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激光及γ射线对有丝分裂染色体畸变感应现象的比较

P. R. Unnikrishna Pillai[1,2], Padma Nambisan[1]①, V. P. N. Nampoori[3], C. P. G. Vallabhan[3]

(1. 印度科钦科学技术大学生物技术系，科钦 682002)

(2. 印度 S. D. 学院植物学系，阿勒皮 688003) (3. 印度科钦科学技术大学国际光学院，科钦 682022)

摘 要 波长 514nm 的激光照射可用于研究激光导致有丝分裂染色体畸变的效应。本文提供了一种新的辐照系统，能用于研究突变的感应现象，并与从 γ-线辐射源获得的结果进行了比较。

关键词 氩离子激光，染色体畸变，有丝分裂变化

Induction of Mitotic Chromosomal Aberrations by Lasers in Comparison to Gamma Radiations

P. R. Unnikrishna Pillai[1,2], Padma Nambisan[1]②, V. P. N. Nampoori[3], C. P. G. Vallabhan[3]

(1. Department of Biotechnology Cochin University of Science and Technology, Cochin-682 022, India)

(1, 2. Department of Botany, S. D. College, Sanathanapuram, Alleppe-688 003, India)

(3. International School of Photonics, Cochin University of Science and Technology, Cochin-682 022, India)

Abstract Laser irradiation at wavelength 514 nm was used to study the effect of lasers in inducing chromosomal aberrations at mitosis. This study offers a new radiation system which could be used for the induction of mutations. Results are compared with those obtained from studies using γ-rays as irradiation source.

Key words Argon ion laser, Chromosomal aberrations, Mitotic changes

1 Introduction

Radiations are known to induce changes in the molecular organisation of chromosomes manifested as gene mutations, chromosomal aberrations or alterations in the physiological activity of the cell (Cohn, 1969). The manner in which the yield of structural changes increases with increase of the dose of radiation has been extensively studied and the results of these studies form the main basis on which theories of the mechanism of induction of these changes are built (Lea, 1946). While radiations such as X-rays, γ-rays and uv-rays have been routinely used as physical mutagens, not much attention has been given to the effect of visible radiation on living organisms. It has been thought that visible radiation will not affect the life process much, since most of the biological molecules have optical absorption in the uv region. However, if lasers are used instead of conventional incoherent light sources, it can be expected that even visible radiation may influence life process because of the coherence and intensity of laser

①通讯作者。

②Corresponding author.

beams which interact with the biomolecules non-linearly. In the present study 514 nm radiation from Argon ion laser was used to study the effect of visible coherent radiation on biological systems. The effect of such coherent sources in inducing chromosomal aberrations in root tip cells of faba bean (*Vicia faba*) was compared with that of gamma irradiation. The effect of lasers was also studied with onion (*Allilzm cepa* L.), in order to compare the effect in diverse species of plants.

2 Materials and Methods

Seeds of *Vicia faba* were obtained from the School of Studies in Botany, Vlkram University, Uuain, Madhya Pradesh, India while bulbs of *Allium cepa* L. var CO_4were obtained from the college of Horticulture, Tamil Nadu, Agricultural University, Coimbatore, India. Dry seeds of *V. faba* (6.64% moisture) were irradiated by 5, 10, 20, 30, 40 and 50 kR's of γ-rays at Bhabha Atomic Research Centre, Bombay, India. Seeds were germinated on moist filter papers in petriplates at room temperature. Root tips were collected after 3~5 days. 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 before irradiation. Old roots and outer most scale leaves were manually removed and the onion bulbs were planted in moist sand overnight to encourage root and shoot initiation.

Seeds and bulbs were subjected to laser irradiation by 514 nm excitations from Argonion laser source (Spectra Physics) at various power levels 200, 400, 600 and 800 mW with different power densities (2.25 $mWcm^{-2}$, 4.49 $mWcm^{-2}$, 6.74 $mWcm^{-2}$ and 8.89$mWcm^{-2}$ respectively) 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 in the sample container.

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

Root tips were pretreated in 0.04% colchicine solution for 3 hours at room temperature, fixed in freshly prepared aceto-alcohol (1:3), hydrolysed in TN HCl at 60℃ 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.

3 Results and Discussion

In control untreated roots of *V. faba* and *A. cepa*, mitosis was normal with only 0.11% and 0.23% of aberrations in total number of cells observed respectively. Both lasers and gamma irradiation caused a significant dose dependent decrease in mitotic index and a rise in mitotic aberrations when compared to control. Mito-depressive effects have been observed for several chemicals and physical mutagens and have been attributed to exposure of cells to stress or inhibition of any metabolic event which causes the arrest of cells in G_1(pre DNA synthesis) and G_2 (pre mitotic) phase (Cummins, 1969) while cells in S (DNA synthesis) phase and M (mitosis) usually complete their progression.

Laser irradiation at higher power densities and longer exposure times resulted in high frequency of globular bodies, the chromatin droplets, followed by disintegration of chromosomes (PlateI, 1~13). Eigsti and Dustin

(1957) suggested the term pseudonuclei for such pieces resulting in chromatin disintegration which remains scattered in the cytoplasm. The frequency of formation of chromatin droplets was higher in *V. faha* than in *A. cepa* and occurred only rarely with γ−irradiation of *V. faba*. We believe that formation of chromatin droplets in very high frequencies in laser irradiated samples is an indication of altered metabolic changes in interphase and early mitotic phase cells.

Table 1　Effect of Argon ion lasers at 514 nm at 200～800mW on mitosis in *Allium cepa* L. var CO_4

Abnormalities (% of total cells)	Treatment (mW)	Time (min.)			
		10	20	30	40
Clumping (Control 0.03)	200	0.56	0.42	0.37	0.47
	400	0.39	0.48	0.43	0.38
	600	0.33	0.25	0.30	0.20
	800	0.32	0.36	0.28	0.32
Stickiness (Control 0.00)	20	0.04	0.05	0.06	0.12
	400	0.06	0.08	0.05	0.18
	600	0.02	0.02	0.01	0.00
	800	0.00	0.06	0.06	0.02
Nondisjunchon (Control 0.09)	200	0.38	0.49	0.33	0.23
	400	0.41	0.08	0.27	0.41
	600	0.03	0.14	0.11	0.12
	800	0.18	0.12	0.09	0.23
Bridges (Control 0.00)	200	0.06	0.04	0.14	0.12
	400	0.10	0.10	0.03	0.03
	600	0.08	0.13	0.14	0.18
	800	0.13	0.21	0.17	0.08
Micronucleate (Control 0.06)	200	0.04	0.02	0.00	0.07
	400	0.00	0.00	0.00	0.00
	600	0.00	0.00	0.00	0.00
	800	0.02	0.00	0.00	0.00
Binucleate (Control 0.05)	200	0.16	0.20	0.45	0.43
	400	0.80	0.42	0.54	0.10
	600	0.46	0.59	0.64	0.67
	800	0.51	0.59	0.57	0.49
Elongated nucleate (Control 0.00)	200	0.26	0.13	0.27	0.23
	400	0.33	0.30	0.02	0.20
	600	0.17	0.06	0.13	0.05
	800	0.03	0.15	0.22	0.30
Total aberrations (%) (Control 0.23)	200	1.50	1.35	1.62	1.67
	400	2.09	1.46	1.34	1.26
	600	1.09	1.19	1.33	1.22
	800	1.19	1.49	1.39	1.44
Total No. of cell observed (Control 6472)	200	5007	5407	5148	5988
	400	4904	5014	6339	3926
	600	6586	6294	7011	6589
	800	6279	5217	5449	5313
Mitotic index (Control 4.74)	200	3.44	2.91	2.43	2.53
	400	3.00	2.73	2.62	2.03
	600	1.94	1.73	1.63	1.37
	800	1.56	1.51	1.41	1.32

The spectrum of mitotic changes induced by lasers and γ−rays included chromosome clumping, stickiness, nondisjunction, bridges, laggards and changes in nuclei such as elongated nuclei, micronuclei and multinucleate conditions (Table 2 & 3).

Table 2 Effect of Argon ion lasers at 514 nm at 200, 400, 600 and 800 mW on mitods in *Vicia faba*

Abnormalities (% of total cells)	Treatment (mW)	Time (min.)			
		10	20	30	40
Clumping (Control 0.03)	200	0.14	0.19	0.24	0.29
	400	0.41	0.31	0.24	0.28
	600	0.27	0.38	0.21	0.22
	800	0.34	0.17	0.20	0.09
Stickiness (Control 0.00)	20	0.20	0.14	0.10	0.02
	400	0.09	0.03	0.02	0.06
	600	0.10	0.10	0.05	0.01
	800	0.09	0.14	0.07	0.06
Nondisjunchon (Control 0.03)	200	0.08	0.15	0.14	0.12
	400	0.19	0.08	0.22	0.12
	600	0.12	0.21	0.07	0.12
	800	0.16	0.13	0.18	0.06
Bridges (Control 0.01)	200	0.06	0.04	0.03	0.06
	400	0.06	0.04	0.06	0.11
	600	0.14	0.01	0.04	0.03
	800	0.04	0.02	0.08	0.03
Micronucleate (Control 0.02)	200	0.06	0.10	0.03	0.14
	400	0.50	0.11	0.25	0.17
	600	0.89	0.21	0.14	0.07
	800	0.18	0.05	0.11	0.09
Binucleate (Control 0.02)	200	0.14	0.08	0.03	0.52
	400	0.23	0.59	0.39	0.39
	600	1.14	0.42	0.72	0.51
	800	0.84	0.33	0.43	0.35
Elongated nucleate (Control 0.00)	200	0.00	0.01	0.08	0.06
	400	0.04	0.00	0.05	0.06
	600	0.05	0.30	0.05	0.09
	800	0.03	0.02	0.05	0.06
Total aberrations (%) (Control 0.11)	200	0.68	0.71	0.65	1.21
	400	1.54	1.16	1.23	1.19
	600	2.71	1.63	1.28	1.05
	800	1.68	0.86	1.12	0.74
Total No. of cell observed (Control 10792)	200	8979	7920	10100	12364
	400	7730	9030	10852	10856
	600	7344	9208	9605	9530
	800	7592	9299	12467	11743
Mitotic index (Control 3.74)	200	2.75	2.23	2.03	1.70
	400	2.20	1.92	1.67	1.58
	600	2.18	1.94	1.54	1.54
	800	1.54	1.55	1.27	0.45

The frequency of changes induced by γ-rays, however, differed from those induced by lasers. For instance, micronuclei were found both in laser and γ-irradiated faba bean but the frequency of occurrence was higher using lasers than γ-rays (data not shown). Similarly, multinucleate condition was more frequent in laser than in γ-irradiated samples. Also, severe nuclear fragmentation due to disturbed polarity occurred more frequently in laser treatments. Metaphase chromosome changes such as stickiness and anaphase changes such as bridges were 2～10 fold lower in dividing cells of laser treated compared to γ−irradiated samples.

Table 3　Effect of γ−radiation (A) and lasers at 514 nm (B) on metaphase and anaphase in dividing cells of *Vicia faba*

Treatment	Metaphase	Anaphase	% abnormal cells
	Stickness (% of dividing cells)	Bridges (% of dividing cells)	
A: γ−rays			
5kR	14.16	13.10	19.84
10kR	8.63	16.75	23.37
20kR	11.9	24.50	34.02
30kR	30	20	62
40kR	60	19.17	67.17
50kR	49.67	18.75	68.75
B: Laser 514 nm			
200mW−30min	4.88	1.46	24.87
400mW−30min	1.10	4.97	32.69
600mW−30min	3.38	2.70	24.32
800mW−30min	5.70	6.33	41.77

Stickiness of chromosomes causing chromosome clumping at metaphase and sticky bridges at anaphase have been attributed to disruption of bonds between protein and nucelic acid constitutions of the chromosome (Cohn, 1969) or physical adhesion due to a proteinaceous matrix (Stephen, 1979) or due to failure of proper chromosome condensation in prophase (Purak and Noor, 1990). In the present context we assume that occurrence of such aberrations in irradiated samples may be attributed to metabolic changes resulting in changes in chromosome condensation and protem association.

Effect of laser irradiation differred significantly from γ−rays in that no chromosomes or chromatid breaks were observed. In γ−rays irradiated samples, chromosome lesions resulting in acentric fragments (Plate Ⅱ, 2 & 4), chromosome rings (Plate Ⅱ, 3 & 6), breakages (Plate Ⅱ, 5), gaps and translocations were very frequent.

We hypothesise that laser irradiation in the visible range at the wave length tested brings about putative metabolic changes which results in aberrant mitosis similar to but less frequently than during gamma irradiation. Lasers however do not disturb chromosome integrity severely enough to cause lesions as do γ−irradiation. We are currently testing the effect of lasers of other wavelength from Argon ion lasers.

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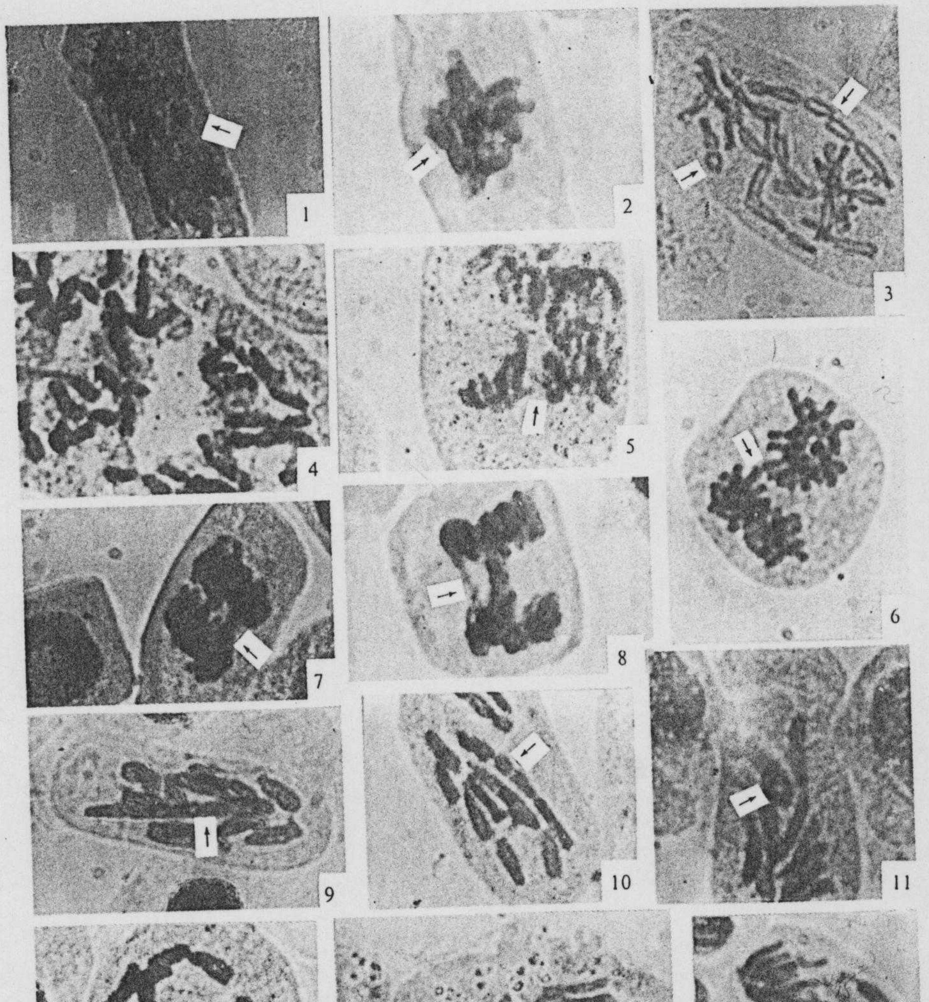
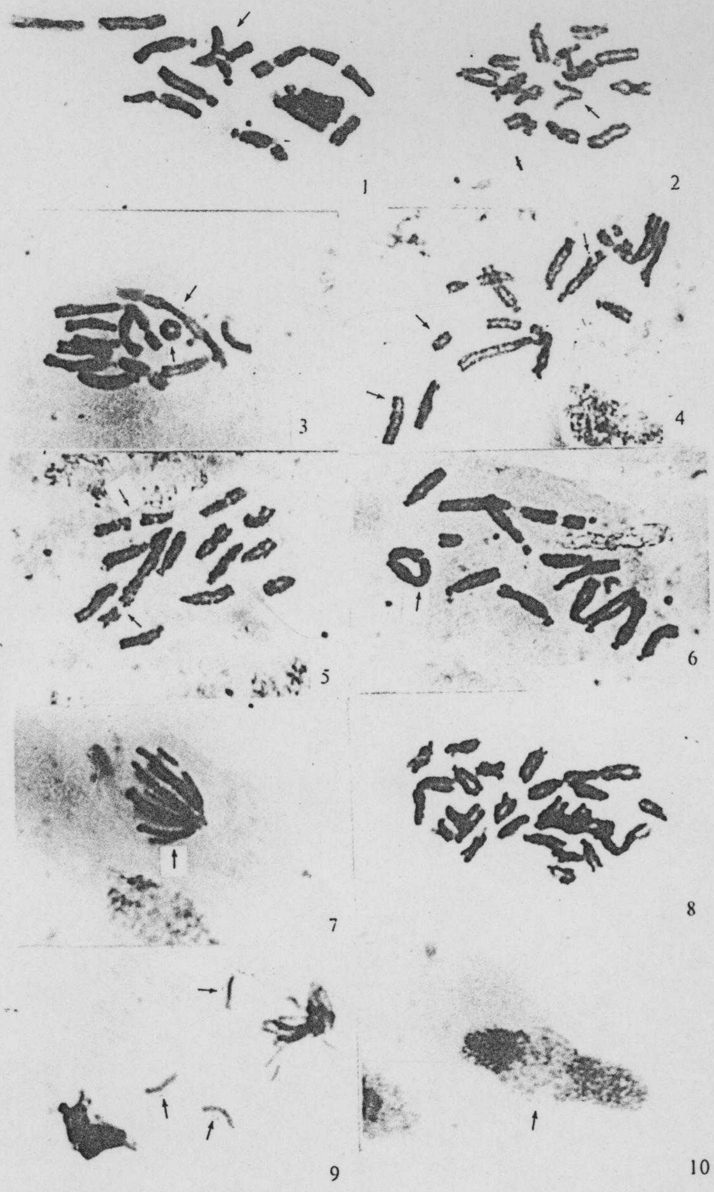


Effect of Argon ion lasers on mitosis in *Allium cepa* root tips (1～8) and *Vicia faba* root tips (9～17).

1. Clumping of chromosomes at prophase; 2. Nondisjunction of chromosomes at metaphase; 3. Metaphase with a tetracentric chromosome and chromatid ring; 4. A tetraploid cell; 5. Disturbed polarity; 6. Star shaped anaphase with a chromosome bridge and disturbed polarity; 7. Anaphase with two chromatic bridges; 8. Anaphase with two unequal chromatic bridges; 9. Metaphase with a dicentric chromosome; 10. Metaphase with a tricentric chromosome; 11. Metaphase with a chromatid ring; 12. A tetraploid cell; 13. Disturbed metaphase with chromatin droplets; 14. Anaphase with multiple chromatic bridges; 15. Telophase with a binucleate cell; 16. Interphase with an unequal distribution of genetic material



Effect of γ–rays on mitosis in *Vicia faba* root tips1. X–type chromatid aberration; 2. Acentric fragment; 3. Chromosome ring, dicentric; 4. Chromatid gap, acentric fragment; 5. Chromosome breakages; 6. Chromosome ring; 7. Only one pole; 8. Tetraploid cell; 9. Three lagging chromosomes; 10. Unusual telophase (× 1000).