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RESEARCH ARTICLE

Mutagenic response of isabgol (*Plantago ovata* L. Forsk) to gamma-rays

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SUMMARY

The fresh seeds of isabgol variety RI-87 were subjected to 15, 30, 45, 60, 75, 90, 105,120 and 135 Kr doses at Co_{60} gamma-rays and lab as well as field experiment was carried out during rabi 2002-03 and 2003-04. The effects of gamma-rays were studied on different parameters such as germination percent, seedling height, seedling dry weight and pollen fertility in M_1 generation and frequency and spectrum of chlorophyll and viable mutants, mutagenic efficiency and effectiveness in M_2 generation with employing nine doses of gamma-rays and one control. Reduction of germination, seeding height, seedling dry weight and pollon fertility in the M_1 generation was observed with increases doses of gamma-rays. In general the frequencies of chlorophyll mutations increased in linear fashion at low and medium doses and were erratic reduction at higher doses. The spectrum of chlorophyll mutants included albina, xantha, chlorina, viridis, tigrina and others. The frequency of these mutants varied with treatments. The 105 kR doses of gamma-rays produce highest frequency of viable mutants. Occurrence of major viable mutants from seedling to adult growth stages were varied *viz*., broad and narrow leaves, paired spikes, coxcomb spikes, ball mutants, gappy spikes, partial and complete sterile. A dose rate of 90 kR and 105 kR were most effective as well as efficient treatments.

Key Words : *Plantago ovata* L., mutations, chlorophyll and viable mutants, mutagenic effectiveness, mutagenic efficiency

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Isabgol (*Plantago ovata* L. Forsk) constituents one of the most important indigenous drug plants of India for their seed and husk which used in stomach disorders such as diarrhea,ulcers, gonorrhoea, pileas, cough, chronic dysenteries of amoebic and bacillary origin and also use for treating constipation and intestinal disorders in ayurvedic medicines. Due to the presence of mucilaginous it has diversified uses not only in medicine but also in small scale industries (Dalal and Sriram, 1995). India is the sole exporter of psyllium to the world market and about 80 to 90 per cent produce is exported but is not able to meet global demand on account of low production and productivity. The possible constraints that limit the yield are many and varied. However, narrow genetic base and account of low chromosome number (n=4) and small chromosome size with 60 per cent constitutive heterochromatic nature are the major constraints (Stack, 1984 and Sareen *et al*., 1999). Because of small size and closely born florets and small stature of the plants, emasculation and artificial pollination is difficult and mutation techniques as a single tool to generate desirable variability (Dalal and Sriram, 1995 and Kumar and Ramesh, 2004). The important and most essential task in mutation breeding is the use of appropriate mutagens. Several investigators have pointed to the usefulness of ionizing radiation for inducing mutation in psyllium (Bhagat and Hardas, 1980, Sareen and Kaul, 1991, Lal and Sharma, 2002 and Jain *et al*., 2005). The usefulness of any mutagens in plant breeding depends not only on its mutagenic effectiveness but also on its efficiency (Krishna *et al*., 1984, Kharkwal, 1998 and Jain, 2004).

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The present study was therefore designed to determined response of psyllium to gamma-rays in respect to frequency and spectrum of chlorophyll and viable mutations, efficiency and effectiveness of gamma-rays.

MATERIALS AND METHODS

Selfed seeds of genotype RI-87 of psyllium were treated with nine doses of gamma-rays ranging from 15 to 135 kR at 15 kR interval by exposing to ^{60}CO source at a dose rate of 3.92 kR per minute at Nuclear Research Laboratory, Indian Institute of Agricultural Research, New Delhi, India. One hundred seeds treated with above doses of radiation along with control were germinated on moist filter paper placed in petridises at room temperature with four replications at PG Lab, Rajasthan College of Agriculture, MPUAT, Udaipur (Raj.), India. Observations were recorded on per cent germination, seedling height (cm) and seedling dry weight (g) after 20 days. Part of irradiated seed material along with control was sown at the experimental field, Rajasthan College of Agriculture, Udaipur. Pollen fertility of M_1 generation was determined by staining the pollen with one per cent acetocarmine stains. For this purpose, plants were selected randomly from each treatment and finally 5 flower buds from each plant were used for microscopic analysis. The pollen grains which failed to stain or showed abnormal shape and improper filling were scored as sterile. Seeds harvested from the main spikes of M_1 plants were raised in properly prepared experimental field for analysis of M_2 generation.

Frequency and spectrum of chlorophyll and viable mutations:

The M_2 generation was raised in compact family block design with three replications during 2003-04. Chlorophyll mutants were scored from fifth to fifteenth day after sowing and classified according to Gutafsson (1940). In all M_1 plant progenies and seedlings were scored for chlorophyll mutations in M_2 generation. Simultaneously these progeny and plants were scored for viable/morphological mutations until the crop was harvested. Frequencies of these mutants were estimated both as per cent of M_2 families segregating (M_1 plant basis) and also as mutations in per cent of M_2 plants (M_2 plant basis).

Estimation of mutagenic effectiveness and efficiency :

Mutagenic effectiveness is defined as measure of frequency of mutations induced by a unit dose of mutagens and mutagenic efficiency indicates proportion of mutations in relation to undesirable effects such as lethality and sterility. Data on seedling injury and pollen sterility recorded in M_1 generation and mutation frequency in M_2 generation were used to determine the mutagenic efficiency and effectiveness according to the formula suggested by Krishna *et al*. (1984) and Kharkwal (1998).

RESULTS AND DISCUSSION

Observations were recorded in the first generation (M_1) after irradiation. First the influence of irradiation doses on the survival of seedlings was described. From Table 1 data were revealed that germination percent, seeding height and seedling dry weight declined due to exposure of gamma-rays upto 135 kR and it was decreased upto 59.89, 54.4 and 62.86 per cent of the control. Like wise pollen fertility was also decreased and decrease has continued with increased dose of gamma-rays. It was indicative from the significant correlation co-efficient of above biological parameters with mutagens. Singh and Rathore (2000) and Lal and Sharma (2002) also reported the decreasing trend of the above characters with increasing dose of gamma-rays.

A common belief is that doses close to LD_{50} for biological parameters such as germination percent, seedling height and seedling dry weight should be taken in to consideration, while planning the mutation breeding experiments. An over doses

Table 1: Effect of gamma-rays on germination, seedling height, seedling dry weight and pollen fertility in M_1 generation

Sr.No.	Doses of gamma-rays	Germination (%)	% reduction from control	Seedling height (cm)	% reduction from control	Seedling dry weight (g)	% reduction from control	Mean of 4,6,8	Pollen fertility (%)	% reduction from control
1	2	3	4	5	6	7	8	9	10	11
1.	Control	100.00	-	4.50	-	0.140	-	-	100.00	-
2.	15 kR	80.00	20.00	3.70	17.77	0.134	4.29	14.02	91.25	8.75
3.	30 kR	65.60	34.40	3.40	24.44	0.111	20.71	26.52	81.25	18.75
4.	45 kR	63.20	36.80	3.33	26.00	0.095	32.14	31.65	71.20	28.80
5.	60 kR	55.25	44.75	3.00	33.33	0.090	35.71	37.93	69.21	30.79
6.	75 kR	50.90	49.10	2.94	34.67	0.085	39.29	41.05	68.25	31.75
7.	90 kR	50.00	50.00	2.25	50.00	0.070	50.00	50.00	61.00	39.00
8.	105 kR	48.71	51.29	2.15	52.22	0.069	50.71	51.41	55.00	45.00
9.	120 kR	45.20	54.80	2.13	52.67	0.061	56.43	54.63	53.25	46.75
10.	135 kR	40.15	59.85	2.05	54.44	0.052	62.86	59.05	48.12	51.88

kill too many treated population while a lower dose produce too few mutations. In the present investigation the LD_{50} measured on the basis of biological parameters like germination per cent, seeding height and seedling dry weight was 90 kR dose of gamma-rays. The LD_{50} at higher doses indicated that the isabgol appeared to be gamma-rays resistant as compared to other angiosperm (Sareen and Kaul, 1999 and Jain *et al*., 2005).

The aim of present investigation was to examine whether and if so to what extent mutations would have originated in the M_2 generation to irradiated seeds. The study this an analysis was made at seedling and later growth stage in M_2 generation especially to estimate frequency of chlorophyll and viable mutations. The frequency and spectrum of chlorophyll mutations calculated as per cent of M_1 and M_2 plants are presented in Table 2 and 3. The classification of chlorophyll mutations as given for barley by Gustafsson (1940) was used. In the present investigation the types albina, xantha, chlorina, viridis, tigrina and some other type of chlorophyll mutants were noticed in all doses tested. The xantha type mutants was observed more frequent at lower doses of gamma-rays whereas albina was more frequent at higher doses of gamma-rays. The results indicated that the photosynthetic activity is more altered at higher doses of gamma radiation. Also some another common groups of chlorophyll mutations like chlorina, viridis and tigrina were found at all the doses of gamma-rays. There was still another group of mutations indicated as "others" which were difficult to place under one of the fore-going head also present but rare in quantity.

In general the medium and higher doses of gamma-rays seem to induce a wider spectrum of chlorophyll mutations. However, Gustafsson (1963) referred those differences in spectrum as conspicuous differences in group mutability by ionizing radiations. Bremer-Reinders (1962) attributed these differences to the metabolic conditions of the seeds during irradiation. In the present study frequency of chlorophyll mutants increased with increasing doses of gamma-rays upto 105 kR and the frequency observed were 90.90 and 11.31 per cent on M_1 and M_2 plant basis, respectively. Beyond 105 kR the frequency of chlorophyll mutants declined. This indicates a saturation effect at higher doses. These results are in accordance with earlier reporters (Sareen and Kaul, 1991 and Jain *et al*., 2005).

Among doses of gamma-rays 90 kR was the most

Table 2: Frequency of chlorophyll mutants in M_2 generation with various doses of gamma -rays

Sr.No.	Doses of gamma-rays	M_1 progenies scored	M_2 plant scored	M_1 segregates for mutation in M_2	M_2 mutants	Mutation frequency (%)	
						M_1 plant basis	M_2 plant basis
1.	Control	60	4560	-	-	-	-
2.	15 kR	55	4400	25	70	45.45	1.59
3.	30 kR	75	5675	40	90	53.33	1.59
4.	45 kR	60	6550	45	150	75.00	2.29
5.	60 kR	45	5000	35	250	77.77	5.00
6.	75 kR	62	6540	50	500	80.64	7.65
7.	90 kR	45	5062	40	560	8.89	11.06
8.	105 kR	55	5215	50	590	90.90	11.31
9.	120 kR	65	5261	55	350	84.62	6.65
10.	135 kR	50	4352	40	400	80.00	9.19

Table 3: Spectrum of chlorophyll mutations in M_2 generations (on M_2 plant basis) with various doses of gamma -rays

Sr. No.	Doses of gamma- rays		Types of chlorophyll mutations						Total M_2 plants scored
			Albina	Xantha	Chlorina	Viridis	Tigrina	Others	
1.	Control	No %	-	-	-	-	-	-	4560
2.	15 kR	No %	5 (0.11)	40 (0.91)	10 (0.23)	5 (0.11)	4 (0.09)	6 0.14)	4400
3.	30 kR	No %	7 (0.12)	45 (0.79)	14 (0.25)	11 (0.19)	5 (0.09)	8 (0.14)	5675
4.	45 kR	No %	10 (0.15)	60 (0.92)	15 (0.23)	20 (0.31)	45 (0.69)	0 (0.00)	6550
5.	60 kR	No %	35 (0.70)	30 (0.60)	60 (1.24)	45 (0.90)	60 (1.20)	18 (0.36)	5000
6.	75 kR	No %	75 (1.15)	9 (0.14)	145 (2.22)	100 (1.53)	170 (2.60)	1 (0.02)	6540
7.	90 kR	No %	11 (0.22)	17 (0.34)	170 (3.36)	165 (3.26)	195 (3.85)	2 (0.04)	5062
8.	105 kR	No %	25 (0.48)	35 (0.67)	165 (3.16)	195 (3.74)	165 (3.16)	5 (0.10)	5215
9.	120 kR	No %	15 (0.29)	20 (0.38)	95 (1.81)	97 (1.84)	120 (2.28)	3 (0.06)	5261
10.	135 kR	No %	25 (0.57)	10 (0.23)	105 (2.41)	115 (2.64)	132 (3.03)	13 (0.30)	4352

effective in inducing mutations followed by 105 kR (Table 4). In general the lower doses were less effective than higher doses for inducing mutations. The estimates of efficiency based on lethality (germination per cent, seeding height and seedling dry weight) and pollen sterility also indicated that 90 kR followed by 105 kR dose was most efficient then lower does of gamma-rays. The lower doses were less effective and efficient than higher doses to inducing chlorophyll mutations which indicated that seeds are more resitant to ionizing radiation at lower does (Krishna *et al*., 1984 and Jain *et al*., 2005).

Except chlorophyll mutations also other changes of

Table 4 : Mutagenic effectiveness and efficiency of different doses of gamma-rays

Sr. No.	Doses of gamma-rays	M_2 seedlings scored	M_2 mutants	% mutant frequency on M_2 plant basis (M)	Effectiveness (M/Doses)	Seedling survival reduction in M_1 (% of control) (L)	Pollen fertility reduction from control (P)	Efficiency	
								M/L	M/P
1.	Control	4560	-	-	-	-	-	-	-
2.	15 kR	4400	70	1.59	0.106	14.02	8.75	0.113	0.182
3.	30 kR	5675	90	1.59	0.053	26.52	18.75	0.060	0.085
4.	45 kR	6550	105	2.29	0.051	31.65	28.80	0.072	0.080
5.	60 kR	5000	250	5.00	0.083	37.93	30.79	0.132	0.162
6.	75 kR	6540	500	7.65	0.102	41.05	31.75	0.186	0.241
7.	90 kR	5062	560	11.06	0.123	50.00	39.00	0.220	0.284
8.	105 kR	5215	590	11.31	0.108	51.41	45.00	0.220	0.251
9.	120 kR	5261	350	6.65	0.055	54.63	46.75	0.122	0.142
10.	135 kR	4352	400	9.19	0.068	59.05	51.8	0.156	0.171

Table 5: Frequency of viable mutations in M_2 generation with various doses of gamma-rays

Sr. No.	Doses	M_1 plant progenies scored	M_2 plant scored	M_1 segregates for mutations in M_2	Number of M_2 mutants	Mutation frequency (%)	
						M_1 pant basis	M_2 pant basis
1.	Control	60	4410	-	-	-	-
2.	15 kR	52	4000	25	150	48.08	3.75
3.	30 kR	72	5074	42	200	58.33	3.94
4.	45 kR	57	6051	45	256	78.95	4.23
5.	60 kR	42	4900	34	330	80.95	6.73
6.	75 kR	60	4920	55	400	91.67	8.13
7.	90 kR	42	4000	39	395	92.86	9.86
8.	105 kR	51	4210	49	425	96.08	10.10
9.	120 kR	61	4111	57	325	93.44	7.91
10.	135 kR	47	4210	45	330	95.74	7.84

Table 6: Spectrum of morphological variants in M_2 generation (on M_2 plant basis) with various doses of gamma-rays

Sr. No.	Type of morphological variants		doses of a-Y									
			Control	15	30	45	60	75	90	105	120	135
1.	Broad leaves	No %	-	15 (0.38)	23 (0.45)	34 (0.56)	0.00 (0.00)	20 (0.41)	10 (0.25)	7 (0.17)	0.00(0.00)	6 (0.14)
2.	Narrow leaves	No %	-	25 (0.63)	18 (0.35)	23 (0.38)	24 (0.49)	63 (1.28)	62 (1.55)	25 (0.59)	0.00(0.00)	8 (0.19)
3.	Coxcomb spikes	No %	-	30 (0.75)	27 (0.53)	45 (0.74)	22 (0.45)	64 (1.30)	61 (1.53)	93 (2.21)	62 (1.51)	4 (0.100
4.	Paired spikes	No %	-	12 (0.30)	35 (0.69)	27 (0.45)	41 (0.84)	39 (0.79)	84 (2.10)	89 (2.11)	34 (0.83)	81 (1.92)
5.	Ball mutants	No %	-	17 (0.43)	25 (0.49)	63 (1.04)	42 (0.86)	41 (0.83)	15 (0.38)	84 (1.99)	0.00(0.00)	83 (1.97)
6.	Horn mutants	No %	-	20 (0.50)	0.00(0.00)	51 (0.84)	71 (1.45)	0.00(0.00)	10 (0.25)	37 (0.88)	32 (0.78)	42 (1.00)
7.	Gappy spikes	No %	-	5 (0.13)	33 (0.65)	5 (0.08)	63 (1.29)	69 (1.40)	69 (1.73)	45 (1.07)	68 (1.65)	67 (1.59)
8.	Partial sterility	No %	-	20 (0.50)	31 (0.61)	0.00(0.00)	30 (0.61)	65 (1.32)	42 (1.05)	0.00(0.00)	91 (2.21)	0.00(0.00)
9.	High sterility	No %	-	6 (0.15)	7 (0.14)	6 (0.10)	37 (0.76)	39 (0.79)	42 (1.05)	45 (1.07)	38 (0.92)	39 (0.93)
	Total M_2 plant scored		4410	4000	5074	6051	4900	4920	4000	4210	4111	4210

phenotype occur in the seedling and later growth stage in M_2 generation are presented in Table 5 and 6. Among all the doses of gamma-rays 105 kR produced highest frequency of viable mutations followed by 75 kR and 90 kR doses (Table 5). The frequency of viable mutations both on M_1 and M_2 plant basis increased with increase in doses of gamma-rays upto 105 kR followed by a slight decline with further increase. The highest frequency of 96.08 and 10.10 per cent on M_1 and M_2 plant basis respectively was observed at 105 kR treatment. The types of viable mutations varied with doses (Table 6) and mainly consist of broad and narrow leaves, coxcomb spikes, paired spikes, ball mutant, horn mutants, gappy spikes, partial and complete sterile.

The frequency of viable mutations estimated on M_1 and M_2 plant basis showed a linear relationship. However, a saturation effect was seen at higher doses. Similar results have been reported by (Anonymous, 1980, Sriram *et al*., 1987 and Jain *et al*., 2005). At higher doses because of high sterility the response of the progeny will be more erratic among treatments depending upon gametic and zygotic elimination (Kharkawal, 1998). A wide spectrum of viable mutations such as leaf mutants, spike mutants and steriles were observed at all the doses of gamma-rays. The frequency of viable mutants was more at medium doses and sterile were more frequent at higher doses of gamma-rays. Similar results were also observed by Sareen and Kaul (1991) and Jain *et al*. (2005).

Present investigation indicated that mutation breeding particularly ionizing radiation hold promise in the improvement of Psyllum and gamma-rays treatment 75 to 105 kR were found most appropriate. Some of the economically important mutants were also isolated like broad leaves, paired spikes, ball mutants and horn mutants and will be test in later generations.

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