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MORPHOGENETIC IMPACT OF GAMMA IRRADIATION IN *LINUM USITATISSIMUM* L.

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Induced mutations are usually restored to create variability not available in the gene pool or to correct specific deficiency of an otherwise outstanding genotype, since gamma-rays have proved to be very effective in inducing variability and also in increasing the mutation frequencies therefore, these are currently being used in gentic improvement programmes of different plant species. In view of that cytological investigations were carried out in experimental set of *Linum usitatissimum* L. irradiated at different doses of gamma rays. The plants showed varying degree of meiotic irregularities, almost at all the treament doses. The various types of meiotic abnormalities included scattering, unorientation, non-synchronous division, secondary association, multivalent-formation etc.

Keywords: Gamma rays; Induced mutations; *Linum usitatissimum;* Meiotic abnormalities; Multivalent-formation; Secondary association.

Introduction

Linum usitatissimum L. belongs to family Linaceae and is commonly known as Linseed. It is an important food- grain grown in most parts of India and since Linseed is richest source of oil and protein, therefore, it is of utmost importance to find out ways to increase qualitative and quantitative characters of plant through induced-mutagenesis.

Material and Method

Seeds of *Linum usitatissimum* L. (variety- Garima) obtained from Chandrashekhar Azad University, Kanpur were irradiated at 5-doses of Gamma- rays viz. 100Gy, 200Gy, 300Gy, 400Gy and 500Gy from Co^{60} source at National Botanical Research Institute, Lucknow. These seeds were sown in 3 replicates alongwith control set for comparision. For cytological analysis, buds were fixed in carnoy's fixative and later stored in 70% alcohal. Slides were prepared using standered acetocarmine-anther-squash-technique.

Results and Discussion

Morphological parameters : In the present investigation, seed-germination percentage was observed to be 95% in control set, while at different doses of gamma- radiation viz. 100Gy upto 500Gy, it displayed a decreasing trend ranging from 80% to 30.6%. (Table 1).

In control set of linseed plant survival was recorded to be 90%, whereas at different doses of gamma-irradiation viz. 100Gy, 200Gy, 300Gy, 400Gy and 500Gy it was observed to be 70%, 60%, 53.3%, and 43.3% and 33.3%, respectively.

Number of cotyledonary leaf and its type varies at different doses of gamma- irradiation. .In contol set of *Linum,* unifoliate- type of cotyledonary leaves were observed, and number of cotyledonary leaves/plant was 2, whereas at 100Gy, 200Gy, 300Gy and 400Gy of Gamma-irradiation, it was observed as 2 (1 bifoliate+1unifoliate), 2+2 (2 trifoliate+2unifoliate), 2+2 (2 tetrafoliate+2unifoliate) and 2 (1bifoliate + 1unifoliate), respectively.

At 500Gy of gamma-irradiation, no specific type or pattern in cotyledonary leaves has been observed. In control set average plant- height was observed as 36 cm, whereas it varied at different doses of gamma-irradiation. Maximum and minimum values of plant-height were recorded to be 38.2 and 20.2 cm at 300Gy and 500Gy doses of gamma irradiation, respectively. Average stem-girth was observed to be 0.8 and 2.4cm at 100Gy and 300Gy dose of gamma-irradiation, respectively.

Average number of branch/plant in control-set of *Linum* was observed as 4, whereas it ranged between 8-3 at various doses of gamma irradiation. Average number of capsule/plant in control set of *Linum* was observed as 36, whereas it ranged between 45 and 18 at different doses of gamma- radiation. Average number of seeds/capsule in control set of *Linum* was observed to be 10, whereas it ranged from (13-6) at various doses of gamma- radiation.

Cytological parameters: In control-set of *L. usitatissimum* L. (Table 2) meiosis was perfectly normal (n=15) (Fig.1-3). However, the plants at different doses of gamma-rays showed varying degree of chromosomal

Table 1. Various morphological parameters recorded at different doses of gamma irradiation in *Linum ussitatisimum* L.

Morphological parameters	Control	100Gy	200Gy	300Gy	400Gy	500Gy
Seed germination (%)	95	80	70	76.6	63.3	39.6
Plant survival (%)	90	70	60	53.3	43.3	33.3
No. of cotyledonary leaf & its type	UF	2 1 bifoliate+1	2+2 (1 BF+2UF)	2+2 (2TF+UF)	2 (1BF+1UF)	No SP
Plant height (cm)	36	35.8	37	38.2	25.2	20.2
Plant girth (cm)	1.4	0.8	2	2.4	1.8	0.95
No. of nodes	43	47	63	76	46	28
Length of internode (cm)	0.5	0.9	0.4	0.3	0.8	0.7
No. branch per plant	4	7	8	8	5	3
No. capsule per plant	36	30	32	45	27	18
No. of seed per capsule	10	11	12	13	8	6

UF= Unifoliale, BF=Bifoliate, TF=Trifoliates, SP = Specific pattern.

Table 2. Various cytological abnormalities induced by gamma rays in *Linum ussitatisimum* L.

Treatment	Total number of PMCs	Types of meiotic abnormalities (%)							Total abnormality (%)
		Un	Ns	St	Sa	Sc	Mv	Bn	
Control	181	-	-	-	-	-	-	-	-
100Gy	170	1.2	4.7	1.42	2.01	1.60	0.82	0.99	12.74
200Gy	169	2.6	3.0	4.18	2.16	1.81	0.9	1.5	16.15
300Gy	95	8.03	9.65	8.10	0.85	1.71	1.7	1.65	21.69
400Gy	191	7.05	4.02	6.02	4.02	3.16	2.15	1.25	27.67
500Gy	160	11.8	2.4	5.41	3.25	2.76	3.2	1.86	32.68

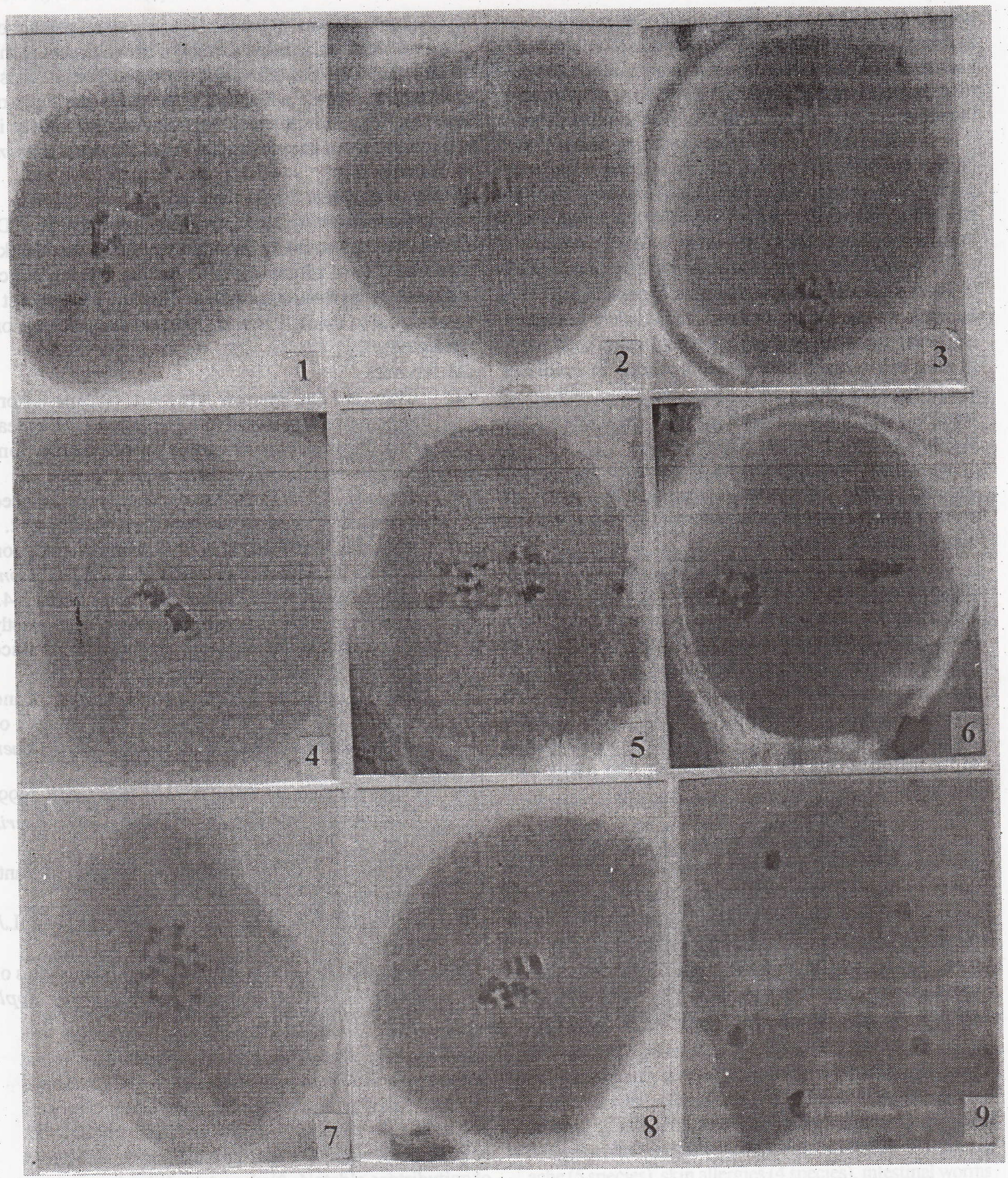
Un= Unorientation, Ns= Non synchronous div, St= Stickiness, Sa = Secondary association, Sc= Scattering, Mv= Multivalent formation, Bn= Binucleate.

abnormalities spread in all the stages of division. A dose-based increase in meiotic abnormalities was observed in gamma- irradiated sets. Highest percentage of abnormality was recorded to be 32.68% at the dose of 500Gy.

Though the most common abnormalities recorded in gamma- irradiated sets were unorientation and secondary-association (Fig.4), scattering (Fig. 5), non-synchronous-division (Fig.6), stickiness, desynapsis (Fig.7), multivalent (Fig. 8) and pentapolarity (Fig. 9) etc., but the most prominent abnormality was unorientation of chromosomes at metaphase, where chromosomes failed to arrange them at equatorial-plate. Its frequency was recorded to be highest (11.8%) at 500Gy dose of gamma-radiation.

Major anaphasic abnormalities include unequal separation or non-synchronous-division (Fig.6). However, other observed abnormalities included stickiness, secondary- association, scattering, multivalent formation and binucleate stage etc.

The results clearly revealed that the percentage of germination in gamma- irradiated sets of *L. usitatissimum* L. exhibited inverse relationship alongwith increasing irradiation doses, whereas, meiotic- anomalies increased showing dose dependent relation. Radiation induced



Figs. 1-9. Different stages of meiotic division in *Linum*.
Fig. 1- Normal diakinesis (n=15); **Fig.** 2- Normal Metaphase-I; **Fig.** 3- Normal Anaphase-I; **Fig.** 4- Unorientation & Secondary- association; **Fig.** 5- Scattering at Metaphase; **Fig.** 6-Non-synchronous-division; **Fig.** 7- Desynapsi; **Fig.** 8- Multivalent- formation; **Fig.** 9- Pentapolarity.

growth inhibition may basically be due to genetic- loss following the formation of chromosomal-aberration[1]. Unorientation of bivalents appears to be due to improper-functioning or breakage of spindle- fibres, which cause the scattering of chromosomes all over the cell -space.

Stickiness has been reported to be the result of partial- dissociation of nucleoprotein and alteration in the pattern of organization[2]. Jayabalan and Rao[3] reported stickiness due to disturbances in the cytochemically-balanced reactions.

Gaulden[4] attributes chemically induced stickiness to direct action of mutagen on the histone proteins leading to improper functioning of DNA. Multivalent formation has been reported in various plants like tomato[5] and Lentil[6]. In most of the cases multivalent formation in irradiated seed progenies has been contributed from reciprocal translocations, which result in segmental homology between non-homologous chromosomes.

Secondary associations of chromosomes in many diploid species have been interpreted as a result of modified chromosome arrangement due to duplication, interchanges or stickiness[7,8].

The record of scattering (Fig.5) and multipolarity (Fig.9) may be due to disturbance in the spindle formation. Desynapsis (Fig.7) is a phenomenon in which the homologous chromosomes come together and synapse at pachytene but fail to remain paired subsequently. In many cases, it is known to be governed by a single pair of recessive genes or induced by either environmental factors or by mutagens or brought about by an interaction of the genotype and the envionment or very rarely by a dominant gene[9].

On the basis of above cytomorphological investigations it can be concluded that all the morphological parameters displayed a trend of suppression at the lower doses of gamma-irradiation as compared to control .The dose of 300Gy was considered to be most beneficial dose of gamma-radiation for mutagenic-effectiveness because morphological parameters viz. height, girth, number of nodes, number of cotyledonary leaves, number of branch /plant, number of capsule/plant etc. showed a trend of enhancement at the dose of 300Gy. Beyond the dose of 300Gy, all these parameters showed a decreasing trend.On the basis of above observation it can be concluded that 300Gy is optimum dose of gamma-irradiation for *L. usitatissimum* L. mutagenesis.

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