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Mutagenic Response of *Macrosperma* Lentils to Gamma Rays

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Abstract: There was a decrease in all the biological criteria studied with an increase in dose of gamma rays in the M_1 generation. A dose dependent response was observed (seedling shoot length, r = - 0.998; root length, r = - 0.985; and pollen fertility, r = - 0.968) and at higher doses the effect was drastic. The LD_{50} (dose at which 50% lethality would occur) values for all biological end points were relatively moderate. In the M_2 generation, the chlorophyll mutation frequency increased in a linear fashion at low to medium (10-20 kR) and was erratic at higher doses. The chlorophyll mutant spectrum included xantha, albina, viridis, tigrina, light green, and dark green. A dose of more or less 20 kR may prove to be an appropriate for inducing chlorophyll mutations in *macrosperma* lentils studied.

Key words: Lentil (*Lens culinaris* Medik.), Mutations, Gamma radiation

Introduction

The diploid lentil (*Lens culinaris* Medik.) (2n = 14) has been classified as *Macrosperma* having bigger flattened seeds and *Microsperma* having small round seeds by Barulina, 1930 and others (Sharma *et al.*, 1995 and Solh and Erskine, 1984). Lentil is the second major pulse crop after chickpea and is an important source of protein in the staple diet of common peoples of Pakistan. The genetic improvement has been mainly through selection from land races. Hybridization is difficult since the florets are very small for hand emasculation and pollination. The generation of new variability is necessary and new techniques are needed to circumvent the present barriers in improvement. Numerous investigations have pointed to the usefulness of ionizing radiation for inducing mutations in crop plants. However, one of the requirements of mutation breeding for achieving effective selection is the determination of appropriate mutagen treatment which induces the highest degree of genetic variability (Sharma and Sharma, 1984). The present study was designed to observe the effect of different doses of gamma rays on seedling growth and to determine the appropriate dose for inducing mutations in lentil. The study included biological parameters such as seedling shoot length, root length and pollen fertility in the M_1 generation. In M generation, the frequency and spectrum of chlorophyll mutations were studied. The mutagenic effectiveness and efficiency of different doses of gamma rays in lentil was also estimated.

Materials and Methods

Dry and well-filled seeds of five *Macrosperma* genotypes of lentil were treated with four doses of gamma rays ranging from 10 to 40 kR with an interval of 10 kR by a ^{60}Co source (2000 seeds). After irradiation, one hundred seeds per Petri dish along with untreated controls were sown in three replications with completely randomized design. The seeds were spread uniformly on filter paper moistened with distilled water. The Petri dishes were placed in an incubator (22 ± 3 °C). After germination, the Petri dishes were filled with autoclaved sand for the nourishment of seedlings. Two weeks after sowing, seedling shoot and root lengths were recorded.

For each treatment, 300 seeds were sown in the field in three replications with randomized complete block design to raise M_1 generation. Each replicate consisted of 100 seeds each planted about 10 cm apart. Pollen fertility was estimated from the flowering buds of five randomly selected plants in each replication under microscope. The flowering buds were excised early in the morning and fixed in acetic alcohol (1 : 3) for 24 hours at room temperature. The fixed buds, stored at low temperature in 70% alcohol, were squashed in 1% acetocarmine stain on glass slide. Three slides were prepared for each plant and were screened for pollen fertility. Pollen stainability was used as a criterion for pollen fertility. Well-filled, darkly stained pollen grains were considered fertile, and partially filled or unstained ones were considered sterile.

To ensure sufficient M_1 plants at each dose, the remaining seeds were sown adjacent. To raise the M_2 generation, seeds from the individually harvested M_1 plants were sown in plant progeny rows alongwith parental controls. Chlorophyll mutants were scored soon after emergence. Chlorophyll mutation frequency was estimated both as percent M_2 families segregating (M_1 plant basis) and as mutations in percent of M_2 plants (M_2 plant basis). Mutagenic effectiveness and efficiency were calculated according to Konzak *et al.* (1965) as follows:

$$\text{Mutagenic effectiveness} = M/kR$$
$$\text{Mutagenic efficiency} = M/L \text{ or } M/P$$

where M is the frequency (in percentage) of chlorophyll mutations in the M_2 generation estimated on an M_2 plant basis, kR represents gamma ray dose, L is the percentage reduction in seedling survival in M_1, and P is the percentage reduction of pollen fertility in M_1.

The data were subjected to an analysis of variance. Correlation coefficient (r) was calculated for gamma ray dose and mutagenic response.

Results and Discussion

Highly significant differences were observed among genotypes as well as among doses for shoot and root lengths (Table 1). Drastic reductions of seedling shoot and root length were observed for 20 kR to 40 kR in all the genotypes. ILL-6024 showed 56.7% reduction in shoot

Table 1: Effect of different doses of gamma rays on laboratory grown *Macrosperma* lentil seedling shoot and root length in the M_1 generation

Accession	Parameter of growth	Growth* (cm) at different doses of gamma rays (k R)					Reduction (%) in growth at different of gamma rays doses (k R)			
		0	10	20	30	40	10	20	30	40
ILL-4605	Shoot	13.3	13.1	11.5	10.0	8.3	1.5	13.5	24.8	37.6
	Root	8.4	8.2	7.2	5.1	4.4	2.4	14.3	39.3	47.6
ILL-5782	Shoot	12.8	12.1	10.0	6.8	5.6	5.5	21.9	46.9	56.3
	Root	6.3	5.7	4.8	3.1	2.4	9.5	23.8	50.8	61.9
L-605	Shoot	14.7	13.2	9.6	5.0	1.6	10.2	34.7	66.0	89.1
	Root	7.5	6.4	4.6	2.5	1.8	14.7	38.7	66.7	76.0
ILL-5748	Shoot	19.8	12.2	7.7	3.3	1.1	38.4	61.1	83.3	94.4
	Root	11.4	5.3	3.4	2.0	1.1	53.5	70.2	82.5	90.4
ILL-6024	Shoot	13.4	5.8	4.0	2.1	1.4	56.7	70.1	84.3	89.6
	Root	6.1	3.4	2.2	1.5	0.1	44.3	63.9	75.4	83.6
LSD 5%	Shoot			0.907						
	Root			0.808						

* Recorded 2 weeks after sowing

Table 2: Effect of differet doses of gamma rays on *Macrosperma* lentil pollen fertility in the M_1 generation

Accession	Dose (k R)	Number of pollen grains studied	Percentage of fertile pollen	Pollen fertility as % of control
ILL-4605	0	3983	94.10	100.00
	10	4106	90.61	96.29
	20	4190	70.60	75.03
	30	4247	55.47	58.95
ILL-5782	0	5504	97.11	100.00
	10	4319	92.96	95.73
	20	3883	66.80	68.79
	30	3657	40.61	41.82
ILL-605	0	5281	93.13	100.00
	10	4901	82.33	88.40
	20	4796	55.23	59.30
	30	3815	37.59	40.36
ILL-5748	0	4861	96.36	100.00
	10	4943	94.34	96.87
	20	4454	51.50	53.45
	30	5278	34.01	35.29
ILL-6024	0	5917	92.01	100.00
	10	4808	84.40	91.73
	20	5177	41.47	45.07
	30	4833	28.76	31.26
LSD 5%			15.31	

length and ILL-5748 showed 53.5% in root length at the lowest dose of 10 kR. Radiation showed highly significant negative correlations with shoot (r = - 0.998) and root length (r = - 0.985). Similar responses of radiation on different crop species were noticed by Tufail *et al.* (1993) and Sharma *et al.* (1993) in lentil; Rafiullah *et al.* (1994) in brassica species. Reduced growth has been explained on the basis of changes in auxin synthesis, ascorbic acid content, and physiological and biochemical disturbances (Gordons, 1957; Gunckel and Sparrow, 1954; Singh, 1974; Usuf and Nair, 1974).

Pollen fertility underwent significant reduction with increasing dose of gamma rays (Table 2). With a dose of 10 kR, the pollen fertility was 96.3, 95.7, 88.4, 96.9, and 91.7% of the control while decreases of 41.0, 58.2, 59.6, 64.7, and 68.7 of the control at the highest dose of gamma rays were observed in ILL-4605, ILL-5782, L-605, ILL-5748, and ILL-6024 respectively. At 20 kR and above, the decrease in pollen fertility was drastic while at lower dose of 10 kR, it was gradual in all the genotypes. The high pollen sterility following exposure to gamma rays (r = - 0.968) may be attributed to meiotic disturbances.

Chlorophyll mutants increased with gamma ray dose up to 20 kR (Table 3). At 20 kR, the frequencies observed were 4.8 and 0.77% on M_1 and M_2 plants basis respectively in ILL-4605, 5.6 and 1.18% in ILL-6024. Beyond 20 kR dose of gamma rays, the frequencies of chlorophyll mutants declined. No chlorophyll mutations were observed in the nontreated (0 kR) controls. The saturation effect at higher doses has been explained by intrasomatic selection (Sreekantaradhya and Madhavamanon, 1979) and rigor of both diplontic and haplontic sieves (Swaminathan, 1961). Ehernberg (1955) has indicated that at very high doses, the dose rate curves may deviate from linearity and thus produce lower than expected mutation yields. The present studies also showed similar results of nonlinearity for chlorophyll mutations with increasing levels of gamma ray exposures. The data on spectra of chlorophyll mutations are

Table 3: Frequency of *Macrosperma* lentil chlorophyll mutations in the M_2 generation following treatment with different doses of gamma rays

Accession	Dose (k R)	No. of M_1 progenies scored	No. of M_2 plants scored	No. of M_1 progenies segregating for mutations in M_2	No. of M_2 mutatnts	Mutation frequency %	
						M_1 Plant basis	M_2 plant basis
ILL-4605	0	125	3498	-	-	-	-
	10	125	3467	3	15	2.4	0.43
	20	125	3392	6	26	4.8	0.77
	30	125	3256	4	21	3.2	0.62
	40	125	3256	2	8	1.6	0.25
ILL-5782	0	125	3446	-	-	-	-
	10	125	3402	4	16	3.2	0.47
	20	125	3327	7	29	5.6	0.87
	30	125	3304	4	22	3.2	0.67
	40	125	3213	3	12	2.4	0.37
ILL-605	0	125	3480	-	-	-	-
	10	125	3419	7	32	5.6	0.94
	20	125	3307	8	42	6.4	1.27
	30	125	3283	3	13	2.4	0.40
	40	125	3116	3	7	2.4	0.23
ILL-6024	0	125	3469	-	-	-	-
	10	125	3304	6	28	4.8	0.85
	20	125	3318	4	39	6.4	1.18
	30	125	3321	8	14	3.2	0.44
	40	125	3016	2	5	1.6	0.17
	Total	3125	82624	104	467		

Table 4: Spectrum of *Macrosperm* lentil chlorophyll mutations in the M_2 generation (M_2 plant basis) following treatment with different doses of gamma rays

Accession	Dose (k R)	Spectrum of chlorophyll mutations (%)						Total plants scored
		Xantha	Albina	Viridis	Tigrina	Light green	Dark green	
ILL-4605	0	-	-	-	-	-	-	3498
	10	0.29	-	-	-	0.09	0.06	3467
	20	0.44	0.15	0.03	-	0.09	0.06	3392
	30	0.21	0.12	0.03	-	0.18	0.09	3405
	40	0.15	-	-	-	0.09	-	3256
ILL-5782	0	-	-	-	-	-	-	3446
	10	0.29	-	-	-	0.12	0.06	3402
	20	0.51	0.09	-	-	0.15	0.12	3327
	30	0.24	-	0.06	-	0.21	0.15	3304
	40	0.09	-	0.03	-	0.16	0.09	3213
ILL-605	0	-	-	-	-	-	-	3412
	10	0.57	-	-	0.06	0.24	0.06	3308
	20	1.10	0.12	0.06	0.03	0.58	0.09	3265
	30	0.52	-	0.03	-	0.23	0.07	2905
	40	-	-	-	0.03	0.45	0.07	2905
ILL-5748	0	-	-	-	-	-	-	3480
	10	0.44	-	0.09	-	0.35	0.06	3419
	20	0.82	-	0.06	-	0.15	0.15	3307
	30	0.09	-	0.06	-	0.21	0.03	3283
	40	0.03	-	0.10	-	0.10	-	3116
ILL-6024	0	-	-	-	-	-	-	3469
	10	0.36	-	0.06	-	0.27	0.15	3304
	20	0.54	0.12	0.06	-	0.30	0.15	3318
	30	0.09	-	0.03	-	0.19	0.12	3221
	40	-	-	0.03	-	0.10	0.03	3016

shown in Table 4. The types xantha, albina, viridis, light green, and dark green were observed in all the genotypes. The type tigrina mutants was noticed only in L-605. The spectra of chlorophyll mutations showed that xantha mutants were frequently induced, which is quite similar to the results of Sarker and Sharma (1989) followed by light green and dark greens. Tigrina and albina mutations were very rare. However, the incidence of the various types of

Table 5: Mutagenic effectiveness and efficiency of different doses of gamma rays in *Macrosperma* lentils

Accession	Dose (k rad)	Effectiveness (M/krad)	Seedling survival reduction in M_1 (% control) (L)	Pollen fertility reduction in M_1 (% control) (P)	Efficiency (%)	
					M/L	M/P
ILL-4605	0	-	-	-	-	-
	10	0.043	0.8	3.7	0.54	0.12
	20	0.038	7.9	24.9	0.10	0.03
	30	0.021	18.8	42.4	0.03	0.02
	40	0.006	35.2	-	0.01	-
ILL-5782	0	-	-	-	-	-
	10	0.047	1.9	4.1	0.25	0.12
	20	0.044	10.0	32.4	0.09	0.03
	30	0.022	54.0	57.9	0.01	0.01
	40	0.009	58.6	-	-	-
ILL-605	0	-	-	-	-	-
	10	0.094	11.9	7.3	0.08	0.13
	20	0.010	50.7	41.2	0.04	0.05
	30	0.028	75.0	61.5	0.01	0.01
	40	0.014	85.4	-	0.01	-
ILL-5748	0	0.094	19.2	3.3	0.05	0.29
	10	0.064	44.4	46.6	0.03	0.03
	20	0.013	79.1	64.7	0.01	0.01
	30	0.006	89.9	-	0.003	-
ILL-6024	0	-	-	-	-	-
	10	0.085	17.9	8.0	0.05	0.11
	20	0.059	59.4	53.7	0.02	0.02
	30	0.015	79.7	68.5	0.01	0.01
	40	0.004	90.1	-	0.002	-

chlorophyll mutants did not follow a dose-related trend. Among doses of gamma rays, 10 – 20 kR were observed more effective for inducing mutations in ILL-6024, ILL-5748 whereas 10 – 30 kR were more effective in ILL-4605 and ILL-5782 followed by L-605 (Table 5). In general, the lower doses were more effective than higher doses for inducing mutations. The estimates of efficiency based on lethality and pollen sterility also indicated that lower to medium doses of gamma rays were more efficient and higher doses were less efficient than lower doses. From the present study, it appeared that a dose of more or less 20 kR may prove an appropriate for inducing mutations in *macrosperma* lentils.

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