Indian J. Genet., **51** (1): 107–111 (1991)

USE OF POLLEN TRAITS FOR EARLY DETECTION OF INDUCED MICROMUTATIONS IN WHEAT

P. K. SUBUDHI, B. K. MOHAPATRA* AND S. K. SINHA

Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Bhubaneshwar 751 003

(Received: June 25, 1988; accepted: February 3, 1990)

ABSTRACT

Two pre-release wheat varieties, namely, OW 32-3 and OW 13-1, were treated with two doses each of ethyl methane sulphonate (EMS) and sodium azide (SA) and the effectiveness of the mutagenic treatments for induction of micromutations was assessed in M_1 using two pollen traits, namely, pollen sterility and size. The presumptive mutants showing only pollen sterility were classified as Type I, those showing only pollen size variation were termed Type II, and those showing both pollen sterility and size variation were called Type III. The Type II mutations occurred in greater frequency in the EMS treatments of both the varieties. Between the varieties, OW 13-1 had more of Type II mutations with lower doses of both the mutagens. EMS was more effective than SA in inducing useful micromutations, while OW 13- 1 showed better response than OW 32-3 for such mutations.

Key words: Wheat, M_1 generation, pollen sterility, pollen size.

Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement. The mutations so induced could be macro- or micromutations. The latter category mutations result in increased variability in quantitative traits and are relatively more useful in plant breeding. Thus, it would be of great help to identify mutagens and their doses that could induce high frequency of micromutations against different genotypic backgrounds. Also, any method that would permit early detection of such mutations would be of great practical value. In the present study, an attempt has been made to assess the effectiveness of some mutagenic treatments for inducing micromutations in wheat using two pollen traits as the indicator parameters in M_1.

MATERIALS AND METHODS

The material comprised the M_1 generation following treatment of two pre-release wheat

*Addressee for correspondence.

varieties, namely, OW 32-3 and OW 13-1, with two chemical mutagens along with the appropriate controls. The mutagens used were ethyl methane sulphonate (EMS) at 0.5% and 0.25% and sodium azide (SA) at 0.02% and 0.01%. The EMS solution was prepared in distilled water and the SA solution in phosphate buffer of pH 3. A distilled-water control and a phosphate-buffer control of each variety corresponding to the two treatment media, were used for comparison. Five hundred healthy seeds were soaked in distilled water for 12 h and then treated with freshly prepared mutagen solution for 6 h at room temperature ($26^{\circ} \pm 1^{\circ}C$). After treatment, the seeds were thoroughly washed in running tap water. The treated seeds were space planted in the field under normal cultural conditions.

Spikelets which were expected to open the next day, were collected from 30 randomly chosen plants from each treatment in 70% alcohol. Two pollen traits, i.e., pollen sterility and pollen grain size, were studied using the basal florets of each spikelet. One anther from each floret was teased in 0.5% IKI solution and the material observed under microscope. Five different microscopic fields under low (10 x 10) magnification were taken for observation on pollen sterility. For pollen size, 50 fertile pollen grains were measured using ocular micrometer under high (10 x 40) magnification. All readings were taken to the nearest micrometer division.Mean and variability of pollen grain size, pollen sterility and a new parameter called the "mutation index" that combined the information on the two pollen traits were calculated for each treatment and the effectiveness of the treatments were assessed in terms of these parameters.

RESULTS AND DISCUSSION

The mutagenic effectiveness of two chemical mutagens and mutational response of two varieties were analysed in terms of variability in pollen grain size, pollen sterility, and an index based on information on both these traits in M_1. The results are presented on these lines.

MEAN AND VARIABILITY OF POLLEN GRAIN SIZE

Mean pollen diameter showed a shift towards the lower side in all the mutagen-treated populations of OW 32-3 compared to the controls (Table 1). The EMS treatments had little effect on mean pollen diameter in OW 13-1, but the higher dose of SA (0.02%) increased the same in this variety.

The SA treatments increased the variability of pollen grain size in both the varieties, whereas the EMS treatments increased variability in OW 13-1 and decreased in OW 32-3. The EMS-treated populations of both the varieties showed a wider range of between-plant variation in pollen size, compared to the controls. As to the SA treatments, the range of variation in pollen size was narrower in the treated populations of OW 32-3 and similar to that of the control in the treated populations of OW 13-1. The results revealed significant

differences among the treatments in respect of pollen size variability. Variation in M_1 pollen size could be a good index of induced mutations, which could be used for predicting mutation frequency in M_2 [1].

Table 1. Effect of EMS and SA treatments on variability of two pollen traits in wheat

Variety and treatment	Pollen grain diameter (ocular division) range	mean	SD	CV(%)	Pollen sterility (%)
Var. OW 32-3:					
EMS 0.50%	10.0–24.5	14.3	1.03	7.12	12.1
EMS 0.25%	10.0–24.0	14.6	1.29	8.71	8.2
Distilled-water control	10.0–23.5	15.7	1.49	9.39	4.6
SA 0.02%	10.0–19.5	14.2	1.11	7.83	9.8
SA 0.01%	10.5–20.5	15.6	1.22	8.35	6.7
Buffer control	10.0–22.0	14.9	1.16	7.78	6.3
Var. OW 13-1:					
EMS 0.50%	10.5–23.5	15.2	1.10	7.18	7.1
EMS 0.25%	11.0–23.0	15.1	1.23	8.12	6.1
Distilled water control	11.0–20.5	15.2	0.98	6.40	4.0
SA 0.02%	11.0–19.5	14.8	1.24	8.38	12.0
SA 0.01%	10.0–20.0	14.5	1.12	7.70	12.9
Buffer control	10.5–19.5	14.5	0.88	6.05	6.5

POLLEN STERILITY

There was significant increase in pollen sterility with increase in dose of the mutagens as compared to the controls. There was, however, an exception to this general trend: the lower dose of SA induced greater sterility than the higher dose in OW 13-1. OW 32- 3 showed greater pollen sterility in EMS treatments and OW 13-1 in SA treatments. The phosphate-buffer controls also showed higher pollen sterility than the distilled-water controls of both the varieties. In a study of the M_1 and M_2 generations following chemical mutagenesis in two greengram varieties, Sahoo and Samolo [2] observed significant positive correlations between pollen sterility in M_1 and mutation frequency in M_2 of both the varieties.

INDEX OF MUTATIONAL RESPONSE

An attempt was made to combine the different aspects of variation in pollen traits to obtain an estimate of the frequency of mutant or affected plants and thus estimate the effectiveness of the mutagenic treatments in each variety. One criterion was the average pollen size of individual plants. If the average pollen size of a plant in a treated population was lower than the minimum or higher than the maximum of the range of pollen size of the 30 plants sampled from the control population, that plant was considered to be an affected plant carrying at least one mutant sector. The other aspects considered were pollen sterility and intraplant variability of pollen size for which the following criteria were applied. A plant was considered to be a mutant if it transgressed the limits of variation in

the corresponding control populations. More specifically, transgression of the upper limit of pollen sterility or intraplant CV of pollen size was used as one criterion, and transgression beyond the lower or upper limits of the overall range of pollen size in the control population was used as another criterion. On the basis of the frequency of mutant plants, a 'mutation index' was calculated for each mutagenic treatment by scoring each mutant plant as 1, 2 or 3, depending on the evidence from one, two or all the three criteria, adding the scores for each treatment, dividing the sum by the number of plants sampled from the treatment, and finally, dividing by 3 to adjust the maximum possible value of the index to 1. The index values ranged from 0.010 to 0.344, where 0.01% SA gave the lowest and 0.5% EMS the highest value, both in OW 32-3 (Table 2). Based on this index, EMS treatments appeared to have induced more mutations than the SA treatments. The index values also revealed differential response of the two genotypes to mutagenic treatments. Genotypic differences in mutational response have been reported by other workers [3–5].

Table 2. Frequency of different types of mutant plants and mutation index in M_1 populations of wheat

Variety and treatment	Number of plants studied	Number of mutant plants			Proportion of mutant plants, %	Proportion of type II mutants, %	Mutation index
		Type I	Type II	Type III			
Var. OW 32-3							
EMS 0.50%	30	12	5	7	80.0	16.6	0.34
EMS 0.25%	30	3	5	4	40.0	16.6	0.18
SA 0.02%	27	3	1	1	18.5	3.7	0.07
SA 0.01%	30	—	1	—	3.3	3.3	0.01
Var. OW 13-1							
EMS 0.50%	30	2	4	1	23.3	13.3	0.09
EMS 0.25%	30	2	6	2	33.3	20.0	0.13
SA 0.02%	20	2	2	1	25.0	10.0	0.10
SA 0.01%	21	1	4	3	38.1	19.0	0.17

Further effort was made to categorize the affected plants of the treated populations into Type I, Type II and Type III mutants, where the Type I mutants were assumed to carry mostly chromosomal aberrations (evidence from pollen sterility), Type II mostly gene mutations (evidence from pollen size variation), and Type III assumed to carry both chromosomal aberrations and gene mutations (evidence from both pollen sterility and pollen size variation). Assuming that gene mutations are more desirable than chromosomal aberrations, the frequency of Type II plants could be a good indicator of the relative efficiency of mutagenic treatments for inducing useful mutations. The frequency of the three types of mutant plants in different treatments is presented in Table 2, which shows that the

lower dose of SA in OW 13-1 and both the EMS doses in both varieties induced appreciable frequency of gene mutations. Both the EMS doses were equally efficient in inducing Type II gene mutations in OW 32-3, while none of the SA doses proved effective for inducing such mutations in this variety. Between the varieties, OW 13-1 showed better mutational response, particularly for Type II gene mutations. Similarly, EMS was superior to SA by inducing more useful mutations, which is conformity with the earlier finding [6].

The results revealed the differences in effectiveness of the mutagenic treatments and the differential response of the genotypes to such treatments. It is reasonable to conclude that pollen traits could be used to exercise selection both between and within M_1 populations. It should also be possible to select plants for specific types of mutations, such as, Type I, II or III, depending on the breeding objectives.

REFERENCES

1. R. C. Mishra, B. N. Samolo and S. K. Sinha. 1981. Relationship of M_1 parameters with M_2 mutation frequency in green gram (*Vigna radiata* (L.) Wilczek.). Haryana Agric. Univ. J. Res., **11**: 481–484.

2. S. Sahoo and B. N. Samolo. 1973. Effect of presoaking on EMS induced pollen sterility and M_2 mutation frequency in diploid and tetraploid green gram. Orissa Univ. Agric. Tech. J. Res., **3**: 28–31.

3. J. V. Goud. 1967. Induced mutation in breadwheat. Indian J. Genet., **27**: 40–55.

4. J. V. Goud, K. M. D. Nayar and M. G. Rao. 1970. Mutagenesis in sorghum. Indian J. Genet., **30**: 81–90.

5. R. Rathnaswamy, S. Krishnaswami and P. V. Marappan. 1978. Radiosensitivity studies in green gram (*Vigna radiata*) (L.) Wilczek). Madras agric. J., **65**: 351–356.

6. H. Heslot. 1977. Review of main mutagenic compounds. *In*: Manual of Mutation Breeding (2nd edn.). FAO/IAEA, Vienna: 51–58.