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Induced polygenic variability in different groups of mutagenic damage in lentil (*Lens culinaris* Medik.)

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Abstract

Dry, healthy and uniform seeds of a *macrosperma* lentil (*Lens culinaris* Medik.) cv., 'Precoz Selection' were treated with three doses (0.005, 0.01 and 0.02%) each of ethylene imine (EI) and N-nitroso-N-ethyl urea (NEU) and gamma rays (5, 10 and 20 kR). In M_1, different groups of mutagenic damage were identified in each treatment *viz.*, low seedling damage and low sterility (LL), high seedling damage and low sterility (HL), low seedling damage and high sterility (LH), and high seedling damage and high sterility (HH). Effective selection was attempted in M_2 based on desired shift in character mean and higher CV than the highest observed in the control, followed by identification of M_3 families with higher mean than the highest in the control. Among the mutagens tested, NEU induced the highest frequencies of mutated and promising progenies with multiple characters in both the M_2 and M_3, followed by EI and gamma rays, and different groups of mutagenic damage were observed to follow the pattern: HH > HL > LH > LL in the M_2 and HH > LL in the M_3.

Key words: Lentil, induced mutations, multiple characters, polygenic variability

Introduction

Lentil (*Lens culinaris* Medik) is an important pulse crop of the Indian subcontinent. As the exploitation of variability through recombination breeding is tedious due to tiny flower, mutation breeding is another advisable approach for lentil improvement. Studies on experimental mutagenesis in lentil are limited and confined to *microsperma* varieties [1-5]. These studies have shown that this crop is highly sensitive to mutagenesis. The success of any mutation breeding programme depends not only on the quality of induced mutations, but also on the screening techniques to identify mutations which occur with a very low frequency among a large number of others with little breeding value. In general, selection for quantitative traits, such as yield, should preferably be carried out in early generations because most of the desired combinations of favourable alleles are likely to be lost in advanced generations due to intensive or even no selection for other traits [6]. However, after Brock [7-9], it became a common practice to advance only normal looking M_2 plants to the M_3 and apply the first cycle of selection not earlier than the M_3. This results in increased volume of non-mutated material and loss of promising variants. Consequently, the proclaimed advantage of rapid progress in breeding through mutation as compared to hybridization was lost. This probably acted as a discouraging factor for the workers to adopt micromutations as a tool for plant improvement on large scale, and most studies terminated at the M_3 itself after demonstrating the increase in variance and genetic advance [10]. However, the efficiency of early generation (M_2) selection has been reported in lentil [4], field pea [11] and mungbean [12]. Therefore, keeping in view the above facts, the present study aims to evolve a technique to increase the frequency of micromutations and ensure greater reliability of early generation selections.

Materials and Methods

Five hundred, 1000 and 1500 dry, healthy and uniform seeds of a *macrosperma* lentil cv., 'Precoz Selection' were treated with three doses each viz., 0.005, 0.01 and 0.02% of ethylene imine (EI) and N-nitroso-N-ethyl urea (NEU), and 5, 10 and 20 kR of gamma rays, respectively. The treated and control (500 untreated) seeds were sown immediately in the field to raise the M_1. The M_1 plants exhibited mutagenic damage as leaf aberrations (a - sectors) at seedling stage and fertility reduction (sterility) at maturity. They were classified into four groups: low seedling damage and low sterility (LL), high seedling damage and low sterility (HL), low seedling damage and high sterility (LH), and high seedling damage and high sterility (HH).

From each group, plants with sufficient seed were taken to raise the M_2. The progeny of each M_1 plant constituted one M_2 family. The spacings between rows and plants were 30 and 5 cm, respectively. Five

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normal looking competitive plants from each M_2 family were taken randomly to record observations on eight quantitative characters (cf Table 1). The M_2 progenies which showed a higher coefficient of variation (CV) than the corresponding highest CV in the control for any of the characters were considered as carrying induced mutations, and the mean values of such progenies were compared with the population mean of the control. These progenies were then classified into groups with lower mean, unchanged mean and higher mean than the control families for each character. From such interfamily selection, it was possible to detect some promising M_2 families having a higher CV as well as a higher mean for various characters, but higher CV and lower mean for days to maturity (considering early maturity as a desirable trait). Further, more rigorous selection was exercised to identify only those M_2 families which showed a higher CV and desired mean (lower for days to maturity, higher for remaining characters) for more than one character, i.e., for multiple characters. These progenies were considered to be 'exceptionally promising' from standpoint of selection for yield improvement.

The M_3 was raised as single plant progenies of the selected M_2 plants (Table 3). Almost all M_2 families were advanced to the M_3 except those showing very poor grain yield. The M_3 consisted of material from only two extreme groups of mutagenic damage (LL and HH) in each treatment. The intermediate damage groups (LH and HL) were not continued beyond the M_2 due to the increased volume of experimental material. Observations were restricted to the same eight quantitative characters as in the M_2 by adopting the same procedure. As the intrafamily variance was expected to decline in the M_3, the comparison of mean values was considered as the most important criterion to estimate the effectiveness of M_2 selection. Therefore, the character mean of each M_3 family was compared with the highest mean value recorded in the control. An M_3 family having a higher mean than the highest in the control was considered as 'promising' to advance further. The mean, variance and CV were computed on a progeny and a population basis using the standard statistical procedures [13].

Results and Discussion

Significant interfamily variance was observed for all characters under study in all nine mutagenized populations. The overall analysis of polygenic variability induced by different mutagens in different groups of mutagenic damage (Table 1) showed that a fairly high number of mutated families for different quantitative characters can be identified in the M_2 itself [12, 14]. It was observed that all mutagens are not equally effective in generating polygenic variability. In general, EI and NEU induced a higher frequency of mutated progenies than gamma rays for all the traits in both the M_2 and M_3. Similar observations have been reported earlier in lentil [1, 4, 14], mungbean [15] and field pea [11]. The higher effectiveness of the alkylating agents over gamma rays in inducing polygenic variability in grain legumes is now confirmed [3, 11, 14, 16]. The lower efficiency of gamma rays than EI and NEU for induction of polygenic variability can be explained by the fact that gamma rays induce a greater proportion of chromosomal aberrations, whereas EI and NEU induce mostly the point mutations. However, Sareen and Koul [17] have reported higher effectiveness of gamma rays over the alkylating agent, ethylmethane sulphonate (EMS) in isabgol (*Plantago ovata* Forsk.). Among chemicals, NEU induced more variability for all the characters than EI in both the M_2 and M_3. Rapoport [18] has called the mutagens belonging to the nitroso group as "supermutagens" in view of their higher mutagenic effects, a consequence of their alkylating ability on the gene directly. Among the four groups of mutagenic damage, HH carried maximum variability for all the eight characters, resulting in the highest frequency of mutated families in the M_2, followed by HL, LH and LL. The most mutable character was pods/plant (32.2%), whereas seeds/pod was the least mutable (12.2%). Similar observations have been reported earlier in field pea [11].

The frequencies of promising progenies for various characters were calculated in different groups of mutagenic damage in the M_2 (Table 1). NEU was the most effective mutagen as it induced the highest frequency (from 4.0% for seeds/pod to 12.4% for pods/plant) of promising progenies, followed by EI (3.3% - 11.3%) and gamma rays (2.7% - 9.7%). The groups of mutagenic damage were observed in the following order with regard to the frequency of promising progenies: HH > HL > LH > LL [11].

The proportion of promising families (Table 2) was the highest (24.9%) in the NEU-treated population, followed by EI (23.4%) and gamma rays (20.1%). A similar trend was observed when promising families with multiple characters (2 - 6) were considered. The proportion of such progenies among the total promising progenies was observed in the order: NEU (67.2%) > EI (66.5%) > gamma rays (63.1%). The proportion of promising progenies with single or multiple characters in different mutagenic damage groups was observed in the order: HH > HL > LH > LL. These observations confirm the earlier report in field pea [11].

In the M_3, the promising progenies were identified by comparing their means with the highest character mean (lowest mean in case of days to maturity) of the control. The highest proportion of promising

Table 1. Frequency of mutated and promising progenies for various characters in different groups of mutagenic damage in M_2 (pooled over all treatments of a mutagen)

Group	Number of progenies in M_2		Mutated progenies (%)							
	Raised	Harvested	Days to maturity	Plant height	Branches/ plant	Clusters/ plant	Pods/plant	Seeds/pod	100-seed weight	Seed yield/plant
Control	174	100	-	-	-	-	-	-	-	-
Gamma rays										
LL	237	218	10.6(1.4)	11.5(0.9)	11.5(01.8)	16.1(03.2)	21.1(05.0)	06.0(0.0)	07.8(0.5)	18.3(03.7)
LH	247	188	16.5(4.3)	16.5(2.7)	17.6(04.3)	22.3(05.9)	26.6(08.0)	10.1(1.6)	11.2(2.1)	23.9(06.4)
HL	219	154	24.7(7.1)	24.7(6.5)	25.3(07.1)	23.9(10.4)	37.0(12.3)	13.6(3.9)	14.3(3.9)	33.8(12.3)
HH	228	182	30.8(9.9)	29.7(8.2)	28.6(09.3)	31.9(12.1)	39.0(14.8)	15.4(6.0)	15.9(6.6)	35.7(14.3)
Overall	931	742	19.9(5.4)	19.9(4.3)	20.1(05.4)	24.3(07.5)	30.2(09.7)	10.9(2.7)	12.0(3.1)	27.2(08.8)
EI										
LL	250	192	12.0(01.6)	12.5(1.6)	14.1(02.6)	18.2(04.2)	24.0(06.3)	08.3(1.0)	09.4(1.0)	20.3(04.7)
LH	257	168	18.4(04.8)	17.9(3.6)	20.8(04.8)	25.0(07.7)	31.0(09.5)	10.7(1.8)	10.7(2.4)	26.2(08.3)
HL	254	146	24.7(08.2)	24.7(6.8)	24.7(07.5)	29.5(11.0)	37.0(14.4)	14.4(4.1)	15.1(5.5)	30.8(11.6)
HH	248	182	29.1(11.0)	25.3(8.2)	29.7(11.0)	33.5(14.8)	39.0(15.9)	16.5(6.6)	17.0(7.1)	34.6(15.9)
Overall	1009	688	20.8(06.3)	19.8(4.9)	22.1(06.4)	26.3(09.3)	32.4(11.3)	12.4(3.3)	12.9(3.9)	27.8(10.0)
NEU										
LL	234	181	18.2(03.9)	16.0(2.2)	18.2(03.3)	23.2(07.2)	29.3(08.8)	09.9(1.1)	10.5(1.7)	26.5(07.2)
LH	230	187	20.9(08.3)	18.7(4.8)	21.4(04.8)	25.7(09.6)	31.0(10.7)	12.3(3.2)	13.9(3.7)	28.3(09.6)
HL	264	206	24.8(08.3)	21.8(6.3)	24.8(08.3)	29.1(12.1)	34.0(12.6)	13.6(4.9)	16.5(5.8)	32.0(13.1)
HH	245	173	30.6(12.1)	27.7(9.8)	30.6(12.7)	34.7(16.2)	41.6(17.9)	17.9(6.9)	19.7(8.1)	38.7(17.9)
Overall	973	747	23.6(07.5)	21.0(5.8)	23.7(07.2)	28.1(11.2)	33.9(12.4)	13.4(4.0)	15.1(4.8)	31.3(11.9)
Overall expt.	2913	2177	21.5(6.4)	20.3(5.0)	22.0(6.3)	26.2(9.4)	32.2(11.2)	12.2(3.4)	13.4(4.0)	28.8(10.2)

Figures in parentheses indicate the frequency of promising progenies.

Table 2. Frequency of promising progenies with multiple characters in different groups of mutagenic damage in M_2 (pooled over all treatments of a mutagen)

Group	Number of progenies studied	Promising progenies		Progenies with various combinations of characters						Promising progenies with multiple characters	
		No.	%	1	2	3	4	5	6	No.	%
Gamma rays											
LL	218	25	11.5	12	7	4	2	-	-	13	52.0
LH	188	30	16.0	12	10	4	3	1	-	18	60.0
HL	154	40	26.0	13	10	9	4	3	1	27	67.5
HH	182	54	29.7	18	10	10	9	4	3	36	66.7
Overall	742	149	20.1	55	37	27	18	8	4	94	63.1
EI											
LL	192	24	12.5	9	8	5	2	-	-	15	62.5
LH	168	35	20.8	13	9	6	5	2	-	22	62.9
HL	146	39	26.7	13	7	7	6	4	2	26	66.7
HH	182	63	34.6	19	13	12	11	5	3	44	69.8
Overall	742	161	23.4	54	37	30	24	11	5	107	66.5
NEU											
LL	181	23	12.7	9	6	4	3	1	-	14	60.9
LH	187	40	21.4	13	10	7	6	3	1	27	67.5
HL	206	58	28.1	20	12	12	7	5	2	38	65.5
HH	173	65	37.6	19	13	12	11	6	4	46	70.8
Overall	747	186	24.9	61	41	35	27	15	7	125	67.2
Overall expt.	2177	496	22.8	170	115	92	69	34	16	326	65.7

progenies (17.6%) over the entire experiment was for pods/plant, followed by yield/plant (13.9%), pod clusters/plant (12.0%), branches/plant (8.6%), plant height (4.9%), days to maturity (3.9%), 100-seed weight (2.9%) and seeds/pod (2.7%). Thus, the characters showed variable response to selection. Among the

Table 3. Frequency of promising progenies for various characters in different groups of mutagenic damage in M3 (pooled over all treatments of a mutagen)

Group	Number of progenies studied	Promising progenies (%)							
		Days to maturity	Plant height	Branches/ plant	Clusters/ plant	Pods/plant	Seeds/pod	100-seed weight	Seed yield/plant
Gamma rays									
LL	600	1.2	1.0	2.5	5.2	7.3	0.3	0.3	5.7
HH	483	6.0	6.8	11.4	16.4	24.6	3.9	4.6	20.3
Overall	1083	3.3	3.6	6.5	10.2	15.1	1.9	2.2	12.2
EI									
LL	552	1.4	2.2	4.2	5.8	9.2	1.1	1.1	6.7
HH	514	5.2	7.8	12.8	17.5	26.1	4.3	4.5	20.4
Overall	1066	3.3	4.9	8.3	11.4	17.4	2.6	2.7	13.3
NEU									
LL	529	2.6	2.6	6.8	9.1	12.1	1.7	1.9	9.6
HH	476	7.8	10.3	16.0	21.0	30.0	5.7	5.9	23.7
Overall	1005	5.1	6.3	11.1	14.7	20.6	3.6	3.8	16.3
Overall expt.	3154	3.9	4.9	8.6	12.0	17.6	2.7	2.9	13.9

mutagens, NEU induced the highest frequency of promising progenies for all the characters, followed by EI and gamma rays. The frequency of promising families for all the characters was several times higher in the HH groups of all the mutagenic treatments than in their corresponding LL damage groups.

The proportion of promising progenies with multiple characters (Table 4) among the promising progenies in the M_3 was the highest in the NEU-treated populations (80.8%). The next in order were EI (79.0%) and gamma rays (76.9%). The damage group HH had much higher proportion (79.8-84.1%) of the promising progenies with multiple characters than the LL group (69.2-74.3%). The proportion of promising progenies with single and multiple characters in different groups of mutagenic damage in the M_2 and M_3 (Fig.1) followed the sequence: HH(10.4%) > HL(9.1%) > LH(7.0%) > LL(5.1%) in case of progenies with single characters and HH (23.5%) > HL (18.0%) > LH (12.3%) > LL (7.1%) for progenies with multiple characters in the M_2. Out of the two damage groups compared in the M_3, HH had much higher frequency of promising progenies with single (7.1%) and multiple characters (32.5%), than LL (4.0 and 10.6%, respectively). A large number of promising progenies isolated from the M_2 was confirmed as promising in the M_3 also, suggesting that the selection exercised in the M_2 was effective. The findings of this study are in agreement with the observations reported earlier in lentil [4], field pea [11] and mungbean [12].

In conclusion, the NEU treatments and the damage group HH carried the highest proportion of mutated

Table 4. Proportion of promising progenies with multiple characters in M3

Group	Number of progenies studied	Promising progenies		Progenies with various combinations of characters						Promising progenies with multiple characters	
		No.	%	1	2	3	4	5	6	No.	%
Gamma rays											
LL	600	65	10.8	20	20	19	6	-	-	45	69.2
HH	483	173	35.8	35	58	40	23	12	5	138	79.8
Overall	1083	238	22.0	55	78	59	29	12	5	183	76.9
EI											
LL	552	77	13.9	21	27	16	12	1	-	56	72.7
HH	514	194	37.7	36	70	45	25	11	7	158	81.4
Overall	1066	271	25.4	57	97	61	37	12	7	214	79.0
NEU											
LL	529	105	19.8	27	24	15	3	-	78	74.3	74.3
HH	476	208	43.7	33	73	52	23	16	11	175	84.1
Overall	1005	313	31.1	60	109	76	38	19	11	253	80.8
Overall expt.	3154	822	261	172	284	196	104	43	23	650	79.1

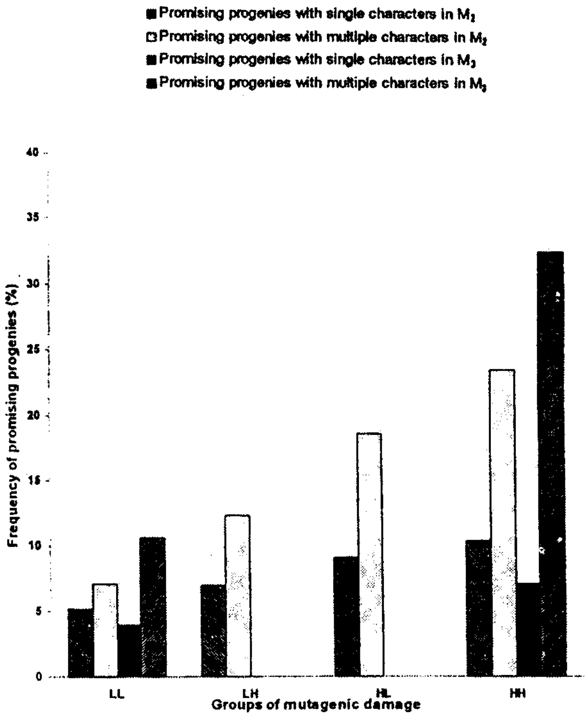

Fig. 1. Frequency of promising progenies in different groups of mutagenic damage

promising progenies. It is, thus, evident that efficiency of confirmed mutations for polygenic traits (micromutations) can be increased in a directed manner by concentrating on the HH damage group and the M_2 progenies showing high CV and higher mean in "positive" direction than the highest mean of the control.

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