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OPTIMIZATION OF SELECTION STRATEGY FOR MICROMUTATIONS IN MUNGBEAN (*VIGNA RADIATA* (L.) WILCZEK)

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Data are presented on leaf abnormalities, pollen sterility, seed sterility and survival in M_1 generation following seed treatment o mungbean cv. 'Dhauli' with gamma-rays (10 and 40kR), EMS (0.1 and 0.8%) and NG (0.01 and 0.05%). The M_1 plant material in each treatment was divided into selected and unselected lots. Selected lots constitute the plants which suffered the above physiological damages. The selected and unselected lots in M_2 were again grouped into non-segregating and segregating (macromutational) families. The normal looking plants from segregating M_2 families of selected and unselected lots were advanced to M_3 generation. Seed yield/ plant was recorded on single plant basis in M_1, M_2 and M_3 generation. The macromutation frequency in selected lot was nearly double than its unselected counterpart. Very high polygenic variability was realised in treatments with higher mutagenic doses (close to LD_{90}) of selected segregating population followed by that of unselected lot in both M_2 and M_3 generations. Selection for micro-mutation can be carried out reliably with higher degree of precision by identifying M_1 plants with maximum damage from these higher dose mutagenic treatments and selection of promising micromutants in their M_2 segregating populations. The selected segregating populations (of M_2) exhibited mean shifted towards negative direction, but a few micromutant plants with exceptionally high yield potential compared to those from selected non-segregating and unselected segregating population, could be recovered due to its very wider range and high induced genetic variability. This saves time, labour,space and cost towards experimentation and paves the way for higher degree of genetic improvement through micro-and macromutation as well.

Key Words: Gamma rays, EMS, NG, selection strategy, micromutaion.

The literature on the induction of polygenic mutations in crop plants has been extensively documented. However, no significant breakthrough has yet been achieved for plant productivity, more plausibly due to lack of efficient handling procedure for effective isolation of polygenic mutations. It is in vogue to advance non-segregating (normal looking) M_2 families to M_3 generation and apply first sieve of selection not earlier than M_3. This undoubtedly increases the plant population, larger part of which is non-mutated material. It is most likely that promising micromutants are lost with the advance of generation. This paper presents a more precise and realistic procedure for reducing a larger part of the non-mutated material and thereby paves the way for effective and reliable recovery of micromutants.

MATERIALS AND METHODS

Six sets of 500 seeds of mungbean, cv. 'Dhauli' were treated with low and high mutagenic doses of gamma-rays (10 and 40kR), EMS (0.1 and 0.8%) and NG(0.01 and 0.05%). The higher mutagenic doses cited here correspond to doses close to LD_{90}. Chemical treatments were administered for three hours at 28 ± 1°C following a pre-soaking of 11.50 hours in distilled water and gamma-rays treatments were given in the 60_{co} cell at IARI, New Delhi. Observations were recorded on M_1 leaf abnormalities e.g., unifoliate, unifoliate with 1-2 lobes, bifoliate, bifoliate with 1-2 lobes, tricotyledonary, trifoliate with asymmetrical position of side leaflets and truncated leaves at 25 days after sowing (DAS). Each M_1 plant was examined for pollen and seed sterility (%). The percentage of survival was recorded at 60 DAS. M_2 and M_3 generations were grown following pedigree method. The entire M_1 population in each treatment was divided into selected and unselected lots to study the effect of physiological damage on induced polygenic variation in M_2 and M_3. The selected lots constitute

Table 1. Effect of mutagenic treatments on M_1-abnormalities and variability in yield/plant.

Mutagen	Dose	Leaf abnormalities (%)	Pollen sterility (%)	Seed sterility (%)	Survival (%)	Seed yield/plant (g)		
						Range	Mean	CV (%)
Gamma-rays	10kR	2.5	0.9	5.3	82.4	3.9-5.2	4.5	19.0
	40kR	23.3	30.5	65.3	18.1	1.5-2.2	1.8	16.5
EMS	0.1 %	1.2	0.8	3.9	85.2	4.0-5.9	5.3	20.3
	0.8 %	10.5	8.2	28.3	25.2	1.7-2.5	2.1	17.3
NG	0.01 %	1.3	1.0	5.2	80.3	4.1-5.9	5.0	19.5
	0.05 %	15.2	10.0	38.8	22.0	2.3-3.0	2.7	17.0
Control	0.0	0.0	0.0	0.0	90.0	4.0-5.5	5.1	18.8

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Table 2. Macro-mutation frequency and effect of M_1 selection on induced variability in M_2 for plant productivity (g).

Mutagen	Dose	Mutation frequency (%)		Unselected (segregating)			Selected (segregating)		
		Unselected	Selected	Range	Mean	C.V. (%)	Range	Mean	C.V. (%)
Gamma-rays	10 kR	1.21	1.75	4.0-6.8	5.3	20.3 (10.3)	3.0-8.7	5.8	25.3 (15.3)
	40 kR	15.3	24.50	2.0-9.7	5.1	30.4 (12.50)	0.9-11.3	4.5	35.8 (22.5)
EMS	0.1 %	2.09	2.31	3.0-8.9	6.0	23.2 (15.2)	3.5-7.8	6.0	28.3 (21.5)
	0.8 %	9.53	16.73	2.5-9.8	6.5	31.1 (18.8)	1.5-13.3	4.8	40.8 (30.0)
NG	0.01 %	1.71	1.93	3.8-8.3	6.3	23.5 (13.5)	3.8-10.3	8.0	31.8 (20.0)
	0.05 %	7.92	18.33	2.0-9.5	6.0	35.8 (15.2)	2.5-15.1	5.3	48.3 (30.2)
Control		0.00	0.00	4.8-6.9	5.9	19.9	4.8-7.0	5.8	20.0

* Figure within parenthesis indicate variability induced in non-segregating population in M_2.

Table 3. Effect of M_2 selection on induced variability in M_3 for plant productivity.

Mutagen	Dose	CV(%) in unselected segregating population	CV (%) in selected segregating population
Gamma-rays	10 kR	28.3	35.3
	40 kR	38.1	55.4
EMS	0.1%	32.2	35.2
	0.8 %	40.0	68.2
NG	0.01 %	36.5	40.3
	0.05 %	45.0	70.8

M_1 plants which suffered from any of the above physiological damages plausibly caused by mutagenic treatment. The selected and unselected lots in M_2 were again grouped into non-segregating and segregating (macromutational) families. The normal looking plants (without any damage and macromutation) choosen randomly from segregating M_2 families of selected and unselected lots were advanced to M_3 generation. Macro-mutation frequency (chlorophyll and morphological) was scored as percent M_2 seedlings. Seed yield/plant was recorded on single plant basis in each treatment in M_1, M_2 and M_3 generation. Data were subjected to a appropriate statistical method to measure the relative efficiency of mutagenic treatments in relation to the quantum of induced genetic variability.

RESULTS AND DISCUSSION

The mature embryo in seed is the target of mutagenic treatments and the initial dividing cells are the independently mutating genome (Keiding. 1970). The authors assessed the extent of damage to these 'initial cells' caused by mutagenic treatments. In general, high mutagenic doses impair both the growth and reproductive phase leading to very low survival at maturity. Further, gamma-rays caused higher mutagenic damage than the chemical mutagen (Table 1). Significant reduction in fertility of seed was realised in the mutagenic treatments particularly at higher doses close to -LD_{90} in each of the mutagens. Hence, seed sterility (ovule abortion) may be considered as reliable criterion for assessing physiological damage caused by mutagenic treatments.

It is reasonable to assume that a few survived M_1 plants (in high dose treatments) expressed maximum physiological damage (s), bore a larger section of chimeral sector and may impact altered character manifestation in the entire mutagenised material in succeeding generations. While, lower doses seem to have lower frequencies of chimeral plants and very small mutated tissue in chimeric plants may not compete successfully with non-mutated tissue during deplontic selection. This paves little scope for haplontic selection and the plants are likely to produce practically very few mutated seeds.

It is evident from Table 1 that marginal increase in variability in lower doses over the control led to the expression of polygenic variability even earlier than M_2. In contrast, masking of genetic effects due to high seed sterility in high mutagenic doses may be responsible for low variability in seed yield compared to control (Sharma, 1986). Plant productivity in high mutagenic doses reduced to approximately half owing to excessive physiological damages particularly seed sterility in M_1.

Irrespective of the kind of mutagens used, the genetic variability induced was higher in selected segregating mutagenised population than its sister unselected population in M_2. It is worth to note that the macromutation frequency in selected lot was nearly double than its unselected counterpart and the frequency was exceptionally high in high mutagenic doses (Table 2).

In general, the segregating population (macromutational) of either of the selected and unselected lot is shown to express about three-fourth times higher induced variability compared to their respective non-segregating population. The selected segregating population exhibited higher variability over its counterpart in the unselected lot in M_2 as well as M_3 generation. Purposefully, promising M_2 families (based on high mean and high standard deviation) of the selected segregating population in high mutagenic doses were identified and stressed upon for single plant selection for assessment of genetic improvement *per se* with regard to seed yield in their M_3 pedigree lines and succeeding generations. This is because, the probability of the so called 'minor' genes also to be hit may be high where macromutational events are already identified and more so in the material where there is clear evidence of genetic damage due to treatment.

Data presented in Table 2 and 3 showed noticeable increase in variability in M_3 over M_2 generation which has often been referred to as release of additional variability. This is ascribable to the mechanism of inheritance in the polygenic system. The exceptionally higher percentage of release of additional variability in selected segregating population of high mutagenic doses than corresponding low doses in M_3 generation can be traced back to the extensive damage in selected plants in M_1 generation. Hence, selection of M_1 plants with maximum damage is a pre-requisite to increase the efficiency of mutation breeding for polygenic traits.

The segregating population involving high mutagenic doses particularly that of 0.05% NG had very wider range but low mean performance with regard to plant productivity in M_2. The shift of mean towards negative direction may be ascribed to the fact that these segregating populations of selected lot contain higher proportion of inferior individuals which may be due to carry-over effects of mutagenic damage by high doses of mutagens. However, a few micromutant plants (one in 0.8% EMS two in 40KR gamma-rays and four in 0.05% NG) with high seed yield had been isolated from some promising families of selected segregating population in high mutagenic doses in M_2 followed by confirmation in M_3 and succeeding generations. Such mutants were subjected to single plant selection based on criterion erstwhile mentioned, in each generation; and were shown to have very high yield potential (10 q/ha) in M_6 generation. This envisaged that some cells of mutant tissues (or mutant sectors) of M_1 plants with maximum mutagenic damage might have experienced activation of sleeping genes or duplication of genes with additive effect and thus led to recovery of transgressive mutants towards positive direction for seed yield. Such a reliable recovery of high yielding mutants has been reported by Tripathy *et al.* (1992) in mungbean and Tripathy (1997) in *Lathyrus sativus*. The selection strategy followed in the present investigation can undoubtedly save time, space, labour and cost towards experimentation with consequent higher degree of genetic improvement through micro- and macromutation as well.

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