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MUTATION BREEDING IN IMPROVEMENT OF *PLANTAGO OVATA* FORSK

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ABSTRACT

Plantago ovata, a medicinal herb, requires improvement in seed yield to meet world demand for its seed husk. Mutation breeding has been attempted on account of the narrow genetic base of the crop. Two mutagens, gamma rays and ethylmethane sulphonate, were tried. The result obtained in all three replicates revealed that 20 kR dose of gamma rays boosted seed yield by more than 200%. The experiments were repeated during years between 1993-1995 and the results were confirmed. These results recorded a breakthrough in a crop not amenable to other techniques for improvement and are of great commercial value.

Key Words : *Plantago ovata*, Gamma rays, EMS, Seed yield

Plantago ovata is a medicinal herb, which yields isabgol that is effective in curing chronic diarrhoea, dysentery, and constipation. The recent finding of its potentiality to lower cholesterol levels in blood serum has increased its importance further [1, 2]. The plant is indigenous to the Mediterranean region and West Asia. In India, the crop is mainly cultivated in North Gujarat, from where huge consignments of seed and husk are exported to Canada, France, UK and USA. Exports made between 1990 and 1992 earned the country foreign exchange worth Rs. 638 million [3]. The country holds monopoly in world trade of *isabgol*. However, the annual production falls short to meet the global demand. Since the area under cultivation of the crop is not likely to increase significantly in the future, seed yields can be raised only by maximizing yields through genetic improvement of the crop.

Conventional methods for plant improvement, like selection, hybridization and induced polyploidy have not caused any major breakthrough primarily for want of genetic variations within the crop. The narrow genetic base of the species is attributed to its number of small chromosomes with a lot of heterochromatin, low chiasma frequency and high selfing rate.

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The crop has very low nutrient requirements. Ordinarily, 25 kg N + 25 kg P/ha are applied as a basa fertilizer dose during the last ploughing; 30 days after sowing 25 kg N/ha is top-dressed. An increase in fertilizer application does not increase seed yield significantly [4]. Moreover, dry matter yield, N concentration in seed and total uptake of N increased, while the swelling factor, which is responsible for its use in the pharmaceutical industry, decreased with an increase in the rate of applied N from 0 to 50 kg/ha [5].

In crops where non-availability of genetic variability is the major bottleneck, mutation breeding can provide a solution. Accordingly, in this study, two mutagens, namely gamma rays and ethylmethane sylphonate (EMS) were evaluated for inducing variations in components of seed yield.

MATERIAL AND METHODS

Seeds of *P. ovata* var. Gujarat Isabgol 1 were procured from the Gujarat Agricultural University, Anand. Dry seeds were treated with gamma rays and EMS. ^{60}Co was used as the source of gamma rays and seven doses (20, 40, 60, 80, 100, 120 and 140 kR) were tried. The different concentrations of EMS applied were 0.01, 0.05, 0.10, 0.25, 0.50 and 1.00% all for duration of 18h. each. The solutions were prepared in distilled-water. Three replicates of each treatment were run simultaneously,

The median lethal dose (LD_{50}) was determined from data on seed germination, seedling height and dry weight. Fifty seeds of each treatment were placed on moist filter paper in petridishes and kept at room temperature to germinate.

Part of the treated seed sample of each treatment was sown in experimental beds along with an untreated control. The experiment was laid out in a complete randomized block design. Each block had eight beds for gamma ray irradiated seeds and seven beds for EMS treated seeds. Only organic manure was added as fertilizer. Each bed had eight ridges for sowing and seeds were covered with soil. Before sowing, light irrigation was applied to ensure rapid germination.

Data on plant height, tiller, spike and seed counts, seed size and seed weight were collected in field from M_1 and M_2 plants. The M_2 progeny was grown from seed collected from individual M_1 plants on a plant to row basis. All quantitative data were subjected to statistical analysis.

RESULTS AND DISCUSSION

Gamma ray treatment up to 60 kR dose reduced the rate of seed germination. However, doses of 60-120 kR had a promotory effect. Still higher doses i.e. 140 kR dose reduced germination rate to a mere 29.68% of the control. Seedling height and seedling dry weight registered a gradual reduction as the dosage of gamma rays was increased (Table 1). All three seedling related characters showed a reduction with an increase in concentration of EMS (Table 1). From data it emerged that the LD_{50} for *P. ovata* lies between 120 and 140 kR dose of gamma rays and between 0.5 and 1.0% of EMS (Figs. 1 & 2). This indicates that *P. ovata* is relatively resistant to gamma rays as compared to other flowering plants.

Table 1. Seed germination, seedling height and seedling dry weight following treatment with gamma rays and EMS

Treatment	% of control		
	Germination	seedling height	dry weight
Gamma rays (kR)			
20	50.17 ± 0.23	88.75 ± 0.50	81.39 ± 0.73
40	49.47 ± 0.54	77.60 ± 0.72	78.72 ± 0.46
60	49.47 ± 0.83	67.09 ± 0.86	64.04 ± 0.59
80	61.48 ± 0.75	63.66 ± 0.93	78.72 ± 0.28
100	62.90 ± 1.02	63.87 ± 0.47	61.37 ± 0.93
120	79.15 ± 0.96	55.73 ± 1.02	50.70 ± 0.84
140	29.68 ± 1.31	43.94 ± 1.31	41.36 ± 0.59
EMS 18 h^{-1} (%)			
0.01	98.94 ± 0.33	58.31 ± 0.73	105.10 ± 0.92
0.05	89.04 ± 0.52	55.52 ± 0.89	96.68 ± 0.63
0.10	86.22 ± 0.09	50.37 ± 1.04	78.40 ± 0.63
0.25	83.39 ± 0.92	48.02 ± 0.96	68.87 ± 0.49
0.50	70.67 ± 0.97	36.01 ± 1.43	63.39 ± 0.69
1.00	46.64 ± 1.54	28.94 ± 1.74	42.10 ± 0.03

The effect of gamma rays on plant parameters was erratic and that of EMS was dose specific for plant height and seed count but erratic for other traits. Following the exposure to mutagens, positive shifts in mean values of some quantitative characters and negative shifts for some other characters have been reported in *P. ovata* and mung bean [6-12]. During the present study, exposure to 20 kR gamma

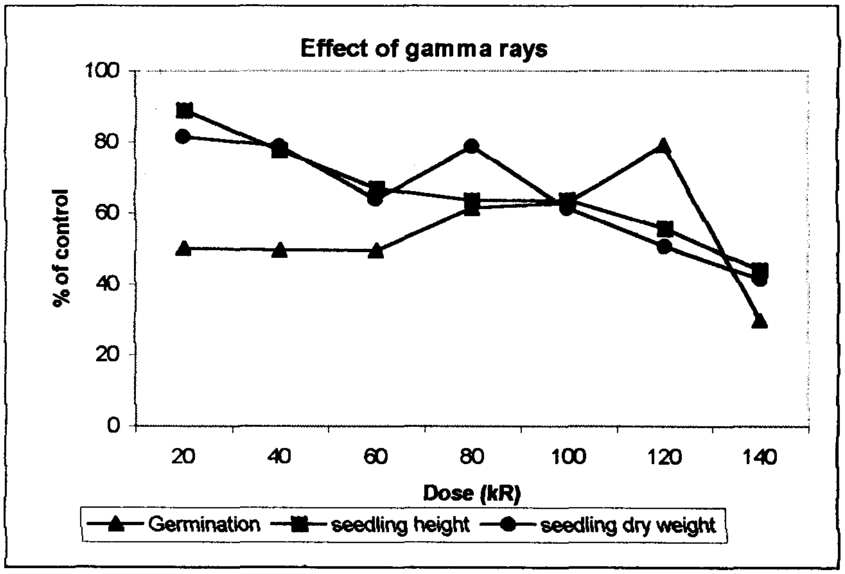

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Fig. 1. Effect of gamma rays on seed germination, seedling height and seedling dry weight

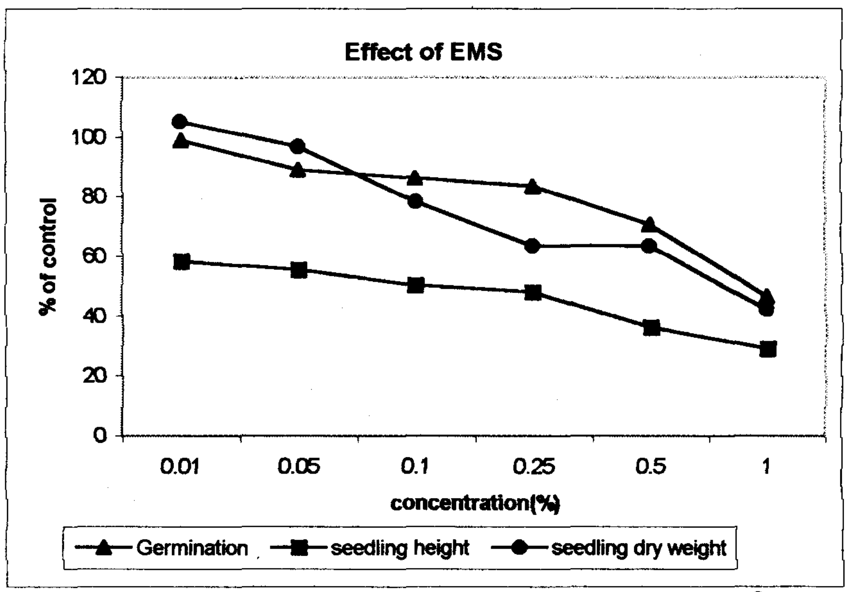

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Fig. 2. Effect of EMS on seed germination, seedling height and seedling dry weight

Table 2. Range, mean and variance of different yield components in the M_2 generation following gamma ray treatment

Treatment (kR)		Plant height (cm).	Tiller/ plant	Spike/ plant	Seed size (mm^2)	Seed/ plant	Seed wt./ plant (g)
Control	Range	36.8-43.5	2-23	6-62	1.52-3.05	214-871	0.01- 6.00
	Mean	40.17 ± 4.3	7.00 ± 1.3	38.70 ± 6.6	2.52 ± 0.01	480.6±7.12	2.47 ± 0.28
20	Range	26.6-43.4	2-19	7-9	2.0-3.0	247-2249	0.69-11.01
	Mean	34.53±0.7**	10.0 ± 0.54	48.3 ± 02.7	2.69±0.04**	867.0 ± 213.77	5.14 ± 0.64*
	Variance	1.31	1.30	1.19	1.99*	10.35**	116.33*
40	Range	30.9-42.6	3-14	15-67	1.3-2.5	84-918	0.85-5.01
	Mean	36.18±0.8*	8.39 ± 0.52	41.96 ± 2.6	1.80±0.01**	634.7±82.88	2.44 ± 0.37
	Variance	2.23	2.11	2.23	10.47**	1.75	29.86**
60	Range	22.7-45.9	3-16	15-86	1.3-2.29	127-2844	0.36-6.25
	Mean	35.28±0.7**	7.01 ± 0.57	35.06 ± 2.9	1.73±0.01**	598.42 ± 44.2	2.50 ± 0.29
	Variance	1.39	1.47	1.58	13.44**	16.85**	33.89**
80	Range	20.0-37.7	4-9	21-46	1.8-3.0	242-831	2.21-5.06
	Mean	29.90±1.5**	5.6 ± 0.29	28.00 ± 1.3	2.41 ± 0.05	375.3 ± 52.77	3.18 ± 0.21
	Variance	1.214	20.46**	25.02**	2.46**	1.27	6.43**
100	Range	24.9-32.9	4-11	21-56	1.8-3.0	221-514	2.08-5.37
	Mean	28.20±0.7**	6.08 ± 0.48	38.40 ± 2.2	2.39 ± 0.05	314.5 ± 30.15	3.12 ± 0.27
	Variance	5.03**	7.64**	9.42**	2.83**	3.89*	12.0**
120	Range	22.5-31.9	2-8	13-40	2.0-3.0	94-534	1.18-5.38
	Mean	27.70±0.6**	4.38±0.72*	21.90±3.2*	2.80±0.04**	269.5±38.45*	3.29 ± 0.36
	Variance	4.57**	3.59*	4.24*	1.51	2.17	23.94**
140	Range	14.7-33.7	3-5	17-26	1.5-3.0	168-341	2.04-3.17
	Mean	24.60±1.1*	4.34±0.21*	21.70±0.9**	2.30±0.05**	253.2±29.51*	2.40 ± 0.19
	Variance	.01	39.91**	48.81**	2.53**	8.16*	2.25

*$P \leq 0.05$; **$P \leq 0.01$

rays caused the mean values for seed size and seed weight/plant to increase significantly while seed count remained almost unaltered in the M_1 generation but increased in M_2 generation. A significant increase in plant height during the M_1 generation was induced by 40 kR dose. The mean value for seed size was increased with higher doses, e.g. 80, 120 and 140 kR, other traits mostly registered no change (when they did it was negative (Table 2). Application of different concentrations of EMS did not alter the mean values of most characters but, whenever they did, the change was deleterious (Table 3). However, mean values for seed size and yield were increased significantly by most treatments.

Table 3. Range, mean and variance of different yield components in the M_2 generation following treatment with EMS for 18 hrs.

Conc. of EMS/ H_2O		Plant height (cm).	Tiller/ plant	Spike/ plant	Seed size (mm^2)	Seed/ plant	Seed wt./ plant (g)
Control	Range	33.5-45.5	1-12	6-55	1.50-3.07	445-575	1.07- 4.31
	Mean	40.0 ± 0.70	5.48 ± 0.42	22.4 ± 02.1	2.52 ± 0.02	489.0±21.41	2.10 ± 0.26
0.01	Range	35.1-43.5	3-6	7-28	1.72-2.98	90-1238	2.35-5.17
	Mean	39.28±0.22	4.12 ± 0.36	15.60 ± 1.8	2.41 ± 0.05	556.9±128.4	339±0.29**
	Variance	1.17	3.26	3.88	1.77	64.7	30.62**
0.05	Range	33.1-42.0	1-6	4-29	1.75-2.95 .	45-842	2.03-4.10
	Mean	37.50±0.75	4.76 ± 0.36	18.80 ± 1.8	2.39±0.04	355.3±84.37	2.97±0.27*
	Variance	1.29	3.10	1.04	1.65	42.32	2.55
0.10	Range	34.4-39.5	2-5	13-25	2.0-3.04	34-714	2.38-4.20
	Mean	36.70±0.60	4.48 ± 0.22	17.40 ± 1.1	2.69±0.03*	279.3±61.39	3.36±0.21**
	Variance	1.94	9.18	9.32**	1.51	16.70	1.98
0.25	Range	31.0-38.9	1-9	8-50	1.2-2.19	25-550	0.82-2.27
	Mean	34.20±0.90	4.48 ± 0.32	22.41 ± 1.5	1.73 ± 0.01	158.2±47.59	1.60 ± 0.13
	Variance	1.04	1.03	3.13*	1.82	3.46	1.19
0.50	Range	28.4-39.3	2-16	7-87	1.4-2.6	1-320	0.0003-1.24
	Mean	33.73 1.23	6.30±1.175	26.75 ± 5.8	1.80 ± 0.01	88.0±29.69	1.21±0.05*
	Variance	1.93	3.96	3.98*	1.43	9.86	1.024

*$P \leq 0.05$; **$P \leq 0.01$

Of the two mutagens used, gamma rays proved more effective in boosting seed yield; EMS increased mean values for tiller number (0.5%) and seed count (0.01, 0.05, 0.10 and 0.25% concentration) but reduced it for seed yield. In contrast, gamma ray irradiation increased the mean values for all seed related characters. This was particularly true of 20 kR treatment in which some genotypes were isolated, which registered 220% increase in seed yield.

The components of seed yield did not respond similarly to different doses of the mutagen. In *P. ovata* seed yield bears a positive correlation with plant height, leaf, tiller, spike and seed counts, and seed size. The multiple correlation coefficient is 0.9197. Different doses of mutagens tried increased mean values for some and reduced them for other characters. Accordingly, the overall seed yield did not show any increase for most of the treatments. Similarly, 0.25% EMS treatment increased both tiller and spike count but reduced seed size. The increase in seed count following 40 kR and 0.01% EMS treatments was nullified by smaller seed size and increase in seed size following 80, 120 and 140 kR gamma rays treatments was nullified due to drastic reduction in seed count. Seed yield was increased over the control by treatments of 20, 60, 80, 100 and 120 kR gamma rays and 0.01, 0.05 and 0.10% EMS. However, only the 20kR treatment resulted in an increase in seed yield over the control since it affected all parameters favourably.

The experiment was repeated between 1993 and 1995, using two doses of gamma rays, 20 and 40 kR. The seed yield/plant obtained following the 20 kR treatment was 180% of the control suggesting that this dose can be used to increase the seed yield potential of *P. ovata*.

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