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Induced Micromutation in Relation to Mutagen Induced Stress in Wheat

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ABSTRACT

Differential response of two wheat varieties in M_1 generation was obtained, when treated with two potent chemical mutagens viz., Ethyl methane sulphonate and Sodium azide. Two parameters such as Mutation Index and Strees Response Index, basing on pollen traits and some quantitative data on growth respectively were used for comparing the response of varieties to mutagens as mutagenic and stress causing agent. It is possible to induce useful mutations with less of physiological damage for which selection of appropriate mutagen, its dose and the genotype is considered most important.

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INTRODUCTION

Mutation breeding is considered as an useful supplement to conventional breeding. Induction of useful genetic variations is a more desirable goal than simply increasing variability. The present study was carried out to determine differential response of genotypes to the mutagens in the form of mutagen induced stress and induced micromutations basing on the variability in some quantitative traits and pollen traits respectively. This work emphasizes the economy in investment by selection of M_1 plants (both within and between treatments) carrying micromutations and selection of proper chemical, its dose and the genotypes with definite response.

MATERIALS AND METHODS

Five hundred uniform seeds of two prerelease varieties viz., OW 32 (two-gene dwarf) and OW 13 (semi tall) were treated with two chemical mutagens such as Ethyl methane sulphonate (EMS) and Sodium azide. (SA). The concentrations of EMS and SA were 0.5%, 0.25% and 0.02%, 0.01% respectively with treatment period of 6 hours and presoaking of 12 hours at room temperature of 26 ± 1°C. Distilled water and phosphate buffer at 3.0 pH were taken as control for EMS and SA treatments respectively. The observations on germination, flowering, seedling survival and young plants survival were noted. Spikelets were collected randomly from 30 plants of each treatment to record observations on pollen sterility and pollen size by using 0.5% IKI solution in low power and high power objective respectively. After the crop was harvested, 25 plants were sampled randomly for observations on some quantitative traits such as plant height, length of 1st, 2nd, 3rd internode (from top), spike length, peduncle length, spikelet number, grain number, length of 1st, 2nd and 3rd leaf sheath.

Three different aspect of variation in pollen traits such as pollen sterility, reduction in pollen size and increase in its size variability compared to control population were used to obtain a rough measure of mutational response in each variety. Former trait was used as an index of chromosomal mutations, while later two indicated occurence of presumable factor mutations. Sampled plants were scored for all the 3 pollen characteristics

individually for occurrence of at least a mutant sector and then Mutation Index (MI) was calculated as follows :

$$\text{Mutation Index (M.I.)} = \frac{\text{Total scores}}{\text{No. of plants} \times 3}$$

Plants within population of different treatments were further categorised as Type I, Type II and Type III, where Type I was believed to carry only chromosomal aberrations (evidence from sterility), Type II plants carrying only factor mutations (evidence from mean pollen size and or coefficient of variability/range of pollen size) and Type III plants carrying both chromosomal aberrations and factor mutations (evidence from the above three aspects).

Similarly all the characters excepting pollen traits were used to calculate Stress Response Index (SRI) for each treatment variety combination after scoring the characters in suitable scale as given in the next section.

RESULTS AND DISCUSSION

Table 1 indicated higher value of Mutation Index in EMS treatments and lower value for SA treatments in OW 32 whereas OW 13 exhibited medium mutational response for both the chemicals.

It is reasonable to consider mutagen as a stress factor since these bring about adverse physiological changes within the plant body resulting in reduction in growth, survival, germination and other traits. Since the mutagenic treatments in general cause two major phenotypic changes viz., (1) Reduction in mean values and (2) increase in variability, it was argued that the same effects could be used as a simple approach to the problem of quantification of stress response. Following this approach a 'stress response index' (SRI) was calculated for each variety combination. The rational was obtained from the observations of Johnson and Frey (1967) in oats and Sinha and Patnaik (1969) in rice regarding phenotypic expression of quantitative characters under 'stress' and 'non-stress' conditions. The steps for calculation of SRI are as follows : (1) For each of the character above, reduction in mean value as well as increase in coefficients of variability (C.V.) compared to control was given an index score of 1 separately. Thus for each treatment the index score would vary from 0 to 2 since in the event of no change or reverse change the score given was zero for both mean and C.V. (2) For flowering appreciable delay in first flowering was given an index score of 1 and increase in range of flowering was also given the score 1. Conversely the score 0 was given for no change or reverse change. (3) For each of the remaining two aspects i.e., germination and survival, reduction to the tune of 10 % of the control was given the index score 0; between 11-30% was given the score 1; between 31-50% was scored 2 and reduction beyond 50% was scored 3. (4) These individual scores were summed up and total index score (Range 0 to 30) divided by maximum value 30 to obtain SRI with maximum value 1. Table 1 (last column) indicates that SRI values were smaller but more variable in OW 32 compared to higher but consistent value in OW 13.

In mutation breeding experiments, mutagens with low physiological effect and strong genetic effects are desirable. A number of M_1 parameters have been recognised as possible indicators of M_2 mutation frequency (Blixt *et al.*, 1964; Rana and Swaminathan, 1967; Savin *et al.*, 1968; Karunakaran and Kiss; 1971). In this study, average pollen grain size and pollen size variation were used as possible indicators of M_2 mutation frequency, based on the previous work in this laboratory (Mishra *et al.*, 1981).

Despite limited number of pollen criteria, differential response of genotypes and different effects of the two mutagens could be clearly distinguished vide Table 1 showing

TABLE 1

Mutation index, Frequencies of Type I, Type II, Type III plants and stress response index in M_1 population.

Treatment	*M.I.*	*No. of Type I*	*No. of Type II*	*No. of Type III*	*Type II frequency*	*Frequency of all types*	*SRI*
$V_1 T_1$	0.344	12	5	7	0.166	0.80	0.37
$V_1 T_2$	0.178	3	5	4	0.166	0.40	0.27
$V_1 T_4$	0.074	3	1	1	0.037	0.19	0.70
$V_1 T_5$	0.010	--	1	--	0.033	0.03	0.23
$V_2 T_1$	0.089	2	4	1	0.133	0.23	0.67
$V_2 T_2$	0.130	2	6	2	0.200	0.33	0.57
$V_2 T_4$	0.100	2	2	1	0.100	0.25	0.63
$V_2 T_5$	0.174	1	4	3	0.190	0.38	0.67

V_1 = OW 32 T_1 = 0.5% EMS T_4 = 0.02 % SA

V_2 = OW 13 T_2 = 0.25 % EMS T_5 = 0.01 % SA

M.I. = Mutation Index

SRI = Stress Response Index

relationship between Type II and overall. If Type II M_1 plants truely represent those carrying only factor (gene) mutations, the increase in this ratio could provide an estimate of the expected M_2 families segregating for mutation exploitable for crop improvement. Applying this criterion Sodium azide (SA) would appear to be more useful in the sense that along with increase in overall frequency, the Type II plant frequency would correspondingly increase. By the same yardstick, OW 13 could be considered responsive to both EMS and SA mutagenesis (at lower dose) in a more desired way than OW 32, which appears to be more sensitive to EMS but not to SA. But with EMS, OW 32 did not show increase in Type II frequency even with about two fold increase in overall M_1 mutant plant frequency.

When the effect of mutagens on the varieties was examined from both mutational and stress response point of view (Table 1), the following picture emerged.

Variety	Response to SA	Response to EMS
OW 32	Low mutational response relative to stress response	High mutational response relative to stress response
OW 13	Medium mutational response relative to stress response	Medium mutational response relative to stress response

It reveals high variability in the response of OW 32 to the chemicals as mutagenic and stress causing agent. On the other hand OW 13 showed high consistency for both categories of response to the two chemicals.

Another point worth emphasizing is the distinct difference between the two potent mutagens from following angles i.e., (i) relation between Type II and all types of M_1 plants, (ii) mutational response relative to the induced

stress. These observations further suggest (a) that the physiological effects of SA are entirely different from those of EMS, (b) that EMS is a broad spectrum mutagen compared to SA, (c) that mutations relative to the induced stress may be relatively less for SA than EMS and (d) that more useful mutations may be obtained from OW 13 by use of both the mutagens at lower doses, whereas OW 32 was responsive to only EMS at lower dose.

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