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Original Research Article

Biostimulating effect of laser beam on the cytomorphological aspects of *Lathyrus sativus* L.

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Abstract: The pure inbred seeds of grass pea (*Lathyrus sativus* L. var Pusa 24) were irradiated with three different doses of laser beams. For the experiment, a helium-neon laser (He-Ne) with the wavelength of 632.8 nm and power density - 1 mWcm-2 was chosen. In theM1 generation, stimulation as well as reduction in morphological and yield-contributing traits was estimated. The results showed that in the M1 generation, lower doses of laser beams (0.5 and 1.0 min) induced biostimulation effect of the analyzed traits. Highest dose (1.5 min) induced reduction of the traits value in comparison to the control. The progeny obtained in M2 generation was comparatively better than M1 progeny for the same traits. Apart from morphological traits, cytological analysis exhibited a number of chromosomal aberrations in both the generations. Stickiness of chromosomes was recorded as one of the prevalent abnormality. The results obtained show that the helium-neon laser with the wavelength of 632.8 nm can be an effective tool for increasing variability of agronomic traits in grass pea.

Keywords: *Lathyrus sativus* L. var Pusa 24, laser beams, M1, M2 generations, biostimulation effect,

Introduction

Induction of mutations by radiations or chemical mutagens increases the spontaneous mutation rate manifolds and thus, also increase the chances of obtaining rare characters. The major benefit with mutagenesis is that each time newer traits may be introduced through it, in high-yielding commercial cultivars. During the last two decades researches on mutagenesis have expanded rapidly to include site-directed mutagenesis in its realms. Although very promising, this technique needs much further studies to produce desirable mutants in one generation, on mass scale. The polygenic traits viz., grain yield, maturity time, grain quality; stress tolerance and disease resistance have been improved in various crops by mutagenesis (Kharakwal, 1996).

In the beginning of 1960, the first work on the application of ruby laser beams in biology was published (Bessis *et al.,* 1962). The above gave inspiration to many investigators to use laser to induce mutation (Dudin, 1990). Other investigators showed, that although longer irradiation with laser beams damages genetic cell material, lower doses exert a biostimulative effect (Koper, 1996) markedly higher than low doses of chemomutagens (Adamska and Ma³uszyñski, 1983).

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Grass pea (*Lathyrus sativus*) commonly known as khesari in India, is an ancient cultigen. It is cultivated as a major crop in India, Nepal, China and Pakistan and is known as "poor man's diet" with remarkable adaptation to extremes of drought, waterlogging, temperature, moisture, etc. and requires low inputs for its cultivation. Besides this, it is highly palatable with rich source of protein (28-32%) and lysine content.

Therefore, the present study is an attempt to induce and screen out the morphogenetic changes occurring due to laser beam in *Lathyrus sativus* L.

Materials and Methods

Pure and inbred seeds of *Lathyrus sativus* var. Pusa-24 were obtained from IIPR, Kanpur. Seed packets were made for the exposure of laser beam to different time durations. Seeds of *Lathyrus sativus* var. Pusa-24 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 24mW and the intensity of light falling on the seeds was about 1mW cm-2. Selected durations for exposure were 0.5, 1.0 and 1.5 minutes. Un-irradiated seeds were treated as control for the comparison with mutagen (laser beam) treated population.

Raising of First/M1 Generation: After the treatment, these treated seeds were sown in earthen pots. These earthen pots contained equal amount of soil manured with dried and decomposed cow dung. The sowing was performed in 2 replicates for each duration of treatment. Along with all the treated sets, control seeds were also sown in replicate corresponding to each treatment for the comparative analysis. All the morphological and cytological parameters were assessed.

Raising of Second/M2 Generation: Seeds from randomly selected plants of M1 generation were sown in the next season. Seeds were again sown in 2 replicates in earthen pots to raise M2 generation. All the morphological and cytological parameters were assessed.

Cytological Analysis: For the fixation, commonly used fixative is Carnoy's fixative (3:1 Absolute alcohol: Acetic acid). At the time of flowering, young floral buds of 10 randomly selected plants from each treated set were fixed in Carnoy's fixative and stored for 24h and then preserved in 70% alcohol at 4ºC. Acetocarmine **(**2%) was used for the staining of chromosomes for the cytological analysis.

Results

Morphological analysis

Data of all the morphological parameters from randomly chosen plants at each treatment dose was recorded. This was studied in first and second generations with reference to growth parameters, fertility parameters and the yield parameters.

Efficiency of treatment was estimated by analyzing the percentage of seeds germinated and plants survived from the population of sown seeds. Germination percentage was observed and recorded after 10 days of sowing, taking into account that upto this time all the potential seeds must have germinated.

$$\textbf{Germination (\%)} = \frac{\textbf{Number of normal seedlings X 100}}{\textbf{Total seeds used for germination test}}$$

Survivability was the next parameter, which was studied. This was observed after 21 days of sowing. Survived plants were recorded in form of survival percentage.

Germination and survival:

M1 generation: The germination and survival in control and laser beam treated seeds of *Lathyrus sativus* were evaluated and have been presented in Table 1 and 2, respectively. The germination and survival percentages of control plants were 95.40% and 93.80%, respectively. Whereas, laser beam treated seeds surprisingly displayed a noteworthy enhancement over the controls (Table 1 & 2). The maximum germination (96.0%) was observed at lowest dose (0.5 min) and minimum (58.33%) at the highest dose of treatment (1.5 min). The survival percentage was also recorded as 94.89% at lowest dose, which gradually decreased to 56.80% at highest dose of treatment.

Table.1: A comparative account of germination (%) due to laser beam in M_1 and M_2 generations in Grass pea.

Treatment	M_1 generation			M_2 generation		
	Mean	S.E.	C.V.	Mean	S.E.	C.V.
con	95.40	0.31	0.75	96.12	0.31	0.75
L 0.5	96.0	3.60	18.99	96.80	0.00	1.75
L 1.0	78.63	1.19	3.19	79.0	0.80	1.99
L 1.5	58.33	0.76	2.43	60.54	0.83	2.28

Table.2: A comparative account of survival (%) due to laser beam in M_1 and M_2 generations in Grass pea.

Treatment	M_1 generation			M_2 generation		
	Mean	S.E.	C.V.	Mean	S.E.	C.V.
con	93.80	1.00	2.51	95.13	0.27	0.69
L 0.5	94.89	2.08	20.33	97.31	0.00	1.76
L 1.0	75.34	1.21	3.35	78.92	0.79	2.06
L 1.5	56.80	0.90	3.22	59.32	0.88	2.75

M2 generation: The seeds collected from M1 generation were sown to raise M2 generation and data regarding germination and survivability with respect to control were examined. Table 1 & 2 gives a comparative account of germination and survival percentages as percent of control in M1 and M2 generations. The germination and survival percentages in M2 generation demonstrated a slight increment over the respective doses in the M1 generation at almost all doses.

Morphological parameters

A comparative data of morphological parameters in M1 and M2 generation has been presented in Table.3 to 7.

1. Plant height (cm)

The mean height of the control plants in M1 generation has been recorded as 55.8 cm. The treated plants exhibited wide range of mean plant height both higher and lower values as compared to that of the control plants. In M2 generation, the mean plant height varied greatly. The mean plant height recorded for control plants was 56.2 cm which was greater in comparison to the M1 generation (table.3 and plate. A).

Table.3: A comparative description of plant height (cm) due to the treatment of laser beam in *Lathyrus sativus* L. for both M_1 and M_2 generations.

Treatment	M_1 generation					M_2 generation				
	Mean	Min	Max	CV	SE	Mean	Min	Max	CV	SE
con	85.00	84.00	86.00	1.176	0.577	87.33	86.00	89.00	1.749	0.881
L 0.5	83.00	82.00	84.00	1.204	0.577	85.00	84.00	86.00	1.176	0.577
L 1.0	86.66	85.00	88.00	1.762	0.881	87.00	86.00	88.00	1.149	0.577
L 1.5	90.66	89.00	93.00	2.295	1.201	89.00	88.00	90.00	1.000	1.123

Plate.A: Morphological plate showing the effect of laser beam on the plant height of Lathyrus sativus L. at different durations of treatment.

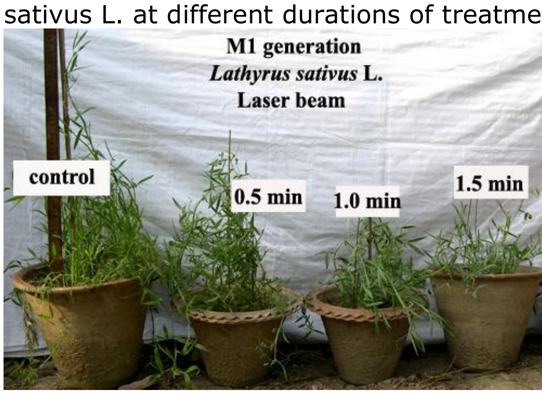

Table.4: A comparative description of days to flowering due to the treatment of laser beam in *Lathyrus sativus* L. for both M_1 and M_2 generations.

Treatment	M_1 generation					M_2 generation				
	Mean	Min	Max	CV	SE	Mean	Min	Max	CV	SE
con	55.83	55.20	56.50	1.165	0.375	56.23	56.10	56.40	0.271	0.088
L 0.5	54.43	54.30	54.60	0.280	0.088	55.40	55.20	55.60	0.200	0.361
L 1.0	50.33	50.10	50.60	0.499	0.145	52.83	52.70	53.00	0.152	0.289
L 1.5	44.50	44.30	44.70	0.449	0.115	50.10	49.90	50.30	0.200	0.399

3. Days to maturity:

In M1 generation, the mean value of days to maturity of control plants was recorded as 147.0 days. The treated sets exhibited increased maturity period as compared to that of control plants. Maximum maturity period in laser treatment was 148.3 days at 1.5min treatment duration.

In M2 generation, the mean values of days to maturity were increased at all the treatment doses as compared to the M1 generation. In the control set it was observed to be 148.00 days. While it reached to as high as 146.33 days at 0.5min treatment (table.5).

Table.5: A comparative description of days to maturity due to the treatment of laser beam in *Lathyrus sativus* L. for both M_1 and M_2 generations.

Treatment	M_1 generation					M_2 generation				
	Mean	Min	Max	CV	SE	Mean	Min	Max	CV	SE
con	147.00	146.00	148.00	0.680	0.577	148.00	147.00	149.00	0.675	0.577
L 0.5	142.00	141.00	143.00	0.704	0.577	141.00	140.00	142.00	0.709	0.577
L 1.0	143.33	141.00	145.00	1.452	1.201	143.00	142.00	144.00	0.699	0.577
L 1.5	148.33	147.00	150.00	1.029	0.881	146.33	145.00	148.00	1.043	0.881

4. Number of pods per plant:

The mean values of number of pods per plant were recorded as 57.0 in control plants. However in the treated sets, maximum number of pods per plant (54) was evaluated at the lowest dose 0.5 min dose. In M2 generation, the mean values of number of pods per plant were increased at all the treatment doses as compared to the M1 generation. In the control set it was observed to be 58.66 (table-6).

Table.6: A comparative description of number of pods per plant due to the treatment of laser beam in *Lathyrus sativus* L. for both M_1 and M_2 generations.

Treatment	M_1 generation					M_2 generation				
	Mean	Min	Max	CV	SE	Mean	Min	Max	CV	SE
con	57.00	56.00	58.00	1.754	0.577	58.66	58.00	59.00	0.984	0.333
L 0.5	54.00	53.00	55.00	1.851	0.577	54.00	53.00	55.00	1.851	0.577
L 1.0	51.00	50.00	52.00	1.960	0.577	50.00	49.00	51.00	2.000	0.577
L 1.5	46.66	45.00	48.00	3.273	0.881	47.00	46.00	48.00	2.127	0.577

5.100 seed weight (gm):

Evaluation of 100 seed weight for control and treated sets displayed wide spectrum of variation. The control set exhibited seeds yield per plant as mean of 58.26gm. However, treatment doses exhibited lower mean values of seeds yield/plant than the control set. The treated set showed a reduction in seed yield as 53.30gm from lowest dose to 42.6gm at highest dose.

The seed yield per plant in M2 generation for control set was enhanced from that of M1 generation. The seed yield of all the treated sets was found to be higher or nearer to the respective mean values of M1 generation. The mean seeds yield/plant in this generation of control plants was recorded to be 59.20gm while, minimum seed count was recorded at 1.5 min treatment dose which was 44.43gm (table-7).

Table.7: A comparative description of 100-seed weight (gm) due to the treatment of laser beam in *Lathyrus sativus* L. for both M_1 and M_2 generations.

Treatment	M_1 generation					M_2 generation				
	Mean	Min	Max	CV	SE	Mean	Min	Max	CV	SE
con	58.26	58.10	58.50	0.357	0.120	59.20	58.70	60.00	1.182	0.404
L 0.5	53.30	53.10	53.60	0.496	0.152	54.33	53.90	54.80	0.829	0.260
L 1.0	50.36	50.10	50.60	0.499	0.145	51.80	51.60	52.00	0.386	0.115
L 1.5	42.60	42.10	42.90	1.023	0.251	44.43	43.90	44.80	1.063	0.272

Cytological analysis:

M1 generation: A wide spectrum of chromosomal aberrations was encountered in all of the treated sets which have been tabulated in table- 8 and 9. Control sets illustrated perfectly normal behaviour of chromosomes with 7 bivalents at diakinesis and metaphase – I and 7:7 separation at anaphase – I. In the treated sets substantial increase in abnormality percentage was recorded. A dose based increase in meiotic abnormalities was observed in all the treated sets.

Table.8: A comparative account of different cytological abnormalities & total abnormality (%) induced by laser beam in M_1 generation in Grass pea.

Treatment	Metaphase I/II abnormalities (%)							Anaphase I/II abnormalities (%)						Tab%	Tco
	St	Pm	Fg	Un	Mv	SA	Oth	St	Lg	Bg	Us	Mp	Oth		
Con	-	-	-	-	-	-	-	-	-	-	-	-	-	-	869
L 0.5	0.91	0.71	0.2	0.4	0.3	0.1	0.3	0.81	0.5	0.4	0.3	0.21	0.81	5.95	981
L 1.0	1.47	1.16	0.73	1.79	1.05	1.79	2	1.37	1.16	0.73	0.52	0.63	2.11	16.51	946
L 1.5	2.7	1.93	1.83	1.44	1.06	1.83	1.44	1.54	2.02	1.35	1.64	1.25	2.02	22.05	1035

Table.9: A comparative account of different cytological abnormalities & total abnormality (%) induced by laser beam in M_2 generation in Grass pea.

Treatment	Metaphase I/II abnormalities (%)							Anaphase I/II abnormalities (%)						T Ab %	Tco
	St	Pm	Fg	Un	Mv	SA	Oth	St	Lg	Bg	Us	Mp	Oth		
Con	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1127
L 0.5	0.37	0.15	-	0.22	-	-	0.15	0.20	0.07	-	0.07	-	0.22	2.23	1341
L 1.0	0.63	0.24	0.16	0.31	0.16	0.24	0.3	0.39	0.31	0.08	0.24	0.16	0.24	3.45	1274
L 1.5	0.96	0.40	0.48	0.56	0.40	0.32	0.48	0.88	0.64	0.32	0.56	0.24	0.72	8.68	1255

Various chromosomal abnormalities such as stickiness, univalents, multivalents, precocious movement, secondary association, laggards, bridges, non-synchronous division etc. were present in high frequency. Whereas, disturbed polarity, multi-polarity, unorientation, fragmentation, micronuclei, unequal separation etc. occurred at lower rate.

A sudden elevation in total abnormality percentage was depicted from 5.95% at lowest dose of 0.5 min to as high as 22.05% at highest dose of 1.5 min. All of the chromosomal aberrations were significantly present at all of the doses. High degree of stickiness was present both at metaphase I/II and anaphase I/II with high percentage. This was the most common aberration with 2.7% frequency at metaphase and 1.54% at anaphase at the highest dose of 1.5 min.

M2 generation:

In this generation also similar types of aberrations were observed but a drastic reduction in percentage was recorded as compared to M1 generation. The frequency of aberrations was recorded as 2.23% at lowest dose and 8.68% at highest dose. Stickiness was still the most prevalent abnormality observed in all the treatments.

Discussion

Induced mutagenesis has now been accepted as a significant tool to break through the limitations of variability and to create variability in a short period of time (Yaqoob and Rashid, 2001; Akgun and Tosun, 2004; Thanga Hemavathy and Raja Ravindran, 2005; Eroglu, 2007). Since 1960s, several collections of mutant lines from different species have been isolated and successfully used in many different areas of plant biology and crop breeding. The main advantage of mutation breeding is the potential for improving one or a few characters without changing the rest of the genotype. Success, however, depends on controlling and directing the induced mutation process for the production of desirable mutations.

Improving the criteria of crop plants by applying physical factors such as laser has the advantage over other physiological and chemical methods which are currently used for this purpose. Seed dressing with various growth regulators, plant hormones, fertilizers etc. are currently considered the most efficient, the best recognized and the most often used practice. However, such substances may modify the chemical structure of the treated seeds, pollute the soil and pose a great danger to the environment. Therefore, more attention has been paid to study physical factors that favorably improve cultivated plants (Barbatni, et al*.*, 2007; Perveen et al*.*, 2010).

Laser is considered one of the physical methods that can be safely applied to improve the quality and yield of crop plants (Inyushin, et al*.*, 1981; Ivanova, 1998; Koper, 1994; Podleoeny, 2002). Laser treatment of seeds was found to achieve biostimulation in many aspects of plant growth and development as it enhanced the rate of germination, seedling growth, in some cereals (Inyushin et al., 1981; Koper, 1994). Treated plants were also less susceptible to disease and other unfavorable environmental conditions (Chen et al., 2010).

Weber, et al*.* (1990) stated that laser primarily resulted in the perforation of the plant cell wall and consequently facilitated the internalization of nutrients essential for plant growth and development; which is likely the apparent reason for the increased growth in the treated plants.

This conclusion is consistent with those of previous studies (Rybiñski and Garczyñski, 2004; Chen et al., 2005; Chen, 2008; Cwental, et al., *2010)* dealing that laser induces large number of morphological and physiological changes in higher plants. In those studies, exposure to laser beam had shown a linear increase in growth, including dry mass gain, leaf size and consequently the yield of many crop plants (Cwintal and Olszewski, 2007; Cwintal, et al., 2010**)**.

Biostimulation effect is induced by many chemical compounds (Powell and Matthews, 1988) as well as different sources of radiation (Olechowiak and Dziamba, 1994; Podleoeny, 1997; Sodkiewicz *et al.,* 1995). The laser offers a pure ecological source of energy that ensures high yield. Using laser beams Bljandur (1987) obtained new, high yielding maize mutants with markedly improved earliness. The laser mutants were successfully introduced in the breeding programme in China (Wang, 1991; Xu, 1988; Zhu, 1988). The results obtained showed that He-Ne laser may be an effective tool for inducing biostimulation processes as well as broader genetic variability of traits in grasspea.

The results obtained showed two different effects-biostimulation and reduction of the analysed traits. Biostimulation was observed for the seeds irradiation with short exposure to the laser (0.5min). Similar effect was noticed after laser application in the study on lupin (Podleoeny, 1997), french bean (Szyrmer and Klimont, 1999), beetroot (Koper, *et al.,* 1996), maize (Lipski and Koper, 1997], faba bean (Podleoeny, 1997), grasses (Sawicki, 1995), soybean (Plesnik, 1993) and tomatoes (Koper, 1997). The increase in germination percentage might have been due to the effect of mutagen on meristematic tissues of the seed.

The induction of cytological disturbances in the meiotic cells of treated plants is of great value, as it results in genetic damage that is handed over to the next generation. Cytogenetic evidence is one of the best documented experimental proofs for the elucidation of the mode of speciation on different groups of plants (Zohary, 1984). The degree of cytological aberrations either in mitosis or meiosis is regarded as one of the dependable criteria for estimating the effect of a mutagen.

In the present study, effects of Laser beam at different doses were tested on the cyto-morphological aspects of grass pea. The increment in meiotic aberrations showed a positive correlation with treatment dose. Similar results with different mutagens were also studied by various investigators like Kumar and Dubey (1998), Dhamyanthi and Reddy (2000). Cytological abnormalities such as stickiness, precocious movement, scattering etc. were more frequent at all the treatment doses. The spectrum of meiotic chromosomal abnormalities is broad and it includes high proportion of stickiness and precocious movements and moderate frequency of secondary associations and laggards. This implies that the mutagen may have brought some alterations in the pattern of organization of chromosomes. The high level of variability in the morphology of the plant was visible due to mutagenic treatments.

The inhibitory effects at high doses leading to a decreased yield was due to prohibitory action of enzymes concerned with the initial growth processes and changes in the enzyme activity (Blixet, *et al.,* 1963). Besides, induction in quantitative characters, the mutagen also caused delay in flowering. The seed count and seed weight was also reduced which may be attributed to high pollen and ovule sterility due to mutagenic effect of interfering with the biochemical changes in the seeds which might have resulted in lowering of the yield. In M2 generation, the chromosomal aberrations were much reduced to their counterparts in M1 generation, which subsequently led to the decline in sterility percentage and thus, yield in terms of seed count was enhanced but still the seed weight was reduced due to biochemical changes influenced by mutagenic effects.

The results compiled on the basis of the present study confirm high effectiveness of laser beam in inducing wide variability of grasspea traits, which was also observed by other investigators (Nerkar, 1976; Waghmare and Mehra, 2000; Rybiñski 2001; Rybiñski, et al. 2006). Kumar and Dubey (2001) reported that plant height has high heritability (74%).

Conclusions

Our results are in agreement with those reported by Kumar and Dubey (2001). For 1000-seed weight of grass pea, heritability was determined as 96% by Milczak, *et al.,* (2001). Therefore, they pointed out that 1000-seed weight was less affected than other yield criteria. Our results confirm the findings of Kumar and Dubey (2001), Kumari and Prasad (2005). The above documentation suggests that the mutagen (laser beam) with high efficiency and lower damage can be selected for enhancing productivity of the plant. The univalents encountered here may result in formation of aneuploids and may be helpful in genetic analysis of plants.

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