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Mutagen sensitivity and mutability in lentil

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Summary. Seeds of two cultivars, each of 'macrosperma' and 'microsperma' varietal groups of lentil were mutagenised with gamma-rays and NMU to determine their mutagen sensitivity and mutability. The increasing doeses of gamma-rays and NMU decreased germination, root and shoot length, pollen fertility and plant survival, but increased the occurrence of leaf spots. The root system was found to be more sensitive to both mutagens than the shoot. The 'macrosperma' varietal group was more sensitive to both the mutagens than 'microsperma' group. In 'microsperma' group, variety 'Pusa-1' was more sensitive to both the mutagens than 'L-259', while in the 'macrosperma' group 'L-1491' showed more sensitivity to the mutagens than 'L-1492'. Radio-sensitivity corresponded positively with chemo-sensitivity in both varietal groups. There was a positive relationship between radio- and chemo-sensitivity of the genotypes and their mutability. The results also revealed the existence of a parallelism between radio-mutability and chemo-mutability. Due to saturation in the mutational events and vigour of both diplontic and haplontic selection in the biological material, the mutation frequency either decreased or remained constant at higher doses of the mutagens.

Key words: *Lens culinaris* – 'Macrosperma' – 'Microsperma' – LD_{50} – Mutagen sensitivity – Mutability – Radiosensitivity

Introduction

It is well known that extreme diversity exists between biological organisms in their response to mutagens. Response to seed treatment with various mutagens provides valuable information for mutation breeding as it facilitates the planning of the experiments designed to get higher mutation frequency. Reports have been published concerning studies on the biological effects of radiations and chemical mutagens and the relative mutagen sensitivity in lentil (Sinha and Godward 1968; Uhlik 1971; Sharma and Kant 1975). However, in the present investigation, more extensive results are presented regarding the mutagen sensitivity of different genotypes, the similarities or differences between genotypes with regards to radio-sensitivity and chemo-sensitivity, radio-mutability and chemo-mutability and the relationship between mutagen sensitivity and mutability following treatment of small-seeded ('microsperma') and large-seeded ('macrosperma') genotypes of lentil (*Lens culinaris* Medik.) with gamma-rays and N-nitroso-N-methyl urea (NMU).

Materials and methods

Microsperma genotypes ('Pusa-1' and 'L-259') and 'macrosperma' ('L-1491' and 'L-1492') were used in this study. Samples of 200 air-dried and well-filled seeds having a 10% moisture content were exposed to 6, 10, 14, 18 and 22 kR gamma-rays using a 5,500 Ci ^{60}Co gamma-chamber 4000 (B.A.R.C., India) at a dose rate of 120 R/s. Seeds were also treated with a 0.00 (control), 0.005, 0.01, 0.02, 0.03 and 0.04% solution of NMU prepared in phosphate buffer (pH 7.0) for 6 h with intermittent shaking at 25 °C. After the chemical treatment, the seeds were washed under running water for a half h. The seeds treated with mutagens along with 200 untreated control seeds (soaked in phosphate buffer) were sown immediately in the germinating trays and in the field without any post-treatment.

Characters such as root and shoot length of 7-day-old seedlings grown at 25 ± 1 °C temperature and 97% relative humidity in the laboratory, germination on 25th day in the field, plant survival at maturity, frequency of leaf spots and

Table 1. LD_{50} of lentil genotypes for different biological parameters in the M_1 generation

Biological parameter (% reduction)	Gamma rays (kR)				NMU (%)			
	Microsperma		Macrosperma		Microsperma		Macrosperma	
	'Pusa-1'	'L-259'	'L-1491'	'L-1492'	'Pusa-1'	'L-259'	'L-1491'	'L-1492'
Germination	13.5	18.6	9.2	10.7	0.015	0.027	0.008	0.012
Root length	17.7	21.8	9.1	11.8	0.024	0.034	0.014	0.091
Shoot length	>22.0	≫22.0	15.2	19.2	0.040	>0.040	0.027	0.029
[a] Leaf spots[c]	10.6	14.6	6.2	8.4	0.014	0.020	0.001	0.008
Pollen fertility[b]	10.8	16.2	2.3	6.1	0.006	0.023	≪0.005	<0.005
Plant survival	>22.0	≫22.0	15.6	17.7	0.028	>0.040	0.021	0.027

[a] % induction; > higher; ≫ much higher; < lower; ≪ much lower
[b] LD_{25}; [c] LD_{10}

Table 2. Relative mutagen sensitivity of lentil genotypes

Biological parameter	Radio- and chemo-sensitivity	
	Macrosperma	Microsperma
Germination	'L-1491'>'L-1492'>'Pusa-1'>'L-259'	
Root length	'L-1491'>'L-1492'>'Pusa-1'>'L-259'	
Shoot length	'L-1491'>'L-1492'>'Pusa-1'>'L-259'	
Plant survival	'L-1491'>'L-1492'>'Pusa-1'>'L-259'	
Leaf spots	'L-1491'>'L-1492'>'Pusa-1'>'L-259'	
Pollen fertility	'L-1491'>'L-1492'>'Pusa-1'>'L-259'	
Plant survival	'L-1491'>'L-1492'>'Pusa-1'>'L-259'	

> higher

pollen fertility were used to assess the dose response. LD_{50} was determined for root and shoot length, germination and plant survival. The induction of leaf spots in M_1 was less than 20% in most of the treatments, therefore, LD_{10} value was computed for this character. Similarly, all the treatments induced less than 40% pollen sterility. Therefore, with respect to this parameter, LD_{25} was determined. LD_{50}, LD_{25} and LD_{10} were calculated using the regression equation of Y (dose) on X (damage) as $Y = a + bX$. The seeds from each M_1 plant were harvested separately. M_1 plant progenies were grown in order to score the chlorophyll mutations in M_2 generation. The frequency of chlorophyll mutations was calculated as percentage of families segregating for any type of chlorophyll mutation (M_2 family basis) as well as the percentage of chlorophyll mutants in the population of a particular treatment (M_2 mutant basis).

Results

Mutagen sensitivity

Germination, root and shoot length, pollen fertility and plant survival decreased progressively with increasing doses of gamma-rays and NMU in both varietal groups of lentil. This is indicative from the significant correlation co-efficients of biological parameters with the doses of the mutagens (Figs. 1 and 2). However, the frequency of leaf spots did not show a significant correlation with the dosages given the mutagens.

Reduction in the 50% germination (LD_{50}) parameter occurred at 13.5 kR gamma-rays and 0.015% NMU in 'Pusa-1', whereas higher doses were needed for the mutagens of variety 'L-259' (Table 1). However, still lower doses were required to induce the same damage in macrosperma varieties. Reduction in root length to the extent of 50% was caused by 17.7 kR gamma-rays and 0.024% NMU in 'Pusa-1' and 21.8 kR gamma-rays and 0.034% NMU in 'L-259', and by much lower doses of both mutagens in the macrosperma varieties. In the microsperma group, LD_{50} for shoot length was higher in variety 'L-259' than 'Pusa-1', whereas it was lower in the macrosperma varieties. The doses of gamma-rays and NMU which produced leaf spots in 10% of the plants (LD_{10}) were 10.6 kR and 0.014%, and 14.6 kR and 0.020% in 'Pusa-1' and 'L-259', respectively, while doses of 6.2 kR and 0.001% and 8.4 kR and 0.008% were required in 'L-1491' and 'L-1492', respectively. In the microsperma group, varieties 'Pusa-1' and 'L-259' showed 25% pollen sterility (LD_{25}) at 10.8 kR gamma-rays and 0.006% NMU and 16.2 kR gamma-rays and 0.023% NMU, respectively. In the case of the macrosperma varieties, a reduction in 25% pollen fertility was not reached even at the minimum doses applied to the mutagens. LD_{50} for plant survival was the highest in variety 'L-259' followed by 'Pusa-1', 'L-1492' and 'L-1491'; following mutagenesis with both mutagens (Table 2).

Mutability within varietal groups

In the macrosperma group, the mutability (% mutated progenies) of variety 'L-259' (Table 3) induced with different doses of gamma-rays and NMU was lower (4.55–9.75 and 8.75–15.23, respectively) than that of

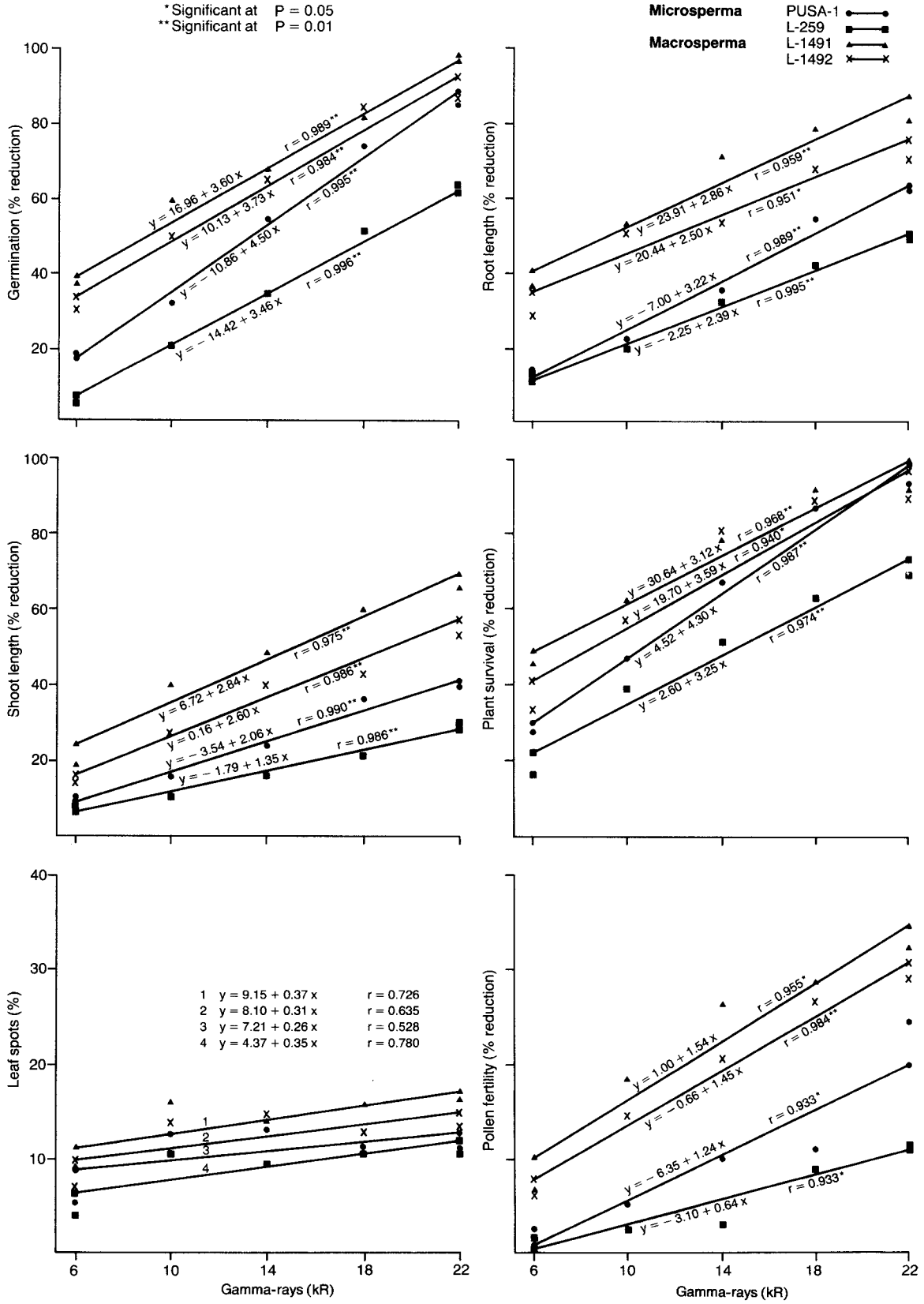

Fig. 1. Effect of gamma rays on different M_1 parameters in lentil

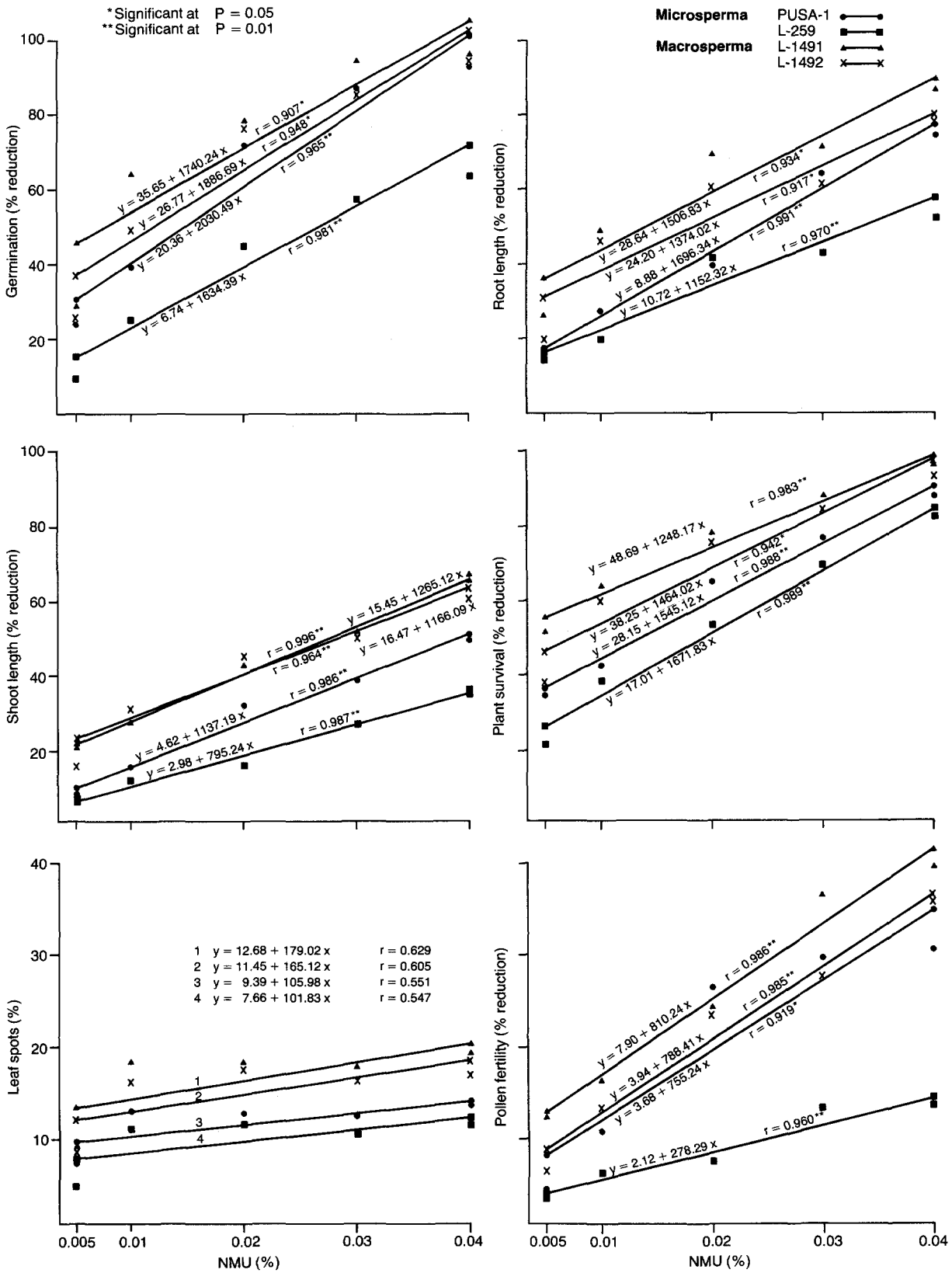

Fig. 2. Effect of NMU on different M_1 parameters in lentil

Table 3. Chlorophyll mutation frequency of lentil genotypes in the M_2 generation

Mutagen treatment	Microsperma				Macrosperma			
	'Pusa-1'		'L-259'		'L-1491'		'L-1492'	
	Mutated progenies (%)	Mutants (%)	Mutated progenies (%)	Mutants (%)	Mutated progenies (%)	Mutants (%)	Mutated progenies (%)	Mutants (%)
Gamma-rays (kR)								
6	5.81	0.85	4.55	0.53	14.63	2.10	9.76	1.23
10	11.11	1.27	9.52	1.26	21.27	2.56	16.28	2.43
14	11.34	1.45	9.09	1.12	19.81	2.06	16.33	2.51
18	10.53	1.11	9.75	1.43	20.12	2.23	15.31	2.15
22[a]	–	–	–	–	–	–	–	–
NMU (%)								
0.005	11.29	1.24	8.95	1.11	24.24	2.87	20.51	2.67
0.01	16.66	2.12	15.15	1.71	39.39	5.47	36.84	4.13
0.02	16.29	2.32	15.23	1.56	40.31	5.87	36.13	4.31
0.03	15.92	2.09	14.81	1.36	38.29	5.31	35.23	4.01
0.04[a]	–	–	–	–	–	–	–	–

[a] Data not included due to high lethality in M_1

'Pusa-1' (5.81–11.34 and 11.29–16.66). Similarly, radio- and chemo-mutability expressed as % mutants in 'L-259' was less (and ranged from 0.53–1.43 and 1.11–1.71, respectively) than that of 'Pusa-1' (0.85–1.45 and 1.24–2.32). The mutation frequency (% mutated progenies) obtained in the macrosperma group with different doses of gamma-rays was lower (9.76–16.33) in variety 'L-1492' (Table 3) than variety 'L-1491' (14.63–21.27). A similar trend was observed for NMU induced mutability of these genotypes. Gamma-ray and NMU induced mutation rate (% mutants) of 'L-1492' was also lower (1.23–2.51 and 2.67–4.31 respectively) than that in 'L-1491' (2.10–2.56 and 2.87–5.87).

Mutability between varietal groups

The mutability calculated as percentage of mutated progenies in the macrosperma varieties (Table 3) following treatment with 6 to 18 kR gamma-rays and 0.005 to 0.03% NMU was higher (9.76–21.27 and 20.51–40.31, respectively) than microsperma group (4.55–11.34 and 8.95–16.66). Likewise, mutation rates (% mutants) of different doses of gamma-rays (1.23–2.56) and NMU (2.67–5.87) were higher than the microsperma group (0.53–1.45 and 1.11–2.32, respectively).

Discussion

The increasing doses of mutagens caused a progressive increase in the biological damage measured in terms of reduction in germination, root and shoot length, plant survival and pollen fertility in the M_1 generation (Figs. 1 and 2).

Similar results have been reported in other leguminous crops (Sjodin 1962; Gregory 1968; Nerkar 1970; Hussein and Disouki 1976). However, the induction of leaf spots or a-sectors was independent of dosages given the mutagens.

The retardation in the root length was more pronounced than that found in the shoots (Table 1). The root system appears to be relatively more sensitive to mutagens. This can possibly be due to an inhibition of division in root cells by mutagens, which exert less effect on the elongation of shoot cells. The shoot growth is reported to be mainly due to the cell elongation while root growth is more dependent on cell division (Ivanova 1968). Mishra et al. (1980) also observed a greater delay in rooting than that of shooting in *Kalanchoe diagremntiana* leaves following irradiation with gamma-rays. Based on several M_1 parameters, other than those studied earlier (Sinha and Godward 1972), in two varieties, each of microsperma and macrosperma groups using gamma-rays and NMU, it was confirmed that macrosperma lentils are more sensitive to mutagens than microsperma ones. As in other food legumes, an increase in seed size is one of the most conspicuous trends under domestication and evolution, macrosperma forms are to be regarded as the more advanced (Zohary 1976). It is probable that during evolution an increase in seed size has been associated with a decrease in seed tolerance to mutagens. Al-Rubeai (1980) found a positive relationship between seed size and mutagen sensitivity in *Phaseolus* species.

At biologically comparable doses for different M_1 parameters, the macrosperma group was relatively more radio- and chemo-sensitive than the microsperma one (Table 2). 'Pusa-1' was more radio- and chemo-sensitive than 'L-259' of the microsperma group. Similarly in macrosperma, 'L-1491' was more radio- and chemo-sensitive than the variety 'L-1492'. A parallelism was observed between radio-sensitivity and chemo-sensitivity of the genotypes under study.

Some of the radio-sensitive mutants have also earlier been found to possess increased sensitivity towards alkylating chemicals (Brendel et al. 1970). Howard-Flanders et al. (1966) and Brendel et al. (1970) have shown that certain types of damage caused by radiations and chemical mutagens have common repair enzymes.

The chlorophyll mutation frequency induced with mutagens was 1.5–2 times higher in the mutagenically sensitive macrosperma group as compared to the less sensitive macrosperma group (Table 3).

Similar observations have been made in some food legumes (Sahai 1974; Venktaswarlu et al. 1978). By and large, the relative radio-mutability showed correspondence with chemo-mutability. The findings are in conformity with the results of Sidorova (1967) in peas.

Chlorophyll mutation rate increased with an increase in the dose of mutagens up to a certain level, beyond which it decreased or remained constant (Table 3). The phenomenon has been attributed to the intra-somatic selection, reduction in the number of M_2 plants produced by high sterile M_1 plants and other processes of gamete as well as zygote elimination (Ehrenberg and Nybom 1954; Ehrenberg et al. 1958; Dellaret 1980).

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