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Mutagenic effectiveness and efficiency of gamma irradiation in lentil cultivars (*Lens culinaris*)

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Abstract
Seeds of the three small seeded lentil (*Lens culinaris* Medik. var. *microsperma*) varieties viz. P-38, P=577 and LL=19 were irradiated with 100 Gy, 200 Gy and 300 Gy doses of gamma rays. The effectiveness and efficiency of gamma rays was higher at low dose levels and gradually decreased with increase in the dose of gamma rays. However the effectiveness of gamma rays (Calculated on the basis of M_2 seedlings) in cv. P-577 was increase with increase in the radiation dose. The radiation treatment of 200Gy and 300 Gy were moderately effective in P-38 and P-577 and least effective in LL-19.

Keywords: irradiation, *Lens culinaris*, mutagenic effectiveness and efficiency

Introduction
Lentil is an important crop of the Indian sub-continent where generally the small seeded (*Lens culinaris* Medik. var. *microsperma*) verities are grown. Lentil is an excellent dietary staple because of there of high protein content and nutrient density that compliments the nutritional deficiencies of cereal based diet, simultaneously its cultivation enhance the soil fertility by adding nitrogen, carbon and organic matter. It has also high level of dietary fiber, Vitamin B_1 and straw is valuable for animal feed. The varieties of lentil under cultivation have low yield levels because of narrow genetic base and susceptibility to various diseases. Studies on experimental mutagenesis in this crop are limited and confined mainly to cereal crops.
Mutation breeding is relatively a quicker method for improvement of crops. It has been observed that induced mutation can increase yield as well as other quantitative traits in plants and in some cases of animals (Verma, 2017) [20]. In these situations, the induced mutagenesis is one of the alternative breeding methods which can be applied to enhance the variability and correct one of more defects of cultivars. The induced mutation technology for the crop improvement have successfully been used to create about 3222 mutant varieties around the world, of which India contributes 330 mutant varieties. Overall 13 mutant lentil varieties released till now with only two from India. The usefulness of mutagens in plant breeding depends not only on its mutagenic effectiveness but also its mutagenic efficiency. Effectiveness means the rate of mutation induction as depended upon the mutagenic dose and efficiency refers to the mutation rate in relation to various biological effects, usually a measure of damage (Nilan *et al.*, 1965) [12]. Analysis of effectiveness and efficiency of mutagen is an imperative tool in mutation breeding for crop improvement. The prior information of effectiveness and efficiency facilitate the selection which is essential to recover high frequency of desirable mutation. The value of effectiveness and efficiency estimation depend on various factors like biological, environmental and chemical that can modify mutation rate of different mutagens. The present paper investigates the results on effectiveness and efficiency of gamma radiation in three small seeded varieties of lentil.

Materials and Methods
Dry, healthy and well filled seeds of uniform size of three *microsperma* varieties viz. P-38, P-577 and LL-19 were subjected to irradiation at 100 Gy, 200 Gy and 300 Gy of gamma rays from a CO^{60} source located at the Division of Genetics, IARI, New Delhi. For each treatment (dose) 200 seeds of each variety were used. Comparable controls were maintained throughout the experiment. Treated seed along with control were sown immediately after irradiation in the experimental field to raise M_1 generation.

Seed from each M_1 plant were harvested individually and separately. M_2 generation was raised following M_1 plant to row method. Twenty seeds from each M_1 plant were sown in a single row. A spacing of 25 x 15 cm was followed between rows and between plants in a row, respectively. Germination data in each treatment were recorded after 30 days of sowing. Pollen sterility was determined by staining the pollen grain from fully developed anther before dehiscence. The population was carefully screened for chlorophyll deficient mutations during early stages of seedling development i.e. after up to 12 days after germination. Various types of chlorophyll mutations were recorded in the M_2 generation and they were classified following Gustafsson (1940) [6] with slight modifications for calculating mutagenic effectiveness and efficiency. Following Konzak *et al.* (1965) [9], the mutation frequencies (% M_2 seedlings) were used to calculated mutagenic effectiveness and efficiency.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (\% } M_2 \text{ seedlings)}}{\text{Dose in Gy}}$$

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (\% progenies)}}{\text{\% lethality or sterility}}$$

Results and Discussion

The data on chlorophyll mutation frequency (both on plant progeny and M_2 seedling bases) were utilized for the determination of the effectiveness and efficiency of different doses of gamma rays and the results are presented in Table 1. Mutagenic effectiveness was calculated as the ratio of mutated families or mutant seedlings in M_2 to the amount (dose) of radiation applied, while the efficiency was determined as the ratio of mutated families to M_1 seedling lethality or pollen sterility in M_1.

The results, in general, indicate that the mutagenic effectiveness of gamma rays was high at lower dose (100 Gy). However, in cv. P-577, the effectiveness of gamma rays (calculated on the basis of M_2 seedlings), was observed to increase with increase in radiation dose (Table 1). 100 Gy gamma rays treatment was highly effective in all 3 lentil varieties whereas 200 Gy and 300 Gy treatments were moderately effective in P-38 and P-577 only and least effective in LL-19 (Table 1).

The data on mutagenic efficiency also reveal that low dose of gamma rays was highly efficient compared to higher doses. However, the efficiency values determined as the ratio of percent mutated families to sterility deviate from the above general trend particularly in cvs. P-38 and P-577 with 200 Gy dose in both cases being highly efficient compared to 100 Gy and 300 Gy gamma rays treatments. Among all the treatments in three varieties, 100 Gy gamma rays dose in LL-19 was highly efficient (0.66) and 200 Gy dose in the same variety was least efficient (0.12). The results, in general, indicate that the effectiveness and efficiency of gamma rays are high at lower dose levels and gradually decrease with increase in the dose of radiation applied.

The mutagenic effectiveness and efficiency is a measure of usefulness of a particular mutagen for mutation breeding in a particular crop genotype. Mutagenic effectiveness is measure of the frequency of mutations induced by the unit dose of the mutagen, whereas the mutagenic efficiency of a treatment indicates the extent of genetic damage recorded in M_2 generation in relation to the biological damage caused in M_1 (Konzak *et al.*, 1965) [9]. Therefore, the mutagenic effectiveness is an indicator of the genotype sensitivity towards the increasing mutagenic concentration, mutagenic efficiency explain the proportion of mutations in relation to undesirable biological effects, such as seedling injuries, pollen sterility and meiotic abnormalities induced by the particular mutagen. Since chlorophyll mutations are most conspicuous and easily detectable they have been extensively utilized to find out the sensitivity of a crop plant to mutagens and also the relative effectiveness and efficiency of different mutagenic treatments (Gaul, 1961; Gustafsson, 1965; Goud, 1967; Gautam *et al.*, 1992) [7, 4, 3]. Effectiveness means the rate of mutation induction as dependent upon the mutagenic dose; efficiency, on the other hand, refers to the ratio of mutations to various biological effects, usually a measure of damage (Nilan *et al*., 1965) [12]. In the present study, in general, it was observed that mutagenic effectiveness (based on M_1 plant progenies and M_2 plants) was higher at low dose levels and gradually decreased with increase in the dose of gamma rays. However, in LL-19 it was positively dose dependent. Mutagenic efficiency based on Mf/S (mutated families/sterility) and Mf/L (mutated families/lethality) of gamma rays was also higher at low doses. Similar results were earlier recorded by many workers (Gupta and Yashvir, 1975; Nerkar, 1977; Sharma and Sharma, 1979a, b; Subramanian, 1980; Tyagi, 1988; Laskar and Khan, 2017) [5, 11, 15, 16, 18, 19, 10]. The decrease in effectiveness and efficiency at higher dose levels of radiations may be due to failure in proportionate increase in mutation frequency with increasing dose and/or due to biological damage (sterility and lethality) occurring at a faster rate than the frequency of mutations (Konzak *et al.*, 1965) [12], while the increase in the mutagenic effectiveness with increased dose of gamma rays observed in LL-19 indicated that the mutation rate per unit dose of radiation has not reached the saturation point (Sharma and Sharma, 1981) [17]. The decline the effectiveness at higher dose apparently showed that the mutations are independent events and their rate of occurrences is not proportional to the strength of mutagen concentrations. The higher efficiencies of lower and moderate doses of mutagen was clearly due to the lower biological damage (lethality and sterility) which increased with the increase in the dose of gamma radiation. Similar observation of higher effectiveness and efficiency at lower and/or moderate doses of mutagens were also reported in *Lathyrus* (Nerkar, 1977) [11], *Pisum sativum* (Sharma, 2010), *Glycine max* (Khan and Tyagi, 2010) [8] and *Vigna radiata* (Wani *et al.*, 2011) [21] The observed increases in the mutagenic efficiency (Mf/S) at higher doses of gamma rays in some cases suggest that the induced pollen sterility was of a lower magnitude than the response for mutation induction by the mutagen. Mutagens induce differential genetic and cytogenetic changes (Fahmy and Fahmy, 1959) [1]. Therefore, the mutagenic effectiveness and efficiency will also depend upon the nature of induced mutations and chromosomal aberrations. The magnitude of effectiveness and efficiency also depends on the method of computing the rate of mutation (M_1 plant progenies or M_2 Mutants) (Sharma, 1990; Wani, 2017) [14, 22]. Effectiveness and efficiency of gamma rays were found to vary with varieties/varietal group used (Sharma and Sharma, 1979a) [15].

Table 1: Mutagenic effectiveness and efficiency of gamma rays in three varieties of lentil

Variety	Dose (in Gy)	% Mutated families	% Mutant M_2 seedlings	Lethality %	Pollen Sterility %	Mutagenic effectiveness		Mutagenic efficiency	
						%MF	%M_2 seedlings	% MF	%MF
						RD	RD	S	L
P-38	Control	-	-	0.00	4.34				
	100	6.59	0.50	16.96	18.07	0.0659	0.0050	0.36	0.39
	200	10.42	0.84	54.46	25.04	0.0521	0.0042	0.42	0.19
	300	15.63	1.46	69.64	41.04	0.0521	0.0049	0.38	0.22
P-577	Control	-	-	0.00	5.15				
	100	5.60	0.25	14.64	14.41	0.0560	0.0025	0.39	0.38
	200	9.18	0.62	30.67	20.27	0.0459	0.0031	0.45	0.30
	300	13.70	1.02	48.67	35.99	0.0457	0.0034	0.38	0.28
LL-19	Control	-	-	0.00	3.93				
	100	6.38	0.58	12.80	9.60	0.0638	0.0058	0.66	0.50
	200	3.57	0.38	30.49	19.91	0.0179	0.0019	0.18	0.12
	300	5.68	0.48	42.68	33.82	0.0189	0.0016	0.17	0.13

% MF = per cent mutated families
L = per cent lethality
S = Per cent pollen sterility
RD = Dose of gamma rays

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