Indian J. Genet., 59(4): 479-485 (1999)

INDUCTION AND ISOLATION OF MORPHOLOGICAL MUTATIONS IN DIFFERENT MUTAGENIC DAMAGE GROUPS IN LENTIL (LENS CULINARIS *MEDIK*)

I. S. SOLANKI* AND B. SHARMA

Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012

(Received: February 6, 1997; accepted: August 25, 1999)

ABSTRACT

Seeds of a large seeded lentil cv., Precoz Selection, were treated with three doses each of gamma rays, ethylene imine (EI) and N-nitroso-N-ethyl urea (NEU). Based on the frequency of morphological mutations, the mutagens were arranged in the order; NEU > EI> gamma rays. Different damage groups induced morphological mutations in the order : HH > HL > LH > LL. The morphological mutations induced changes for growth habit (compact, bushy and prostrate), foliage (narrow, broad, curly, laciniata and tendrillar), plant height (tall and dwarf), and maturity and flowering behaviour (early, late and sterile). A mild relative mutagenic specificity, and differences in mutability of genes for different traits were observed. In general, the spectrum of morphological mutations was not influenced by the groups of M_1 damage, except that some mutation types occurred more frequently than others (quantitative difference) in certain groups.

Key Words : Morphological mutations, mutation frequency & spectrum, lentil

In the Indian subcontinent, generally the small seeded varieties of lentil (*Lens culinaris* Medik. var. *microsperma* Zhukovsky) are grown. Studies on experimental mutagenesis in this crop are limited and confined mainly to *microsperma* varieties [1-5]. The varieties of lentil under cultivation have low yield levels because of narrow genetic base and susceptibility to various diseases. In these situations, the induced mutagenesis is one of the alternative breeding methods which can be applied to enhance the variability and correct one or more defects of a cultivar. For any mutation breeding programme, selection of effective and efficient mutagen(s) is very essential to recover high frequency of desirable mutations. The large seeded varieties of lentil are comparatively found to be more mutable than the small seeded varieties [1, 6]. In the present investigation undertaken on a large seeded lentil cv., Precoz Selection,

*Present address : Department of Plant Breeding, CCS Haryana Agricultural University, Hisar 125 004

the M_1 material was classified into four different mutagenic damage groups with an idea to identify the group that would carry maximum mutations in later generation(s).

MATERIALS AND METHODS

Dry and healthy seeds of uniform size of a *macrosperma* lentil cv., Precoz Selection, were treated with three doses (5, 10, and 20 kR) of gamma rays and three doses (0.005, 0.01 and 0.02%) each of ethylene imine (EI) and N-nitroso-N-ethyl urea (NEU). The seeds were irradiated with gamma rays in a Co^{60} gamma cell delivering 5000 R/min, and for treatment with chemicals, they were soaked in freshly prepared aqueous solution of EI and NEU with different concentrations for 6h at 21°c and then washed in running water. The treated seeds alongwith control were sown immediately in the field to raise the M_1 generation. The M_1 material in each treatment was classified on the basis of total biological damage to the plants at seedling stage expressed as leaf aberrations (a-sectors) as well as fertility reduction as a consequence of mutagenic treatment. The four mutagenic damage groups thus formed were: low seedling damage and low fertility reduction (LL), high seedling damage and low fertility reduction (HL), low seedling damage and high fertility reduction (LH) and high seedling damage and high fertility reduction (HH).

In M_2 generation, the treated progenies as well as control were screened for morphological mutations several times throughout the crop duration. Mutation frequency was calculated as the percentage of mutated M_2 progenies and plants. The mutations affecting different morphological features of the plant were grouped according to the classification proposed by Blixt [7]. The frequencies of morphological mutations in similar type of mutagenic damage groups in a particular mutagen were pooled over all the three doses.

RESULTS AND DISCUSSION

The frequency of morphological mutations in different mutagens and mutagenic damage groups in M_2 generation is given in Table 1. The overall frequency of morphological mutations in this study was 4.6% mutated progenies and 0.45% mutated M_2 plants. No morphological mutant was obtained in the untreated (control) material. Chemical mutagens induced higher frequency of morphological mutations than radiations (gamma rays). This is in agreement with earlier reports [1-5, 8-10]. NEU was most effective in inducing mutations with 5.5% mutated M_2 progenies and 0.56% mutated plants, followed by EI (4.3% and 0.42% mutated progenies and plants, resp.) and gamma rays (3.9% and 0.37%). Therefore, the general trend of

Table 1. Frequency of morphological mutations in different damage groups in M_2 generation (pooled over three doses of a mutagen)

Treatment	% mutated	
	progenies	plants
Control	—	—
Gamma rays:		
LL	2.1	0.18
LH	3.3	0.33
HL	4.5	0.46
HH	6.0	0.56
Overall	3.9	0.37
EI:		
LL	2.7	0.25
LH	3.5	0.35
HL	4.7	0.49
HH	6.4	0.63
Overall	4.3	0.42
NEU:		
LL	3.4	0.37
LH	5.0	0.48
HL	6.1	0.62
HH	7.4	0.75
Overall	5.5	0.56
Total Mutations (%)	4.6	0.45

morphological mutation frequency with different mutagens was, NEU > EI > gamma rays. Rapoport [11] for the first time referred to the nitroso compounds as "supermutagens" because of their high mutagenic effects. The superiority of NEU to induce the highest frequency of morphological mutations has been demonstrated by several workers in different crops [1, 2, 8-10, 12]. The frequency of morphological mutations was the lowest with gamma rays. This conforms to the results of Sharma [13], Zenelov [14], Mohan [9], Singh [10], and Tripathi and dubey [5].

Significant group differences with regard to morphological mutation induction were observed with all the mutagens (Table 1). The highest frequency of morphological mutations was recorded in group HH, and the lowest in group LL with all the mutagens. Among the remaining two groups, HL was found inducing higher mutation frequency than LH group. The HH group in NEU induced the highest frequency of morphological mutations (7.4% mutated M_2 progenies and 0.75% mutated plants),

Table 2. Spectrum morphological mutations in different damage groups in M_2 generations (pooled over three doses of a mutagen)

Damage group	Distribution of different morphological mutations (%)													
	Growth habit			Foliage						Plant height		Maturity		
	compact	bushy	prostrate	narrow	broad	rogue	curly	laciniata	tendrillar	tall	dwarf	early	late	sterile
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gamma rays														
LL	0.06	-	0.03	-	0.03	-	-	-	-	0.06	-	-	-	-
LH	0.06	-	0.06	0.06	0.06	-	0.03	-	-	0.03	-	0.03	-	-
HL	-	0.03	0.03	0.07	0.03	-	0.07	-	0.07	-	0.03	0.03	0.07	0.03
HH	-	0.07	0.03	0.10	-	0.10	-	-	0.03	-	0.07	0.10	0.07	-
Overall	0.03	0.02	0.04	0.05	0.03	0.02	0.02	-	0.02	0.02	0.02	0.04	0.03	0.01
EI:														
LL	0.08	0.03	-	-	-	-	-	0.03	-	0.08	-	0.03	-	-
LH	0.03	0.03	0.03	-	0.08	-	0.03	0.05	-	0.05	0.03	0.03	-	-
HL	-	0.05	0.03	0.08	-	0.05	0.08	-	-	0.03	0.08	0.05	-	0.03
HH	-	0.06	0.06	0.06	-	0.06	0.03	-	0.09	-	0.09	0.09	0.06	0.03
Overall	0.03	0.04	0.03	0.03	0.02	0.03	0.03	0.02	0.02	0.04	0.05	0.05	0.01	0.01
NEU:														
LL	0.12	0.06	0.03	0.03	-	-	-	-	-	0.09	-	0.03	-	-
LH	0.03	0.09	0.06	0.06	0.06	-	0.03	0.06	-	0.03	0.03	0.03	-	-
HL	-	0.08	0.05	0.08	0.05	0.05	0.08	0.03	0.02	0.03	0.03	0.05	0.05	0.02
HH	-	0.03	0.03	0.09	0.06	0.08	0.09	0.06	0.08	-	0.08	0.06	0.06	0.03
Overall	0.04	0.06	0.04	0.06	0.04	0.04	0.05	0.04	0.03	0.04	0.04	0.04	0.03	0.01
Total mutations(%)	0.03	0.04	0.04	0.05	0.03	0.03	0.04	0.02	0.02	0.03	0.04	0.04	0.02	0.01

followed by HH groups of EI (6.4% and 0.63% mutated progenies and plants, resp.) and gamma rays (6.0% and 0.56%). Likewise, with other damage groups, a similar trend of morphological mutation frequencies was observed. Different mutagenic damage groups based on the frequency of morphological mutations were arranged in the following order : HH > HL > LH > LL. This indicates that seedling damage (leaf-aberrations) is a better measure of the genetic changes induced by mutagenic treatments than seed sterility. Blixt [7] found leaf-aberrations to be the most effective index among all the M_1 parameters. Supporting the findings of the present report, Blixt *et al.* [15] and singh [10] also found positive response between the degree of leaf-aberrations in M_1 and morphological mutation rate in M_2 generation. With regard to sterility in M_1, several reports can be cited supporting the results of the present study [10, 16]. These studies showed positive correlation between sterility level in M_1 and frequency of mutations in M_2 generation.

The morphological mutations affecting different plant parts included changes for growth habit (compact, bushy and prostrate), foliage (narrow, broad, rogue, curly, laciniata and tendrillar), plant height (tall and dwarf), and maturity and flowering behaviour (early, late and sterile). The spectrum of morphological mutations induced by different mutagens and groups of mutagenic damage is presented in Table 2. Some of the morphological mutations, viz. mutations affecting foliage and growth habit, appeared more frequently (0.19% and 0.11%, resp.) than plant height and maturity (0.07%). Similar results have been reported earlier in lentil [1-5] and peas [9, 10, 14]. A mild relative mutagenic specificity was observed in relation to morphological mutations. Although different spectra following treatments with EI and NEU (14 types) and gamma rays (13 types) were obtained, they were not significantly different in these two groups of mutagens used. The differences observed in the spectra of morphological mutations were more of quantitative nature rather than qualitative [17-19].

Relative differences in the mutability of genes for different traits have been observed, as some of the mutation types appeared with distinctly higher frequencies in some mutagens. For instance, mutations affecting maturity appeared more frequently with gamma rays and NEU (0.08%), whereas EI induced more mutations for plant height (0.09%). NEU induced comparatively more mutations affecting foliage (0.26%) and growth habit (0.14%) than EI and gamma rays. The more frequent induction of certain mutation types by a particular mutagen may be attributed to the fact that the genes for these characters are probably more responsive to either alkylating compounds or ionizing radiations the mutagens with different modes of action. This could be due to differential mode of action of the mutagens on different base sequences in various genes. Nilan [19] concluded that different mutagens and treatment procedures may also change the relative proportion of different mutation types. Differences in the frequency of various morphological mutations have been reported in the published literature [1-5, 8-10, 20]. In general, the spectrum of morphological mutations was not influenced by the groups of M_1 damage, except that some mutation types occurred more frequently than others (quantitative difference) in certain groups [10]. This may be due to a relatively high frequency of mutation induction in that particular group.

ACKNOWLEDGEMENTS

The award of Senior Research Fellowship by the Indian Council of Agricultural Research, New Delhi, to the first author is gratefully acknowledged.

REFERENCES

1. S. K. Sharma. 1977. Induction of Mutations for Qualitative and Quantitative Characters in Lentil (*Lens culinaris* Medik.) Unpubl. Ph.D. Thesis, IARI, New Delhi.
2. A. Sarker. 1985. Efficiency of Early Generation Selection for Polygenic Mutations in Lentil (*Lens culinaris* Medik.). Unpubld. Ph.D. Thesis, IARI, New Delhi.
3. P. Dixit and D. K. Dubey. 1986. Mutagenic efficiency of gamma rays, NMU and their combinations in lentil (*Lens culinaris* Medik.) var. T.36. Indian J. Genet., **46**: 501-505.
4. B. S. Tyagi and P. K. Gupta. 1991. Induced macromutations in Lentil. LENS, **18**: 3-7.
5. A. Tripathi and D. K. Dubey. 1992. Frequency and spectrum of mutations induced by separate and simultaneous application of gamma rays and ethyl methane sulphonate (EMS) in two *microsperma* varieties of lentil. LENS, **19**: 3-8.
6. S. K. Sharma and B. Sharma. 1981. Induced chlorophyll mutations in lentil. Indian J. Genet., **41**(3): 328-333.
7. S. Blixt. 1972. Mutation Genetics in *Pisum*. Agri. Hort. Genet., **30**: 1-293.
8. S. Sahai. 1974. Cytogenetic, Mutational and Seed Protein Analysis of some Pulse Materials. Ph.D. Thesis, IARI, New Delhi.
9. D. Mohan. 1983. Combined Effect of Hybridization and Mutagenesis on Increasing Genetic Variability in Pea. Unpubld. Ph.D. Thesis, IARI, New Delhi.
10. N. P. Singh. 1988. Mutation Studies with Early Generation selection for Polygenic Variability in Peas (*Pisum sativum* L.) Unpubld. Ph. D. Thesis, IARI, New Delhi.
11. I. A. Rapoport. 1966. Supermutagens : Peculiarities and Mechanism of Action of Supermutagens. Publ. House, Nauka, Moscow, USSR, pp 9-23.
12. R. M. Desai and C. R. Bhatia. 1975. Mutagenecity of N-Methyl-N- nitroso urea and N-ethyl-N-nitroso urea. Mutation Res., **27**: 119- 121.
13. B. Sharma. 1965. A comparative study of physical and chemical mutagens on the basis of variation appearing in second generation. Izvstia Timiriazev Agric. Acad., Moscow, USSR, **4**: 127-140.
14. A. N. Zenelov. 1966. The mutagenic effects of gamma rays in treatments of immature pea seeds. Izvestija Timirjaz Sel' skoch. Acad. (Moskva), **6**: 162-167.

15. S. Blixt, O. Gelin, R. Mossberg, G. Ahnstrom, L. Ehrenberg and R. A. Lofgren. 1964. Studies of induced mutations in peas. Agri. Hort. Genet., **22**: 186-194.
16. E. I. Kivi. 1965. Some aspects of sterility effect of radiation on the basis of gamma- and x-rays treated barley. *In*: The Use of Induced Mutations in Plant Breeding: FAO/IAEA, Pergaman Press, pp. 151-158.
17. S. Blixt, L. Ehrenberg and O. Gelin. 1963. Studies of induced mutations in peas. VII. Mutation spectrum and mutation rate of different mutagenic agents. Agri. Hort. Genet., **21**: 178-216.
18. S. Blixt. 1964. Studies on induced mutation in peas. VIII. Ethylene imine and gamma rays treatments of the variety Witham Wonder. Agri. Hort. Genet., **22**: 171-183.
19. R. A. Nilan. 1967. Nature of induced mutations in plants. In: Induzierb Mutationen und ihre Nutzung, Erwin-Bauer-Ged. Vorl. IV. Abb. dentsch. Acad. Wissensch, Berlin, K1. Mediz., **2**: 5-20.
20. Vandana, A. Tripathi and D. K. Dubey. 1994. Frequency and spectrum of mutations induced by ethyl methane sulfonate (EMS) and diethyl sulfate (DES) in lentil var. K-85. LENS, **21**: 16-19.