Annals of Plant and Soil Research 18(3): 196-202 (2016)

GAMMA RAYS AND EMS INDUCED VIABLE MUTANTS AND MUTAGENIC FREQUENCY IN ADT (R) 47 RICE

D. RAJARAJAN*, R. SARASWATHI AND D. SASSIKUMAR [1]

Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India.

Received: April, 2016; Revised accepted: August, 2016

ABSTRACT

The present study was carried out to induce viable mutants in ADT (R) 47 rice. Seeds were subjected to different treatment levels of gamma rays and Ethyl Methane Sulphonate (EMS). The treated and untreated plants were self-fertilized in M_1 generation to observe different morphological characters in M_2 generation. A wide spectrum of unique macro mutants was isolated in this investigation. These independent mutants exhibited varied phenotypes from wild type. The mutagenic frequency was highest at 300Gy (0.88%) of gamma irradiation and gamma rays were found to be most effective in inducing viable mutations compared to EMS. Four broad types of viable mutants could be witnessed. The most distinct mutants isolated were different leaf characters, plant stature, duration and altered grain characters.

Key words: Rice, Gamma rays, EMS, Viable mutants

INTRODUCTION

Rice (*Oryza sativa* L.) is an ideal system for studying plant architecture and is the staple food source among cereals consumed by more than half of the world's population. India has the largest area under rice in the world and ranks second in production (Anonymous, 2013). The induction of mutations as a technique for crop improvement occupies a significant place among modern methods of plant breeding. Nilan *et al.* (1977) have suggested induced mutations as a best supplement to natural genetic variability. In plants, ionizing radiations and chemicals have proved to be most efficient in inducing new genetic variability. A striking example of their role has been in rice breeding, where induced mutants have not only been released directly as improved cultivars but more importantly have been used as donor parents in standard hybridization programs. Mutation breeding is also envisaged to enlarge the frequency and spectrum of mutations and to increase the incidence of viable mutations as an approach towards directed mutagenesis (Singh *et al.,* 1998). Plants showing morphological deviations and survival up to maturity were scored as viable mutants. The occurrences of morphological mutations after treatments with physical and chemical mutagens have been reported in rice crop (Chakravarti *et al.* (2012; Vasline and Sabesan, 2011). In general any mutational event may bring large or small changes in the phenotype of the plant. The changes in macro mutants have highest significance in plant breeding because they may sometimes give a desired phenotype. The present study has been made to assess the frequency and spectrum of viable mutants in M_2 generation of the Rice cultivar ADT (R) 47.

MATERIALS AND METHODS

In the current investigation, the seeds of rice cultivar ADT (R) 47 were selected to induce viable mutations. The genetically pure seeds of ADT (R) 47 rice (*Oryza sativa* L.) variety were obtained from Tamil Nadu Rice Research Institute, Aduthurai of Tamil Nadu Agricultural University. The seeds were irradiated with different doses (200, 250 and 300Gy) of gamma rays from ^{60}CO at Centre for Plant Breeding and Genetics, Coimbatore. For EMS treatment, healthy seeds were treated with varied concentrations of (100, 120 and 140mM). The treated seeds were carefully removed from the solution and they were thoroughly washed in running tap water for two to three times to eliminate the residual effect of the chemical. The field experiment was carried out in the south farm, Tamil Nadu Rice Research Institute, Aduthurai without replication. Thirty days after sowing, the seedlings were transplanted to the main field with the single

Corresponding author e-mail: rajarajandeva@gmail.com
[1] *Tamil Nadu Rice Research Institute, Aduthurai - 612101*

seedling per hill. Normal agronomic practices were followed to raise a good crop. The spacing was maintained at 20 cm (between the rows) and 10 cm (between plant to plant) in the field. The M_1 plants were harvested separately and the plant progenies were raised in M_2 generation. From each treatment of gamma irradiation and EMS, 100 M_1 plants individually, totalling to 600 plants were forwarded to M_2 generation based on M_1 seed fertility. The primary panicle of individual M_1 plant was forwarded to M_2 as per plant to progeny method. The control and treated progenies were screened several times for viable mutations throughout the M_2 crop duration. The frequency and spectrum of different types of viable mutants were scored at various developmental stages of M_2 plants particularly from flowering to maturity period. All visible changes in comparison to the control were classified for deviation from the normal and the most conspicuous characters taken into consideration were leaf characters, plant stature, crop duration and grain characters. The frequency and spectrum of viable mutants were calculated in M_2 population on seedling basis. The frequency of viable mutations was calculated on the basis of total number of M_2 plants in the respective mutagen. The frequency of each mutant type was also calculated on the basis of the total number of M_2 plants screened in all the treatments. Finally, the overall frequency of viable macro mutations isolated in each mutagen was calculated on the basis of the total number of M_2 plants screened.

The formula proposed by Konzak *et al.* (1965) was followed for the calculations of mutagenic effectiveness and efficiency by incorporating the mutation frequency values recorded for each mutagenic treatment.

Mutagenic effectiveness = Mutagenic Frequency / Dose or (concentration (c) x time (t))

Mutagenic efficiency = Mutagenic Frequency / Biological damage

RESULTS AND DISCUSSION

Economic potentialities of identified viable mutants in the M_2 generation

Swaminathan (1965) classified mutations into two major groups: (1) those which can be detected in a group of plants requiring testing by biometrical analysis as micro mutations, (2) others which can be recognized by a study of individual plants involving gross changes in the phenotype as macro mutations. Induced macro mutations in many cases may not be suitable for being released directly for cultivation, but they may prove excellent initial breeding materials (Gustafsson and Tedin, 1954). The great merit of such mutants is that the variability would be available in well adapted variety. Gaul (1965) defined macro mutations as those involving gross changes in the phenotype which could be recognized with certainty in a single plant. In the present investigation various types of viable mutants such as Tall, Dwarf, Narrow leaf, Early maturity plants, Late maturity plants, Bold seed and Small seed could be detected at seedling stage in M_2 generation. The data pertaining to the mutation spectrum and frequency of viable mutants was recorded in all the treatments. In the current study, some of the morphological (viable) mutants were observed in M_2 generation with different dose/concentration of mutagens. An increase in the number of viable mutants was realized with the increase in the dose/concentration of gamma rays and EMS treatments in the present study.

Spectrum of viable mutants

In the present study, four broad types of viable mutants could be witnessed in the M_2 populations of ADT (R) 47 treated with physical and chemical mutagens. They consisted of 22 leaf mutants - short leaf (1), narrow leaf (14) and broad leaf (7); 44 stature mutants – tall (24), dwarf (20); 31 duration mutants – early (9), late (22) and 59 grain type mutants - long slender grains (28), medium slender grains (12), grain with beaks (14) and awned type (5) (Fig.1). The mutagenic frequency was highest at 300Gy (0.88%) of gamma irradiation and gamma rays were found to most effective in inducing morphological and viable mutations compared to EMS. Vasline and Sabesan (2011) also concluded that 0.8% EMS was the most effective dose in inducing a wide range of viable mutants in rice varieties ADT 43 and ADT (R) 45.

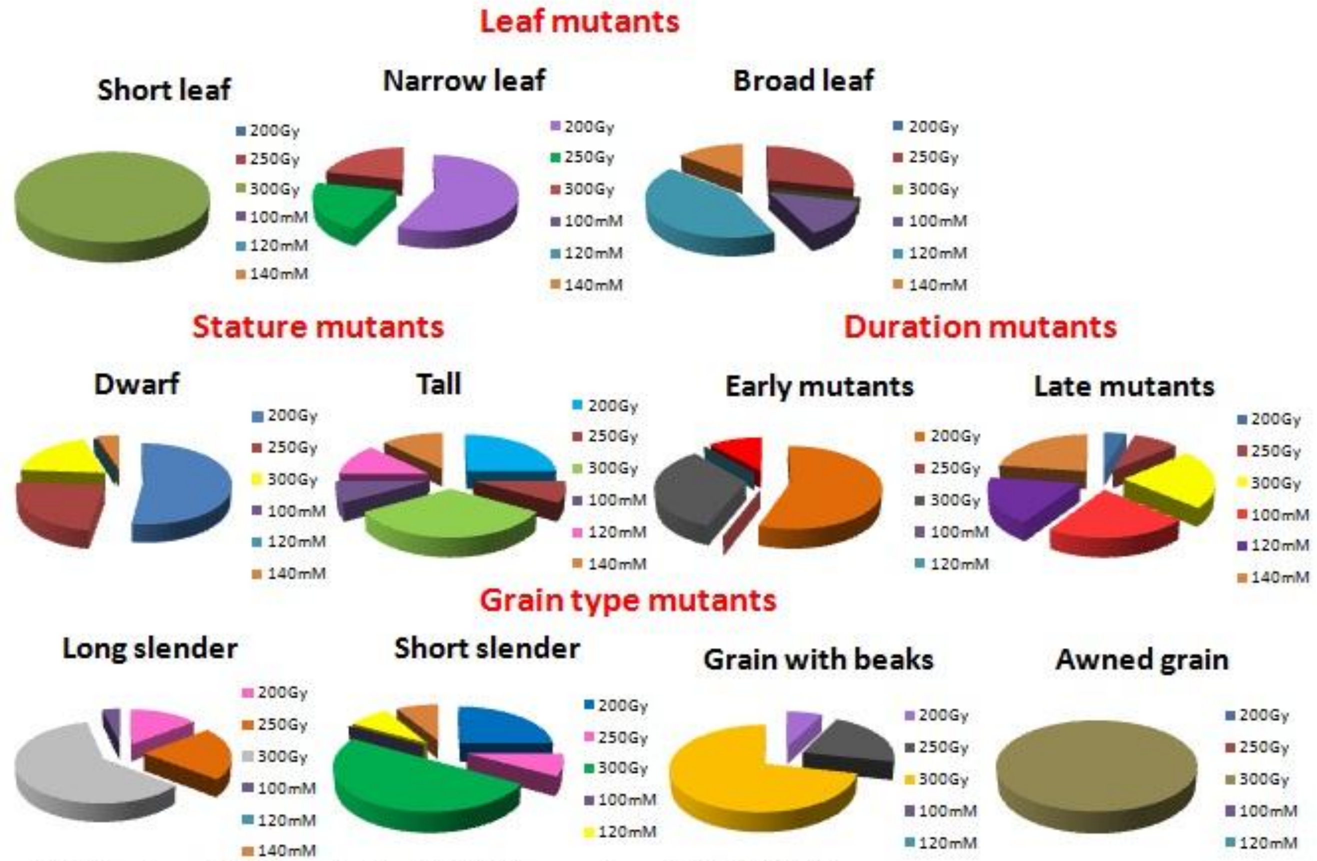

Fig 1. Spectrum of viable mutants isolated in M_2 generation of ADT (R) 47 Rice

Leaf mutants

Among the 22 leaf mutants, three categories *viz.,* short leaf, narrow leaf and broad leaf could be seen in this study. One short leaf mutant was observed at 300Gy dose of gamma rays. Out of 14 narrow leaf mutants, eight mutants were noticed in 200Gy, three in 250Gy and three in 300Gy gamma treatments. Broad leaf mutants were observed both in gamma and EMS treatments *viz*., two at 250Gy dose, one each at 100mM and 140mM, three at 120mM concentrations. Broad leaf mutants had more number of productive tillers and grains per panicle; medium in duration with increased panicle length. Maximum narrow leaf mutants were observed at 0.8 % EMS in IR 64 by Singh *et al.* (1998) Singh and Singh (2003) and Vennila (2005).

Stature mutants

The mutants with short stature (less than 75cm in height) and tall stature (those having more than 110cm plant height) in comparison to ADT (R) 47 variety were encountered. Twenty dwarf mutants *viz.*, 11 under 200Gy, five under 250Gy and four under 300Gy were found in gamma irradiated populations and only one was observed at 140mM of EMS. Out of 24 tall mutants, 16 mutants *viz.*, six in 200Gy, two in 250Gy, and eight in 300Gy and eight mutants *viz.,* three each at 120mM and 140mM and two at 100mM were recovered in gamma and EMS induced mutated populations. Mutagenesis has proved very effective in reducing plant height (Baloch *et al.,* 2003; Chakravarty, 2010). The induction of dwarf mutants by radiation is considered to be one of the most promising applications of mutations in plant breeding. In some of the crops, erectoides mutants characterized by shorter height with dense ears would be of a progressive value not only because of their improved lodging resistance but also due to their concomitant high yielding ability under high nitrogen response. Such modifications in plant height could be developed with short culm without impairing their yield with considerably high efficiency (Kawai *et al.,* 1961).

Among the plant stature mutants, the frequency of dwarfs was the maximum in gamma ray treated population. The dwarf mutants were early with less number of grains per panicle and low yield. Dwarf mutants in ADT (R) 47 were obtained to an extent of 10-15cm reduction in height. However, single plant yield was low and hence these mutants could be utilized in the hybridization programme to transfer the desirable character. Dwarf mutants were developed through utilization of the induced mutation method in rice breeding by Alionte and Alionte (1995), Singh *et al.* (1998), Shadakshari *et al.* (2001), Singh and Singh (2003) and Qin *et al.* (2008). Tall mutants were of higher proportion in EMS treatments in this study. The tall mutants showed an increased height of 25-30cm and had longer maturity period but with increase in panicle length and single plant yield compared to the control. In M_3 generation of Akshaya rice variety, Krishnaveni and Niveditha (2014) isolated eight different types of morphological mutants, of which dwarf plant type occupied the top position (21.0%) followed by tall types (18.8%).

Duration mutants

There were two classes *viz.*, early (110-119 days) and late (>140 days) mutants in comparison with the control. Early mutants were less in number *viz*., five at 200Gy, three at 300Gy of gamma rays and one at 140mM of EMS treatments compared to 22 late mutants. These late mutants comprised of five each at 300Gy, 100mM and 140mM, four at 120mM, two at 250Gy and one at 200Gy. Several mutants affecting the maturity period were obtained which ranged from early (110-119 days) to late (>140 days) in duration. As early as in 1968, Tanaka reported a large number of early flowering and early maturity mutants which have been selected in rice after using different physical mutagens. The degree of earliness varied considerably in the different genotypes. In exceptional cases, an extra ordinary high shift of the flowering was realized. For instance, a Japonica rice mutant was about 60 days earlier than that of parent variety. More early mutants were isolated in gamma treatments than EMS in the present study.

A reduction of vegetative growth duration offers several advantages such as diminution of the damage caused due to natural disaster and advancing the harvesting time. In the present study, the magnitude of late mutants was of higher proportion than early mutants in gamma rays and EMS treatments. Frequent occurrence of lateness than earliness was evidenced from the studies conducted by Gopinathan Nair (1971), Rao and Reddi (1986) and Singh *et al.* (1998) and Singh and Singh (2003).

Table 1: Mutagenic frequency of viable mutants in M_2 generation of ADT (R) 47 Rice

Viable mutants	Gamma rays (Gy)			EMS (mM)		
	200	250	300	100	120	140
Total of no. of plants isolated	39	24	62	9	11	12
Seedlings examined	7333	7357	7014	6728	7135	7631
Mutagenic frequency (%)	0.53	0.33	0.88	0.13	0.15	0.16

Grain type mutants

Seed size and seed shape mutants were found in both gamma and EMS treated populations of the genotype ADT (R) 47. Among the different categories, 28 mutants had long slender grains, 12 had medium slender grains, 14 had beaks in the grain, five were of awned type. The mutants were classified based on changes in size. Long, medium, bold, grain with beaks as well as awned grain mutants were also noticed in different treatments. Of the 59 mutants, only three were observed in EMS treatments and the rest were isolated in gamma irradiation. Grain type mutants were in greater proportion in gamma ray treatments than in EMS treatments. This might be due to the genotypic effect which showed greater sensitivity to irradiation treatments. Mutants with alterations in grain size were also reported by Kaul *et al.* (1980); Shobharani *et al.* (2004); Patnaik *et al.* (2006); Bhat *et al.* (2007) and Chakravarti *et al.* (2012). A wide range (15 types) of viable macro-mutations was observed by Chakravarti *et al.* (2012) *i.e.,* 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. 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). The differences observed in the spectra of morphological mutations were more of quantitative nature rather than qualitative. 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%). The more frequent induction of certain mutations might be attributed to the fact that the genes for these traits were probably relatively more vulnerable to mutagenic treatments.

Table 2: Mutagenic effectiveness and efficiency based on viable mutations in M_2 generation of ADT (R) 47 rice

Mutagen	% seedling height in M_1(I)	% survival reduction in M_1 (L)	% pollen sterility in M_1 (S)	Mutation frequency (M)	Effectiveness	Efficiency		
					(M x100) / Gy or cxt	(M x 100) / I	(M x100) / L	(M x 100)/ S
Gamma rays (Dosage)								
200Gy	89.00	41.94	18.07	0.53	0.27	0.60	1.27	2.94
250Gy	79.73	56.77	22.16	0.33	0.13	0.41	0.57	1.47
300Gy	73.54	73.76	28.58	0.88	0.29	1.20	1.20	3.09
				1.74	0.69	2.21	3.04	7.51
EMS (Concentration)								
100 mM	86.67	35.76	13.45	0.13	0.022	0.15	0.37	1.00
120 mM	81.67	52.89	16.03	0.15	0.021	0.19	0.29	0.96
140 mM	78.33	62.10	22.23	0.16	0.019	0.20	0.25	0.71
				0.45	0.062	0.54	0.92	2.66

Mutagenic frequency, effectiveness and efficiency based on viable mutations

The mutagenic frequency of gamma rays to induce viable mutants was highest at 300Gy (0.88%) followed by 0.53% and 0.33% at 200Gy and 250Gy doses (Table 1 & 2). The chemical mutagen EMS exhibited highest mutagenic frequency of 0.16% at 140mM concentration. The lower concentrations of 120mM and 100mM recorded 0.15% and 0.13% mutagenic frequency. The effectiveness of gamma rays in inducing morphological and viable mutations ranged between 0.13 % (250Gy) and 0.29 % (300Gy). The effectiveness of EMS in inducing viable mutations was in the range of 0.019 % (140mM) to 0.022 % (100mM). The physical mutagen *viz.,* gamma irradiation displayed higher effectiveness (0.69) when compared to EMS (0.062). In gamma irradiated populations, based on injury, the mutagenic efficiency ranged between 0.41 % (250Gy) and 1.20 % (300Gy). In terms of lethality, efficiency of gamma rays in inducing viable mutations was in the range of 0.57 % (250Gy) to 1.27 % (200Gy). Its efficiency to induce viable mutations based on sterility ranged from 1.47 % (250Gy) to 3.09 % (300Gy). Highest efficiency of gamma rays in inducing viable mutants on the basis of injury and sterility was observed at 300Gy and lethality was noticed at 200Gy. The efficiency of EMS in inducing viable mutations based on injury, ranged between 0.15 % (120mM) and 0.20 % (140mM). Based on lethality, the values ranged from 0.25 % (140mM) to 0.37 % (100mM). On the basis of sterility, efficiency values ranged from 0.71 % (140mM) to 1.00 % (100mM). Maximum efficiency of EMS in inducing viable mutants on the basis of lethality and sterility was observed at 100mM concentration. Highest efficiency of gamma rays in inducing viable mutants on the basis of injury (1.20%) and sterility (3.09%) was observed at 300Gy and lethality (1.27%) was noticed at 200Gy. Likewise in EMS, at 100mM concentration, viable mutants were induced on the basis of lethality (0.37) and sterility (1.00%).

The induction of early flowering, dwarf and semi-dwarf mutants could be useful in recombination breeding program indirectly for developing high yielding lines, while high yielding mutants could be of immediate use directly as varieties. Further, these mutants can also be used to identify genes responsible for different developmental stages of plants.

Acknowledgement

The authors are grateful to Government of India, Department of Atomic Energy, Bhabha Atomic Research Centre (BARC), Board of Research in Nuclear Sciences (BRNS) for providing financial assistance to perform this research.

REFERENCES

Alionte, G. and Alionte, E. (1995) Results obtained in rice breeding (*Oryza sativa* L.) by induced mutation method. Romanian Agric. Res., 4: 53-61.

Anonymous (2013) World rice production. www.advaceagriculturalpractice.in

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

Bhat, R. S., Surendra, P., Hanumaratti, N. G and Nayak, G. V. (2007) AM3- an induced rice mutant with improved grain size and yield. International Rice Research News letter, 32(1): 18-19.

Chakravarti, S. K. R., Kumar, H., Lal, J. P and Vishwakarma, M. K. (2012) Induced mutation in traditional aromatic rice - frequency and spectrum of viable mutations and characterization of economic values. The Bioscan 7(4): 739-742.

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

Gaul, H. (1965) The concept of macro and micro mutations and results on induced micro mutations in biology. Radiation Botany 5: 407- 426.

Gopinathan Nair, G. V. (1971) Studies on induced mutations in rice (*Oryza sativa* L.). Ph.D. Thesis, University. of Madras.

Gustafsson, A. and Tedin, O. (1954) Plant breeding and mutations. *Acta Agric. Scand.*, 4: 633-639.

Kaul, M. L. H., Matta, N. K. and Kumar, V. (1980) Mutation genetic studies in rice-Development of protein rice mutants. Agric. Res. J. Kerala 19(1): 41-47.

Kawai, T., Sato, H. and Masina, I. (1961) Short culm mutation in rice by P32.Prac. Symp. on the effects of ionizing radiation on seeds (IAEA, Vienna): 565-579.

Konzak, C. F., Wickham, I. M. and Dekock, M. J. (1972) Advances in methods of mutagen treatment. Induced Mutations and Plant Improvement, IAEA, Vienna Pp. 95-119.

Krishnaveni, B and Niveditha, M. S. (2014) Effect of mutagens on quantitative and qualitative characters in M_3 generation of rice variety ‘Akshaya’ (BPT 2231). Journal of Rice Research 7 (1 & 2): 16-24.

Nilan, R. A., Kleinhofs, A and Konzak, C. F. (1977) The Role of Induced Mutation in Supplementing Natural Genetic Variability. Ann. N.Y. Acad. Sci. 287: 367-384.

Patnaik, A., Chaudhary, D and Rao, G. J. N. (2006) Genetic Improvement of long grain aromatic rices through mutation approach. Plant Mutation Reports 1: 11-15.

Qin, R., Qiu, Y., Cheng, Z., Shan, X., Guo, X., Zhai, H and Wan, J. (2008) Genetic analysis of a novel dominant rice dwarf mutant 986083D. Euphytica **160**: 379-387.

Rao, D. R. M. and Reddi, T. V. V. S. (1986) Azide mutagenesis in rice. Proceedings Indian Academy of Science **96**: 205-215.

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

Shobharani, 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. (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 ray induced mutations in rice (*Oryza sativa* L.). Indian Journal of Geneties **58** (4): 455- 463.

Swaminathan, M. S. (1965) Report of the meeting of the symposium in the use of induced mutations in plant breeding. Radiat. Botany **5**: 65- 69.

Tanaka, S. (1968) Radiation- induced mutations in rice. In: Rice Breeding with Induced Mutations. IAEA, Vienna. pp. 53- 64.

Vasline, A. and Sabesan, T. (2011) Assessment of chlorophyll and viable mutation in M_2 generation of rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding **2**(1): 107- 111.

Vennila, S. (2005) Studies on induced mutagenesis in rice var. Jeeraga Samba (*Oryza sativa* L.). M.Sc., (Ag.) Thesis, Annamalai Univ., Annamalai Nagar.