in total phenolic and flavonoids content are considered as a common response for high radiation doses, **Elizar et al. (2018)**. The phenols paramount for the plant is due to a fair correlation among antioxidant and free- radical scavenging activity and its phenolic content and protects the plant against irradiation - induced oxidative stress, **Elizar et al. (2018)**.

Total carbohydrates.

Total carbohydrates content exhibited a profound surge, reaching 69.29 and 70.55 under the 2.5 Gy treatment. This accumulation is a direct consequence of enhanced photosynthetic efficiency linked to higher pigment concentrations (as shown in Table 4). The same result was obtained by **Mounir et al. (2022)** who found that low doses of gamma irradiation increase the total carbohydrates percentage of Jerusalem artichoke.

The reduction of carbohydrates percentage with the increase in gamma ray doses might be related to the enhancement of the metabolic activation of hydrolyzing enzymes (**Maity et al. 2004**).

Phytohormonal Dynamics.

Endogenous growth-promoting GA3 content rais sharply to 18.13 and 18.95, while a concurrent increase was observed in the stress-regulating hormone ABA, which advanced to 8.87 and 7,53 under the highest dose, as presented in Table 4. The present results are generally in agreement with those of (**Patil et al., 2017**) on Dendrathema grandiflora; (**Wang et al.,2020**). On *Chrysanthemum monrifolium* Donglinruixue; **Asgari Lajayer., (2019)** on *Ocimum basilicum*, **Hajizadeh et al (2022)** on *Lilium longiflorum*; and **Ghosh et al. (2020)** on *J. auriculatum*. The effect of radiation on increasing endogenous hormones in plants and the involvement of auxin and cytokinin in regulating growth and photosynthetic attributes are known **(Xiong et al., 2020**). The enhanced plant growth in response to low gamma doses could be related to altering the hormonal signaling network and increased cells' anti-oxidative capacity (**Kim et al., 2004**). Also, gamma irradiation causes modifications in transcriptional control of genes related to signal transduction, transcription factors, antioxidant system, phytohormones, metabolism transport, energy, morphogenesis and cell cycle (**Gicquel et al. 2012 and Gudkov et al. 2019**).

Table 4. Effect of Gamma irradiation on Chemical characters of Zamioculcas zamiifolia L. leaves.

Chemical characters / Treatments	Total phenolic (mg/100g F.W)		Total Carbohydrates (mg/g F.W)		GA3 (µg / 100g F.W)		ABA (µg / 100 g F.W)	
	2023	2024	2023	2024	2023	2024	2023	2024
Control	21.80 c	26.15 c	54.99 c	59.71 c	9.99 c	11.51 c	4.97 c	3.83 b
1Gy	31.60 b	34.38 b	62.79 b	67.48 b	14.21 b	14.76 b	6.89 b	6.56 a
2.5Gy	36.21 a	37.86 a	69.29 a	70.55 a	18.13 a	18.95 a	8.87 a	7.53 a
LSD	**3.93**	**1.42**	**3.44**	**2.44**	**0.71**	**0.50**	**0.24**	**2.27**

Anti-oxidant enzymes activity

The estimation of antioxdant enzymes Superoxide dismutase, Catalase and Peroxidase was determined during the second season, as shown in Figure1. Upon exposing the plants to a low-to- moderate dose of 1Gy, a sharp and significant spike in the activities of antioxidant enzymes superoxide dismutase, catalase and peroxidase was recorded compared to the control group. Results indicated that 2.5 Gy scored an increase in the activity of peroxidase and super oxide dismutase. Also, **Shena et al. (2010)** reported that low dose of γ-radiation stimulates the peroxidase activity; **Sreedhar et al. (2013)** found that a variable degree of stimulation in the activities of POD in leaves of groundnut (Arachis hypogaea L.) according to the dose of γ-rays. **Kebeish et al. (2015)** found a gradual increase in CAT activity in *Allium sativum* L. with an increasing level of irradiation up to 100 Gy. Exposure to γ-irradiation causes changes in biochemical activity through various metabolites produced as a function of γ-irradiation. The most important of these metabolites are peroxyl radicals. The radiation protective effect of peroxidase is due to removal of H_2O_2, peroxides, and especially lipid hydrogen peroxides.

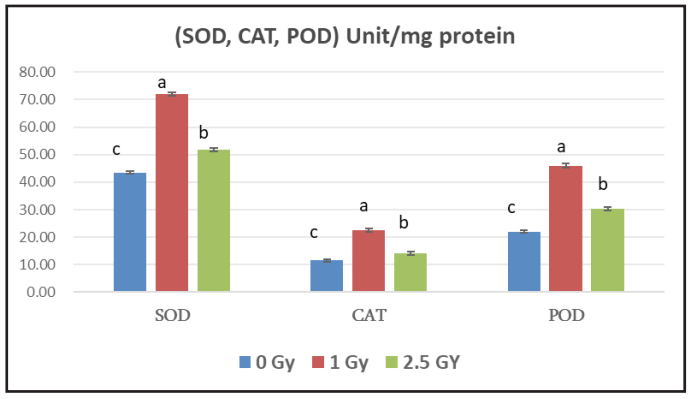


Figure (1): Effect of Gamma radiation doses (0 Gy, 1 Gy, 2.5 Gy) on Antioxidant enzymes activity (SOD, CAT and POD) Unit/ mg protein of Zamioculcas zamiifolia leaves during 2024 season. Values are presented as mean ± SD . Means with different letters within the same enzyme category are significantly different according to Duncan´s multiple range test (p≤ 0.05)

This accounts for the greater effectiveness of peroxidases than catalase. The induction of POD by 175176the irradiation would be one of the defense systems activated through the ROS-mediated cellular signaling (**Wi et al., 2007**). It was suggested therefore that the increase in gamma doses lead to an increase in the specific activity of peroxidase enzyme (**Sah et al., 1996**).

3. Anatomical study

Based on the results figures (2 A& B) showed, transverse sections of Zamia leaflets revealed noticeable anatomical variations among the different irradiation treatments. Thickness of leaflets was significantly affected by gamma-ray irradiation treatments. In the control, leaflet thickness reached approximately 688.38 µm in the midrib region and about 612 µm in the lamina, showing well-organised and compact mesophyll tissues. A marked reduction in thickness was observed by gamma ray irradiation at a rate of 1 Gy, where the midrib measured about 401.196 µm and the lamina 207.491 µm, reflecting a decline in tissue organization and structure measurements compared to the control. The thinnest midrib was produced by a rate of 2.5 Gy (192.470 µm), whereas lamina measured about 181.056 µm under the same Gray, indicating severe reduction associated with pronounced structural deterioration of leaf tissues. Overall, the results demonstrated a progressive decline in anatomical integrity with an increasing irradiation dose. The present study clearly demonstrates a dose-dependent effect of irradiation on the anatomical structure of Zamia leaf tissues. The higher values observed in the control samples (A and B) reflect intact tissue organization, normal cellular arrangement, and stable structural integrity in the absence of irradiation stress. In 1 Gy (Fig. 2C and D), the reduction in measurements suggests the beginning of tissue disorganization, which may be attributed to partial inhibition of meristematic activity and altered cellular expansion. This indicates an early stage of structural disturbance without complete breakdown of tissue architecture. A more severe response was observed in the high irradiation treatment (Fig. 2 E and F), where the

lowest values were recorded. This may indicate marked tissue disruption and partial tissue collapse, likely resulting from excessive radiation stress. High irradiation levels are known to induce DNA damage, oxidative stress, and metabolic dysfunction, which collectively contribute to structural breakdown of leaf tissues and loss of normal anatomical organization.

The observed pattern in mesophyll and midrib thickness indicates a clear dose-dependent response to irradiation, where reductions in leaf blade thickness are directly proportional to increasing dose intensity. According to **Sakr *et al.* (2017)**, mesophyll thickness in *Brunfelsia pauciflora* increased significantly at 5 and 10 Gy, reaching a maximum at 10 Gy (225 µm), followed by 5 Gy (175 µm), representing increases of 80% and 40% over the control, respectively. This enhancement suggests that moderate irradiation doses may stimulate cellular expansion and/or division within mesophyll tissues. In contrast, higher doses (20 and 30 Gy) led to reductions of 11.11% and 25%, respectively, below control levels, indicating the onset of detrimental effects at elevated irradiation levels. Overall, these findings support the concept that irradiation exerts a biphasic effect on tissue development: moderate doses, particularly 10 Gy, enhance anatomical traits, whereas higher doses beyond an optimal threshold negatively affect tissue structure. This pattern is consistent with findings by **Sakr *et al.* (2013),** who reported that gamma irradiation at 5, 10, and 15 Gy altered Epidermal and mesophyll cell size, as well as vascular tissue diameter in leaves, stems, and roots. These anatomical changes were generally associated with shifts in physiological activity. Additionally, gamma irradiation induced noticeable color changes in the leaves and stems of handeuleum.

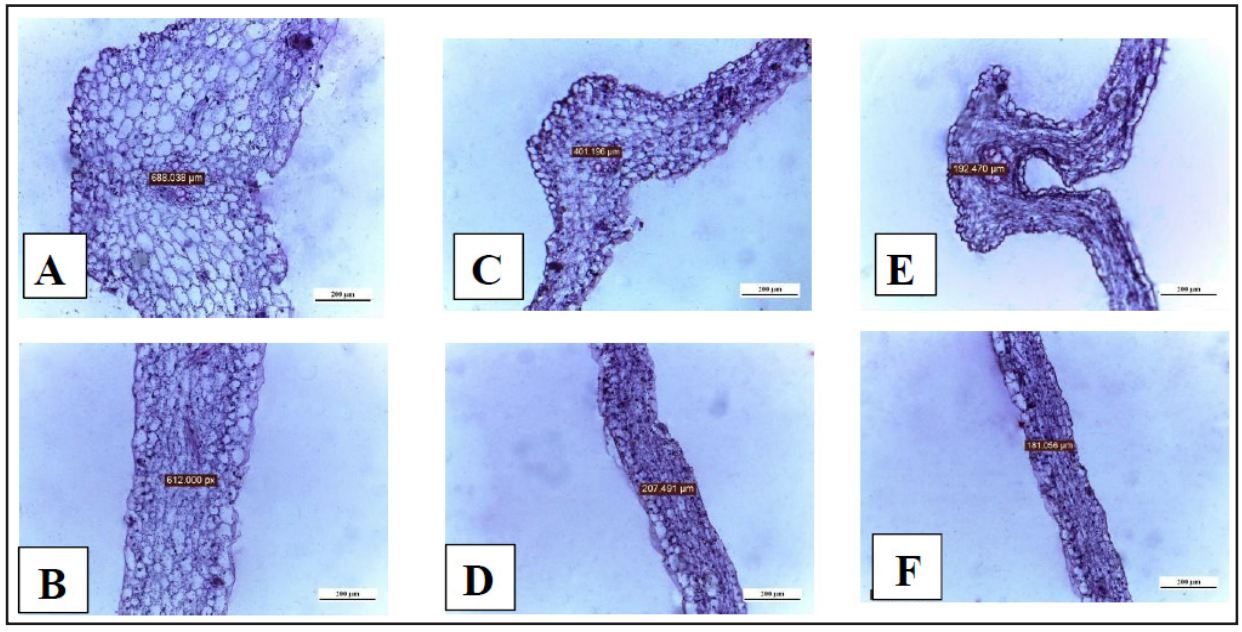


Figure (2): Leaflet anatomical structure of Zamia at 0 Gy (control; A, B), 1 Gy (C, D), and 2.5 Gy (E, F). Midrib and lamina thickness decreased with increasing radiation intensity

4. Molecular genetic identification:

ISSR profile analysis of genetic variation

Analysis of the ISSR data (Fig. 3, Table 5) revealed that five primers generated a total of 32 amplified bands, ranging in size from 181 to 1614 bp. Of these amplified products, 28 bands were monomorphic, while 4 were polymorphic, resulting in an overall polymorphism rate of 12.5% across the studied genotypes.

Primer-specific polymorphism varied significantly (Table 1). Primer 17898A yielded the highest polymorphic profile (22.2%), followed by primers UBC 807 (20.0%) and UBC 811 (12.5%). Conversely, primers HB-10 and HB-12 generated entirely monomorphic banding patterns. The identification of distinct polymorphic ISSR bands effectively demonstrates that gamma irradiation induced limited, yet detectable, genetic variation in *Zamioculcas zamiifolia* relative to the non-irradiated control. The emergence of these polymorphic bands in irradiated treatments implies that gamma radiation induced DNA alterations, potentially mutations or structural rearrangements. This phenomenon aligns with previous reports characterizing radiation-induced polymorphism as a consequence of DNA strand breaks and subsequent repair mechanisms, which collectively generate novel genetic variability applicable to plant improvement programs (**Beyramizadeh et al., 2023; Hanafy and Akladious, 2018**).

Hierarchical cluster analysis

Hierarchical cluster analysis using the average linkage method based on rescaled distance (**Everitt et al., 2011**) revealed a distinct two-cluster topology, elucidating robust grouping patterns among Zamia treatments under gamma irradiation (Fig. 4). The Control (CK) and 1 Gy treatments coalesced early to form a highly homogeneous primary sub-cluster, characterized by strong statistical similarity and negligible variance. This visual evidence of limited divergence reinforces that low-dose irradiation minimizes phenotypic and physiological impacts on Zamia tissues relative to baseline parameters (**Wi et al., 2005**). Conversely, the 2.5 Gy (G2.5) treatment displayed an independent, strongly segregated trajectory, remaining highly isolated and aggregating only at the maximum rescaled distance threshold of 25. This pronounced divergence explicitly identifies G2.5 as a definitive statistical outlier with characteristics significantly altered compared to both the control and lower-dose treatments. The delayed agglomeration profoundly signifies that escalating gamma radiation exposure to 2.5 Gy surpasses a critical physiological or genetic threshold in Zamia tissues, inducing substantial divergence (**Kim et al., 2004**).

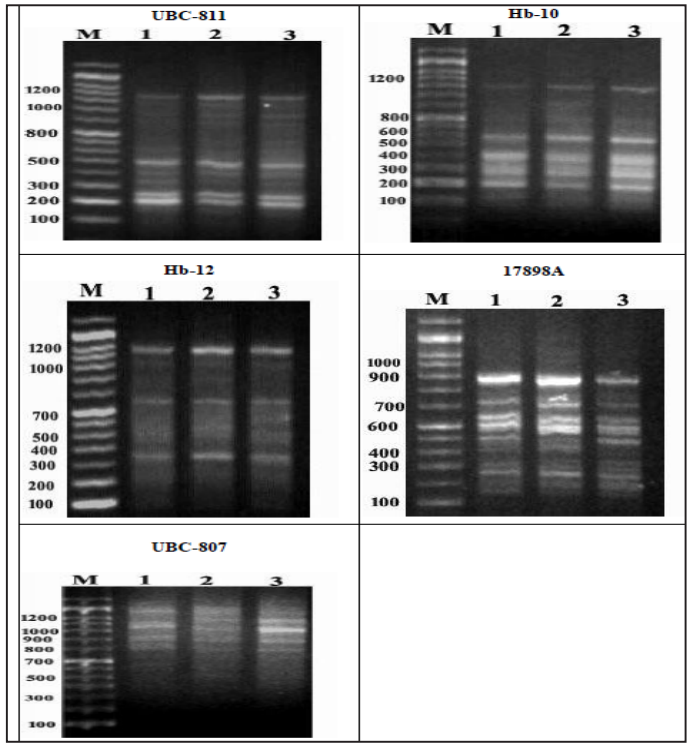


Figure (3): ISSR-PCR banding patterns of Zamioculcas zamiifolia under gamma irradiation using primers (UBC 811; HB-10, HB-12, 17898A, UBC 807). Lane M kb molecular-size ladder, (1: control, 2: 1 GY, 3: 2.5 Gy)

Table 5. Type and number of amplified DNA generated by five DNA-ISSR primers used for the identification of the three Zamioculcas zamiifolia (1: control; 2: 1 GY; 3: 2.5 Gy).

Primer	No. of Bands	Band Size Range (bp)	Monomorphic Bands	Polymorphic Bands	Unique Bands	Monomorphism (%)
HB-10	5	181–1268	5	0	0	100%
HB-12	5	509-1614	5	0	0	100%
17898A	9	182.2- 959	7	2	0	78%
UBC 807	5	727–1158	4	1	0	80%
UBC 811	8	191–1372	7	1	0	88%
Total	32	-	28	4		87.5

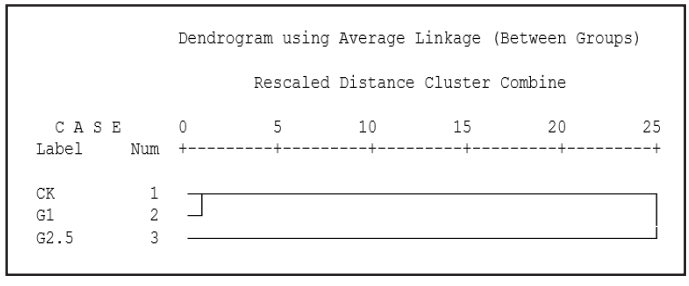


Figure (4): Presents a hierarchical cluster analysis dendrogram using the average linkage (between groups) method to evaluate the statistical relationships among CK, G1, and G2.5

Conclusions

Gamma radiation (1 Gy) significantly enhanced leaflet area, number of leaves and photosynthetic pigments, indicating positive physiological modulation. Higher radiation (2.5 Gy) also increased pigment synthesis but caused a slight reduction in growth. Therefore, the 1 Gy dose is recommended for potential improvement of *Zamioculcas zamiifolia* growth and ornamental value under controlled irradiation programs.

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