J. Phytol. Res. **19**(2) : 215-220, 2006

INDIVIDUAL AND COMBINED EFFECTS OF LEAD AND GAMMA IRRADIATIONS ON GENETIC RECOMBINATION IN *LATHYRUS SATIVUS* L.

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The effects of ionizing radiations, lead (Pb) and combination treatments on chiasma frequency were studied in the M_1 plants of *Lathyrus sativus* (2n=14) raised from the treated seeds. All the treatments caused a decrease in the chiasma frequency as compared to control. There were differences in the actions of different mutagens individually and in combination. The treatment with lead (Pb) caused higher reduction in chiasma frequency than gamma rays, while the combination treatment of both, showed positive response on chiasma frequency.Several structural changes including univalents, multivalents etc. were observed and discussed in the light of present study.

Keywords: Chiasma frequency; Gamma rays; *Lathyrus sativus*; Lead.

Introduction

Genetic recombination is considered to play a central role in bringing out the variations in all the sexually reproducing eukaryotes. Chiasma frequency characterizes the pairing of homologous chromosomes at meiosis and controls the degree of recombination besides influencing fertility. Atleast one chiasmata per bivalent is necessary for orderly disjunction otherwise some homologues may migrate to same pole and form aneuploid gametes. The pairing and recombination of chromosomes is under genetic control[1].The recombination index of a species can be estimated as the sum of haploid number of chromosomes and mean chiasma frequency per cell[2].

In the last three decades, reports have been coming on the treatment of dry seeds with gamma radiations. Most of these reports claim reduction in chiasma frequency, e.g.in *Pisum sativum*[3,4], *Lens culinaris*[5] and in *Sorghum durra*[6].It has also been shown that the chiasma frequency following mutagenic treatments increased in some cases[7,8].From the studies on the effect of gamma irradiations on chiasma frequency in *Lilium* and *Tradescantia*[9], it is evident that sensitive periods to mutagenic treatment are restricted to late zygotene-early pachytene stages.

Besides the ionizing radiations, heavy metals also have some genotoxic potentials[10]. Among various heavy metals, lead (Pb) has been taken into consideration. Although, the mechanism by which lead induces mutation is unknown, but studies during present investigations, suggest that lead induced mutations may not be the result of direct damage to DNA but may occur via indirect mechanisms including disturbances in enzyme functions important in DNA synthesis. Being aware of these informations endeavour was made by the present authors to explore the possibilities of improvement in grasspea by manipulations in cytogenetic consequences resulting through gamma irradiations and lead treatment.

During the present study therefore attempts have been made to assess the effects of gamma irradiations and lead individually and in combination on the chiasma frequency in *Lathyrus sativus*. The purpose of this study is to correlate the chiasma frequency with genetic recombination

Materials and Method

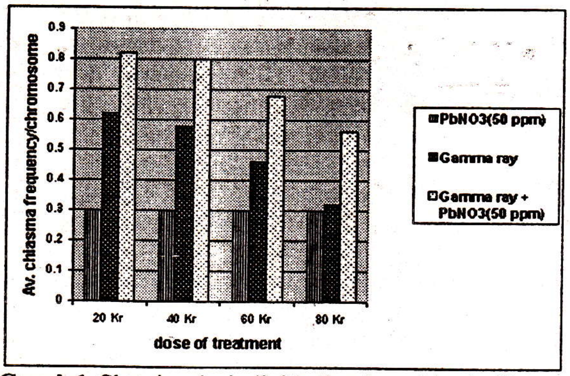
The seeds of *Lathyrus sativus* var.Pusa-24 were obtained from National Bureau of Plant Genetic Resources, New Delhi. For the study of chiasma frequency, four different experimental sets were planned. Set-1 was considered as control. In set-2 healthy seeds of *L.sativus* were irradiated at different doses of gamma irradiation viz.20, 40, 60 and 80 Kr at National Botanical Research Institute, Lucknow and sown in normal garden soil. Set-3 comprised of unirradiated seeds sown in lead amended soil with concentrations viz.25, 50, 100, 200 and 300 ppm. In set-4 gamma irradiated seeds were sown in lead amended soil with above mentioned doses and concentrations. At the time of bloom, young buds were fixed in Carnoy's fixative for 24 hours and transferred into 70% alcohol. Anthers were squashed in 2% acetocarmine to study the chiasma frequency in each set.

Results and Discussion

Cytological studies in control plants of *L.sativus* exhibited the presence of 7 bivalents at diakinesis and metaphase-1 (Fig.1 & 2) and 7:7 separation at anaphase-1. Scoring and analysis of chiasma frequencies at various doses of gamma irradiation, lead nitrate treatment and combination treatment have been presented in Table-1.

The data clearly reveal that there was a marked decrease in the frequency of chiasmata per chromosome in the plants of M_1 generation at different concentrations of lead nitrate as compared to that of control (Table-1). The chiasma frequency gradually decreased alongwith the increasing doses of lead nitrate, hence dose-dependant. Various types of meiotic abnormalities were scored at the stages from diakinesis to telophase-II alongwith the increasing doses. Non-synchronization in divisional stages of PMCs, precocious movement and late seperation of bivalents were noticed at higher doses of $PbNO_3$ (100-300 ppm). Stickiness was the most pronounced anomaly to be recorded at 200 and 300 ppm. The percentage of meiotic aberrations was found to be increased tremendously from 100-300ppm of $PbNO_3$, whereas, the chiasma frequency showed a marked decline within the range of 100-300ppm. Besides this, the concentration of 50 ppm showed comparatively lower degree of abnormalities and increased frequency of multivalents. Hence, this dose was selected for further proceedings in combination treatment with gamma rays. Regarding the gamma ray treatment, Table-1 shows a marked regression in the chiasma frequency alongwith the increasing doses. In the present investigation, besides normal ring and rods, 'V' shaped bivalents with one chiasmata (Fig.3) and univalents with zero chiasmata were recorded at higher doses of gamma radiation. Highly irregular microsporogenesis was observed at metaphsae-1 and anaphase-1 with precocious movement of chromosomes and chromatin bridges, respectively. These abnormalities tend to increase at higher doses. Multivalents of closed ring (Fig.4) and open chain (Fig.5) were analysed at lower doses. Control as well as treated sets were studied for chiasma frequency and their average chiasma frequency per chromosome was calculated and presented in Table-1.

Table-1 also explicits the data scored at the combination treatment of 50ppm $PbNO_3$ and different doses of gamma rays. Results pertaining to the frequency of chiasmata per chromosome in combined treated sets as compared to their respective controls, clearly indicate a marked decline in chiasma frequency. Multivalents, secondary associations (Fig.6), open and closed chains of bivalents were prominent at lower doses. In combination treatment, 40Kr + 50ppm $PbNO_3$ dose caused an increase in the frequency of chiasmata per chromosome (0.80%) as compared to individual treatment of gamma rays and lead nitrate. The treatment of 20Kr + 50ppm dose however caused an



Graph 1. Showing the individual and combined effects of $PbNO_3$ and gamma rays on the chiasma frequency of *L.sativus*.

intermediate effect on chiasma frequency/chromosome (0.82%) as compared to control (1.01%). Other meiotic abnormalities at higher doses were recorded such as precocious movement (Fig.7), unoriented anaphase-I (Fig.8), bridges (Fig.9), etc. (Table-2). The order of potentialities of mutagens to cause reduction in chiasma frequency can be summarized as follows- -
Combination treatment < Gamma irradiation treatment < Lead nitrate treatment (50ppm). (presented in the graph-1).

Many factors such as temperature, season, age and amount of heterochromatin on the chromosomes are known to affect recombination and therefore chiasma[11]. Chiasma frequency is a genetically controlled character[12-14]. Stebbins[15] states that "since chiasma frequency is in part genotypically controlled, diploids which contain genes for low chiasma frequency are likely to produce polyploids forming few or no multivalents".

The reduction in chiasma frequency due to $PbNO_3$ treatment can be explained in the terms of chromosomal morphology. This reduction might have occurred due to shortening of chromosomes alongwith a change in the morphology. The high frequency of rod bivalents recorded at lower doses viz. 25 and 50 ppm indicated the possibility of low affinity amongst their chromosomes. Although some PMCs showed the formation of 7 bivalents, their orientation on equatorial plate was abnormal. Such a behaviour may be explained on the basis of heterogenisity. The tendency of formation of few univalents and rod bivalents observed in control material is the indication of the fact that distal chiasma localization attendant in the bivalents is of weak nature which may be under genetic control.

Formation of bridges and laggards at anaphase-I at higher doses of $PbNO_3$ can be mainly attributed to the instability of the multivalents to separate properly. Adjacent orientations seem to be more responsible for the

Table 1. Mean chromosomal associations and chiasma frequencies at diakinesis in M_1 plants after individual and combination treatments.

Treatment	Doses (Kr/ppm)	No.of PMCs observed	Mean frequency						Average chiasma frequency/ chromosome
			I	II	III	IV	V	VI	
Control	-	55	-	0.6	-	-	-	-	1.01
Gamma Rays	20 Kr	42	0.97	2.91	2.77	4.87	5.55	1.95	0.62
	40 Kr	60	0.99	3.02	2.92	4.92	5.25	2.01	0.58
	60 Kr	46	1.21	3.21	3.01	4.61	5.52	2.62	0.46
	80 Kr	37	1.61	3.41	3.24	4.52	5.62	3.10	0.32
$PbNO_3$	50ppm	69	2.12	3.62	3.48	4.62	5.71	3.28	0.30
Gamma Ray+$PbNO_3$	20Kr+50ppm	62	2.41	3.71	3.54	4.78	5.82	4.12	0.82
	40Kr+50ppm	55	2.53	3.92	3.71	4.98	5.92	4.23	0.80
	60Kr+50ppm	52	2.91	4.12	3.94	5.02	5.96	4.36	0.68
	80Kr+50ppm	38	3.21	4.24	4.21	5.62	5.98	4.40	0.56

Abbreviations:I-univalents,II-bivalents,III-trivalents,IV-quadrivalents, V-pentavalents,VI- hexavalents.

Table 2. Meiotic abnormalities in M_1 plants of *Lathyrus sativus* after individual and combination treatment.

Treatment	Doses (Kr/ppm)	Percentage of metaphasic abnormalities			Percentage of anaphasic abnormalities		
		Precocious movement	Stickiness	Scattering	Irregular seperation	Bridges	Laggards
Control	-	0.10	-	-	-	-	
Gamma Rays	20Kr	0.46	-	0.52	-	0.97	0.23
	40Kr	0.62	0.12	0.48	0.28	1.24	0.56
	60Kr	0.82	0.27	0.42	0.38	1.33	0.68
	80Kr	0.96	0.34	0.38	0.47	1.47	0.86
$PbNO_3$	50ppm	0.72	0.26	0.45	0.26	1.31	0.48
Gamma Ray+ $PbNO_3$	20Kr+50ppm	0.28	0.19	0.43	0.31	0.92	0.22
	40Kr+50ppm	0.38	0.29	0.58	0.47	1.31	0.58
	60Kr+50ppm	0.41	0.37	0.68	0.62	1.42	0.74
	80Kr+50ppm	0.53	0.58	0.73	0.71	1.56	0.92

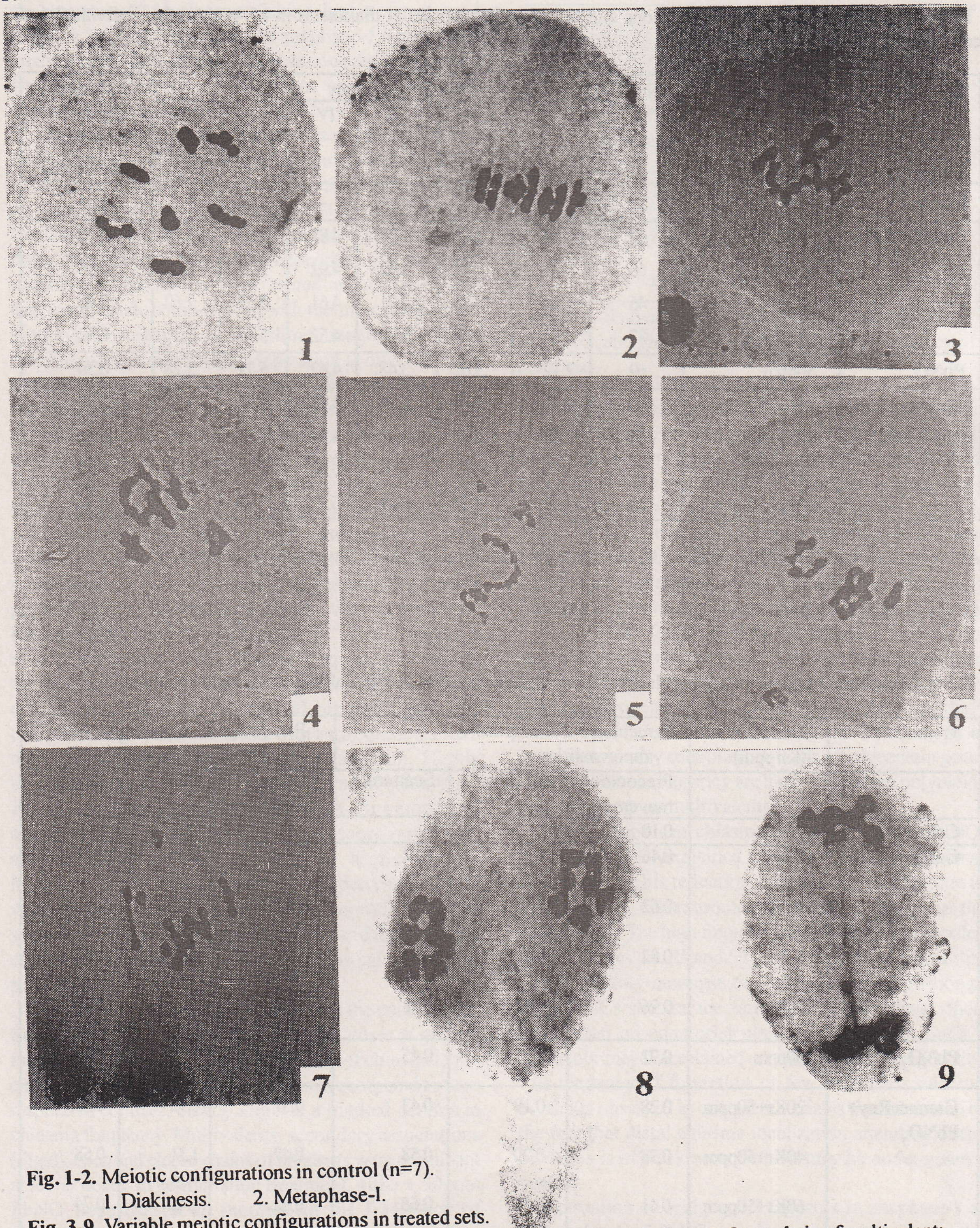


Fig. 1-2. Meiotic configurations in control (n=7).
1. Diakinesis. 2. Metaphase-I.

Fig. 3-9. Variable meiotic configurations in treated sets.
Metaphase-I - 3. "V" shaped multivalent; 4. Closed chain of multivalent; 5. Open chain of multivalent; 6: Secondary association of multivalent; 7. Precocious movement.
Anaphase-I- 8. Unorientation; 9. Single bridge.

formation of laggards where there is always a possibility of unequal and delayed seperation. Random movement of univalents to any pole at higher doses of $PbNO_3$ may lead to unequal seperation of chromosomes. Besides these abnormalities, a lower degree of damage was observed at 50ppm dose. This suggests that lower dose causes small amount of damage to the structure of DNA.

The occurrence of five bivalents and one quadrivalent at metaphase-1 was frequently observed at lower doses of gamma rays. The quadrivalents appeared both in chains and ring configurations. The close association of quadrivalents with the nucleolus indicates that a reciprocal translocation might have occurred between non-homologous chromosomes belonging to different pairs following breakage induced by gamma irradiation.

High frequency of ring shaped quadrivalent suggests that reciprocal translocation had occurred between two relatively large sized chromosomes following breakages at a site closer to centromere[16]. When open chain is formed as an outcome of adjacent type of translocation followed by delayed segregation; laggards may appear which ultimately give rise to micronuclei at telophase-II[17].

Frequent formation of multivalents at lower doses of combination treatment has been attributed to irregular pairing and breakage followed by translocation and inversions. According to Choudhary[18] the realized frequency of such multiple associations will of course be limited by mechanical interference and length of interchanged segments. These suggestions explain the low percentage of quadrivalents observed at higher doses in the present investigation. Chromatin bridges observed at anaphase-I & II can be explained on the basis of crossing over within an inversion[19]. Jain and Basak[20] in *Delphinium* have suggested that reduction in chiasma frequency after irradiation could be due to cryptic structural changes in some of the chromosomes which restrict pairing and univalents, hence reduce the chiasma frequency.

A comparative account on individual and combination treatment, clearly revealed that combination treatment is more efficient in increasing chiasma frequency per chromosome as compared to individual treatments. In the individual treatments, decrease in chiasma frequency can be attributed to the disturbance in chromosome coiling, failure or restricted pairing at pachytene and delay in DNA synthesis. On the other hand, the increase in chiasma frequency in combination treatment was attributed to either failure of terminalization or increase in crossing over following mutageneic treatments.

Hence, it can be concluded that lead nitrate proved to be more potent mutagen in reducing the chiasma frequency leading to the formation of aneuploid gametes, whereas gamma irradiation gave intermediate effect on chiasma frequency and in combination treatment, gamma rays acting additively with $PbNO_3$ created less damage to the nuclear apparatus and increased the chiasma frequency leading to increased genetic recombination.

Acknowledgements

The authors are highly indebted to N.B.P.G.R, New Delhi for providing seeds and N.B.R.I, Lucknow for providing gamma irradiation facility. Authors are thankful to all the laboratory members for their valuable guidance and help.

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