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Effect of Gamma Irradiation on Morphological Biochemical and Cytological Attributes of *Salvia hispanica* L.

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Abstract

The present study focuses on investigating the interaction between radiation and biological systems, specifically examining the detrimental effects of radiation. This approach has proven to be effective in determining the optimal tolerance range of radiation on plants, which can lead to the development of beneficial characteristics and help improve the narrow genetic base. The study was conducted on Salvia hispanica L., a medicinally important plant. Inbred seeds of Salvia hispanica L. were subjected to five different doses of gamma rays (50, 100, 150, 200, 250 Gy) using a Co-60 source with a gamma irradiator of 7.247 k rate. The seeds were sown in triplicate, along with a control set. Various characteristics were assessed, including germination percentage, survival percentage, presence of chlorophyll mutants, and plant height. The results indicate that lower doses of gamma irradiation (<LD50) positively influence plant growth, including plant height, inflorescence size, and productivity. However, higher doses of gamma radiation (>LD50) have detrimental effects on several plant attributes, such as seed germination, survivability, inflorescence size, and pollen fertility. Chromosomal abnormalities were also observed, with an increase in their occurrence corresponding to higher doses of gamma radiation. Germination percentage and survivability were found to decrease as the dose of gamma radiation increased. The dose of 50 Gy resulted in the highest level of genetic variability. Additionally, compared to the control group, a significant percentage of chromosomal abnormalities, particularly stickiness and scattering, was observed at a dose of 250 Gy. Lower doses of gamma radiation (specifically 50 and 100 Gy) showed statistically significant (p>0.5) positive responses in Salvia plants, including plant height, leaf area, leaf mutants, inflorescence axis, and seed size. On the other hand, higher doses of radiation proved to be fatal for the plants.

In summary, this study provides valuable insights into the effects of gamma radiation on Salvia hispanica L., highlighting the optimal dose range for inducing beneficial changes in plant characteristics while also emphasizing the harmful consequences associated with higher doses of radiation.

Keywords- *Salvia hispanica* L., Gamma rays, chromosomal aberrations, Genetic Variations, LD_{50}.

1. Introduction

Salvia hispanica L. most preferably referred to as "*chia*" is a medicinally important plant of the Lamiaceae family. It is native to central and southern Mexico and Guatemala and distributed over several countries of South America. Chia seeds are high in oil contents, rich in polysaccharides and fatty acids mainly omega-3 fatty acids and omega-6 fatty acids. In ancient times, "chia" was used highly by Mayan and Aztec populations as a plant of medicine.

China is merging it as a new "superfood" that offers a great source of antioxidants, dietary fiber, and omega-3 fatty acids. Seeds of plants were used as food since 3500 B.C. and functional as a commercial crop in central Mexico between 500 and 900 B.C. (De Falco *et al.,* 2017).

"Chia" also helps to enhance the satiety index and prevent nervous system disorders, inflammation, cardiovascular disease, and diabetes. Gutierrez *et al.* (2014) highlight that chia seeds contain a significant amount of alpha-linolenic acid, comprising approximately 68% of their composition. Additionally, according to Jimenez *et al.* (2013), polyunsaturated fatty acids like omega-3 and omega-6 are considered crucial for human health since they cannot be synthesized within the human body.

Black chia, a crop that has been known since pri-Cortesian times and is considered a pseudocereal, is utilized as both food and medicine due to its high content of alpha-linoleic and omega-3 acids, which hold great importance to conduct genetic improvement work on the species to enhance certain agronomic attributes (Lopez, e.d.2020).

In the study conducted on Salvia hispanica L. plants, it was observed that the irradiation dose had an impact on the composition of chia seed oil. Specifically, there was a decrease in the levels of oleic acid (18:1) and linoleic acid (18:2), while an increase in the levels of palmitic acid (16:0) and stearic acid (18:0) was observed. This shift in fatty acid composition was directly proportional to the increase in the irradiation dose. Furthermore, a reduction in the overall content of monounsaturated and polyunsaturated fatty acids was also noted (Akyol *et al.,*

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2022). These findings indicate that gamma irradiation treatment influences the fatty acid profile of chia seed oil, potentially impacting its nutritional properties and applications.

Mutation breeding serves as an effective tool for plant breeders to introduce variability in crop plants without altering the original genetic makeup of the cultivar. It offers the potential to obtain desirable characteristics that may not exist in nature or have been lost during the course of evolution (Novak and Brunner, 1992). Artificial mutations can be induced using specific mutagens, including physical agents such as gamma rays, X-rays, fast and slow neutrons, and chemical substances such as ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), sodium azide, base analogs, and acridine dye. These mutagens facilitate the induction of mutations in the plant's genetic material.

Mutation induction has become a well-established and widely accepted approach in plant breeding programs. It allows for the integration of novel traits into existing germplasm and the improvement of cultivars with desired characteristics (Kiani *et al.,* 2022). By harnessing the power of mutation breeding, plant breeders can enhance the genetic diversity of crops and develop improved varieties that exhibit specific qualities.

A lot has been accomplished in crop improvement through the development and official release of thousands of crop varieties with the help of mutation breeding programs. Variation in living organisms on Earth planet was ultimately sourced from mutations. Gamma rays are the most used physical mutagens and breeders have adhered to them for crop improvement programs (Celik and Atak, 2017). The interaction between gamma rays and atoms or molecules of the biological material can be direct or indirect. In direct action, DNA was hit by the rays, thereby disrupting the molecular/genome structure while in indirect action the rays hit the water and cause radiolysis of water that eventually results in the generation of free radicles (Limoli *et al.*,2001;Desouky *et al.,* 2015).

The use of mutagenesis has been widely employed to enhance variability for crop improvement (Acharya *et al.,* 2007). The stimulatory attributes of gamma rays at lower concentrations/doses have been vigorously used by breeders for enhancing the vigorous of numerous crop varieties which were either dealing with problems of or bearing poor qualitative, polygenic, and other stress-tolerant traits (Dwivedi *et al.*,2021). Gamma radiation was the foundation for gamma spectrometry, a key technique used for analyzing radioactive materials qualitatively and quantitatively in various ambient environments. Gamma rays are the most energetic form of electromagnetic radiation and they possess an energy level from 10 keV (Kilo Electron volt) to several hundred KeV. They are considered the most penetrating radiation source compared with other sources such as alpha and beta rays. It falls into the category of ionizing radiation and interacts with atoms or molecules to produce free radicles in cells.

Here in the experimental setup, we have installed an experimental design of triplicates and sowed the seeds of gamma treatments with a control set. We have tried to get a remarkable change in the treated sets to enhance their genotypic diversity. As in previous studies, it was observed that Gamma irradiation had a stimulatory effect on primary branches, including the number of pods/plants, number of flowers per plant, seed index, etc. Gamma-irradiation treatment regulates proteins of crop plants by altering their conformation, oxidation of amino acids of the particular protein, rupture, and breakage of covalent bonds, and by the generation of protein-free radicles that can be beneficial for application in breeding programs. The use of gamma irradiation was found to be an effective method for inducing cross-linking and improving both the barrier and mechanical properties of the edible films and coatings based on proteins (Mastro *et al*., 2016).

A significant response in plants was reported to be caused by gamma irradiation compared to the control. It was found that phenolic and tocopherol levels decreased, and free fatty acid and peroxide count increased with the increase of irradiation dose, and these changes were statistically significant ($p<0.01$). The ratio of palmitic acid and steric acid increased, while the ratio of oleic acid and linoleic acid decreased with increasing irradiation dose (Akyol, 2019).

The purpose of this study was to find out and determine the harmful effects of radiation and to establish the radiation quality and dose range in which benefits, in terms of more productive or in general more suitable plant systems, would be obtained.

2. Materials and Methods

2.1. Procurement of materials

First of all, we have purchased the inbred seeds of *Salvia hispanica* L. from NutriPlanet Private Limited, Bengaluru-520068, Karnataka, India. The rest needs and requirements were fulfilled by Naithani Plant Genetics Laboratory, Department of Botany, University of Allahabad-211002, UP, India.

2.1.1. Treatment of seeds through different Doses of gamma rays

First of all, the seeds of *Salvia hispanica* L. were filled in small plastic packets and sent to NBRI, Lucknow for gamma irradiation. The doses given to the seeds were 50, 100, 50, and 200&250 Gy respectively. The irradiation process was carried out in a Cobalt-60 at a rate of 7.247k gamma irradiator.

2.1.2. Weather conditions and optimum temperature for seed sowing

After the treatment of the seeds, they were sown in earthen pots in triplicates along with control sets. Replicates were planted in a completely randomized block design (CRBD) at a temperature of 25℃±2℃ and relative humidity 76% in outdoor conditions.

2.1.3. Morphological study

For the morphological study the germination percentage was calculated after seven days of sowing, survival was noted after thirty days, and plant height trend was noted after 45 days of sowing. Inflorescence size was also measured after 7 days of its emergence.

2.1.4. Meiotic study

For the meiotic analysis young floral buds of control and variant plants of *Salvia hispanica* L. with appropriate size were fixed in Carnoy’s fixative (Alcohol 3: Glacial Acetic Acid 1) for 24 hrs and then transferred in 90% alcohol to preserve the buds. Anthers were teased and stained in 2% acetocarmine, followed by squash preparation.

Further, the process of squash and the slides were observed under a Nikon phase-contrast microscope (Eclipse iE200, Japan). The observation was made to calculate the total abnormality percentage (TAB %) of treated sets and control as.

$$\text{Total Abnormality (\%)} = \times 100 \frac{\text{Total number of actively dividing cells}}{\text{Total number of aberrant cells}}$$

2.1.5. Pollen fertility

For this study, mature flowers were taken and dusted over glass slides to remove the mature pollen grains of anthers from them. After this, the pollens were stained with acetocarmine and mounted with glycerin to observe under the microscope. The pollen fertility of each treated set was calculated by staining them with acetocarmine. The darkly stained pollens are considered viable while those unstained are considered non-viable. A common method for assessing pollen viability was by staining and direct counting, as described by Heslop-Harrison in 1992.

$$\text{Pollen fertility (\%)} = \times 100 \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}}$$

2.2. Biochemical Study

Fresh leaves of *Salvia* have been taken to extract Chlorophyll a, b, and carotenoids from it with 80% acetone and the amount and estimation of it were determined according to the Lichtenthaler method (1987).

$$chlorophyll\ a : \frac{12.25(A_{663}) - 2.79(A_{646}) \times volume}{weight\ of\ leaf\ tissue(mg)}$$

$$Carotenoids : \frac{[1000(A_{470}) - 1.82(Chla) - 85.02(Chlb)]/198 \times volume(ml)}{weight\ of\ leaf\ tissue(mg)}$$

$$chlorophyll\ b : \frac{21.5(A_{646}) - 5.1(_{663}) \times volume}{weight\ of\ leaf\ tissue(mg)}$$

2.3. Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software. A one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT, $p < 0.05$) was conducted for mean separation and the graph was plotted by using sigma plot 10.0 software. The actual mean and standard error were calculated and the data were subjected to analysis of variance.

3. Results

3.1. Morphological Results

3.1.1. Germination and Survival

In the current study, the germination of the seeds of *Salvia hispanica* L. reduced with increasing doses of gamma rays while at low doses of treatment viz. 50 Gy there was an increase in germination compared to the control plant. **Figure 1(A)** depicts that initial doses of gamma (50 and 100 Gy) are causing significant effects compared to higher doses of gamma irradiation, i.e 150, 200, and 250 Gy. The germination percentage of the control seeds was 94.66±1.33and at 50 Gy recorded 96±2.30 the highest among all the treated sets, while the higher doses showed a continuous decline in the germination percentage. The control set showed the highest survivability i.e. 92.34±1.29, while the highest gamma dose (250 Gy) showed the lowest survivability percentage (i.e. 58.45±1.94) among all the treated sets.

3.1.2. Plant Height

The plant height trend of all the treated sets was taken accordingly, and after the observation it was found that the initial doses of gamma (i.e. 50 and 100 Gy) have shown a positive effect on plant height that helps enhance the vigor and production of the plants. Figure 1(B) and Figure 2 (A) depict that the plants treated with lower doses of gamma have better height and vigor in comparison to control plants and the plants treated with higher doses of gamma (i.e. 150, 200, and 250 Gy).

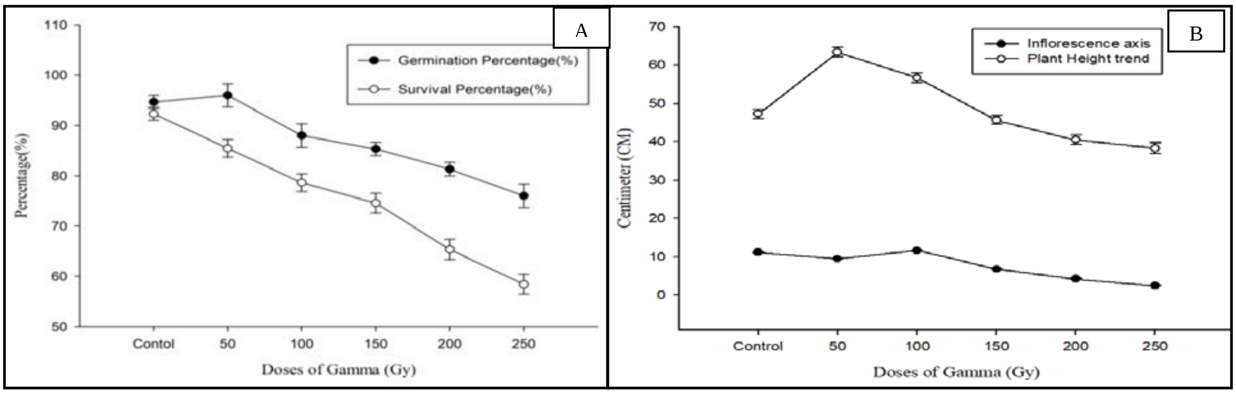

Figure 1: Graphs showing morphological parameters i.e. Germination and Survival % (A) and Inflorescence and plant height trend (B)

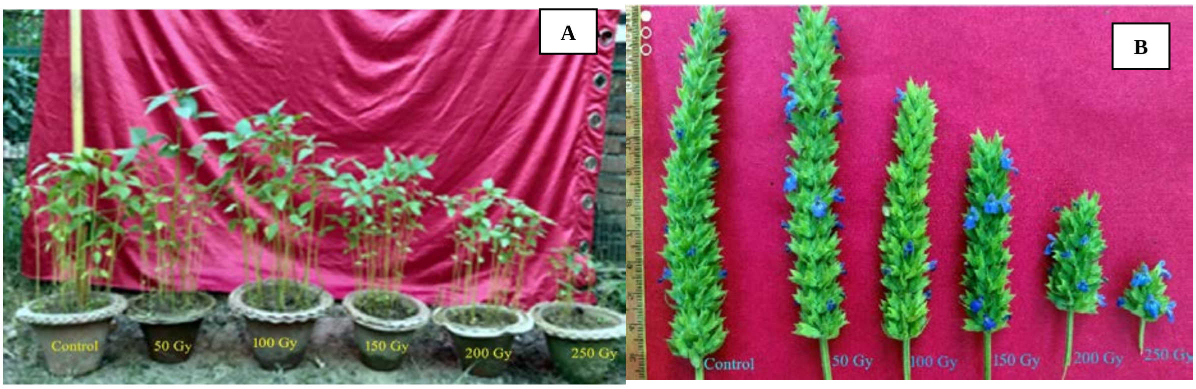

Figure 2 (A) and (B): Images showing plant height trend and inflorescence size respectively in treated sets

3.1.3. Inflorescence size

In the experimentation, variability in the inflorescence has also been observed. It was found that at initial doses of gamma, the inflorescence was larger and more vigorous in comparison to the control set, while higher doses proved disastrous to them as they are causing hindrances in the growth and development of the inflorescence. The most vigorous inflorescence was observed at 50 Gy treatments, and the frailest one was observed at a 250 Gy dose of gamma. Figure 1 (B) and 2 (B) clearly shows that the inflorescence of the 50 Gy treated set was larger and stronger than to all other sets including control plants.

3.2. Leaf variants

During the experimentation with gamma irradiation treatment, several leaf variants were observed. The treated sets exhibited abnormalities in terms of leaf color, shape, and patterns. Color variants included Xantha and Albina, representing abnormal colors compared to the control group. Shape variants comprised leaf bifurcation, rolling, and the development of leathery leaves. Additionally, pattern variants were observed in the study such as; presence of three leaves at a single node or bunches of leaves clustered at a node. Figure 3 illustrates the different types of leaf variants identified in the experimental setup, contrasting them with the control group

Figure 3: Leaf variants observed during the study: A. Control; **B.** Bifurcated leaf; **C.** Leathery leaf; **D.** Deformed leaf; **E.** Bifurcation of leaf axis; **F.** fleshy leaf; **G.** Tricotyledonous leaf; **H.** Differential development of leaf at same axis; **I.** Semi-xantha; **J.** Xantha; **K.** Semi-albina; **L.** Albina.

3.3. Cytological Results

3.3.1. Cellular abnormalities

In *Salvia hispanica* L. the haploid chromosomal set **n=6** (2n=12 in the control set, normal divisions were observed, while abnormalities were recorded in the treatment. In the cytological preparation, it was observed under the microscope that the percentage of abnormal division was increasing simultaneously with the increasing dose of gamma. In other words, the abnormality percentage was directly proportional to doses of gamma. As the dose of gamma radiation increased, there was a corresponding elevation in the total abnormality percentage (TAB%). This trend was evident from the data presented in Table 1 and illustrated in Figure 5. Specifically, the lowest TAB% (6.20±0.28) was recorded at the lowest administered dose of 50 Gy, whereas the highest TAB% (12.74±1.00) was observed at the highest dose of gamma radiation, namely 250 Gy.

Cellular abnormalities were recorded during the experiment, observed at both metaphase and anaphase stages. These abnormalities encompassed a range of irregularities, including stickiness, unorientation, scattering, forward movement, asymmetric division, and disturbed polarity. Stickiness, metaphasic scattering, precocious movement at metaphase, and stickiness at anaphase were the most frequently observed abnormalities among the recorded instances.

It was quite articulate by the contemplation of cytological preparations of the treatment that gamma irradiation treatment was injurious for the health of cellular development and it was potentially inhibiting systematic cell proliferation of the pollen mother cells (PMCs) and readily causing numerous chromosomal abnormalities mentioned in the above paragraph.

3.3.2. Pollen fertility

In the above-mentioned experimental setup, pollen fertility was also taken into account and it was observed that the fertility of pollen grains was decreased as the doses of gamma are increased. Based on the data obtained in the experiment, the pollen fertility was found to be inversely proportional to the doses of gamma (Figure 5). The rate of pollen fertility was highest (97.65%) the in control set the lowest fertility of the pollen (67.46%) was observed at the highest dose of gamma i.e.250 Gy.

According to the observation, it was pretty sure that gamma irradiation was hazardous for the health and development of potent pollen grains which fertilize the egg and play its foreknown role in the plant’s life cycle.

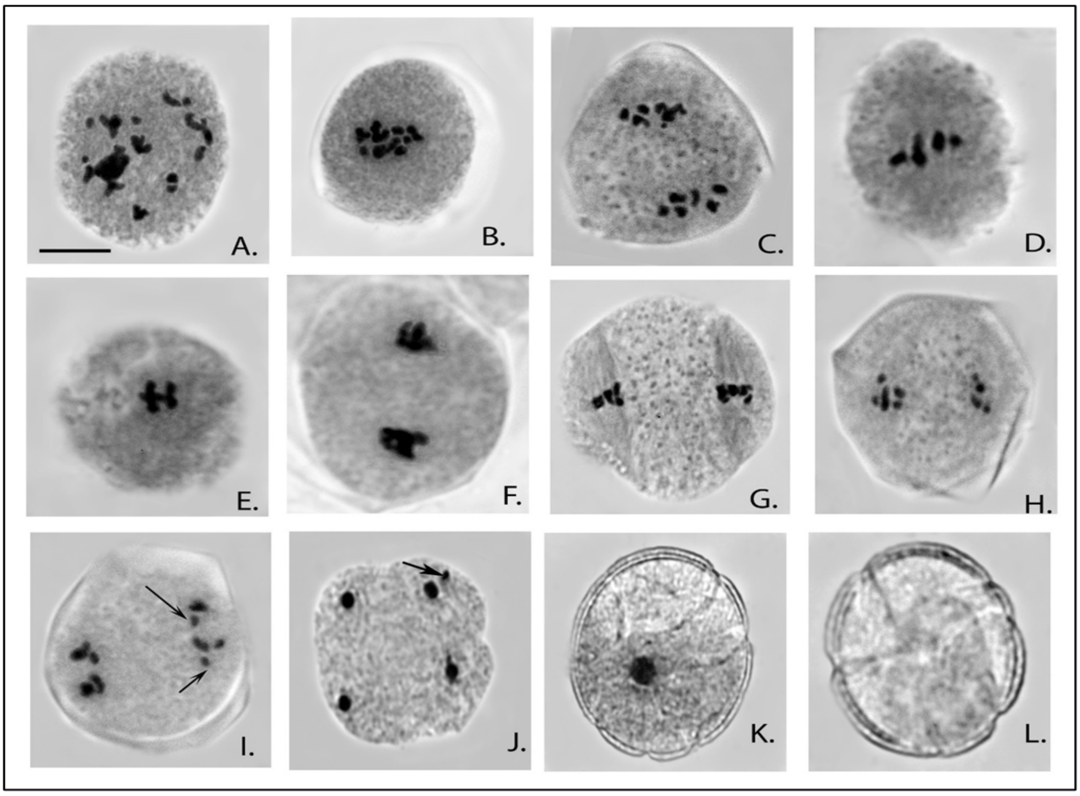

Figure 4: Cytological Observation: A. Diplotene; **B.** Normal Metaphase; **C.** Normal Anaphase; **D.** Stickiness at Metaphase I; **E.** Multivalent formation; **F.**Stickiness at Anaphase I.; **G.** Normal Metaphase II; **H.** Scattering at Metaphase II;; **I.** One laggard and one forward movement of chromosome at Anaphase II; **J.** Telophase; **K.** Fertile with (nucleus) and **L.** Sterile pollen (without nucleus). (Scale bar = 5µm)

3.4. Biochemical Results

3.4.1. Chlorophyll a

The impact of gamma irradiation treatment on the main photosynthetic pigment, chlorophyll a, was investigated in the experimental plants. The results demonstrated that gamma irradiation had a detrimental effect on the development of this crucial photosynthetic pigment. It was evident that the amount of chlorophyll a decreased progressively with increasing doses of gamma irradiation, in comparison to the control group (Figure 6). The observation clearly establishes an inverse relationship between the amount of chlorophyll a and the doses of gamma irradiation

3.4.2. Chlorophyll b

Similar to Chlorophyll a, the content of chlorophyll b also exhibited a declining trend in response to increasing treatment doses, ranging from 50 Gy to 250 Gy, as depicted in Figure (6).

3.4.3. Carotenoid

Carotenoids, which are photosensory pigments found in plants, were also important considerations, similar to treatments. In comparison to the control group, all the treated groups exhibited a declining trend (Figure 6). As the doses of gamma irradiation increased, the carotenoid content decreased in a similar manner to that observed for chlorophyll a and b.

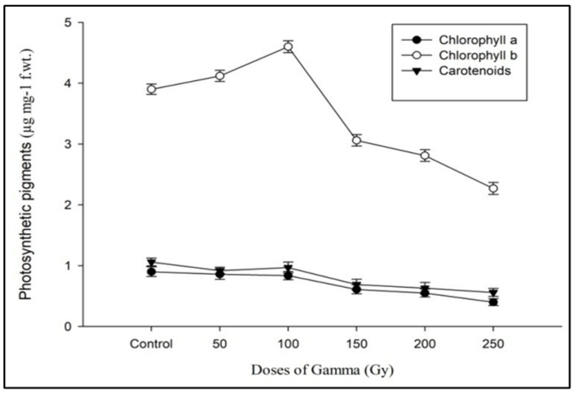

Figure 6: Graph representing photosynthetic pigments content in control as well as treated sets of Gamma

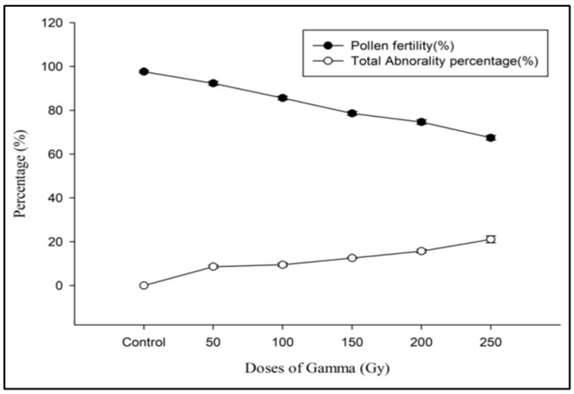

Figure 5: Graph representing pollen fertility and TAB percentage with increasing doses of irradiation.

Table 1: Gamma irradiation-inducedcytological abnormalities and their percentage in *Salvia hispanica* L.

Treatment	No of PMC’s Observed	Metaphasic abnormality (%) (Mean±SE)					Anaphasic abnormality (%) (Mean±SE)					Oth	Tab	Pollen fertility %
		Sc	Pm	St	Un	Asy	St	Un	Fr	Sc	Dp			
Control	276	-	-	-	-	-	-	-	-	-	-			97.65 ± 0.50^{a}
50 Gy	263	0.51 ± 0.12^{a}	0.25 ± 0.13^{a}	1.14 ± 0.21^{a}	0.51 ± 0.26^{a}	0.25 ± 0.25^{a}	1.52 ± 0.21^{a}	0.51 ± 0.26^{a}	0.50 ± 0.33^{a}	050 ± 0.25^{a}	0.38 ± 0.22^{a}	1.13 ± 0.13^{a}	6.20±0.28	92.34 ± 0.88^{b}
100 Gy	253	0.26 ± 0.26^{a}	1.19 ± 0.25^{ab}	1.45 ± 0.14^{ab}	0.66 ± 0.34^{a}	0.12 ± 0.12^{ab}	1.58 ± 0.22^{b}	0.40 ± 0.24^{b}	0.80 ± 0.24^{a}	0.26 ± 0.26^{b}	0.39 ± 0.23^{a}	0.26 ± 0.13^{a}	7.26±0.54	85.64 ± 0.89^{c}
50Gy	248	0.53 ± 0.26^{a}	052 ± 0.34^{ab}	1.71 ± 0.13^{bc}	0.66 ± 0.36^{a}	0.40 ± 0.23^{ab}	1.45 ± 0.29^{b}	0.26 ± 0.13^{b}	0.54 ± 0.36^{a}	0.66 ± 0.13^{b}	0.65 ± 0.45^{a}	0.39 ± 0.23^{a}	7.78±0.24	78.57 ± 0.88^{d}
200 Gy	239	0.67 ± 0.35^{a}	0.83 ± 0.25^{ab}	2.08 ± 0.29^{bcd}	0.42 ± 0.25^{a}	0.54 ± 0.12^{b}	1.67 ± 0.28^{b}	0.55 ± 0.37^{b}	1.09 ± 0.24^{a}	0.69 ± 0.14^{b}	0.42 ± 0.24^{a}	0.54 ± 0.12^{a}	9.50±0.22	74.63 ± 1.05^{e}
250 Gy	220	0.31 ± 0.15^{a}	0.60 ± 0.30^{b}	2.73 ± 0.28^{cd}	1.07 ± 0.55^{a}	0.91 ± 0.25^{b}	2.72 ± 0.51^{b}	1.36 ± 0.25^{b}	0.76 ± 0.15^{a}	1.67 ± 0.33^{b}	0.46 ± 0.26^{a}	0.15 ± 0.15^{a}	12.74±1.00	67.46 ± 1.08^{f}

Where,**PMC’s-** Pollen mother cells, **SE-** Standard error,**Sc-** Scattering of chromosomes, **Pm-** Precocious movement of chromosomes, **St-** Stickiness of chromosomes, **Un-** Unorientation in chromosomal sets, **Asy**-Asynchronisation, **Fr-** forward movement in chromosomes, **Dp-** Disturbed polarity; **Oth-** Others, **Tab-** Total abnormality percentage ($p= <0.5$)

4. Discussion

Germination was the most important phenomenon of the life cycle of any plant to maintain the genotype in existence in the system. In the present experiment, it has been found that the germination displayed a decreasing trend as the doses of gamma increased compared to the control. A group of scientists (Raina *et al.* 2016) has also reported the delay in the initiation of metabolism following germination, resulting in a uniform delay in mitotic activity and hence seedling growth. This phenomenon may be due to the detrimental effect of high energy beams of gamma and the chromosomal aberrations, disturbance in DNA and auxin synthesis, and to impaired cell metabolism (Kirtane and Dhumal, 2004). The same results were also found in Bhringraj by Kumar and Mishra (2019). They also found a decreasing pattern of the rate of germination with increasing doses of irradiation. Rifnas *et al.* (2019) also reported a progressive reduction in the germination of *Calotropis gigantea* seeds with increasing doses of gamma. The same pattern of the rate of germination *Zea mays was* reported by Yadav *et al.* (2015).

Survivability was the second important thing for the growth and development of the plants, which showed the same pattern as of rate of germination. The survivability of the plants also decreased with increasing doses of gamma irradiation. Reduced survival rate at higher mutagenic levels has been attributed to various factors, such as chromosomal damage leading to meiotic arrest (Khursheed *et al.,* 2008). Kumar and Singh, 2020, also reported that at metabolic levels, higher doses of gamma rays may disrupt chloroplast membrane and metabolism, due to which photosynthesis was affected which ultimately reduces survivability and causes the death of the plants.

The trend observed in plant height indicated that lower doses of irradiation were beneficial and resulted in more favorable responses. Similar findings have been reported by Kumar and Mishra (2020) in Bhringraj and Kumar & Singh (2020) in Artemisia annua. Ali *et al.* (2016) have proposed that lower doses of gamma rays can stimulate plant growth by enhancing the antioxidative capacity of cells or by modulating hormonal signalling. Wi *et al.* (2007) put forward a hypothesis suggesting that lower doses of gamma irradiation can induce growth by influencing hormonal activities in plant cells or by bolstering antioxidant defences, enabling plants to better cope with daily stress factors such as temperature fluctuations and light intensities.

In contrast, higher doses of irradiation were found to have inhibitory effects on plant growth, potentially attributed to disturbances in hormonal balance, leaf gas exchange, water exchange, and enzyme activity (Kiong *et al.,* 2008). These factors may lead to a reduction in internodal length due to insufficient water and mineral supply to the plants. Additionally, as the radiation dose increases, the rate of DNA mutations also increases, which can disrupt bud development and interrupt cell differentiation, ultimately inhibiting plant growth (Ali et al., 2016). The increase in inflorescence size could potentially be attributed to the same underlying consequences described by Wi *et al.* (2007) and Ali et al. (2016).

Chlorophyll mutants reported (Fig-3) viz., semi-xantha, Xantha, semi-Albina, and Albina were observed in the gamma treatment. Fig-3 (I) Xantha wasstraw-coloured yellow leaves(Arul Balachandran and Mullainathan, 2009). Chlorophyll mutant, Albina (Fig. 3D) was pale dull white color and its lifespan (10-20 days) was comparatively shorter than usual leaf (Kumar *et al.* 2022). Several authors have reported the occurrence of different types of chlorophyll mutations such as Xantha, Albina, Viridis, Chlorine, etc. (Kolar *et al.,* 2011; Arisha *et al.,* 2015; Verma *et al.,* 2018). The reason behind these chlorophyll mutants and the genes and proteins involved in it was still a matter of research (Ahumada-Flores *et al.,* 2021).

In the case of cytological observations, the major abnormalities recorded were stickiness, scattering, precocious movement, unorientation, asynchronisation, etc. These chromosomal abnormalities in the PMCs are attributed to the change in their organization, imbalance in their protein signaling, and disturbance in microtubule formation and functioning. The inhibitory effect on the cell cycle of gamma irradiation at higher doses has also been reported earlier in *Hordeum vulgare* by Eroglu *et al.* (2007), *Allium cepa* by Ahirwar, (2015), and *Triticum aestivum* by Borzouei *et al.* (2010).

The abnormalities observed in the experimentation may be attributed to numerous problems related to spindle fibers, microtubules, histone proteins, and a few other chromosomal proteins. The major chromosomal anomalies viz. Stickiness (Fig. 4 D, F, H) in the chromosomes arise due to an imbalance of spindle fibres caused by mutagenic treatment (Jabee *et al.,* 2008). Gaulden (1987) predicted that the stickiness in chromosomes has been seen due to the malfunctioning of one or two types of specific non-histone proteins which are responsible for the proper organization and compaction of chromosomes. In one more study, the sticky chromosomes resulting in the cells may be due to the increased chromosomal contraction and condensation [Ahmed and Grant (1972)]. In a study, Kuras *et al.* (2006) observed that this abnormality of chromosomes was the outcome of an imbalance in histones or other proteins which can control the proper structure of nuclear chromatin.

Precocious movement (Fig. 4 I) in chromosomes at metaphase was observed because of the migration of chromosomes to the poles, which may be attributed to early chiasma terminalization in diakinesis or metaphase I (Srivastava and Kapoor, 2008) or due to disruption of spindle formation (Kumar and Dwivedi, 2015). Precocious movement noted in the cells of experimental plants may be due to early terminalization of the chromosome or due to the chemical breaking of the protein moiety of the nucleoprotein backbone. (Kumar and Pandey, 2017)

Levan (1938) studied that the scattering of chromosomes (Figure 4G) was a result of the problematic function of spindle fibers and was generated in the process due to the loss of microtubules, disruption of the spindle fibers, etc.in *Allium cepa* L.

Unorientation in the cells may be due to the disturbed microtubule orientation or disturbed polarity of the cells. Disturbed polarity (Fig. 1K) or tri polarity might be due to spindle disfunctioning (Kumar and Dwivedi, 2012).

Declinement in pollen fertility was because of the formation of sterile pollen due to the side effects of mutagens on male reproductive organs. Pollen viability was considered to be an important parameter of pollen quality (Dafni and Firmage, 2000). Pollen sterility was also a major finding reported in the study. Here it was found that the rate of pollen sterility was increasing with increasing doses of irradiation which ultimately leads to non-viable pollen formation and creates a danger over the survival of the genotypes of the plants in the system. Muthusamy &Jayabalan (2002) also reported the same in the *Gossypium* plant. They reported that the rate was directly proportional to the irradiation dose. Kumar and Singh (2020) also reported the same pattern in *Artemisia annua*. Jagtap & More (2014) have analyzed the same output on *Lablab purpureus* and concluded that sterility in the plants enhances vigorously as the dose of physical or chemical mutagen increases.

Biochemical studies revealed the same pattern of decline meant as germination, survival, and pollen fertility. Here, major photosynthetic pigments including Chl*a* & Chl*b* have shown a narrowing from the control plant's mean value of photosynthetic pigment. A sharp deterioration in the amount of both the photosynthetic pigments (Chl a &Chl b) was observed. Carotenoids, the accessory photosynthetic pigment have also shown the same phenomenon of decline, such as main photosynthetic pigments. These findings may be attributed to the hazardous nature of high-energy gamma rays. Gamma radiation alters photosynthetic apparatus by damaging the photosystem complexes, but at lower doses of gamma rays these complexes allow photosynthesis by capturing light energy, protect photo-oxidative damage of chlorophyll from ROS, and release excess energy as heat (Kovacs and Keresztes, 2002; Kim *et al.*, 2004). Jing *et al.* (2008) also found that photosynthetic pigments decreased in gamma-treated sets compared to the control set. Kumar and Singh (2020) also proposed that gamma irradiation was detrimental to the photosynthetic pigments of Bhringraj and the effect was strengthened with increasing doses of gamma irradiation.

5. Conclusion

In terms of cytology, the study revealed a clear association between increasing doses of irradiation and the occurrence of numerous cytological aberrations. It was noteworthy that the percentage of total abnormalities increased with higher doses of gamma radiation. However, alongside the adverse effects, lower doses of irradiation also provided an opportunity to generate new mutant varieties of the plant with enhanced genetic variability and improved vigor compared to the parent plant. This offers the potential to broaden the narrowed genetic base of the plant caused by continuous inbreeding depression.

Overall, these findings highlight the complex effects of gamma-ray irradiation on plant traits, with lower doses showing potential for beneficial changes in morphology and genetic variability, while higher doses induce detrimental effects across multiple aspects of plant development and productivity.

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