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Comparative Effect of X-Rays and Laser Beam on the Genetic Architecture of *Lathyrus sativus* L.

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Abstract: The cytogenetic effects of two physical mutagens viz. X-rays [150, 300 and 450 Gy] and Laser beam [0.5, 1.0 and 1.5 minutes] were investigated on the root tip cells of *Lathyrus sativus*. Various types of chromosomal aberrations were observed during the cytological analysis of the root tip cells of both the treatments. Bridges and fragments were the pronounced abnormalities noticed in the X-ray treated seeds whereas stickiness was dominant in laser beam treatment. It was found that X-rays were more deleterious showing a marked mito-depressive effect on mitosis of the concerned plant whereas Laser beams were comparatively less effective in damaging the genetic structure.

Key words:

INTRODUCTION

Mutations are used to create variations among the populations that show narrow genetic base. Among various mutations, irradiation is the most promising process to bring out significant variations. It is a process of exposing the crops to carefully controlled amount of energy [1, 2]. X-rays and Laser beams have been found to be very useful both for surgical and medicinal purposes. The effects of irradiation on the meiotic chromosomes were observed by using X-rays on the inflorescence [3], X-rays and U.V. irradiations on pollen of tomato [4]. Some studies of X-rays and Laser beams have been reported on the seeds of wheat [5].

The aim of the present study is to evaluate the effects of these two physical mutagens on grasspea, as it is a very tolerant plant under adverse environmental conditions. The study can further depict the harmful effects of these mutagens on plants as well as humans.

MATERIALS AND METHODS

The experiment was conducted with the healthy seeds of *Lathyrus sativus* var. Pusa-24 which were irradiated separately with two physical mutagens viz. X-rays and Laser beam. For X-rays treatment, seed packets were exposed to X-rays irradiated from the controlled source (at the rate of 1.6 KR/min) at Alka hospital, Allahabad. Selected doses for experiments were 200Gy, 400Gy, 600Gy and 800Gy (Gy-Gray unit). For Laser beam treatment, seed packets were irradiated at the wavelength of 632 nm with helium-neon laser (especially prepared for irradiation of biological materials) in Physics Department, University of Allahabad. The light power of the laser was 24 mW and the intensity of light falling on the seeds was about 1 mW cm-2. Selected durations for exposures were 0.5, 1.0 and 1.5 minutes.These irradiated seeds were kept for germination on moist filter paper in the petridishes separately. Non-irradiated seeds were considered as controls and were maintained separately. Root tips of 1-2 cm in size were fixed in Carnoy's fixative [3:1 alcohol: acetic acid] for 24 hour and then transferred in 70% alcohol. Slides were prepared using chromosome squash technique and desired photomicrographs were taken.

The whole experiment has been performed in three replicates and data in Tables 1, 2 and 3 are calculated by using the following formulae:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated X 100}}{\text{Total number of seeds used}}$$

10 seeds were taken at a time for each germination test.

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$$\text{Mitotic Index (M.I.)} = \frac{\text{Number of actively dividing cells X 100}}{\text{Total number of cells observed (in one view)}}$$

Actively dividing cells are the cells of metaphase and anaphase stage.

$$\text{Total abnormality percentage (T.ab)} = \frac{\text{Number of abnormal cells X 100}}{\text{Total number of actively dividing cells (in one view)}}$$

$$\text{Standard Error of Mean: SE} = \frac{d}{N}$$

Where,

SE = Standard Error

* = Standard deviation

N = Sample size

RESULTS

Germination tests for the irradiated seeds and the controls were made. A decreasing trend was noticed in the germination percentages [Table 1] among the root tips of X-rays and Laser beam treated seeds. Germination frequency recorded in controls was maximum (99.6%±0.15), whereas minimum at the highest doses of X-rays (66.4%±0.14) and Laser beam (70.5%±0.21), respectively.

Mitotic observations made from the root tip cells of control seeds showed normal metaphase [2n=14] and normal anaphase [14:14 separation] [Fig.1 and 2]. Mitotic index was recorded to be 13.6% with no aberrations.

However, the treated root tip cells showed mitotic depression and various types of chromosomal aberrations [Table 2, 3]. Mitotic index in X-ray treated set was found to range from 10.2% to 5.8% and in the Laser treated set, the range was from 11.0% to 7.2% along with the increasing dose [Table 2].

Table 1: Germination percentage of the seeds of grasspea (*Lathyrus sativus)* irradiated with x-rays and laser beam.

STreatment	Dose	Germination Percentage (%) ±S.E.
Control	-	99.6±0.15
X-rays	150 Gy	90.0±0.12
	300 Gy	87.6±0.11
	450 Gy	66.4±0.14
Laser beam	0.5 min	94.5±0.16
	1.0 min	82.6±0.18
	1.5 min	70.5±0.21

Table 2: Mitotic index and frequency of chromosomal aberrations at different stages of division in the root tip cells of irradiated seeds of grasspea.

			Percentage of abnormal cells at different stages			
Treatment	Dose	No. of cells observed	Metaphase (%)	Anaphase (%)	Telophase (%)	Mitotic Index %
Control	-	816	-	-	-	13.6
X-rays	150 Gy	812	6.2	5.8	2.6	10.2
	300 Gy	786	7.1	6.2	3.5	8.5
	450 Gy	757	8.1	7.6	5.0	5.8
Laser beams	0.5 min	802	4.2	3.6	2.0	11.0
	1.0 min	703	6.0	4.1	2.8	8.8
	1.5 min	715	7.2	5.6	3.3	7.2

Table 3: Percentage (%) and nature of different types of chromosomal aberrations in the root tip cells of irradiated seeds of grasspea.

		Different types of abnormalities (%)										
Treatment	Dose	un	pm	sc	fg	lg	bg	us	st	mc	bc	T.ab (%)
Control	-	-	-	-	-	-	-	-	-	-	-	-
X-rays	150 Gy	0.52	-	0.52	1.56	-	1.04	-	-	-	-	4.68
	300 Gy	0.53	0.53	0.53	1.61	1.07	1.61	0.53	1.61	-	-	8.02
	450 Gy	2.01	1.98	3.96	4.95	1.98	4.95	1.98	3.96	0.99	0.99	29.7
Laser beams	0.5 min	0.46	-	0.46	-	0.46	0.46	-	0.90	-	-	2.74
	1.0 min	0.50	1.01	0.50	-	1.01	0.50	-	1.51	-	-	5.03
	1.5 min	1.33	1.33	2.00	1.33	2.00	2.66	3.33	-	1.33	0.66	17.97

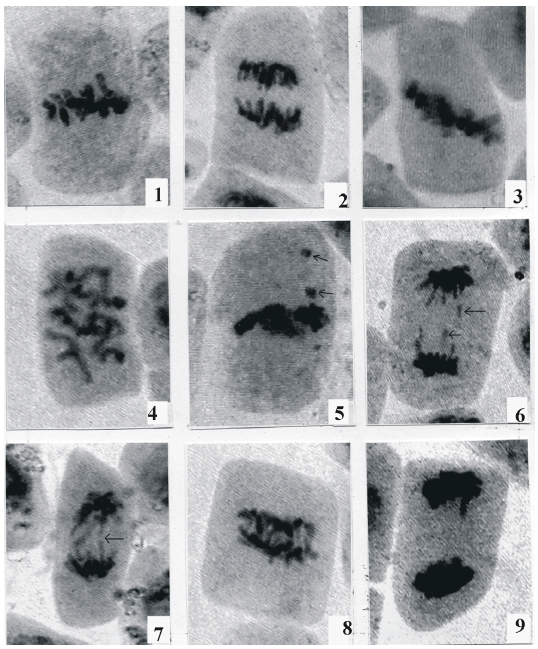

Fig. 1: Normal metaphase (2n=14)
Fig. 2: Normal anaphase (14:14 separation)
Fig. 3: Unorientation at metaphase
Fig. 4: Scattering at metaphase
Fig. 5: Precocious movement at metaphase
Fig. 6: Laggard at anaphase
Fig. 7: Laggard and incomplete bridge at anaphase.
Fig. 8: Multiple bridges at anaphase
Fig. 9: Stickiness at anaphase

The most common visible chromosomal irregularities were unorientation [Fig. 3], scattering [Fig. 4] at metaphase, bridges [Fig. 8], stickiness [Fig. 9], etc. at anaphase in both the treatments. However, the frequency of fragments and scattering was found to be more in X-ray treatment while stickiness and bridges were dominating in Laser beam treatment [Table-3]. Moreover their relative frequencies were dose-dependent. Other chromosomal aberrations were precocious movement [Fig. 5], laggards [Fig. 6, 7], disturbed anaphase, bi-nucleated cells, etc. in both the treatments.

DISCUSSION

Internal and external factors, including environmental pollutants induce various kinds of genetic damages. These lead to the disorders in the generational and regulational functions of the genetic apparatus that cause much pathology and diseases in plants, animals and humans [6].

Reduction in germination can be attributed to inhibitory effects of mutagens on physiological and biological processes necessary for germination. It includes enzyme activity, hormonal imbalances and changes in the balance of growth regulators.

A regression was found in the mitotic index along with the increasing doses of the X-rays and Laser beams. Similar results were obtained after the treatment of different physical mutagens on *Tradescantia* [7], barley [8], *Lens* [9], *Cicer* [10], Soybean [11], *Lathyrus sativus* [12-14].

These chromosomal irregularities can affect the vigour, fertility, yield or competitive ability of the exposed crops [15]. They are the indicators of the clastogenic effects of their inducers [16]. The inhibition of spindle formation has also been shown to lead to severe abnormalities such as stickiness, unequal distribution, bridges, laggards, etc [17]. Similar results were obtained while studying the effects of age and X-rays on *Allium* [18] and on hexaploid wheat using X-rays, Laser rays and U.V. radiations [5].

On the basis of these results, it can be concluded that both the physical mutagens (X-rays and Laser beams) are capable of inducing chromosomal aberrations in the concerned plant, but X-rays are much more chromotoxic as well as cytotoxic than Laser beams, since they induce a greater percentage of abnormalities. Mutagenic data from the plant assays are thus, very important for genetic research and may serve as the basis of means for maintaining a stable ecosystem.

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Legends of Table 3- un-unorientation, pm-precocious movement, sc-scattering, fg-fragmentation, lg-laggard, bg-bridge, us-unequal separation, st-stickiness, mc-micronuclei, bc-binucleate cell, T.ab-Total abnormality.

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