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EFFECT OF MUTAGENS ON POLLEN TRAITS IN M_1 AND THEIR POSSIBLE ROLE AS INDICATORS OF MICROMUTATIONS IN LATER GENERATIONS IN KHESARI (*LATHYRUS SATIVUS* L.) VAR. P-505

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Seeds of khesari (*Lathyrus sativus* L.) were treated with gamma rays, EMS and DES seperately as well as with combinations of gamma rays and EMS/DES. An effort was made for early detection of induced micromutations in M_1 using two pollen parameters i.e. pollen sterility and pollen size. Some plants showed only pollen sterility and 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 grouped in Type III. Assuming that type II carry mostly gene mutations, these could be a good indicator for expecting useful micromutations rather than chromosomal aberrations. Type II variants occured in greater frequency in the individual application of both the chemical mutagens. While lowest dose of gamme rays in combination with DES/EMS also increased Type II mutations. However, individual applications of chemical mutagens were more effective in inducing Type II variants.

Key Words : Pollen sterility, Pollen size, Early detection of mutations.

Induced mutagenesis plays a very important role in enhancing genetic variability for crop improvement by way of inducing micro-mutations in addition to the visible macro-mutations. It would be of great help to identify mutagens and their suitable doses that could induce high frequency of micro-mutations in a crop. Also, any method that would permit early detection of such micro-mutations i.e. in M_1 generation, would be of great practical value. In the present study, an attempt has been made to forecast the effectiveness of some mutagenic treatments for inducing micro-mutations in khesari var. P-505 using two pollen traits as the indicator parameters in M_1.

MATERIALS AND METHODS

Dry seeds of uniform size and shape of khesari var. P-505 were treated with 15,25 and 35 Kr doses of gamma rays. Some of irradiated and some fresh seeds were also treated with 0.125% and 0.5% of aquous solutions of Ethyl methane sulfonate and Diethyl sulfate respectively for six hours at 20±1°C. A sample of untreated seeds was soaked in water for the same period to serve as soaked control. Thus there were a total of 13 treatments as listed in the first column of table 1. After the chemical treatments, the seeds were thoroughly washed in running tap water. Seeds of all the treatments were planted in the field under normal cultural conditions.

Flowers which were expected to open the next day, were collected from randomly selected plants from each treatment in 70% alcohol. Two pollen traits, i.e. pollen sterility and pollen grain size were studied. One anther from each flower was teased in 45% acetocarmine and the material observed under microscope. Ten different microscopic fields under low (10 x 10) magnification were taken for observations on pollen sterility. For pollen size, 50 healthy 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 effectiveness of gamma rays, two chemicals and their combinations on mutational response 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.

Mean pollen diameter showed a shift towards the lower side in all the gamma irradiation doses as compared to the control (Table -1). However, a slight increase was found in individual applications of EMS or DES. The results revealed significant differences among the treatments in respect of pollen size variabi-

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Table 1. Effects of EMS, DES and gamma rays treatments on variability of two pollen traits on khesari (*Lathyrus sativus* L.) var. P-505.

Treatments	Pollen grain diameter (Ocular division)				Pollen sterility (%)
	Range	Mean	SD	CV(%)	
Unsoaked control	7.0-12.0	8.99	0.50	5.69	10.95
Soaked control	7.0-12.8	8.92	0.44	5.06	15.20
15kR	6.0-13.0	8.78	0.49	5.76	37.71
25kR	6.0-13.8	8.81	0.54	6.29	55.55
35kR	6.0-14.5	8.79	0.58	6.81	74.06
EMS 0.125%	6.6-13.9	9.06	0.37	4.19	15.72
DES 0.5%	6.0-18.1	9.23	0.49	5.44	22.28
15kR+EMS 0.125%	6.0-15.2	9.02	0.59	6.72	38.00
25kR+EMS 0.125%	6.8-16.1	8.94	0.57	6.57	55.47
35kR+EMS 0.125%	6.4-16.2	8.96	0.52	5.95	62.09
15kR+DES 0.5%	6.0-13.5	8.95	0.46	5.30	31.84
25kR+DES 0.5%	6.0-14.5	9.02	0.64	7.56	60.55
35kR+DES 0.5%	6.0-14.2	8.76	0.69	8.11	76.79

lity. According to Mishra *et al.* (1981) 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.

Varying degree of pollen sterility was induced in different mutagenic treatments. However, some plants showing very high pollen sterility (50 to 99.99%) were recorded in all the mutagenic treatments. Combination of gamma rays with DES or EMS induced higher sterility than the individual application of DES or EMS. Individual applications of varying gamma ray doses also induced higher pollen sterility than those of the chemical mutagens. DES was found to be more toxic than EMS for pollen fertility. Significant correlations between pollen sterility in M_1 and mutation frequency in M_2 has been observed in a number of crop plants (Sahoo and Samolo, 1973; Mishra *et al.*, 1981; Dixit and Dubey, 1985; Tripathi and Dubey, 1990 and Vandana, 1990).

Different aspects of variations in pollen traits were used to obtain a combined estimate of the effectiveness of mutagenic treatments. Some plants showed only pollen sterility and were classified as Type I, those showing only pollen size variation were termed as Type II and those showing both pollen sterility and size variation were placed in Type III. Type I variants based on pollen sterility alone were assume to carry mostly chromosomal aberrations, Type II based on pollen size variation could be assumed to be gene mutation while Type III may be due to both chromosomal aberrations and gene mutations. Since the gene mutations are more desirable than the chromosomal aberrations, frequency of Type II plants could be a good indicator for expecting useful mutations in later generations. Data summarised in Table-2 reveals that individual application of either of the two chemical mutagens induced appreciably higher frequency of Type II variants in comparision to individual application of gamma rays as well as the combined applications of gamma rays with either chemical mutagens.

A mutation index was calculated after Subudhi *et al.* (1992) for each mutagenic treatment by scoring each variant plant as 1, 2 or 3 depending on the evidence from 1, 2 or all the 3 criteria, adding the scores for each treatment, dividing the sum by the number of plants sampled from the treatment and finally dividing by three to adjust the maximum possible value of the index to one. The index values ranged from 0.63 to 0.84. A perusal of table-2 shows that EMS in combination with

Table 2. Frequency of different types of variant plants and mutation index in M_1 population of khesari (*Lathyrus sativus* L.) var. P-505.

Treatments	Number of plants studied	Number of variant plants			Proportion of variant plants (%)	Proportion of Type II variants (%)	Mutation* index
		Type I	Type II	Type III			
15kR	58	12	12	18	72.41	20.69	0.71
25kR	36	9	5	20	92.44	13.89	0.77
35kR	53	18	4	30	98.11	7.55	0.74
EMS 0.125%	49	2	28	5	71.43	57.14	0.69
DES 0.5%	47	2	21	8	65.96	44.68	0.73
15kR+EMS 0.125%	43	2	12	21	81.39	27.91	0.84
25kR+EMS 0.125%	41	13	4	19	87.80	9.76	0.72
35kR+EMS 0.125%	47	21	5	16	89.36	10.64	0.63
15kR+DES 0.5%	37	9	11	8	75.67	29.73	0.65
25kR+DES 0.5%	43	11	7	23	95.35	16.28	0.76
35kR+DES 0.5%	41	16	1	24	100.00	2.44	0.73

*After Subudhi *et al.* (1991)

15 KR gamma dose is most effective on inducing variations. On the other hand highest dose of 35Kr gamma rays in combination with EMS proved to be least effective.

The results revealed the differences in effectiveness of the mutagenic 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 upon the breeding objectives.

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