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ORIGINAL ARTICLE **OPEN ACCESS**

Assessment of Gamma irradiation induced genetic variability in *Lepidium sativum* Linn.

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ABSTRACT
Mutation breeding is one of the oldest methods in plant breeding programs. Mutagenic effectiveness and efficiency are the most important factors to determining the success of mutation breeding. It is coherent tool for quickly enhancing the genetic makeup of any crop. The purpose of the study is to explore the mutagenic effects of ionizing radiation (Physical mutagen) on Garden cress (Lepidium sativum Linn.) to create a genetic variability with most influential mutagen that is gamma radiation. The seeds were irradiated at four different doses of gamma rays that is 150Gy, 300Gy, 450Gy and 600Gy respectively along with control ones to study the effect of mutagen on root meristem of Lepidium sativum Linn. The remarkably effect can be seen on the parameters such as germination percentage, survival percentage and root length due to the exposure of seeds to the gamma radiation. The Active Mitotic Index found to be decreased with the increased gamma ray dose and considerably increase in Total Abnormality percentage with the increasing dose. The various cytological abnormalities were also observed in irradiated seed including stickiness, laggard formation, scattering, bridge formation, loop formation etc. and the frequency of stickiness was highly observable in mitotic slides.
Keywords: Active mitotic index, Cytological anomalies, Gamma rays, Mutation, Lepidium sativum Linn, Total Abnormality Percentage.

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INTRODUCTION

Mutagenesis is an outcome of mutations in DNA molecules that leads to a permanent change in a DNA sequence. Mutation in DNA occur due to error in DNA replication, exposure to ionizing radiation, exposure to chemicals called mutagens. Mutations are of various types that can be Spontaneous and induced [1]. Spontaneous change can occur spontaneously due to 'mistakes' in DNA replication or mitosis whereas induced mutagenesis require mutagen that can be physical, chemical, and biological. Induced mutagenesis has escalated the mutation breeding by developing new varieties with improved agronomic character [2]. It involves treatment of crops with different chemicals mutagens and radiations to produce variants with desirable characters. A well-known example is the development of high yielding variety of barley having a high protein content and stiff straws [3].The utmost mutagen known for inducing high mutation and variability is Gamma radiation that has maximum electron volts[4]. The exposure of radiation produces a various effect that can be direct and indirect. The direct effect generates a water molecule that are ionized and cause H^-, OH^- radicals that react with various macromolecules (Lipids, proteins, DNA) and also leads to cell damage and cell death. These effect leads to the production of reactive oxygen species (ROS) that is $H_2O_2^-$ and O_2-. Different DNA alterations can be arise due to oxidative damage by Roldan-Arjona and Ariza [5]. The major effect is the induction of DNA break leading to the chromosomal abnormality that are harmful for species. According to the World Health Organization (WHO), 80% of the populations relies on herbal plants for medicines and for their basic need. One of a plant with high medicinal value is *Lepidium sativum* Linn. (2n=16), commonly known as "garden cress" edible herb growing to height of 50 cm belongs to Brassicaceae family enriched in high medicinal value with a curative property for the treatment of asthma, tumors in uterus, hemorrhoidal hemorrhage, coughing, wounds etc. Paranjape and Mehta [6]. elucidated the usefulness of garden cress in the form of traditional tonic to increase height of children, to increase the milk content in female. Sushruta, in Ayurveda, it is being used in bone healing. To meet the demand of the natural products, there is a huge exhaustion of herbs, therefore induced mutagenesis is a replenishing technique to increase the productivity of a crop including the development of the desired new traits with the improvement of the qualitative and quantitative traits. Treatment with ionizing radiations might be beneficial and of vast

economic importance. There are many studies that proves mutation breeding in improving crop improvement. Shah et al., [7] developed a new oil seed *Brassica napus* L cv. ABASIN-95 by induced mutation. They exposed seeds of *B. napus* L. cv. Tower to 1.0, 1.2 and 1.4KGy gamma rays and the resulting new variety was high yielding, resistant to Alternaria blight and white rust. The aim of the experiment is to study the morphological and cytological effect on different doses of Gamma. Sushruta, in Ayurveda, it is being used in bone healing. Hence the overall positive and negative outcomes to be analyzed with help of morphological and cytological aspects of garden cress after the exposure of gamma irradiation.

MATERIAL AND METHODS

Seed Acquisition

The seeds of the Garden Cress (*Lepidium sativum* Linn.) were obtained from DMAPR of Gujarat center, India.

Treatment and climatic conditions

The designed experiment has been performed in the area of Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Prayagraj, U.P., India, during the Rabi season. The exact experimental location is 25°27"43.01'N, 81°51"10.42'E. Prayagraj is situated 98 above the sea level. It is situated in sub-tropical climate zone with average rainfall 1027 mm rainfall and 59% of relative humidity. Temperature in November is 25ºC. Firstly the fresh seeds of Garden cress were systematically arranged in plastic or poly bag and divided into four group. The fresh seeds of each group were individually irradiated in Floriculture Laboratory of National Botanical Research Institute (NBRI), Lucknow (India) and the source for irradiation with gamma rays was from Cobalt-60. The selected dose for irradiation were 150, 300, 450 and 600 Gy. Secondly for the mitotic study the irradiated seeds with control seeds group with control ones were placed on a purified petri plates on a moistened filter paper in a seed germinator (PLT- 147; model SGSC-1) at optimum temperature of 14ºC and humidity. After 2 days of germination, the germinated seeds with root tips were fixed in fixative solution known as Carnoy's fixative in the ratio of 3:1 of absolute alcohol and glacial acetic acid for preserving tissue for future experiments.

Slide Preparation

For cytological study the slides were prepared in a sequential step. Firstly, the root tips were hydrolyzed in 1N HCL for 2 to 3 seconds in water bath having a maintained temperature at 60ºC. Then the root tips washed with distilled water 3 to 4 times and dried with a blotting paper. After drying the root tips were stained with 2% acetocarmine. After 30 minutes of staining the slides were prepared by excising the dark stained root tips and mounting it with 2% acetocarmine followed by squashing technique [8,9]. For every dose five slides were prepared with control seeds and five microscopic fields were analyzed. The mitotic slides were observed at 40X resolution and suitable stages were captured in Nikon phase contrast research microscope (Nikon Eclipse, E200, Japan) through a PCTV software.

Morphological Analysis

Seeds of treated Garden cress with respect to control were sown in the pots and each pot containing the 20 seeds per treated dose. The parameters such Germination percentage, Active Mitotic Index (AMI%), Total Abnormality Percentage (TAB%) were calculated by using the following formula:

$$\text{Germination Percentage} \quad \frac{\text{No. of Germinated seeds}}{\text{Total no. of seeds}} \times 100$$

$$\text{Active Mitotic Index (AMI\%)} = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cells}} \times 100$$

$$\text{Total Abnormality Percentage (TAB\%)} = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cells}} \times 100$$

Data Analysis

Statistical analysis was performed by using SPSS 16.0 software. A One- way analysis of variance (ANOVA) and Duncan's Multiple Range test DMRT ($p<0.05$) were performed for mean separation. By using Sigma Plot 10.0 software the graphs were plotted. The actual mean and standard error were calculated and the data were subjected to analysis of variance.

RESULTS

The final results showed the Gamma rays effect on somatic cell division and morphological parameters in *Lepidium sativum* Linn.

Cytological

Effect of gamma irradiation on AMI% (Active mitotic index) and TAB% (Total Abnormality Percentage).

The effect of gamma irradiation on a plant was dependent on a dose given to seed. The cytological studies show the highly progressive decrease in active mitotic index (AMI %) as the dose of gamma irradiation increased but TAB % increases with doses dependent manner. The calculated AMI for varying Gamma doses revealed an inverse relation between respected doses and AMI (Fig. 1). In comparison to treated plant there was normal value of active mitotic index that is in untreated plant is 12.52±0.18 and the lowest AMI was observed at 600Gy that is 8.65±0.12. The reduction in AMI may be due to the inhibition of DNA synthesis at S- phase the most probably occur due to the reduced ATP level and the pressure from the functioning of the energy production center [10]. The lowest tab was observed in lowest dose (150Gy) that is 2.79±0.09 and the highest tab was observed at highest dose (600 Gy) that is 5.53±0.13. The AMI and TAB can be summarized from the figure 1. Therefore the result shows the inverse relationship between AMI and TAB%. The resultant reduction in AMI (active mitotic index) that gamma irradiation has a suppressive effect on progressive dose of gamma irradiation.

Effect of gamma irradiation on Chromosome organization

The various chromosomal abnormality % induced by gamma irradiation in abnormal cells of root meristem has been shown in Table 1. The level of aberrant cell was elevated with the concentration of Gamma. The rate of chromosomal aberrations gets elevated from 2.79% (150Gy) to 5.53% (600Gy), respectively. Chromosomal aberrations such as stickiness, unorientated chromosomes, loop formation, laggards, precocious movement, bridges were observed which has been shaped in Fig 2.

Morphological

Gamma irradiation effect on seed germination and survivability percentage: The effect of gamma irradiation shows the delayed seed germination at 150Gy, 300Gy, 450Gy, 600Gy dose as compared to control plant. The seed germination in control plant was found to be 94.6± 1.33%. Whereas seed germination decreases with increasing dose of mutagen. The lowest value of germination % was observed at 600Gy (56.0 ±2.30%) (Figure3). The similar result were also observed in *Triticum aestivum* Linn reported by Azam Borzouei et al. [11]. Data of survivability percentage has showed that the higher dose has negatively affected the germination percentage. This result displays the compelling effect of gamma irradiation treatment on survivability % of *Lepidium sativum* Linn. (Figure 3). All treated seed (150, 300, 450 and 600Gy) shows the reduced survivability percentage as compared to control.

The result of gamma rays on shoot and root length was remarkably inhibitory as it can be noticeable from data in Figure 4. The seed treated with higher dose (600Gy) produced a dwarf seedling with reduced roots. Value found to be lowest was 0.5 cm at higher dose (600Gy) and highest value found to be at 2.8 cm at lower dose (150 Gy).

Different types of leaf variation were observed due to abnormality caused by chemical mutagen Chlorophyll development seems to be controlled by many genes located on several chromosomes which could be adjacent to centromere and proximal segment of chromosomes [12]. Chlorophyll variation provides the indication of genetic effect due to the effect of mutagen. The similar result has been shown in various pulse crops by Pandey and Dhanasekar, [13]. Gamma rays induces a nuclear gene mutations or extra chromosomal deficient mutations that might result in chlorophyll deficient mutations. Chlorophyll mutations can be used as genetic markers in basic applied research to unlock the effect of different doses of mutagen in the treated plants for viable mutations [14].

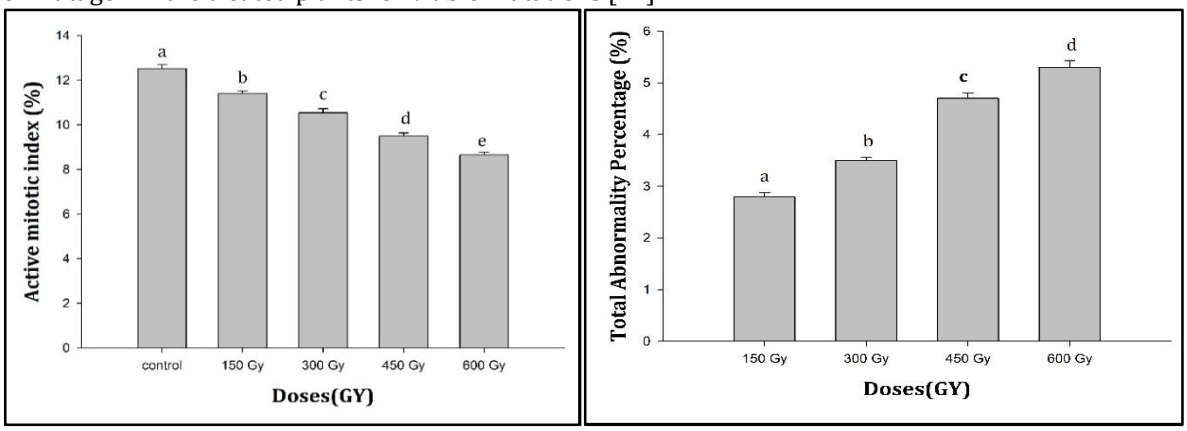

Figure1 (A): Effect of Gamma irradiation on AMI%. 1(B): TAB % in root meristem of *Lepidium sativum Linn.*

TABLE 1: Effect of Gamma irradiation on AMI(%), TAB(%) and various chromosomal anomalies induced by Gamma treatment in *Lepidium sativum Linn.*

Treatment	AMI (%) (Mean ± SE)	Metaphasic abnormalities % (Mean ± SE)				Anaphasic Abnormality % (Mean ± SE)				TAB % (Mean ± SE)
		Sc	St	Un	pr	Sc	St	Lg	Br	
Control	12.52±0.18	-	-	-	-	-	-	-	-	
150Gy	11.40±0.12	1.00±0.00	2.33±0.33	1.67±0.33	1.00±0.00	0.33±0.33	-	0.67±0.33	-	2.79±0.09
300Gy	10.54±0.18	1.00±0.00	2.33±0.33	1.67±0.33	1.33±0.33	0.67±0.33	1.00±0.00	1.33±0.33	-	3.50±0.06
450Gy	9.49±0.14	2.00±0.00	2.67±0.33	2.00±0.00	1.67±0.33	1.33±0.33	1.00±0.00	2.00±0.00	0.67±0.33	4.75±0.10
600Gy	8.65±0.12	2.00±0.00	3.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	1.33±0.33	1.33±0.33	1.00±0.00	5.53±0.13

Sc- Scattering, Pr- Precocious movement, St- Stickiness, Un- Un orientation, Br- Bridge formation, Lg- Laggard formation.

Means followed by lowercase letters are statistically significant at $p < 0.05$ in Duncan's Multiple Range Test.

DISCUSSION

CYTOLOGICAL ANALYSIS

Mitosis is a stage of cell cycle that leads to the quantifiable increase in the organism's cells. From the above studies it can be analyzed that the effect of gamma irradiation seeds significantly suppresses mitotic index and induces chromosomal abnormalities that can be observed through the decrement in active mitotic index with a progressive doses of gamma radiation. The observed reduction in AMI was due to the inhibition of DNA synthesis at S- phase or DNA replication inhibition or inhibition of spindle formation. Blockage or inhibition of the DNA synthesis id due to decreased ATP level and the pressure from the functioning of the energy production center [17, 18] and Control seeds shows the normal distribution of chromosomes at metaphase and anaphase whereas in treated plant there were a chromosomal aberration found at metaphase and anaphase stage that includes inhibitory effect on mitosis due to gamma radiation have also reported by Borzouei et al. [18] and Ahirwar [19]. As gamma radiation has lowest wavelength and highest energy, therefore its penetration power is high causing mutagenic changes severe alterations in living cells. These alterations are the result of the formation of reactive oxygen species (hydrogen peroxide, hydroxyl ions) that cause high functional changes in metabolic activities. The microscopic view shows the normal and abnormal positions of the chromosomes. It can be analyzed from the cytological study that mitosis was normal in the control set. The chromosomal aberrations are lethal but some of them cause genetic effects [20]. The most common and dominant chromosomal abnormality observed is stickiness at metaphase which occurs due to the disturbance in the balance of quantity of histones or other proteins responsible for controlling the proper structure of nuclear chromatin [21]. Another reason of stickiness may be due to the increased contraction and condensation of nucleoprotein leads to the cell death [22]. Precocious movement of a chromosomes seems to be a demonstration of improper spindle functioning. The presence of spindle and multiple bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage fusion bridges cycle [23]. Production of laggard chromosome might be due to the moving speed of the

chromosome differing from normal ones. It may be due to the weak association of chromsomes with spindle fibers. Das and Roy [24] also concluded that due to the consequence of mutagen, spindle fibers were ineffective to carry the chromosomes resulting in lagging chromosome appeared at anaphase. Formation of bridge chromosomes is due to the formation of acentric and dicentric chromosome due to the furrow regression or due to the chromosomal inversion [25].

MORPHOLOGICAL ANALYSIS

On the basis of complete experiment, it can be analyzed that the growth and survivability has been reduced along with the increasing concentration of gamma. There was a postponement of seed germination on higher dose gamma radiation. The forbid effect on seed dormancy causes the delayed effect on seed germination. There was a maximum germination percentage on lowest dose (150Gy) in comparison to control plant that were calculated statistically. In majority of the plant such as chickpea [26]and soyabean [27] has been reported a stimulatory effects of lower doses to seed germination. It can be analyzed that there was a negative effect on survivability % on the higher dose of gamma irradiation. The observed reduction in the survivability of plants is due to the carcinogenic effect gamma radiation. The reduction in the survivability is due to the damage in plant tissues and breakdown of meristematic cells [27]. The LD50 calculated on the basis of seed survivability was observed at the dose of 600Gy. The LD50 is a dose where there is a 50% survivability of a plant. The repressive effect of gamma radiation on Root and Shoot length was more in higher doses (450 Gy and 600 Gy). The mitotic activity got reduced due to reduced root length at higher doses of gamma radiation.

The chlorophyll mutants/variants *viz.,* Aurea (Fig. 5B), Leaflet with Xantha (Fig. 5C) that is straw- colored yellow leaves [28] and Leaf tip Albina were observed in Gamma treatment (Fig. 5). Chlorophyll mutant, Leaf tip Albina (Fig. 5D) is observed with whitish tips of leaves which cause lethal effect and its life span is comparatively shorter than normal leaf (Fig. 5A) [29].

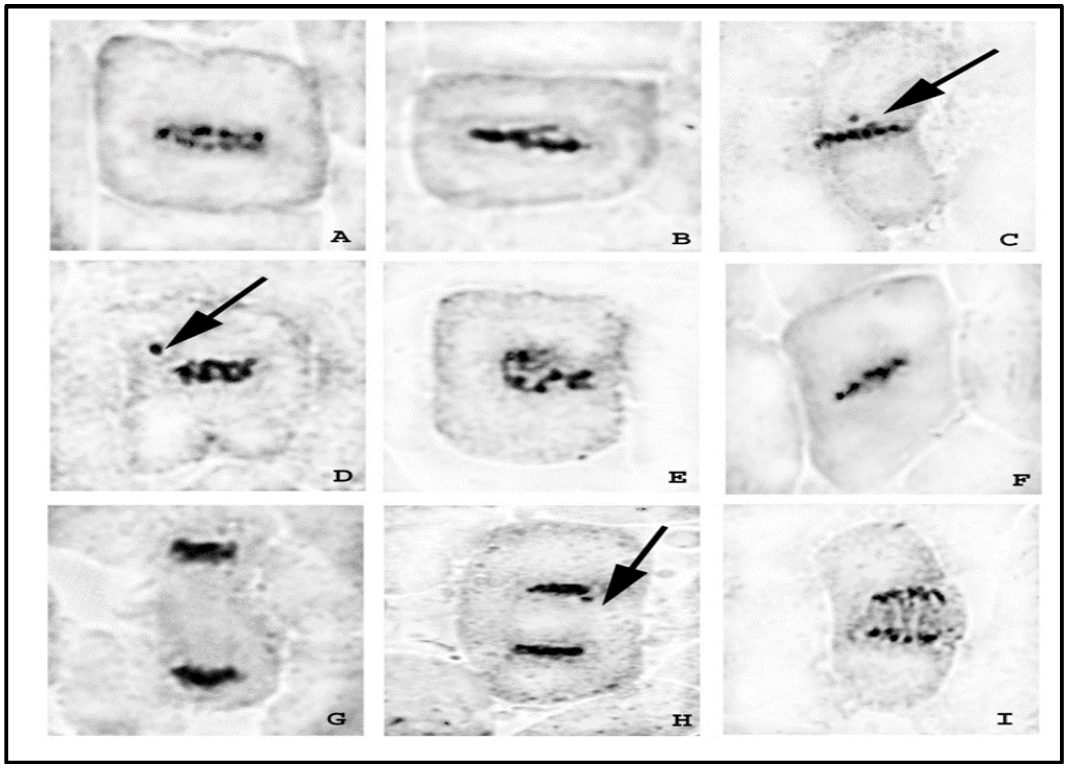

Figure 2: Gamma induced abnormalities in root meristem of *Lepidium sativum* Linn.
Normal Metaphase (2n = 16).
B- Stickiness at Metaphase. C-D- Precocious movement at Metaphase.
E- Loop formation at Metaphase. F- Un orientation at Metaphase. G- Normal Anaphase
H- Laggard formation at Anaphase. I- Bridge formation at Anaphase.

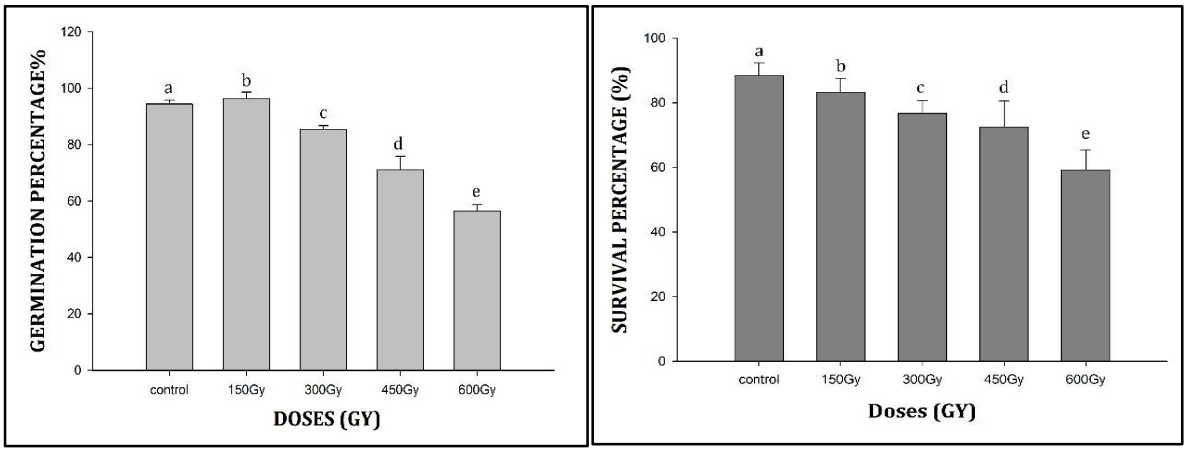

Figure 3: Effect of Gamma irradiation on Germination and Survival % in root meristem of *Lepidium sativum* Linn.

Figure4: Effect of Gamma irradiation on root and shoot length in *Lepidium sativum* Linn.

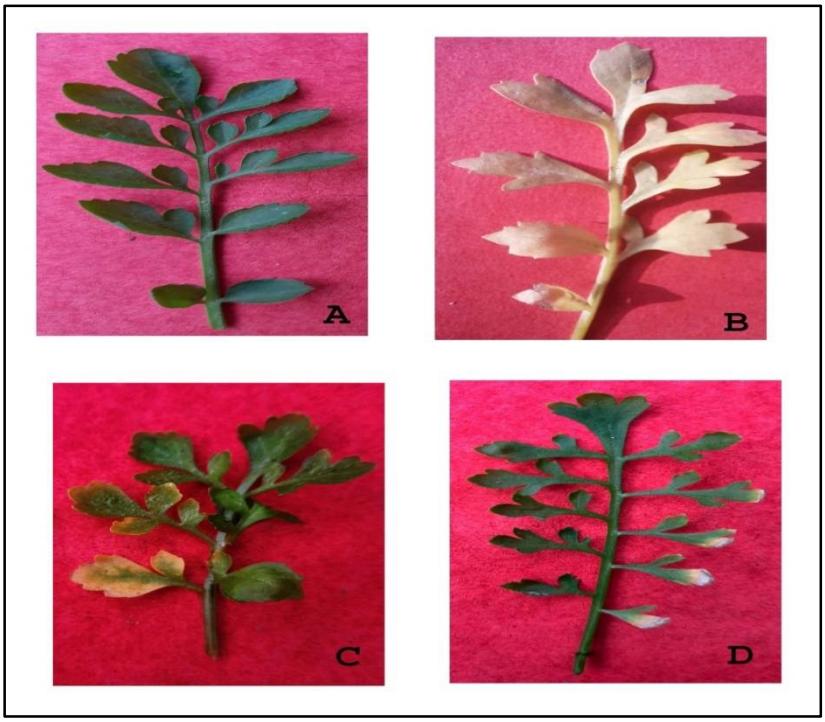

Figure 5: Showing chlorophyll variation (A – Normal; B – Aurea; C – Leaflet with Xantha; D- Alternatively well arranged with Leaf stripes of Albina at tip.) induced by Gamma treatment in *Lepidium sativum* Linn.

CONCLUSION

The above outcome of the experiments indicate that higher dose of gamma shows negative effect and lower dose found to be cause positive effects which may be used for improving the qualitative and quantitative traits of Garden cress to meet the demand of this medicinal plant. Gamma rays shows stimulatory effect at lower dose which influences the genetic variations and creating a new trait.

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