The Bioscan 8(1): 49-53, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

STUDY OF INDUCED POLYGENIC VARIABILITY IN M_1 AND CHLOROPHYLL MUTATIONS IN M_2 GENERATIONS IN AROMATIC RICE

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KEYWORDS
Gamma rays
Polygenic variability
Chlorophyll mutations
Aromatic rice

Received on :
15.08.2012

Accepted on :
24.11.2012

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ABSTRACT

Variability induced for quantitative characters (days to 50% flowering, days to maturity, plant height, panicle length, panicle bearing tillers per plant and grain yield per plant.) were assessed on individual plant basis in M_1 generation. The maximum frequency of chlorophyll mutations per $100M_2$ plants obtain in M_2 generation in 20kR gamma rays + EMS (0.2%) in *Kalanamak* following by *Badshah Bhog*, respectively. The Frequency and spectrum of viable mutations per $100M_2$ plant maximum in 40kR gamma rays+ EMS (0.2%) in Badshah Bhog genotypes were observed in M_2 generation. Among chlorophyll mutations, Albino mutant was most frequent in both the genotypes. The different types of chlorophyll mutations observed in M_2 generation were Albino, Xantha, Striata and Viridis.

INTRODUCTION

Rice is diploid and self-pollinated important and extensively grown food crop ranking at 2nd in area and production in world (Anonymous, 2007) place after wheat. It is the staple food of more than 60 per cent of the world population; India has largest area under paddy in the world and ranks second in the production after China. The use of physical and chemical mutagens or a combination of both has been an important tool for the increase of variability in agronomic and economic improvement of traits in several crop plants including rice. (Jana and Roy, 1973; Agrawal *et al.*, 2000; Singh and Singh, 2003; Sharma *et al.,* 2008). The potentialities of ionizing radiation and chemical mutagens are different and their ability to induce mutation varies from crop to crop and genotype to genotype. The present study was designed to create genetic variability in two non-Basmati aromatic rice viz., Kalanamak (Medium Slender Grain) and Badshah Bhog (Short and flattened grain).

MATERIALS AND METHODS

Two thousand pure, healthy and dry seeds (moisture, 12%) of the two rice varieties, namely, Kalanamak and Badshah Bhog were irradiated with 10, 20, 30 and 40 kR doses of 60Co. gamma rays at National Botanical Research Institute, Lucknow, Uttar Pradesh on 7.6.2005. Irradiated and unirradiated seed lots of each variety were divided into two equal parts (one thousand each). First lot was used as gamma rays treatment alone and other for combined treatment of gamma rays + Ethyl methane sulphonate (0.2%) and EMS (0.2%) alone. For chemical mutagen treatment, seeds were submerged for six hours in distilled water to insure complete hydration of the seeds at 30ºC in incubator. Soaked seeds were blotted for removing surface water before transferring them into Ethyl methane sulphonate (0.2%) prepared with phosphate buffer solution having the pH 7.0 for a period of 6h. in incubator (25ºC) and were given intermittent shaking throughout the period of treatment to maintain uniform concentration. After EMS treatment, the seeds were thoroughly washed in running tap water for 6h to remove residual chemicals.

Nine hundred twenty five seeds of each treatment of both the rice varieties with their respective control were sown in raised nursery beds during rainy (Kharif) season of 2005. All the 21 days old seedlings were transplanted in the well prepared puddle field at the distance of 20cm x 10cm between row to row and plant to plant, respectively. Intercultural operations were done as per need to raise good crop. Observations on pre-and post-harvest characters on individual plants of each treatment were taken for both the rice varieties, namely, Kalanamak and Badshah Bhog. Days to 50% flowering, days to maturity, plant height (cm), panicle length (cm), panicle bearing tillers/plant were record before harvesting, whereas grain yield per plant were taken after harvesting.

The bulked seeds of remaining M_1 plants (the plants showing pollen sterility greater than 40% or more) from each treatment

Table 1: Range, mean and coefficient of variation (CV) for Days to 50% flowering and Days to maturity in M1 generation

Treatment	Days to 50% flowering						Days to maturity					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	137-148	140.27	4.61	139-150	140.60	3.79	160-173	169.18	4.81	159-174	170.58	3.46
Gamma-ray												
10kR	137-154	138.02	7.19	132-150	139.07	6.02	156-178	168.50	7.31	159-180	169.45	6.81
20kR	135-156	137.30*	9.03	129-154	138.80	7.13	160-181	169.33	10.04	155-182	168.83	6.28
30kR	130-157	136.57*	10.11	125-155	136.50*	9.18	158-184	166.02*	11.87	150-184	167.55*	8.14
40kR	129-158	136.03*	13.61	118-156	135.72*	11.23	155-189	165.48*	8.29	148-187	167.02*	10.14
EMS												
0.2%	134-155	138.87	8.14	133-152	143.75*	7.51	164-180	170.67	9.34	156-180	171.07	5.14
Gamma-ray +EMS												
10kR+0.2%	130-154	138.05	10.12	129-153	138.77	7.41	154-182	167.75	7.66	156-181	169.87	6.29
20kR+0.2%	110-157	137.15*	11.22	125-154	137.77*	9.31	140-184	165.88*	8.26	154-183	166.55*	8.16
30KR+0.2%	130-154	136.28	16.19	120-155	137.60*	10.77	152-183	166.88*	10.20	150-185	167.20*	9.39
40kR+0.2%	101-156	134.88*	17.20	114-157	135.45*	14.81	137-186	166.03*	15.61	146-188	165.57	12.78
S.Em±		0.960			0.900			0.983			0.920	
CD at 5%		2.02			1.89			2.06			1.93	

Table 2: Range, mean and coefficient of variation (CV) for Plant height (cm) and Panicle length (cm) in M_1 generation

Treatment	Plant height (cm)						Panicle length (cm)					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	156-175	170.13	5.29	156-174	167.95	5.18	23-28	26.57	7.40	22-28	25.60	7.76
Gamma-ray												
10kR	150-180	168.95	6.67	148-174	165.70	7.02	20-30	26.33	10.10	20-29	25.08	9.71
20kR	144-179	165.02*	8.30	143-170	163.88*	8.58	19-29	25.78	8.61	20-28	24.97	9.64
30kR	141-180	166.53-	10.71	133-168	163.04*	10.74	19-29	25.05*	13.46	19-28	23.50*	10.87
40kR	129-174	163.55*	18.46	130-178	162.48*	16.22	20-30	24.58*	14.80	18-27	23.12*	13.06
EMS												
0.2%	151-179	166.81*	8.99	143-174	166.73	9.14	18-29	26.75	7.41	20-28	25.13	9.38
Gamma-ray +EMS												
10kR+0.2%	147-179	167.65*	7.66	145-173	164.93	7.05	20-29	25.78	10.91	20-27	24.90	10.44
20kR+0.2%	139-173	165.03*	9.09	145-169	163.07*	7.76	19-29	25.03*	12.98	18-27	24.25*	12.80
30KR+0.2%	129-170	164.95*	11.33	141-167	162.65*	14.21	19-28	24.98*	13.06	18-26	24.10*	13.34
40kR+0.2%	120-181	163.12*	19.25	127-177	161.27*	17.49	20-32	23.85*	15.66	20-29	23.00*	16.23
S.Em±			1.22		1.37			0.437			0.423	
CD at 5%			2.56		2.89			0.918			0.890	

were sown in raised nursery beds treatment-wise separately during the rainy (Kharif) season of 2006. All the M_2 population of each varieties were screened carefully treatment-wise for the spectrum of chlorophyll mutations. Lethal chlorophyll mutations were scored between 7-15 days from seeding, whereas viable ones were scored throughout the life span of the plants.

RESULTS AND DISCUSSION

Days to 50% flowering

The values of range mean and coefficient variation for days to 50% flowering in M_1 generation of *Kalanamak* and *Badshah Bhog* are presented in Table 1. Mutagenic treatments caused decline in mean values coupled with increased variability, as judged from high range and CV in all the treatments in both the genotypes. The induced variability was noted to be relatively more with the genotype, *Kalanamak* as compared to *Badshah Bhog,* as evident from the coefficient of variations (CV). The mutagenic treatments, such as, 10kR gamma-ray and 10kR gamma-ray+EMS did not cause significant reduction in days to flowering from the respective control in both the genotypes. Induced variability for early flowering was more pronounced in the treatment 40kR gamma-ray+EMS followed by 20kR gamma-ray+EMS in *Kalanamak,* while it was in the treatment 40kR gamma-ray+EMS followed by 30kR gamma-ray+EMS in *Badshah Bhog,* as evident from the lower values of the treatment range as compared to their respective controls. The magnitude of variability, as evident form range and (CV) was induced maximum by the treatments 40kR gamma-ray+EMS followed by 30kR gamma-ray+EMS in both the genotypes.

Days to maturity

The data for range mean and coefficient of variation for days to maturity in treated as well as control population in *Kalanamak* and *Badshah Bhog* in M_1 generation are presented in Table 1. Mutagen treatments caused decline in mean values coupled with increased variability, as judged form the range and CV, in all the treatments and genotypes. The induced variability was noted to be relatively more in the genotype, *Kalanamak* as compared to *Badshah Bhog,* as evident from the coefficient of variation. The mutagenic treatments, such as, 10kR gamma-ray and 10kR gamma-ray+EMS did not cause significant change in mean days to maturity form the control in both the genotypes. Induced variability for early maturing were more frequent in the treatments 40kR gamma-ray+EMS

Table 3: Range, mean and coefficient of variation (CV) for Panicle bearing tillers/plant and Grain yield /plant (g) in M_1 generation

Treatment	Panicle bearing tillers/plant						Grain yield /plant					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	4.12	6.88	16.91	4-12	6.90	17.62	9.15-15.65	12.81	12.37	8.35-14.35	12.74	13.01
Gamma-ray												
10kR	3.13	6.78	28.88	2.14	6.78	26.38	5.21-18.66	11.85	29.9	4.36-15.63	11.90	29.93
20kR	2-14	6.57	32.95	3-13	6.55	36.58	4.09-17.15	8.18*	38.03	2.25-14.36	7.25*	40.86
30kR	2.-12	6.08*	35.41	2-15	6.35*	39.20	3.12-16.13	6.53*	48.23	1.92-14.36	6.67*	47.86
40kR	1-14	5.98*	41.81	2-14	5.95*	41.19	1.13-15.51	5.44*	55.75	1.85-12.65	5.18*	58.23
EMS												
0.2%	2-13	6.93	28.32	2-13	6.70	28.09	5.36-18.12	11.84	37.42	2.35-16.32	11.82	33.49
Gamma-ray+EMS												
10kR+0.2%	3-13	6.88	29.8	3-14	6.60	31.56	5.12-19.01	9.85*	31.17	5.26-16.32	9.97*	29.96
20kR+0.2%	1-16	6.62	39.17	2-15	6.25*	38.68	3.03-17.04	7.78*	42.57	2.24-14.36	7.78*	45.16
30KR+0.2%	2-15	6.12*	41.21	2-13	6.15*	33.81	2.31-16.19	5.93*	49.47	1.35-11.21	5.06*	55.37
40kR+0.2%	1-12	5.50*	36.21	2-16	5.68*	35.61	1.02-12.88	5.06*	49.47	1.23-10.99	4.54*	64.61
S.Em±		0.321			0.224			0.47			0.46	
CD at 5%		0.674			0.470			0.98			0.96	

Table 4: Frequency and Spectrum of induced chlorophyll mutations in M_2 generation in two aromatic rice genotypes

Treatment	Frequency of chlorophyll mutations						Spectrum of chlorophyll mutations						
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog			
	M_2 seedlings studies	Total No. of Mutants	Mutation frequency per 100 M_2 plants	M_2 seedlings studies	Total No. of Mutants	Mutation frequency per 100 M_2 plants	*Albino*	*Xantha*	*Striata*	*Albino*	*Xantha*	*Striata*	*Viridis*
Control	4000	-	-	4000	-	-	-	-	-	-	-	-	-
Gamma-ray													
10kR	3860	127	3.29	4169	170	4.08	2.72	0.57	-	3.42	0.65	-	-
20kR	3240	218	5.99	3914	277	7.08	4.79	1.19	-	6.61	0.46	-	-
30kR	3482	148	4.25	3766	309	8.20	3.91	0.33	-	6.99	0.55	0.29	0.37
40kR	3470	120	3.46	3545	136	3.84	2.38	0.74	0.33	3.31	0.53	-	-
EMS													
0.2%	3927	184	4.69	4080	195	4.78	3.61	1.06	-	3.34	0.89	-	0.55
Gamma-ray +EMS													
10kR+0.2%	3700	197	5.32	4015	292	7.27	4.65	0.68	-	6.47	0.50	0.15	0.15
20kR+0.2%	3740	245	6.55	3804	378	9.94	4.76	1.58	0.22	7.25	2.39	-	-
30KR+0.2%	3531	143	4.05	3737	300	8.03	2.29	1.76	-	5.21	1.74	1.07	-
40kR+0.2%	3320	110	3.31	3645	108	2.96	1.59	1.37	0.03	1.78	0.80	-	0.38

followed by 20kR gamma-ray +EMS in *Kalanamak,* while it was in the treatments 40kR gamma-ray +EMS followed by 30kR gamma-ray +EMS in *Badshh Bhog,* as evident from the lower values of the treatment range as compared to the respective control. The magnitude of induced variability was high in the treatments 40kR gamma-ray +EMS followed by 30kR gamma-ray +EMS and 40kR gamma-ray in *Kalanamak*, while it was high in 40kR gamma-ray +EMS followed by 40kR gamma-ray and 30kR gamma-ray +EMS in *Badshah Bhog*.

Plant height

The data pertaining to range mean and coefficient of variation for plant height in treated as well as control population of *Kalanamak* and *Badshah Bhog* in M_1 generation are presented in Table 2. Mutagenic treatments caused decline in mean values associated with increased variability, as judged from high range and CV, in all the treatments and genotypes. The induced variability was noted to be relatively more in the genotype, *Kalanamak* as compared to *Badshah Bhog,* as evident from the coefficient of variation. The mutagenic treatments caused significant decline in mean plant height in all the treatments of both the genotypes except in treatment 10kR gamma-ray in both the genotypes and EMS in *Badshah Bhog* as compared to their respective control. The magnitude of reduction in plant height was more frequent in the treatment 40kR gamma-ray +EMS followed by 30kR gamma-ray+EMS and 40kR gamma-ray in both the genotypes; all these three treatments also showed high variability, as evident from coefficient of variation.

Panicle length

The data on range mean and coefficient of variation for panicle length in *Kalanamak* and *Badshah Bhog* in M_1 generation are presented in Table 2. Mutagenic treatments caused decline in mean values of panicle length, while increased variability, as judged form the high range and CV in all the treatments and genotypes, although significant in certain cases only. The mutagenic treatments, such as, 10kR and 20kR gamma-ray, EMS and combined treatment of 10kR gamma-ray+EMS did not show significant reduction in panicle length form the control in both the genotypes. The maximum variability was noted in the treatments 40kR gamma-ray +EMS followed by 30kR gamma-ray +EMS and 40kR gamma-ray in both the genotypes. Increased variability towards positive side was also noted in treatment 40kR gamma-ray +EMS irrespective of genotypes.

Panicle bearing tillers per plant

The values of range mean and coefficient of variation for panicle bearing tillers per plant in *Kalanamak* and *Badshah Bhog* in M_1 generation are presented in Table 3. Mutagenic treatment caused significant decline in mean values with increased variability, as judged from high range in both the direction and coefficient variation in the treatments with 30kR and 40kR gamma-ray alone and in combination with EMS in both the genotypes. The induced variability was more or less parallel in two genotypes. The treatment with 20kR gamma-ray +EMS appeared to be more promising among the treatments as judged from the higher values of range in both the genotypes. There was an increase in number of panicle bearing tillers per plant but insignificant in EMS treatment alone in *Kalanamak*. Induced variability for total number of panicle bearing tillers per plant was high as judged from CV in the treatment 40kR gamma-ray followed by 30kR gamma-ray +EMS in *Kalanamak*, while it was in the treatments 40kR gamma-ray followed by 30kR gamma-ray in *Badshah Bhog*.

Grain yield per plant

The data related to range, mean and coefficient of variation for grain yield in treated population as well as control of *Kalanamak* and *Badshah Bhog* in M_1 generation are presented in Table 3. Mutagenic treatments except 10kR gamma-ray caused significant decline in mean grain yield per plant coupled with increased variability, as judged from the range and CV, in all the treatments and genotypes. The damaging effects of the treatments was noted to be relatively more for grain yield per plant and were more pronounced in the genotype *Badshah Bhog* as compared *Kalanamak* at higher doses of gamma-ray as well as their combination treatments with EMS. As judged from CV, the magnitude of variability was maximum at 40kR gamma-ray +EMS followed by 40kR gamma-ray in both the genotypes, while the maximum, mean reduction was observed at 40kR gamma-ray +EMS in both the genotypes. Treatments with 10kR gamma-ray and EMS alone were ineffective in both the genotypes. In all the treatments, shift in mean towards (negative direction) lower values of range were most common in both the genotypes. The observation recorded for quantitative traits in M_1 generation showed a decline in mean as compared to control values along with an increase in variability, as judged from range and CV, in most of the treatments in both rice genotypes, *Kalanamak* and *Badshah Bhog*. The magnitude of decline in mean coupled with high CV was more pronounced in combined treatments. Of the several traits, grain yield showed major drastic effects of the treatments, as judged from high range and CV. Similar results were also reported in different crops including rice (Vandana and Dubey, 1990; Tripathi and Dubey, 1992).

On the other hand, Scossiroli *et al.* (1961) observed a decline in the mean values for polygenic traits in *Triticum* sp. in M_1 generation, which was not always accompanied with increase in variability. Moreover, the results of Borojevic (1963) showed that irradiation failed to cause an increase in variability for certain polygenic traits in wheat. Reduced plant height and maturity duration coupled with high range and CV in present investigation is of great significance as they are expected to yield short stature and early maturing mutants. Similarly, shift towards positive side, as evident from upper values of range in certain treatment, for panicle length, panicle bearing tillers per plant, total numbers of tillers per plant and grain yield per plant are of also great importance in both the genotypes.

Frequency and Spectrum of Chlorophyll Mutation

In *Kalalnamak*, the highest frequency (6.55%) was observed in the treatments 20kR gamma-ray+EMS followed by 10kRgamma-ray (5.99%) and 10kR gamma-ray + EMS (5.32%) (Table 4). In *Badshah Bhog*, the highest frequency (9.94%) was observed in 20kR gamma-ray+EMS treatment followed by 30kR gamma-ray, (8.20%), 30kR gamma-ray+EMS (8.03%) and 10kR gamma-ray+EMS (7.27%). Chlorophyll mutation frequency was relatively more in the genotype *Badshah Bhog* as compared to *Kalanamak*. Further greater numbers of chlorophyll mutations were induced in the combined treatment than the single treatments of gamma-ray and EMS.

The different types of chlorophyll mutations observed in M_2 generation were as follows. *Albino*: - It is characterized by entirely white leaves and did not survive beyond 8 to 10 days after sowing (DAS). *Xantha*: - It is characterized by yellow to whitish-yellow coloured leaves and survived up to 15 to 20 DAS. *Striata*: - It is characterized by longitudinal strips of white/ yellow colour on leaves and was viable. *Viridis*: - It is pale dull greenish, yellowish-green and is distinguished from *Xantha* by presence of green colour. Most of them are either lethal or if recovered turned green after 20-25 DAS and survived up to maturity and is sterile.

Among chlorophyll mutations, Albino mutant was most frequent in both the genotypes. Further the genotype *Badshah Bhog* exhibited relatively high frequency of Albino as compared the genotype *Kalanamak*. Amongst the treatments, the 20kR and 30kR gamma-ray alone and in combination with EMS gave high frequency of Albino. Next common chlorophyll mutant observed was *Xantha* irrespective of the genotypes. *Striata* and *Viridis* mutants were less frequent and found only in certain treatments; *Viridis* mutant did not occur in *Kalanamak*. Chlorophyll mutations though of various types, viable to non-viable, provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in rice (Awan *et al.*, 1980).

In the present study, the frequency of chlorophyll mutations varied with the genotype as well as mutagen doses in M_2 generation. Total frequency of chlorophyll mutations was relatively higher in *Badshah Bhog* than *Kalanamak*. Similar reports of genotypic differences in rice as well as other crops were reported by many workers (Singh *et al.*, 1998; Shadakshari *et al.*, 2001). The differential response of genotypes to induction of chlorophyll mutations was possibly due to differences in the genetic makeup of the varieties used for mutagenesis.

During the present study, *albino* mutant (Table.4) occurred in higher frequency than *Xantha* or *Striata* or *Viridis*. Several workers also reported a higher frequency of *albino* mutant (Singh *et al.*, 1998; Singh and Singh, 2003). However, Reddi and Suneetha (1992) observed a higher frequency of *Viridis* than *Albino* or *Xantha* in their studies in rice by involving physical and or chemical mutagens.

ACKNOWLEDGEMENT

The first author is grateful to the University Grants Commission (Rajiv Gandhi National Fellowship), New Delhi for the award of JRF and SRF for the present works.

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