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Early generation selection of polygenic mutations in lentil (*Lens culinaris* Medik.)

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Abstract

Healthy seeds of a *macrosperma* lentil 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). The M_1 material in each treatment was classified into four groups of mutagenic damage. On the basis of macromutations induced and intra- and interfamily selection in M_2 generation, different progenies were classified into three broad groups and raised as macromutational, selected and unselected populations in M_3. Among mutagens, NEU and between damage groups, HH (high seedling damage and high sterility) contributed maximum to the total M_3 promising selections. The contribution of M_2 and M_3 generations to the total selections was 80.9% and 19.1%, respectively. This suggested that although new promising selections (about one-fifth of the total) were added in M_3 to those already selected in M_2 the quantum of material nearly trebled, suggesting that promising progenies can be identified with high degree of confidence in M_2 on the basis of mean and variance.

Key words: Early generation selection, induced mutations, *Lens culinaris*, mutagens, polygenic variability.

Introduction

Among all the *rabi* pulses, lentil is second only to chickpea in area (1.1 mha) and production (0.9 mt) [1]. Cultivated lentils belong to two broad groups : the small seeded (*lens culinaris* Medik. var. *microsperma* Zhukovsky) and the large seeded (*Lens culinaris* Medik. var. *macrosperma* Zhukovsky). The large seeded varieties of lentil are comparatively found to be more mutable than the small seeded varieties [2]. Majority of the studies on mutagenesis in lentil have been conducted on the *microsperma* varieties [3-6].

Conventionally, it has been suggested that selection for polygenic traits should normally be practised in M_3 generation when the magnitude of variability is expected to be the highest [7, 8]. A good screening technique is a prerequisite for the identification and isolation of polygenic mutations which occur with a very low frequency in a huge mass of unmutated material. Sneepe [9] suggested that the selection for quantitative trais, 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. Attempts have been made in the past to explore the possibility of selecting for polygenic variability in earlier (M_2) generation with a view to economise time and effort in selection [5, 10, 11]. However, some studies have demonstrated that selection in M_3 is more effective than in M_2 [6, 12, 13].

A comparison of different studies on selection in M_2 and M_3 generations in lentil [2, 5], peas [10], mungbean [14] and chickpea [15] revealed that the two generations did not differ much in respect of selection response. However, Sharma [16] reviewed the work on mutation breeding in different food legumes and suggested that promising progenies can be identified with high degree of confidence in M_2 on the basis of mean and variance. Therefore, with a view to reach a sound conclusion on whether selection should be exercised in M_2 or M_3 the present investigation was undertaken in lentil.

Materials and methods

Dry and healthy seeds of uniform size of a *macrosperma* lentil 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). The treated seeds along with control

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(untreated seeds) were sown immediately in the field to raise the M_1 generation. The M_1 material in each of the nine treatments, on the basis of total biological damage to the plants at seedling stage expressed as leaf aberrations (a - sectors) as well as fertility reduction (sterility) at maturity as a consequence of mutagenic treatment, was classified into four groups of mutagenic damage 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). From each group and control, plants were taken to raise individual M_2 progenies in 4 m long rows at spacing of 30 cm between rows and 5 cm between plants in a row. All the treated as well as control progenies were screened for lethal chlorophyll mutations from emergence till the age of 4 week, whereas the viable chlorophyll and morphological mutations were scored several times throughout the crop duration. The procedures proposed by Blixt [17] for the identification and classification of macromutations in peas were followed.

Those M_2 progenies which showed segregation for macromutations (chlorophyll and morphological) were treated as a separate class, i.e., macromutational and the remaining progenies constituted the non-macromutational class. Five normal looking competitive plants from each control and M_2 progenies (belonging to both macromutational and non-macromutational classes) were taken randomly to record observations on eight quantitative characters (cf. Table 1). The M_2 progenies which showed higher coefficient of variation (CV%) than the corresponding highest CV in control for any of the characters were considered to be the progenies carrying induced mutations. Although a large number of progenies were identified as mutated (with higher CV) for the characters studied, all of them were not expected to be of equal selection value. The mutated progenies for each character were further divided into three groups, i.e., mutated progenies with higher, unchanged and lower mean than control. The character means of the mutated progenies were compared with the mean of control progenies in each mutagenic damage group. From such interfamily selection, it was possible to detect some promising M_2 progenies having higher CV as well as higher mean for various characters, except higher CV and lower mean for days to maturity (considering early maturity as a desirable character). Further, more rigorous selection was exercised to identify only those M_2 progenies which showed higher CV and desired mean (lower for days to maturity, higher for remaining characters) for more than one character. These progenies were considered to be exceptionally promising from selection point of view for yield improvement. Intrafamily selection was practised simultaneously with interfamily selection in the non-macromutational class. Three best plants were selected from each promising M_2 progeny, and finally, all selected as well as unselected M_2 plants were used to raise the M_3 generation.

The M_3 generation was raised as single plant progenies (4 m length) of M_2 plants with spacings of 30 and 5 cm between rows and plants, respectively, to study the micromutations in detail. The M_3 consisted of material from only two extreme groups of mutagenic damage (LL and HH) in each treatment. The remaining two groups (LH and HL) were not continued beyond M_2 in view of the increased volume of experimental material. Observations were restricted to the same eight quantitative characters as in M_2 taking five normal looking competitive plants at random from each progeny. As the intrafamily variance was expected to decline in M_3 the comparison of mean values was considered as the most important criterion to estimate the effectiveness of M_2 selection. Therefore, the mean value of each M_3 progeny was compared with the mean value recorded in control for different traits, and an M_3 progeny having higher mean than control was considered as promising to advance further. The mean, variance and CV were computed on progeny and population basis using the standard statistical procedures [18].

Results and discussion

In the present study, the M_2 material in different treatments was first divided into two classes, i.e., macromutational (comprising progenies which segregated for macromutations) and non-macromutational (comprising progenies which did not segregate for macromutations). In the M_2, selection was applied to those progenies which did not segregate for macromutations, and promising progenies were identified exclusively on the basis of higher CV and greater shift in mean in the desired direction. Thus, the entire M_2 material of macromutationally nonsegregating progenies was divided into "selected" and "unselected" lots. In previous studies [2, 5, 7, 8, 9, 19, 20], only the nonsegregating M 2 progenies were used to grow the M_3 generation. Selection for polygenic traits among the normal looking plants has definite advantage since a large number of progenies not carrying mutation(s) can be easily rejected. On the other hand, there are also progenies which have already segregated for macromutations ("macromutational") in the M_2 generation, in which the mutagen has definitely affected the genetic material (loci) for different morphological traits. The chances of the so-called "minor" genes also being hit in such cases can be expected to be higher than in the material where there is no evidence of genetic damage due to treatment. Therefore, the normal looking plants from the macromutational progenies were also harvested separately and raised in the M_3 along

with the plant progenies from the nonsegregating "selected" and "unselected" lots described above. The progenies again segregating for macromutations in the M_3 were rejected and observations for polygenic characters were recorded only in the nonsegregating families. Thus, the M_3 comprised three types of population, viz., macromutational, selected and unselected.

The comparison of the CV of different populations revealed some very interesting facts (Table 1). The highest CV was observed for seed yield/plant, followed by pods, clusters and branches/plant, whereas the lowest CV was depicted by days to maturity. The results reported earlier in lentil [11] support the findings of the present investigation. For all the polygenic traits studied, without exception, the CV decreased in the selected material as compared to the unselected population, because selection in M_2 shifted the mean in the desired direction (lower values in case of days to maturity, higher in the remaining seven traits), and thus, reduced its variability (CV). The unselected M_2 lots carried the entire induced variability in positive as well as negative directions which was further magnified in M_3 generation. The most interesting was the third group, i.e., the macromutational population which showed higher CV for all the characters than even the unselected material from the nonsegregating M_2 progenies. Similar results supporting the findings of the present study were also obtained in peas [10].

Table 1. Effect of M_2 selection on induced polygenic variability in M_3 generation for different characters

Character	CV in M_2		CV in different M_3 populations			
	Control	Mutagenized population	Control	Macromutational	Selected	Unselected
Days to maturity	1.3	2.4	2.5	6.1	5.1	5.6
Plant height (cm)	11.2	16.4	10.0	22.0	15.4	19.2
Branches/plant	42.2	59.8	26.3	66.2	37.2	64.7
Clusters/plant	45.2	73.4	24.4	75.6	40.0	73.9
Pods/plant	54.0	74.8	30.3	77.8	47.3	75.4
Seeds/pod	17.2	19.9	12.5	20.8	16.6	20.5
100-seed weight (g)	17.7	24.7	27.0	31.2	21.7	27.5
Seed yield/plant (g)	66.8	84.6	48.4	86.7	39.4	86.2

The highest frequency of promising progenies with multiple characters for all the polygenic traits was obtained in the selected population, followed by macromutational and unselected populations (Table 2). NEU induced the highest frequency of promising progenies with multiple characters in all the populations, followed by EI and gamma rays (Table 3). Solanki and

Table 2. Promising progenies with single and multiple characters in different M_3 populations (pooled over all treatments)

Population	Total progenies	Promising progenies		Promising progenies with various combinations of characters						Promising progenies with multiple characters	
		No.	%	1	2	3	4	5	6	no.	%
Macromutational	424	73	17.2	25	19	16	11	2	-	48	65.8
Selected	762	616	80.8	90	228	158	80	37	23	526	85.4
Unselected	1968	133	6.8	57	37	22	13	4	-	76	57.1
Overall experiment	3154	822	26.1	172	284	196	104	43	23	650	79.1

Table 3. Promising progenies with multiple characters in both groups of mutagenic damage in M_3 populations (pooled over three treatments)

Group of mutagenic damage	Macromutational population			Selected population			Unselected population		
	Total progeneis	Promising progenies		Total progenies	Promising progenies		Total progenies	Promising progenies	
		no.	%		no.	%		no.	%
Gamma rays									
LL	35	3	8.6	57	33	57.9	508	9	1.8
HH	85	9	10.6	168	113	67.3	230	16	7.0
Overall	120	12	10.0	225	146	64.9	738	25	3.4
EI									
LL	41	4	9.8	66	42	63.6	445	10	2.3
HH	102	12	11.8	180	129	71.7	232	17	7.3
Overall	143	16	11.2	246	171	69.5	677	27	4.0
NEU									
LL	51	6	11.8	93	60	64.5	385	12	3.1
HH	110	14	12.7	198	149	75.3	168	12	7.1
Overall	161	20	12.4	291	209	71.8	553	24	4.3
Overall experiment	424	48	11.3	762	526	69.0	1968	76	3.9

Sharma [11] reported that the proportion of promising progenies with multiple characters in M_2 and M_3 generations was higher for the chemicals than the gamma rays, and that among chemicals, NEU induced higher frequency of promising selections than EI. As far as the selection efficiency for promising progenies with multiple characters in different populations is concerned, the trend observed was selected > macromutational > unselected. HH group was observed to carry higher number of promising selections than LL among all the mutagens (NEU > EI > gamma rays) and populations (selected > macromutational > unselected). Table 4 shows the contribution of M_2 and M_3 generations and also of different populations to the total promising M_3 selections. The contribution of M_2 selections (selected population) to the total M_3 selections was the highest, followed by unselected and macromutational populations. Among mutagens, NEU contributed the highest frequency of promising progenies, followed by EI and gamma rays in all the populations. The contribution of HH group toward total promising selections in M_3 was higher than LL group in all the mutagens and populations. Earlier results obtained in peas [10] and lentil [11] strongly support the findings of the present study.

Therefore, in contrast to the earlier practice that the macromutational progenies should be excluded from any analysis for polygenic variability, the present study strongly suggests that the chances of success increase in the macromutational population for isolation of promising progenies even for polygenic variants. In fact, selection for polygenic traits can also be applied to the normal looking M_2 plants of the segregating progenies, for concentration on such progenies can lead to a great economy of effort with simultaneous improvement in selection efficiency. The heterozygotes for macromutations among such promising selections can be discarded on the basis of segregation in M_3. One more cycle of selection on the basis of progeny mean and variance in M_3 would confirm the potential of these M_2 selections. Such close watch on the material and rigorous selection can lead to the isolation of confirmed promising strains in M_3 which can be put to initial testing in the form of small plot trials in the next (M_4) generation itself.

As can be seen from Table 1, induced variability for polygenic traits was discernible even in M_2 which can be exploited by appropriate selection technique. However, the variability increased in M_3 over M_2 generation. This phenomenon has been often termed as "release of additional variability". The question arose: where from this additional variability was released? Obviously, the mutagenic treatment was the only cause of all the variability, as a similar increase in variance was not observed in the control. At the same time, much of this induced variability was recorded in the M_2 generation. This provided sufficient basis to start selection in M_2 itself. It is reasonable to assume that the variability generated by the mutagenic treatment starts manifesting in M_2 itself, which is the first segregating generation. Further segregation for the polygenic systems in M_3 is expressed as "release of additional variability". This being the mechanism of induction and inheritance of micromutations, selection in M_2 can certainly help in identifying progenies that are likely to show more variability and better response to selection and simultaneously reduce the volume of unwanted material by rejecting the unmutated "roughage" [16].

A perusal of Tables 3 and 4 revealed some interesting features of the present investigation. As can be seen from Table 3, 69.0% M_2 selections were confirmed as promising in M_3 generation. This suggests that selection in M_2 was very effective and dependable. Although, the characters showed increase in variance with the advancing (M_3) generation (Table 1), which is confirmed by the fact that 19.1% promising progenies with multiple characters were added in M_3 to the total number of promising selections, nevertheless, early generation selection is of great help in reducing the volume of work and saving time and effort. The contribution of M_2 and M_3 generations to the total selections was 80.9% and 19.1%, respectively (Table 4). This suggests that although new progenies with multiple characters (about one-fifth of the total) were added (which were not identified in M_2) in M_3 to those already selected in M_2 the quantum of material nearly trebled (the total progenies studied in LL and HH

Table 4. Contribution of M2 and M3 generations to the total promising M3 selections with multiple characters

Group of mutagenic damage	Promising progenies in M3 population						
	Total	Macro-mutational		Non-macromutational			
				Selected		Unselected	
		no.	%	no.	%	no.	%
Gamma rays							
LL	45	3	6.7	33	73.3	9	20.0
HH	138	9	6.5	113	81.9	16	11.6
Overall	183	12	6.5	146	79.8	25	13.7
EI							
LL	56	4	7.1	42	75.0	10	17.9
HH	158	12	7.6	129	81.6	17	10.8
Overall	214	16	7.5	171	79.9	27	12.6
NEU							
LL	78	6	7.7	60	76.9	12	15.4
HH	175	14	8.0	149	85.1	12	6.9
Overall	253	20	7.9	209	82.6	24	9.5
Overall experiment	650	48	7.4	526	80.9	76	11.7

groups in all the nine treatments in M_2 were 1128 as against 3154 studied in M_3). Similar observations have also been reported in lentil [5] and peas [10]. All the above observations conclusively prove the importance of early generation (M_2) selection of polygenic mutations in lentil.

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