The Bioscan 7(4): 739-742, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

INDUCED MUTATION IN TRADITIONAL AROMATIC RICE – FREQUENCY AND SPECTRUM OF VIABLE MUTATIONS AND CHARACTERIZATIONS OF ECONOMIC VALUES

SATISH KR. CHAKRAVARTI *, H. KUMAR, J. P. LAL AND MANISH K. VISHWAKARMA
Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi - 221 005, INDIA
e-mail: satish_genetics@rediffmail.com

KEY WORDS
Traditional aromatic rice
Kala Namak
Badshah Bhog
Induced mutants
Gamma rays, EMS
Macro-mutations

Received on :
28.06.2012

Accepted on :
17.10.2012

***Corresponding author**

ABSTRACT

With a view to including viable mutations of economic importance in two traditional non–Basmati rice varieties, namely, Kala Namak and Badshah Bhog, dry seeds were treated with 10, 20, 30 and 40kR doses of gamma rays and EMS (0.2%), alone and in combination. Bulk seeds of M_1 plants (showing pollen sterility greater than 40% or more) from each treatment were sown for generation advancement. Both frequency and spectrum of viable mutants and characterizations of economic importance varied with the treatment as well as genotypes. The treatment 40kR + EMS followed by 20kR + EMS in *Kala Namak* while EMS followed by 20kR + EMS and 40kR + EMS in *Badshah Bhog* were most desirable in including viable mutants; combination treatments were most effective. The genotypes *Kala Namak* was more sensitive than the *Badshah Bhog* with regard to the frequency of viable mutants. As regards to spectrum of viable mutations, a total of 15 types of macro- mutations were observed; 10 types in *Kala Namak* and 12 types in *Badshah bhog*. Out of 313 viable mutants in two genotypes, the frequency of different mutants over genotypes were 135 (semi-dwarf), 29(early maturing), 24(increased tillering), 9(bushy and stiff stem), 12(broad and narrow leaf), 4(pigmented node), 13(short slender grain), 38(straw colour grain), 11(change in grain size) and 29(high yielding). Isolation of few semi- dwarf mutant coupled with earliness have assumed great significance in improving traditional aromatic rice.

INTRODUCTION

Rice is the stable food crop of millions of mankind from the dawn of civilization. It is consumed by half of the world's population and cultivated in more than 100 countries on every continent except America. Asia alone produces over 95% of global rice with China (194.3million t) and India (148.3million t), ranked first and second, respectively is by far the largest producers of rice, producing half of the worlds rice (Upadhyay *et al.*, 2011). Rice has enormous genetic variability amongst them aromatic rice has a crucial position in the world rice market. There is a rising pressure for the production of high grain quality aromatic rice in our country for fulfilling demand and export market. Many non-Basmati traditional aromatic rice cultivars are famous in which Badshah *bhog* and *Kala namak* are very popular in North East Plain Zone of India due to their excellent grain quality and aroma. Unfortunately these cultivars are localized to certain pockets and are poor yielder having lodging susceptibility and tall plant height (>170cm). Hence, there is need to improve the yield potential of such cultivars by reducing the plant height and increasing nitrogen responsiveness without losing the aroma and other quality characters of the parental variety. Since, mutagenesis has proved very effective in reducing plant height (Balotch *et al.*, 2003; Chakravarty, 2010), the present work has been carried out using different doses of gamma-rays and ethyl methane sulphonate and their combined treatment in two cultivars *Badshah bhog* and *Kala namak* and results on frequency and spectrum of viable mutations including semi-dwarf mutants are presented here.

MATERIALS AND METHODS

Both the traditional non-Basmati aromatic rice genotypes, namely, *Kala Namak* and *Badshah Bhog* under present investigation are very tall (> than 170cm) and late maturing (> than 170 days from seedling). The kernel contains blackish lemma and palea in *Kala Namak*, while straw coloured in *Badshah Bhog*. Two thousand pure, healthy and dry seeds (moisture 12%) of were irradiated with 10, 20, 30 and 40kR doses of ^{60}Co. gamma rays at National Botanical Research Institute, Lucknow, Uttar Pradesh on June, 2005. Irradiated and un-irradiated 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 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.

M_2 Generation

The bulked seeds of remaining M_1 plants (the plants showing pollen sterility greater than 40% or more) from each treatment were sown in raised nursery beds treatment-wise separately during the rainy (Kharif) season of 2006. Different types of viable mutations and their frequency were scored in each treatment starting from the germination to maturity of the crop. Viable morphological mutations and their frequency were scored up to maturity in all the treated population. The mutations affecting growth habit (erect, spreading, semi-dwarf, bushy), leaf characteristics (large, narrow), tillering, flowering and maturity, seed size, seed coat colour and aroma were scored and selected. The plant showing these features were harvested separately.

RESULTS AND DISCUSSION

The data on the frequency and spectrum of viable macro-mutations per 100 M_2 plants for both the rice genotypes (Table 1 and 2) revealed that the viable mutations occurred at random irrespective of genotypes and mutagenic treatments. The frequency of viable mutations per 100 M_2 plants in case of *Kala Namak* was highest at 40 kR gamma-ray + EMS (1.91) followed by 20 kR gamma-ray + EMS (1.83), 10 kR gamma-ray + EMS (1.60) and 40 kR gamma-ray (1.36). The lowest frequency (0.88%) of viable macro-mutations was observed in single treatment of EMS in *Kala Namak*. While in case of *Badshah Bhog*, the highest frequency of viable macro-mutations was recorded in EMS (1.99%) followed by 20 kR gamma-ray + EMS (1.60%) and 40 kR gamma-ray + EMS (1.60%), while the lowest frequency (0.88%) was observed at 10 kR gamma-ray + EMS. In general, mutagenic treatments induced fairly high frequency of viable mutations in both the genotypes, though varied with the treatments. Combined treatments of gamma-ray + EMS produced more viable (6.85% in *Kala Namak* and 5.44% in *Badshah Bhog*) mutants as compared to single treatment in both the genotypes.

Several workers (Agrawal *et al.*, 2000; Shadakshari *et al.*, 2001; Singh and Singh, 2003; Sharma *et al.*, 2008), also favoured combined treatments to get high frequency of viable mutants in rice. Mutagenic specificity might be explained in terms of the concept of selection sieve (Auerbach, 1967) or that the active genes were more accessible to certain mutagens (doses) than repressible or inactive genes. It could be presumed that

Table 1: Frequency and spectrum of induced viable mutations in M_2 generation in rice genotype *Kala Namak*

Particulars	Gamma-ray				*EMS*	Gamma-ray + EMS (0.2%)				Total Mutant
	10kR	20kR	30kR	40kR	*(0.2%)*	10kR+EMS	20kR+EMS	30kR+EMS	40kR+EMS	wise*
Total M_2 plants studied	1258	1257	1259	1254	1260	1256	1257	1259	1258	11378
Spectrum of mutations	Frequency of mutations									
1 Early maturing	2	-	3	3	-	2	4	1	-	15 (9.38)
2 Semi-dwarf	1	6	9	7	5	8	12	11	13	72 (45.00)
3 Increase tillering	-	3	-	3	-	2	3	2	-	13 (8.13)
4 Bushy	-	1	-	1	-	2	-	-	-	4 (2.50)
5 Broad leaf	-	1	-	-	1	-	-	2	-	4 (2.50)
6 Short slender grains	2	-	2	-	2	1	-	-	1	8 (5.00)
7 Straw colour (black to straw)	4	2	1	-	3	2	1	-	7	20 (12.50)
8 Reduced grain size	2	-	-	1	-	1	2	-	-	6 (3.75)
9 High yielding	3	2	2	-	-	2	-	3	1	13 (8.13)
10 Complete spikelet sterility	-	-	-	2	-	-	1	-	2	5 (3.13)
Total No. of mutants	14	15	17	17	11	20	23	19	24	160
Frequency/100 M_2 plants	1.12	1.20	1.35	1.36	0.88	1.60	1.83	1.51	1.91	1.41

*** = Values given in parentheses relate to percent over total number of viable mutations**

Table 2: Frequency and spectrum of induced viable mutations in M_2 generation in rice genotype *Badshah Bhog*

Particulars	Gamma-ray				*EMS*	Gamma-ray + EMS (0.2%)				Total Mutant
	10kR	20kR	30kR	40kR	*(0.2%)*	10kR+EMS	20kR+EMS	30kR+EMS	40kR+EMS	wise
Total M_2 plants studied	1257	1260	1256	1254	1259	1260	1257	1256	1255	12374
Spectrum of mutations	Frequency of mutations									
1 Early maturing	2	2	-	-	2	-	5	2	1	14 (9.15)
2 Semi-dwarf	4	9	6	7	8	2	9	9	9	63 (41.18)
3 Increase tillering	1	2	-	3	-	-	-	3	2	11 (7.19)
4 Stiff stem	-	-	2	-	1	-	2	-	-	5 (3.27)
5 Narrow leaf	-	-	-	-	1	-	1	-	-	2 (1.31)
6 Broad leaf	1	-	-	-	2	2	-	-	1	6 (3.92)
7 Pigmented node	1	-	1	-	2	-	-	-	-	4 (2.61)
8 Short slender grains	1	-	1	-	2	-	-	1	-	5 (3.27)
9 Red grains	2	3	-	3	4	3	-	-	3	18 (11.76)
10 Increased grain size	-	-	1	-	2	1	-	1	-	5 (3.27)
11 High yielding	2	-	2	2	1	3	3	1	2	16(10.46)
12 Complete spikelet sterility	-	-	2	-	-	-	-	-	2	4(2.61)
Total No. of mutants	14	16	15	15	25	11	20	17	20	153
Frequency/100 M_2 plants	1.12	1.27	1.20	1.20	1.99	0.88	1.60	1.36	1.60	1.24

*** = Values given in parentheses relate to percent over total number of viable mutations**

in combination treatments, as in present case, one mutagen (gamma-ray) may sensitize previously protected sites to the second mutagen (EMS) and these one could expect more additive effects from sequential application of the mutagens. Payez and Deering (1972) reported that the synergism between two mutagens might be firstly because of the first mutagenic treatment making accessible, as much as possible, to mutate leaving non-available site for reaction to the second mutagen and secondly as the per mutational lesions induced by the first mutagen become fixed due to an inhibitory effect of the second mutagen as repair enzymes. Bhatia (1970) reported that changes induced by radiations in the properties of biological membrane (s) would enhance the penetration of chemical mutagens administrated after irradiation treatment leading to synergistic effects. Genotypes specific mutations as noted in present case were also reported (Tyagi and Gupta, 1991).

Spectrum of viable mutants

A wide rang (15 types) of viable macro-mutations were observed (Table 1 and 2); 10 types in *Kala Namak* and 12 types in *Badshah Bhog*. Among these, semi-dwarf, early maturing, increased tillering and grain yield mutant were more frequent. Viable morphological mutations induced and recorded in the present study, such as semi-dwarf, early maturing, increased tillering, bushy, grain characteristics, grain yield and other desirable traits. The differences observed in the spectra of morphological mutations were more of quantitative nature rather than qualitative; high frequency of semi-dwarf mutants (about 43.1 % of viable mutations) were observed in the present case. Several workers also found high frequency of mutants for plant height in rice (Shadakshari *et al.,* 2001; Singh and Singh, 2003; Sharma *et al.,* 2008).

Relative differences in mutability for different traits were observed in the present study; mutability was found to be high (43.1%) for plant height followed by grain mutants (19.80%), early maturity (9.3%) and increased tillering (7.7%), while lowest (1.28%) mutability was noted for pigmented node and bushy plant type irrespective of the genotypes. The more frequent induction of certain mutations might be attributed to the fact that the genes for these traits were probably relatively more responsive to mutagen. Nilan (1967) concluded that different mutagens and treatments might also change the relative proportion of different mutation types. The two aromatic traditional non-basmati genotypes *Kala Namak* and *Badshah Bhog* are matchless in cooking quality and aroma, but it possess two detrimental aspects, which are: tall growing (more than 170cm) habit with weak straw and late maturing. Both these attributes make the cultivar incapable of responding to high fertility or withstanding lodging. In such situation, isolation of early maturing, semi-dwarf mutants and others as described below have assumed great significance. Total number of 29 early maturing mutants with maturity duration of 115-135 days as compared to control (170 days) were isolated in two rice genotypes, namely, *Kala Namak* (15; 9.38 %) and *Badshah Bhog* (14; 9.15 %). Overall early maturing mutants were maximum at combined treatment of 20 kR gamma rays + EMS (0.2%). Several workers also reported early maturing mutants in rice after mutagen treatments (Shadakshari *et al.*, 2001; Singh and Singh, 2003; Domigo *et al.*, 2007), *B. napus* L. (Kumar *et al.* 2012). A total of 135 semi-dwarf mutants with plant height less than 130 cm as compared to control (170 cm) were isolated in both genotypes *Kala Namak* (72; 0.303 %) and *Badshah Bhog* (63; 0.265 %) out of 23,752 M_2 plants; about 43 % of viable mutants were semi-dwarf. The maximum (13) semi-dwarf mutant was observed at 40 kR gamma rays + EMS (0.2%) followed by 20 kR gamma rays + EMS (0.2 %), (12) in *Kala Namak*, while in case of *Badshah Bhog* the maximum (9) semi-dwarf mutant was found in each at 20 kR gamma rays and combined treatment of 20kR, 30kR and 40kR gamma rays with EMS. Several workers reported dwarf and semi-dwarf mutants in rice after mutagenic treatments (Shadakshari *et al.,* 2001; Domigo *et al.,* 2007). These semi-dwarf mutants bred true in M_3 generation. A total of 24 out of 23,752 M_2 plants for increased tillering (tiller more than 16 as compared to 5-10 tillers/plant in normal) were observed in both rice genotypes. Several workers also reported such mutants in rice after mutagenic treatments (Rashid *et al.,* 2003; Maity *et al.,* 2005 and Domingo *et al.,* 2007). Mutants for other characters, such as, bushy, narrow and broad leaf were also observed. Total 4 (2.50 %) bushy mutant only in *Kala Namak*, broad leaf mutant (3.19 %) in both genotypes, while narrow leaf mutant 2 (1.31 %) in only *Badshah Bhog* were observed. Bushy mutant was also reported in rice (Agrawal *et al.,* 2000; Singh and Singh, 2003; Domingo *et al.,* 2007), Black Gram (Thilagavathi and Mullainathan, 2009) and narrow leaf mutant was also reported in rice (Singh *et al.,* 1998; Singh and Singh (2003).

Grain size and colour mutants

Plants with less than 6 mm kernel length coupled with 3 and above L/B ratio were considered as short slender grain mutants. The frequency of this mutant was 8 (5.00%) in *Kala Namak* and 5 (3.27%) in *Badshah Bhog*,. Short slender grain mutant decorticated by Shobha rani *et al*. (2004). Total 20 (12.50%) mutants with straw colour having brown glum and apiculate as compared to dark brown grain in normal *Kala Namak* were observed. Maximum (7) straw coloured grain mutant was noted in 40kR gamma-ray + EMS.A total of 18(11.76 %) mutants only in *Badshah Bhog* (straw coloured grains) showing red grains were observed; four mutants were noted in EMS followed by three each in four treatments namely, 20kR gamma-ray, 40kR gama-ray, 10kR gamma-ray + EMS and 40kR gamma-ray + EMS. A total of 6(3.75%) mutants having 25-30% reduced grain size as compared to control were observed only in *Kala Namak;* maximum two mutants each at 10kR gamma-ray and 20kR gamma-ray + EMS were observed. Earlier reduced grain size mutant found (Shobha rani *et al.* (2004). Total 5 (3.27%) mutants having 15-20% increased grain length and breadth as compared to control were observed only in *Badshah Bhog;* two mutants in EMS and are each in 30kR gamma-ray, 10kR gamma-ray + EMS and 30kR gamma-ray + EMS were observed. Increased grain size mutant earlier studied (Shobha rani *et al.*, 2004).

Several mutants showing change in the length and breadth of grain were noted in the present study. Short slender grain mutant in both the genotypes were of great significance as they add to the quality of grain. Large grain mutants in rice after mutagenic treatments were reported (Shadakshari *et al.,* 2001; Singh *et al.,* 2003; Singh *et al.,* 2004; Patnaik *et al.,*

2006) Mutants for straw colour grain in genotype, *Kala Namak* (Black coloured grains) and red grains in *Badshah Bhog* (straw coloured) were of great significance (Singh, 2000; Shobha Rani *et al.,* 2004), also reported such types of grain mutants in rice after mutagenic treatments. The high yielding mutants showing 15-20 % more grain yield than the normal were observed in both genotypes. Many workers reported high yielding mutants in rice after mutagenic treatments (Shadakshari *et al.,* 2001; Singh and Singh, 2003; Domingo *et al.,* 2007; Bughio *et al.* 2007). Whereas, 9(2.87%) mutants having complete spikelet sterility (more than 90%) in both the genotypes were found in the present study. Complete sterile mutants in rice after mutagenic treatments were also reported by Luo and Zhang (1998); Shadakshari *et al.* (2001); Singh and Singh (2003).

ACKNOWLEDGEMENT

The first author is thankful to Dr. V. K. Mishra of BHU, Varanasi and to University Grant Commission, New Delhi for provided Rajiv Ghandi National fellowship.

REFERENCES

Agrawal, P. K., Sidhu, G. S., Gosal, S. S. 2000. Induced mutation for bacterial blight resistance and other morphological characters in indica rice. *Oryza.* **37(4):** 277-280.

Auerbach, C. 1967. The chemical production of mutations. *Science.* **158**: 1141-1147.

Baloch, A. W., Soomro, A. M., Javed, M. A., Bughio, H. R., Alam, S. M., Bughio, M. S., Mohammed, T. and Mastoi, N. N. 2003. Induction of salt tolerance in rice through mutation breeding. *Asian. J.Pl. Sci.* **2(3):** 273-276.

Bhatia, C. R. 1970. Radiation and Radiomimetirc Substances in Mutation Breeding. Departement of Atomic Energy. *Geneva.* **27:** 299-304.

Bughio, H. R., Odhano, I. A., Asad, M. A. and Bughio, M. S. 2007. Improvement of grain yield in rice variety basmati-370 (*Oryza sativa* L.) through mutagenesis. *Pak. J. Bot.* **39(7):** 2463-2466.

Chakravarty, S. K. 2010. Mutagenesis for genetic improvement of Rice (*Oryza sativa* L.). Ph.D. Thesis, Banaras Hindu University, India (Unpubl.).

Domingo, C., Andres, F. and Talon, M. 2007. Rice cv. Bahia mutagenized population; a new resource for rice breeding in the Mediterranean Basin. *Spanish J. Agr. Res.* **5(3):** 341-347.

Kumar, H., Srivastava, A., Vishwakarma, M. K. and Lal, J. P. 2012. Genetic enhancement of variability through mutagenesis in two genotypes of Brassica. napus L. *Madras Agric. J.* **99(4-6):** 228-231.

Luo, R.T. and Zhang, M. X. 1998. Study on mutagenic effects of rice seeds irradiated by Co_2 laser. *C.R.R.N.* **6 (1):**6-7.

Maity, J. P., Mishra, D., Chakraborty, A., Saha, A., Santra, S. C. and Chanda, S. 2005. Modulation of some quantitative and qualititative characteristics in rice (*Oryza sativa* L.) and mung (*Phaseolus mungo* L.) by ionizing radiation 2005. Elsevier Ltd.

Nilan, R. A. 1967. Nature of induced mutation in higher plants. In: Induced Mutations and their utilization. Proc. Symp. Erwin-baur Gedachtnisvoriesungen-IV, Gaterstleben, 1966 Akademic- Verlag, Berlin, pp. 5-18.

Patnaik, D., Chaudhary, D. and Rao, G. J. N. 2006. Genetics improvement of long grain aromatic rices through mutation approach. *Plant Mutation Reports.* **1(1).**

Payez, J. F., Deering, R. A. 1972. Synergistic and antagonistic effects of caffeine and two strains of cellular slime mould treated with alkylating agent. *Mutation Res.* **16:** 318-321.

Rashid, M., Cheema, A. A., Ashraf, M., Qamar, Z. U. and Mahmood, Z. 2003. Development of basmati rice varieties through the use of induced mutations and related techniques. *Pak.J. Bot.* **35(5):** 811-818.

Shadakshari, Y. G., Chandrappa, H. M., Kulkarni, R. S. and Shashidhar, H. E. 2001. Induction of beneficial mutant in rice (*Oryza sativa* L.). *Indian J.Genet.* **61(3):** 274-276.

Sharma, R. K., Gupta, B. B. and Bijral, J. S. 2008. Frequency and spectrum of viable mutations induced by physical and chemical mutagens in basmati rice (*Oryza sativa*. L) mutations in basmati rice (*Oryza sativa* L.) *J. Res., SKUASTJ.* **7(1):** 33-39.

Shobha, R. N., Subba, R. L. V., Viraktamath, B. C. and Mishra, B. 2004. National guideline for the conduct of tests for Distinctness, Uniformity and Stability Rice (Oryza sativa L.). DRR (ICAR), Rajendranagar Hyderabad.

Singh, S. and Singh, J. 2000. Induced mutations for the improvement of yield and quality traits in Basmati rice (*Oryza sativa* L.) PhD. Theses BHU., Varanasi.

Singh, S. and Singh, J. 2003. Mutation in Basmati rice induced by gamma rays, ethyl methane sulphonate and sodium azide. *Oryza.* **40(1&2):** 5-10.

Singh, S., Richharia, A. K. and Joshi, A. K. 1998. An assessment of gamma rays induced mutations in rice (*Oryza sativa* L.) *Indian J. Genet.* **58(4):** 455-463.

Singh, V. P., Singh, A. K., Zaman, F. U. and Hari, P. A. S. 2004. Extra long slender grain aromatic rice: A new strategy. In: National Symposium on Strategies for enhancing export of quality rice - compilation of Contributory papers. DRR (ICAR), Hyderabad, India. pp. 60 - 65.

Thilagavathi, C. and Mullainathan, L. 2009. Isolation of Macro Mutants and Mutagenic Effectiveness, Efficiency in Black Gram (*Vigna mungo* (L.) Hepper). *Global J. Molecular Sciences.* **4(2):** 76-79.

Tyagi, B. S. and Gupta, P. K. 1991. Induced macro-mutations in lentil. *LENS Newsletter.* **18**:3-7.

Upadhyay, P., Singh V. K. and Neeraja, C. N. 2011. Identification of genotype specific alleles and molecular diversity assessment of popular rice (*Oryza sativa* L.) varieties of India. *Int. J. Plant Breed. Genet.* **5:** 130-140.