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INDUCE MUTATION BREEDING FOR DOWNEY MILDEW TOLERANCE IN ISABGOL (*PLANTAGO OVATA* FORSK.)

***Dinesh Kumar Jain*[1] *& S. K. Jain*[2]**

[1]*Plant Manager, Rajasthan State Seeds Corporation Ltd, Baran, Rajasthan, India*

[2]*Associate Professor, College of Agriculture, Sri Karan Narendra Agriculture University, Jobner, Rajasthan, India*

ABSTRACT

Twenty four mutagenic progenies of isabgolwere selected from different families of M_2 generation of gamma rays irradiated variety RI 89. These lines were grown in a sick plot along with infector rowsin randomized block design with three replications at the experimental farm of Rajasthan College of Agriculture, Udaipur. The observations were recorded on stage of disease occurrence (crop age), per cent disease index andtotal phenolic compounds in leaves at 55, 75 and 95 DAS. The observations for other quantitative and qualitative characters were also recorded. Out of 24 lines 14 lines showed disease resistance to downy mildew. It was also noted that disease tolerant lines were having a higher concentration of total phenolic compounds in leaves. Further, with increasing severity of disease the depletion of total phenolic compound in leaves was less intolerant lines. In the present study it is clear that total phenolic compounds in leaves play an important role in disease resistance and reducing the downy mildew disease of the host plants. The 4 mutant lines viz., L7, L19, L2 and L15 were having less than 8 percent disease infestation and may be used for developing downy mildew disease tolerant varieties for commercial cultivation.

KEWWORDS: *Isabgol, Mutation Breeding, Phenolic Compounds, Disease Index*

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INTRODUCTION

Isabgol or Blond Psyllium (*Plantagoovata*Forsk.) is a winter season medicinal plant. It belongs to the family of*Plantaginaceae* and genus *Plantago*.The seeds of psyllium are mainly valued for mucilaginous rosy white husk which have been used in the indigenous medicines for many centuries (Singh and Virmani, 1982 and Lal*et al.*, 1998).In recent years, the demand of isabgol has been increased in the western countries and it is traded in the major medicinal drug market of the world. In India it is cultivated traditionally in north Gujarat and south western Rajasthan. India holds a monopoly in the production and export of isabgol to the world market. The productivity of isabgol is far below the desired level and India is not able to meet the global demand. If India is to retain monopoly in production and export of this important foreign exchange earning commodity, then intensive efforts will have to be made to breed high yielding varieties with good swelling capacity of husk and tolerant to downy mildew disease. Downy mildew of Isabgol is most important and endemic disease in Rajasthan. It is caused by *Pseudoperonosporaplantaginis*in southern Rajasthan and *Peronosporaalta* in western Rajasthan. It occurs in all isabgol growing areas and causes considerable loss and an increase

in disease intensity was found directly correlated with the reduction in the seed yield. Available varieties of isabgol are lacking in tolerance to downy mildew disease.

Looking to the flower morphology and breeding behavior of isabgol, mutation breeding appears to be the only way of inducing genetic variability and thereafter significant improvement can be achieved. The prime strategy in mutation based breeding is to upgrade the well-adapted plant varieties by altering one or two major traits, which improve their productivity or enhance their quality and value (Ahloowalia*et al.*, 2004).

The present study was undertaken by using RI-89 variety of isabgol as the base material. The RI-89 variety is well adapted to the climatic condition of south west Rajasthan, but it is susceptible to downy mildew disease caused by *Pernosporaplantaginis*resulting in the drastic loss in the yield. Different graded doses of gamma rays were used to induce the genetic variability toidentify and isolate high yielding and downy mildew disease tolerant lines in M_3 generation.

MATERIALS AND METHODS

The seed material used in the present investigation was RI-89 variety of isabgol. Which is somewhat early and well adapted to the climatic conditions of south west Rajasthan, but susceptible to downy mildew disease. Two generation self-fed dry seeds of this variety were irradiated with nine doses of gamma rays *viz*., 15 kR, 30 kR, 45 kR, 60 kR, 75 kR, 90 kR, 105kR, 120 kR and 135 kR at the Nuclear Research Laboratory, Indian Agriculture Research Institute, New Delhi is using ^{60}Co as a source of gamma rays. M_1, M_2 and M_3 generations were raised in the Experimental farm of Rajasthan College of Agriculture, Udaipur. Irradiated seeds of each treatment were sown in the field, along with the control in randomized block design with three replications to rise M_1 generation. Each treatment was planted in four rows of two meter length at 30 cm apart, each line having about 40 plants at 5 cm inter plant distance. Normal appearing twenty plants selected at random and self-fed in each treatment in M_1 generation were advanced to raise the M_2 generation in compact family block design with three replications. Each treatment was taken as family and within treatment the individual plant was taken as progeny. Progenies were sown in single row plot of four meters length at 30 cm apart. The plant-to-plant distance was 10 cm. All the recommended agronomical practices were adopted to raise the healthy crop. No fungicide was used on the crop to assess the disease infestation. For assessment of downy mildew disease a score of 0-5 scale was used. Five plants were selected and tagged in each progeny and disease ratings were taken on 0-5 scale for each plant at 90 days after sowing (DAS). The disease index was calculated by using the formula of Wheeler (1969).Based on disease index 24 plants of different families having no infestation of downy mildew at 90 DAS were selected, self-fed and harvested separately to raise M_3 generation. Observations on various quantitative and qualitative characters were also recorded of selected plants. In M_3 generation experiment was conducted to screen and confirm the disease reaction of 24 downy mildew disease free plants selected in M_2 generation. The 24 plant progenies and a susceptible check, RI 89 were grown in a sick plot along with infector rows. The sick plot was prepared by the artificial inoculation of disease residues collected from the last year's crop. The bulk seeds of highly susceptible/infected progenies for downy mildew disease in M_2 generation were used as infector rows. The experiment was conducted in randomized block design with three replications. Each plot had four meter row length placed at 30 cm apart. The infector rows were sown before and after every two rows of testing lines. All the agronomical practices except spray of fungicides for controlling the disease were followed to raise a good crop. The observations were recorded on stage of disease occurrence (crop age), per cent disease index at 55, 75 and 95 DAS and total phenolic compounds in the leaves at 55, 75 and 95 DAS (mg/g). Apart from the above characters, observations were also recorded on other quantitative and qualitative characters. The analysis of variance was done as per

the method suggested by Panse and Sukhatme, 1978.

RESULTS AND DISCUSSIONS

From the M_2progenies,24 plants having zero disease infestation were retained to evaluate in M_3 generation. The magnitudes of various quantitative characters of these selected plants are presented in the Table 1. Under completely disease free plants the maximum 5 plants were from 120 kR family followed by 105 kR, 75 kR, 60 kR and 30 kR(4 plants from each family). Seed yield per plant ranged from 3.87 gm to 13.65 gm with an average yield of 7.92 gm.The other traits of these plants were also having higher values than control.

In M_3 generation, 24 disease free plants were evaluated in plant to progeny row along with a susceptible check, RI-89. Analysis of variancerevealed that the testing lines have significant difference among them for all the characters studied (Table 2). Table 3 revealed that downy mildew disease appearance was first reported in control at 43.33 DAS and it was delayed significantly in 12 lines. The maximum delaying in the disease appearance was observed in the line 7 (71.67 DAS). Three lines viz., L2, L7 and L10 were completely free from downy mildew disease at 55 DAS. At this stage the overall disease infestation was 6.15 per cent. The disease infestation increased in all the lines with crop age and at 75 DAS the average disease infestation recorded was upto 16.88 per cent. However, L7 was free from disease at this stage. With an increase in crop age, severity of disease increased and it reached to 75.33 percent in control at 95 DAS. At this stage, 14 lines had shown a tolerant reaction to downy mildew disease in which disease infestation was below 20 per cent. The minimum disease index at this stage was in L7 (4%). The phenolic compound in leaves at 55 DAS ranged from 0.16 mg/g (L9) to 0.62 mg/g (L22) with an overall mean of 0.34 mg/g. It increased with crop age in all the lines with an overall mean of 0.37 mg/g with a range from 0.21 mg/g (L6, L9 & L11) to 0.56 mg/g (L19) at 75 DAS. But thereafter it started to decline and it was 0.30 mg/g at 95 DAS. It was maximum in L19 (0.52 mg/g) followed by L2 (0.45mg/g), L16, L22 and L23 (0.43 mg/g) at 95 DAS.A view of normal and downy mildew disease infested plant and field view of downy mildew disease resistant and susceptible lines are presented in the Figure 1 and 2, respectively.

It was interesting to note that disease tolerant lines were having a higher concentration of total phenolic compounds in leaves. Further, with increasing severity of disease the depletion of total phenolic compound in leaves was less intolerant lines. The decrease in total phenols in mung bean infected with *Rhizoctoniasolani* was also reported by Arora (1983), while Luthra*et al.* (1988a) observed the increase in total phenolic compound after disease infection. Lily and Ramadasan (1979) and Sharma *et al.* (1983) also observed that tolerant genotypes had a higher content of total phenols than the susceptible ones. Further Gupta *et al.* (1995) explained that resistance in plants is expressed by oxidation of phenols to quinines which are more toxic to microorganisms. Hence, it is clear that total phenolic compounds in leaves play an important role in disease resistance and reducing the downy mildew disease of the host plants.

In the present study 14 lines showed disease resistance to downy mildew. Among them, 4 lines viz., L7, L19, L2 and L15 were having less than 8 percent disease infestation and these lines may be used for developing downy mildew disease tolerant varieties for commercial cultivation.

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APPENDICES

Table 1: Mean Values of Various Quantitative Characters of Identified Diseases Tolerant Plants Selected in M_2 Generation

Plant/ Line No.	Pedigree	Disease Index	Seed Yield/ Plant (g)	Biological Yield/ Plant (g)	Harvest Index (%)	Plant Height (cm)	Number of Tillers/ Plant	Number of Spikes/ Plant	Spike Length (cm)
1	12.3.2	0.00	5.10	25.00	20.40	34.00	5.00	35.00	6.00
2	12.3.1	0.00	10.61	40.00	26.53	37.00	6.00	70.00	5.00
3	17.7.4	0.00	7.24	20.00	36.20	36.00	5.00	35.00	5.00
4	3.15.1	0.00	3.87	16.00	24.19	34.00	3.00	22.00	5.80
5	14.13.1	0.00	6.01	20.00	30.05	41.00	4.00	37.00	4.50
6	14.14.2	0.00	10.19	35.00	29.11	36.00	4.00	55.00	6.00
7	515-2	0.00	11.38	40.00	28.45	35.00	7.00	52.00	5.00
8	5.10.1	0.00	5.51	30.00	18.37	34.00	5.00	43.00	5.80
9	5.8.1	0.00	7.71	36.00	21.42	38.00	6.00	49.00	7.00
10	5.4.3	0.00	5.86	30.00	19.53	32.00	4.00	42.00	5.00
11	16.6.4	0.00	13.24	40.00	33.10	38.00	6.00	76.00	5.00
12	6.12.1	0.00	13.65	40.00	34.13	37.00	5.00	58.00	6.00
13	6.13.6	0.00	6.10	26.00	23.46	34.00	6.00	44.00	5.30
14	6.8.7	0.00	5.27	22.00	23.95	33.00	5.00	37.00	4.00
15	13.5.3	0.00	5.74	20.00	28.70	34.00	3.00	35.00	4.00
16	18.11.1	0.00	12.00	40.00	30.00	37.00	4.00	65.00	6.00
17	18.18.2	0.00	5.18	20.00	25.90	33.00	3.00	45.00	3.50
18	18.9.1	0.00	8.46	30.00	28.20	35.00	6.00	54.00	3.80
19	417-1	0.00	4.76	20.00	23.80	33.00	5.00	27.00	5.80
20	15.17.1	0.00	6.39	27.00	23.67	37.00	4.00	49.00	6.00
21	15.17.2	0.00	4.41	22.00	20.05	38.00	4.00	27.00	5.50
22	300-5	0.00	6.37	22.00	28.95	32.00	4.00	26.00	4.00
23	20.17.1	0.00	12.58	34.00	37.00	34.00	4.00	55.00	5.00
24	456-2	0.00	12.54	45.00	27.87	33.00	7.00	73.00	5.50
GM		**0.00**	**7.92**	**29.17**	**26.79**	**35.21**	**4.79**	**46.29**	**5.19**

Table 2: Mean Squares for different Characters Observed on Disease Resistant Progenies in M_3Generation

Character	Replication [2]	Genotype [24]	Error [48]
Days to 50% flowering	2.413	3.777**	1.274
Days to 75% maturity	0.413	3.722*	1.802
Reproductive phase	3.640	2.944*	1.501
Plant height	8.579	9.463**	2.951
Number of tillers per plant	0.232	0.878**	0.156
Number of spikes per plant	1.258	69.884**	20.1
Spike length	0.046	0.641**	0.111
Biological yield per plant	0.643	23.819**	5.241
Seed yield per plant	0.029	1.877**	0.391
Harvest index	0.034	26.237*	13.49
Test weight	0.008	0.051**	0.0114
Swelling factor	0.280	2.717**	0.53
Total phenolic compounds at 55 DAS	0.001	0.047**	0.0021
Total phenolic compounds at 75 DAS	0.005	0.035**	0.003
Total phenolic compounds at 90 DAS	0.001	0.027**	0.002
Stage of disease occurrence (crop age)	64.813	138.000*	71.52
Per cent disease index at 55 DAS	73.768	86.938**	39.25
Per cent disease index at 75 DAS	191.520	306.524**	80.96
Per cent disease index at 95 DAS	252.373	684.435**	95.43

***, **Significant at 5% and 1 per cent level of significance**

Table 3: Mean Values of different Characters for Disease Resistant Progenies in M_3Generation

S. No.	Genotype	Days to 50% Flowering	Days to 75% Maturity	Reproductive Phase (Days)	Plant Height (cm)	Number of Tillers/ Plant	Number of Spikes/Plant	Spike Length (cm)	Biological Yield/Plant (g)	Seed yield/ plant (g)	Harvest Index (%)
1	L1	72.33	110.00	37.67	34.90	3.93	18.97	3.40	14.35	2.59	18.07
2	L2	71.67	111.67	40.00	35.67	3.73	26.60	4.52	16.75	3.56	21.28
3	L3	73.00	112.00	39.00	29.60	3.87	26.30	3.11	17.48	2.75	15.85
4	L4	73.33	112.67	39.33	34.53	3.60	28.50	3.36	18.88	3.66	19.32
5	L5	73.33	111.00	37.67	36.47	3.00	14.20	2.89	10.28	1.77	17.18
6	L6	74.67	112.33	37.67	33.27	3.70	28.60	3.76	18.82	3.16	16.70
7	L7	71.00	110.33	39.33	33.73	4.07	20.47	3.20	14.88	2.86	19.17
8	L8	72.00	110.67	38.67	34.97	4.27	26.77	4.52	19.22	3.90	20.49
9	L9	73.00	111.00	38.00	34.43	4.80	30.40	4.01	19.95	3.86	19.18
10	L10	72.67	111.33	38.67	33.83	3.67	22.03	3.50	14.92	2.40	16.27
11	L11	73.67	111.67	38.00	33.23	4.10	33.90	3.64	20.08	4.50	21.50
12	L12	74.33	113.00	38.67	34.10	2.60	24.83	3.23	14.15	2.89	21.11
13	L13	73.33	111.00	37.67	33.03	4.30	26.90	3.66	18.62	3.42	18.49
14	L14	74.33	114.33	40.00	30.00	4.40	31.07	3.57	17.82	3.39	18.98
15	L15	72.67	111.00	38.33	35.17	4.67	26.00	3.80	19.55	3.57	18.66
16	L16	74.00	111.67	37.67	34.03	4.40	29.70	4.40	17.08	3.15	18.29
17	L17	73.67	111.00	37.33	29.60	3.87	21.93	3.28	14.48	2.48	17.11
18	L18	73.67	111.00	37.33	33.47	3.60	18.60	3.11	13.05	2.14	16.41
19	L19	71.33	111.00	39.67	33.13	4.27	27.07	4.13	17.22	3.49	20.26
20	L20	73.33	109.33	36.00	34.53	4.60	33.27	4.23	22.75	4.66	20.51
21	L21	72.67	111.00	38.33	31.90	4.00	24.60	3.32	15.68	2.53	16.31
22	L22	73.67	111.33	37.67	32.60	5.20	31.53	4.14	21.75	4.08	18.88
23	L23	71.67	110.00	38.33	32.43	4.07	22.93	3.39	17.15	4.56	28.75
24	L24	73.00	110.00	37.00	35.40	4.00	25.77	3.48	18.02	2.43	13.46
25	(Control)	76.00	113.00	37.00	34.00	3.98	21.33	3.82	15.75	2.30	14.60
GM		**73.13**	**111.33**	**38.20**	**33.52**	**4.03**	**25.69**	**3.66**	**17.15**	**3.20**	**18.67**
SE		**0.65**	**0.78**	**0.71**	**0.99**	**0.23**	**2.59**	**0.19**	**1.32**	**0.36**	**2.12**
CD5%		**1.85**	**2.20**	**2.01**	**2.82**	**0.65**	**7.36**	**0.55**	**3.76**	**1.03**	**6.03**

Table 3 Contd.,

Genotype	Test Weight (g)	Swelling Factor (cc/g)	TPC at 55 DAS	TPC at 75 DAS	TPC at 95 DAS	Stage of Disease Occurrence	PDI at 55 DAS	PDI at 75 DAS	PDI at 95 DAS	Disease Reaction
L1	1.27	11.67	0.23	0.32	0.24	61.00	3.07	6.67	10.67	T
L2	1.44	12.33	0.44	0.51	0.45	64.33	0.00	8.00	7.33	T
L3	1.30	10.50	0.37	0.41	0.34	59.00	1.33	27.33	20.00	T
L4	1.43	10.33	0.33	0.37	0.31	55.67	4.67	20.67	30.00	MT
L5	1.30	9.67	0.22	0.26	0.17	59.33	6.67	22.00	33.33	MT
L6	1.16	9.50	0.20	0.21	0.17	51.67	5.33	28.00	43.33	MS
L7	1.48	12.00	0.37	0.43	0.34	71.67	0.00	0.00	4.00	T
L8	1.38	11.17	0.34	0.42	0.33	65.67	2.67	14.67	16.67	T
L9	1.53	10.67	0.16	0.21	0.19	50.00	14.00	22.00	26.67	MT
L10	1.38	10.17	0.46	0.44	0.32	63.67	0.00	16.00	26.67	MT
L11	1.46	10.67	0.21	0.21	0.20	55.67	3.87	26.00	23.33	MT
L12	1.44	10.17	0.23	0.23	0.24	53.00	6.67	25.33	23.33	MT
L13	1.53	11.83	0.31	0.37	0.36	63.00	2.00	11.33	12.67	T
L14	1.27	11.17	0.18	0.22	0.24	49.00	14.33	20.00	16.67	T
L15	1.55	12.33	0.23	0.28	0.25	58.33	4.00	4.00	7.33	T
L16	1.25	12.33	0.55	0.50	0.43	67.33	3.33	4.00	10.00	T
L17	1.40	11.50	0.25	0.33	0.31	56.33	4.00	8.00	14.67	T
L18	1.49	11.50	0.36	0.40	0.36	49.67	14.67	14.67	14.67	T
L19	1.54	12.67	0.49	0.56	0.52	57.00	3.33	4.67	6.67	T
L20	1.57	10.17	0.40	0.44	0.22	53.00	7.33	20.00	29.33	MT
L21	1.33	10.17	0.46	0.46	0.28	51.00	12.80	22.00	30.00	MT
L22	1.26	11.17	0.62	0.50	0.43	57.33	2.00	16.00	18.67	T
L23	1.68	11.83	0.44	0.49	0.43	53.67	6.00	10.67	13.33	T
L24	1.22	9.50	0.44	0.41	0.25	49.67	11.67	25.33	36.67	MT
Control	1.29	10.50	0.23	0.28	0.23	42.33	20.00	44.67	75.33	S
GM	**1.40**	**11.02**	**0.34**	**0.37**	**0.30**	**56.73**	**6.15**	**16.88**	**22.05**	
SE	**0.06**	**0.42**	**0.03**	**0.03**	**0.03**	**4.88**	**3.62**	**5.20**	**5.64**	
CD at 5%	**0.18**	**1.20**	**0.08**	**0.09**	**0.07**	**13.88**	**10.28**	**14.77**	**16.04**	
CD at 1%	**0.23**	**1.59**	**0.10**	**0.12**	**0.10**	**18.53**	**13.73**	**19.71**	**21.40**	
CV	**7.66**	**6.61**	**13.68**	**14.47**	**14.87**	**14.91**	**101.88**	**53.31**	**44.30**	

T = Tolerant, MT = Moderately tolerant, MS = Moderately susceptible, S = Susceptible

Figure 1: A View of Normal and Downy Mildew Diseased Plants

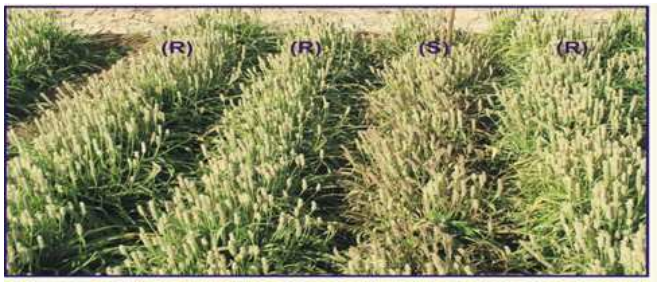

Figure 2: A View of Downy Mildew Diseased Resistant and Susceptible Lines