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Prediction of M_2 Macro and Micro-Mutation Frequency Based on M_1 Effect in Greengram [*Vigna radiata* (L.) Wilczek]

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Abstract: *Mutation breeding requires handling of large population as chances of induction and detection of mutation in a particular gene is rare. This increases the cost of breeding and makes the selection procedure time consuming and tedious. Detection of effective mutagenic treated population in early generations, particularly in M_1 generation is no doubt reduce the population load in subsequent generation and thus cost of breeding and provide better scope of selection. Hence, the present investigation was undertaken in greengram [Vigna radiata (L.) Wilczek] using two varieties and the widely used physical mutagen gamma ray at five different doses to find out the possibility of existence of any relationship between M_1 estimates and M_2 mutation frequency. Results of the study indicated positive relationship between M_1 injury and lethality with M_2 macro- and micro-mutation frequency which may help in early prediction of mutation frequency.*

Keywords: *Gamma ray, Greengram, Induced Macro-mutation, Micro-mutation*

I. Introduction

Mutation breeding requires handling of large population as chances of induction and detection of mutation in a particular gene is rare. This increases the cost of breeding and makes the selection procedure time consuming and tedious. Detection of effective mutagenic treated population in the early generations, particularly in M_1 generation would no doubt reduce the population load in subsequent generation and provide better scope for selection. However, study in this respect is limited and needs more investigation.

Leaf spotting in M_1 had a positive correlation with M_2 mutation frequency in pea [1]. Similar result was also observed in barley [2]. Positive relationship between M_1 injury and M_2 macro-mutation and micro-mutation frequency was also reported in blackgram [3]. Based on the above, in the present induced genetic study in greengram, relative effectiveness of mutagenic treatments was evaluated in terms of their overall effect on different M_1 parameters and their relationship with those of total macro-mutation frequency as well as pooled variability for all the quantitative traits observed in M_2 generation.

II. Materials and methods

For the purpose, genetically pure, uniform and dry seeds of two greengram varieties Sujata and TARM-1 were each irradiated with five different doses (20, 30, 40, 50 and 60 kR) of gamma ray. Treated seeds were sown along with their controls to rise the M_1 generation. In the M_1 generation, two sets of experiments were conducted. One set of experiment was sown in the Laboratory in the earthen pots and the other set was conducted in the field in Randomized Block Design (RBD) with three replications. The laboratory experiment was replicated thrice and an observation on germination, seedling height, hypocotyl length and root length was recorded on all surviving seedlings. The field experiment was conducted in RBD with three replications following all the recommended agronomical package of practices. To study the mutagenic effect on the adult plants traits, observations on plant stand at germination, days to 50% flowering, plant height, number of fruiting branches/plant, number of pods/plant, pod length, number of seeds/pod, 100-seed weight, yield/plant and plant stand at harvesting were recorded. Data on fourteen characters (including seedling and adult plant traits) were analysed following the methods of Numerical taxonomy [4]. Similarity coefficients between treatments were estimated following Gower's similarity coefficient (S_G) formula. Dendrograms were constructed from the matrices of S_G values following SHAN (Sequential Hierarchial Aglomerative and Non-overlapping) clustering strategy. At 70 phenon level, mutagenic treatments were classified into different clusters based on mutagenic effect. Clusters were characterized on the basis of pooled mean for all the characters (brought over to one scale) of M_1 population(s) included in each cluster

M_2 generation was raised in RBD with three replications from the bulked seeds of M_1. Observations on macro-mutations frequency including chlorophyll and morphological mutations in different treatments were recorded from 5^{th} day till physiological maturity. Further attempt was made to estimate macro-mutation frequency in respect of different clusters identified in M_1.Observations on seven quantitative traits *viz*, plant

height, branches/plant , number of pods/plant, pod length, number of seeds/pod, 100-seed weight and yield/plant were also recorded on forty randomly chosen normal looking plants per replication/treatment. Attempt was also made to estimate standard deviations (SD) in respect of different mutagenic treatments in M_2 and pooled SD in respect clusters identified in M_1. Mean and SD of each treatment for each character were standardized after bringing them in to one scale and pooled over characters and treatments to estimate pooled mean and SD of different clusters. The relationship between M_1 pooled mean and M_2 mutation frequency (macro and micro) was ascertained through estimation of correlation of coefficients following the methods of [5].

III. Results and Discussion

At 70 phenon level, three distinct clusters were identified in the dendrogram of variety Sujata (Fig 1). Cluster I included moderate mutagenic treatment 30 kR and 40 kR whereas cluster II included lowest mutagenic dose 20 kR and the control. Cluster III contained both the higher doses of mutagenic treatments. Inclusion of mutagenic treated populations in different clusters indicated variable effect of mutagenic treatments on M_1 parameters. The pooled mean of all M_1 estimates in respect of clusters I to III in order were 0.602, 0.885 and 0.170 respectively (Table 1) indicating decrease in M_1 pooled mean with increase in dose of mutagenic treatment. In case of variety TARM-1, three distinct clusters were identified (Fig. 2). Cluster I included the mutagenic treatments 20 kR, 30 kR and control whereas cluster II included 40 kR and Cluster III included both the higher dose treatments (50 kR and 60 kR). The cluster means of all M_1 estimates in respect of cluster I, II and III were 0.821, 0.529 and 0.159, respectively which indicated decrease in M_1 pooled mean with increase in dose of mutagen. The decrease in cluster means with increase in dose of mutagenic treatment signified more of physiological damage (M_1 injury and lethality) in higher doses of treatment and the results were in conformity with the findings of earlier workers [6, 7]. The inverse relationship between M_1 pooled mean of different clusters and the doses of treatment was also noticed by several workers [8 to 15].

Table 1: M_1 parameter based cluster and their M_1 Pooled mean, M_2 macro-mutation frequency and pooled SD

Variety	Cluster number	Cluster Composition	Pooled M_1 mean	Pooled M_2 mean	M_2 chlorophyll mutation frequency (%)	M_2 viable macro-mutation frequency (%)	Pooled M_2 SD
Sujata	I	30 kR 40 kR	0.602	0.582	3.041	4.476	0.718
	II	Control 20 kR	0.885	0.700	4.000	3.840	0.328
	III	50 kR 60 kR	0.170	0.433	3.206	5.797	0.739
TARM-1	I	Control 20 kR 30 kR	0.821	0.946	4.740	2.445	0.502
	II	40 kR	0.529	0.697	2.690	3.250	0.616
	III	50 kR 60 kR	0.159	0.310	2.900	4.110	0.768

Estimations of pooled cluster mean in M_1 and average macro-mutation frequency as well as pooled variability (represented by SD) in M_2 in respect of these clusters was presented in Table 1. A close look at the result indicated an increase in average viable macro-mutation frequency as well as pooled SD (indicator of induced polygenic variability/micro-mutations) for the traits in M_2 generation in both the varieties with decrease in M_1 pooled mean or increase in M_1 physiological damage. This trend exhibits positive association between M_1 physiological damage and M_2 viable macro- as well as micro-mutation frequencies which is in conformity with the findings of earlier workers [1, 2, 3].

Relationship between pooled M_1 estimates based on mutagenic effect and M_2 mutation frequency was further examined by correlation coefficients. Correlation coefficient of M1 estimates *viz.*, lethality (indicated by per cent reduction in germination) and injury (indicated by per cent reduction in biometrical traits) with frequency of macro-mutation and population SD (indicator of micro-mutation) were worked out (Table 2).

Table 2: Correlation coefficients of M_1 estimates with M_2 macro-mutation frequency and population SD

M_1 estimates	Chlorophyll mutation frequency in M_2 (%)	Viable macro-mutation frequency in M_2 (%)	Population SD in M_2
Lethality	0.143	0.773^{**}	0.781^{**}
Injury	-0.007	0.652^{*}	0.557^{*}

* Significant at P=0.05, ** Significant at P=0.01

M_1 injury and lethality showed strong positive association with viable macro-mutation and micro-mutation (M_2 SD). Chlorophyll frequency showed positive association with M_1 lethality but negative association with M_1 injury but in a very low magnitude. Thus, M_1 injury and lethality could be considered to be one of the possible indicators of induced viable macro- and micro-mutation in M_2 generation. These observations were in broad agreement with earlier findings [16, 2, 3].

IV. Conclusion

In mutation breeding where large populations are handled, estimation of mutagenic effect in M_1 *viz*., plant injury and lethality may help the breeders in identifying effective treated populations in early generation for reduction in cost of breeding and enhancing scope of selection.

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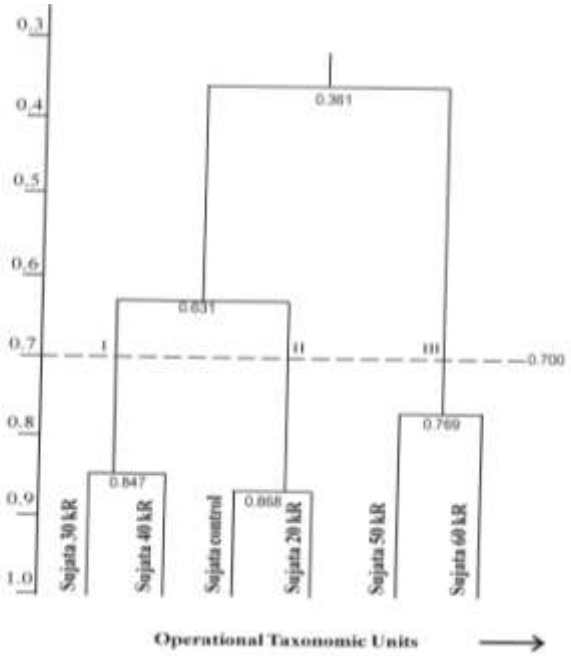

Fig. 1: Dendrogram showing clusters of OTUs (mutagenic treatments) of variety Sujata

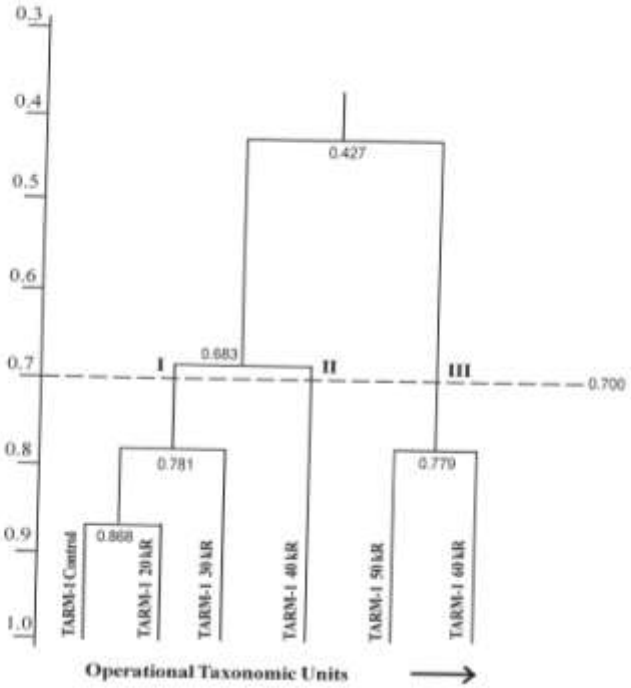

Fig. 2: Dendrogram showing clusters of OTUs (mutagenic treatments) of variety TARM-1