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Induction of mitotic aberrations by Argon ion lasers in *Vicia faba* L. and *Allium cepa* L.

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ABSTRACT. A comparative study of the effect of laser in inducing chromosomal aberrations at 488 nm was done in *Vicia faba* L. (faba bean) and *Allium cepa* L. (onion) with Argon ion laser (Spectra Physics Model 171). Seeds and bulbs of *V. faba* and *A. cepa* were subjected to laser irradiation by 488 nm excitations from Argon ion laser source at power levels 200 and 400 mW with power densities 2.25 mW cm^{-2} and 4.49 mW cm^{-2} and different exposure times (10, 20, 30 & 40 minutes). Similar to the effect of other physical and chemical mutagens, laser caused a dose dependent decrease in mitotic index and a rise in mitotic aberrations when compared to the control. In both plant species, mutations were observed in all stages of mitotic cell cycle. The total percentage of aberrations was two fold higher at 400 mW than at 200 mW exposure.

Introduction

Radiations such as X-rays, γ-rays and UV-rays have been routinely used as physical mutagens and are known to cause dose dependent changes in the molecular organisation of chromosomes resulting in gene mutations, chromosomal aberrations, or alterations in the physiologycal activity of the cell (Lea, 1946; Cohn, 1969). As most biological molecules have optical absorption in the UV range, it has often been assumed that visible radiations are non-mutagenic. However, with the development of lasers, the idea of visible radiations of mutagenic nature has been mooted, because

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lasers by virtue of their coherence and intensity can possibly interact with biomolecules non-linearly. Preliminary studies indicate that laser beams have a biologically stimulant influence resulting in increased germination velocity as well as in related dynamics in the beginning of growth in a number of crop plants such as winter wheat, spring barley, peas (Zubal, 1990), *Vigna radiata* (Govil et al., 1991), *Gossypium* sps. (Akhmedova, 1993), alfalfa, red clover and burr reed (Nanova, 1992). Cytogenetic effects of lasers were reported in *Allium fistulosum, Hordeum vulgare* (Dragan and Khrapunov, 1993) and in *Pisum sativum* (Vasileva et al., 1991). Laser irradiation in the visible range (514 nm) also apparently causes metabolic changes which results in aberrant mitosis similar to but less frequently than under gamma irradiation (Pilli et al., 1997). In the present study, the induction of chromosomal aberrations by Argon ion laser (Spectra Physics Model 171) at 488 nm radiation in *Vicia faba* and *Allium cepa* root tips was assessed with the aim of determining the mutagenicity of laser radiation in the visible region.

Materials and methods

Seeds of *Vicia faba* (faba bean) were obtained from the N.B.P.G.R., New Delhi, India while bulbs of *Allium cepa* var. CO_4 (onion) were obtained from the College of Horticulture, Tamilnadu Agricultural University, Coimbatoore, India. Laser irradiation of plant material was done at the International School of Photonics, Cochin University of Science and Technology, Cochin, India. *V. faba* seeds were soaked overnight in tap water and then decoated before irradiation. Old roots and outermost scale leaves were manually removed and the onion bulbs planted in moist sand overnight to enhance root and shoot initiation.

Seeds and bulbs were subjected to laser irradiation by 488 nm excitations from Argon ion laser source (Spectra Physics Model – 171) at power levels 200 and 400 mW with power densities 2.25 mW cm^{-2} and 4.49 mW cm^{-2} and different exposure times (10, 20, 30 and 40 minutes). The laser beam size was adjusted using proper optical elements so as to get uniform illumination of the sample.

After irradiation, the onion bulbs were planted in moist sand and faba bean germinated on moist filter paper in petridishes at room temperature. Onion root tips about 1-2 cm were collected between 8 and 9 a.m. on the 3rd day, while in faba bean, root tips were collected between 10 and 11 a.m. after 3-5 days of irradiation.

Root tips were pretreated in 0.04% colchicine solution for 3 hrs. at room temperature, fixed in freshly prepared aceto-ethanol (1:3), hydrolysed in 1 N HCl at 60°C for 5-6 seconds and squashed in 2% acetocarmine. At least six actively dividing root tips were scored for each treatment. From each slide 15-20 fields were studied.

Results and discussion

In control untreated roots of *Vicia faba* and *Allium cepa*, mitosis was normal with only 0.11% and 0.23% of aberrations observed, respectively.

Similar to the effect of other physical and chemical mutagens, laser irradiation caused a dose dependent decrease in mitotic index and a rise in mitotic aberrations when compared to control. In both plant species mutations were observed in all stages of the mitotic cell cycle. The total percentage of aberrations was two fold higher at 400 mW than at 200 mW exposure (Tables 1 and 2).

At prophase clumping of chromosomes was the most common type of aberration noted, although this was present in control (unirradiated) samples and did not show dose dependence in irradiated samples. Metaphase aberrations such as stickiness and nondisjunction were several fold higher in irradiated than in control samples. As appearance of stickiness and nondisjunction has been attributed to the presence of breaks and chromosome exchanges during prophase contraction

Table 1

Effect of Argon ion laser at 488 nm at 200 and 400 mW on mitosis in *Allium cepa* L. var. CO_4:

Abnormalities (% of total cells)	Control
Clumping	0.03
Stickiness	0.00
Nondisjunction	0.09
Bridges	0.00
Micronucleate	0.06
Binucleate	0.00
Elongated nucleate	0.00
Bridges (Telophase)	0.00
Total aberrations (%)	0.23
Total No. of cells observed	6472
Mitotic index	4.74

Abnormalities	488 nm-200 mW			
(% of total cells)	10 Minutes	20 Minutes	30 Minutes	40 Minutes
Clumping	0.10	0.05	0.10	0.06
Stickiness	0.224	0.31	0.21	0.20
Nondisjunction	0.317	0.22	0.19	0.26
Bridges	0.018	0.009	0.03	0.08
Micronucleate	0.028	0.00	0.009	0.00
Binucleate	0.214	0.35	0.34	0.21
Elongated nucleate	0.71	0.60	1.31	0.84
Bridges (Telophase)	0.09	0.119	0.06	0.07
Total aberrations (5)	1.7	1.66	2.249	1.72
Total No. of cells observed	10709	10946	11569	12440
Mitotic index	2.28	2.09	2.05	2.068

Abnormalities	488 nm-400 mW			
(% of total cells)	10 Minutes	20 Minutes	30 Minutes	40 Minutes
Clumping	0.06	0.098	0.05	0.052
Stickiness	0.138	0.098	0.087	0.17
Nondisjunction	0.24	0.39	0.24	0.23
Bridges	0.25	0.06	0.25	0.04
Micronucleate	0.00	0.00	0.00	0.00
Binucleate	0.00	0.214	0.23	1.68
Elongated nucleate	0.74	0.60	0.94	0.17
Bridges (Telophase)	0.00	0.04	0.07	0.06
Total aberrations (5)	1.428	2.46	2.64	2.4
Total No. of cells observed	12321	11209	13788	15424
Mitotic index	2.45	1.92	1.79	1.73

(Klasterka et al., 1976) or due to disruption of bonds between protein and nucleic acid constituents or physical adhesion of proteinaceous matrix resulting in failure of chromosome condensation in prophase (Cohn, 1969; Stephen, 1979; Purak and Noor, 1990), apparently lasers affect the process of normal chromosome condensation. Lasers also apparently cause chromosome breaks, as dicentric chromosomes, which are usually formed as result of breaks in each arm of two adjacent chromo-

Table 2

Effect of Argon ion laser at 488 nm at 200 and 400 mW on mitosis in *Vicia faba*:

Abnormalities (% of total cells)	Control
Clumping	0.03
Stickiness	0.00
Nondisjunction	0.03
Bridges	0.01
Micronucleate	0.02
Binucleate	0.02
Elongated nucleate	0.00
Bridges (Telophase)	0.11
Total aberrations (%)	0.00
Total No. of cells observed	10792
Mitotic index	3.74

Abnormalities	488 nm-200 mW			
(% of total cells)	10 Minutes	20 Minutes	30 Minutes	40 Minutes
Clumping	0.18	0.23	0.16	0.196
Stickiness	0.15	0.13	0.16	0.14
Nondisjunction	0.17	0.27	0.099	0.28
Bridges	0.047	0.034	0.099	0.028
Micronucleate	0.26	0.34	0.23	0.29
Binucleate	0.38	0.44	0.40	0.40
Elongated nucleate	0.13	0.02	0.045	0.028
Bridges (Telophase)	0.013	0.013	0.22	0.00
Total aberrations (5)	1.33	1.48	1.413	1.362
Total No. of cells observed	14949	14834	11085	10665
Mitotic index	2.53	2.21	2.17	2.14

Abnormalities	488 nm-400 mW			
(% of total cells)	10 Minutes	20 Minutes	30 Minutes	40 Minutes
Clumping	0.232	0.08	0.28	0.05
Stickiness	0.069	0.08	0.06	0.10
Nondisjunction	0.356	0.086	0.1	0.11
Bridges	0.008	0.52	0.007	0.056
Micronucleate	0.95	2.37	1.72	0.99
Binucleate	0.526	0.14	0.58	1.52
Elongated nucleate	0.039	0.42	0.47	0.48
Bridges (Telophase)	0.00	0.17	0.23	0.00
Total aberrations (5)	2.18	3.87	3.447	2.306
Total No. of cells observed	12925	11590	14030	12573
Mitotic index	2.34	2.05	2.06	1.86

somes and their reunion, was the most commonly observed type of stickiness (Fig. 1). Multiple kinetochore chromosomes such as tricentric, quadricentric and pentacentric have been rarely observed (Figures 1, 2, 13 and 14), which apparently arise from breaks in more than two chromosomes. The other type of stickiness observed rarely in laser irradiated samples were the chromosome rings (Figures 4, 16 and 17). Inter-arm asymmetric type of chromosome interchanges are known to lead to cen-

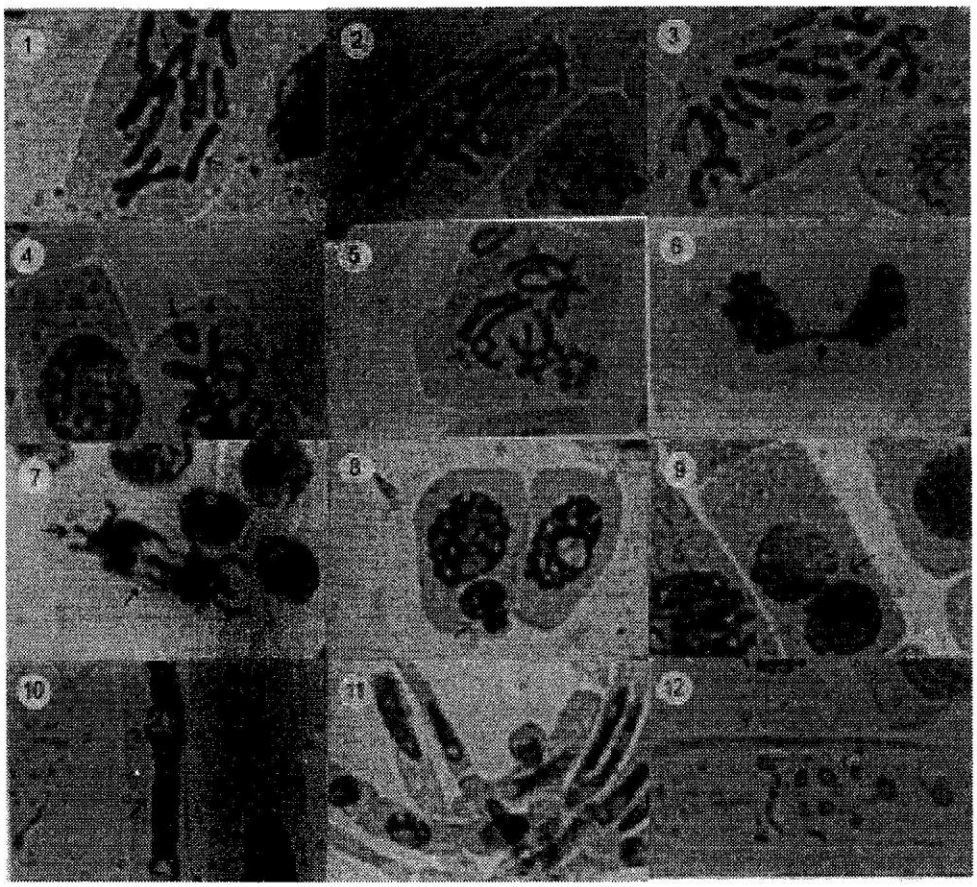

Fig. 1-12. Effect of lasers in *Allium cepa* root tips:
Metaphase with one tricentric, one dicentric and two translocation chromosomes (1), one tetracentric and one translocation chromosomes (2), three translocation chromosomes and an acentric fragment (3), chromosome ring (4), chromatid ring (5). Anaphase with single chromatic bridge (6), multiple chromatic bridge and laggards (7). Interphase with two micronuclei (8). Telophase with a binucleate cell (9), highly elongated and strap shaped nucleus (10). Nuclear polymorphism (11). Cell with nuclear disintegration (12). × 1000

tric ring of varying sizes and a compound fragment. The rings may separate freely or open to a single dicentric ring or the two centric rings may be interlocked (Conger, 1965). In very rare cases late condensation of chromosomes at metaphase were also observed in *V. faba* (Fig. 15). Several reciprocal translocations were observed at metaphase in these materials (Figures 1, 3 and 14). Symmetrical complete chromosome interchanges lead to reciprocal translocations. According to Cohn (1969), they are difficult to detect unless chromosome morphology is drastically changed. Chromatid rings were very rarely observed at metaphase in both cases (Figures 5 and 18). Two breaks within the same chromosome may lead to symmetrical or asymmetrical intrachange and the latter lead to chromatid ring (centric or acentric) and a fragment.

The most common type of aberration seen in anaphase was the formation of chromosome bridges which occurred in a dose independent fashion in the laser irra-

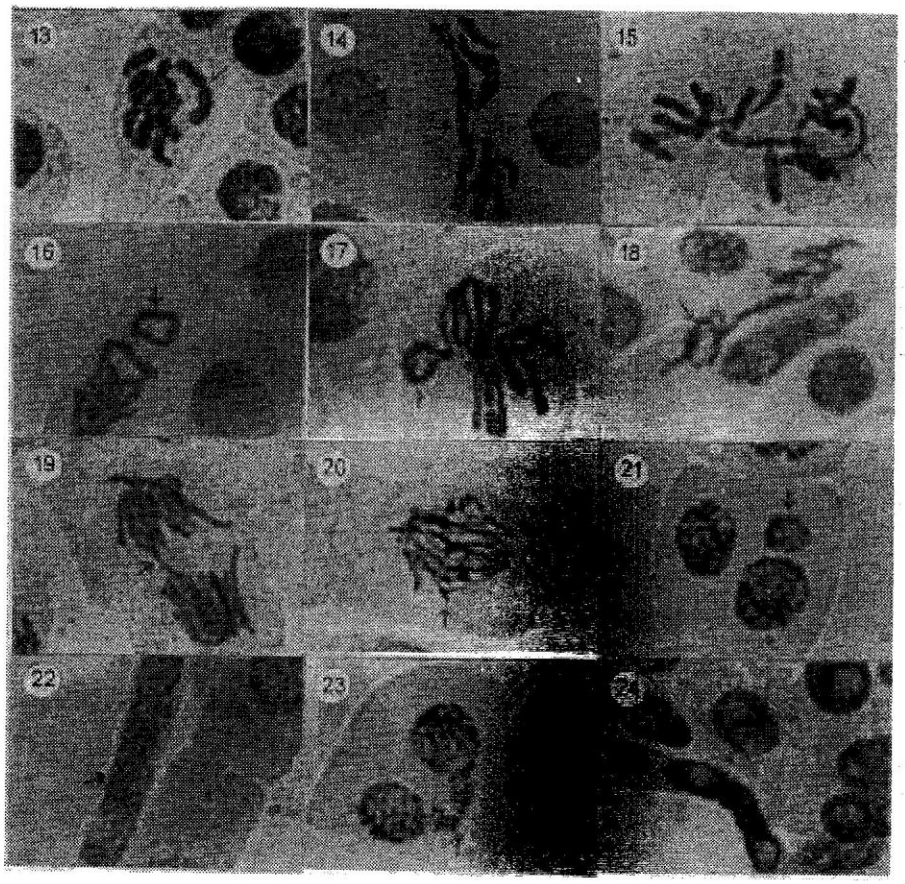

Fig. 13-24. Effect of lasers on mitosis in *Vicia faba* root tips:
Metaphase with tricentric chromosome (13), tetracentric chromosome (14), late condensation in three chromosomes (15), chromosome ring (16), chromosome ring and nondisjunction (17), chromatid ring (18). Anaphase with single chromatic bridge (19), multiple chromatic bridge and laggard (20). Telophase with binucleate cell and micronuclei (21), strap shaped nucleus (22), bridge (23). Nuclear polymorphism (24). × 1000

diated samples. These probably result from laser induced stickiness and bridge breakage fusion cycles (Cohn, 1969) occurring at metaphase. Single as well as multiple bridges were observed (Figures 6, 19, 7 and 20).

In telophase the aberrations noted were the formation of micronuclei, binucleate or elongated nucleate cells and nuclear bridges. In *A. cepa* micronuclei were rarely present under low doses of laser irradiation (200 mW, 10 min.) and were absent under higher doses (400 mW). However in *V. faba* micronuclei were very frequent (Figures 8, 21) and its frequency was 3-10 fold higher at 400 mW as compared to 200 mW. These micronuclei probably form after double time of irradiation at metaphase. According to Levan and Levan (1978), the micronuclei formed after double time of irradiation may survive to the next mitosis and be recovered, although a part of them will be lost at each mitosis.

The formation of binucleate cells was 7-8 fold higher at 40 min. of 400 mW irradiation in *A. cepa* over the same at 200 mW, but showed no dose dependence at

other exposure times in *A. cepa* or in *V. faba* (Figures 9, 21). Binucleate cells are usually formed due to the absence of cytokinesis (Eigsti and Dustin, 1957), possibly due to inhibition of cell plate formation. Lasers possibly affect this process only at high doses in certain plants.

Elongated cells with highly elongated and strap shaped nuclei were frequently observed in *A. cepa* at both 200 and 400 mW, although these were seen in *V. faba* only under higher irradiation doses (400 mW, 20, 30 and 40 min.) (Figures 10, 11 and 22). Changes in cell volume or nucleus volume in mammalian cells have been attributed to the action of inhibitors of nucleic acid and protein synthesis (Walum et al., 1990). Different plant species possibily differ in their sensitivity to inhibition of protein and nucleic acid synthesis by lasers.

Other aberrations noticed at telophase included nuclear bridge formation (Figure 23), nuclear polymorphism (Figures 11, 24), pycnotic nuclei and nuclear disintegration (Figure 12).

The results reported in this paper are part of an ongoing study of the effect of lasers of various wavelengths on mitosis. In an earlier experiment Argon ion lasers of wavelength 514 nm at 200, 400, 600, 800 mW were tested for mutagenicity and compared to γ-irradiation. The frequency of aberrations caused by lasers at 514 nm is lower than that caused by 488 nm reported in this paper. However laser radiations differ significantly from γ-irradiation both in frequency and spectrum of variations caused. Futher studies are being carried out to define the mutagenicity of laser irradiations.

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