Pak. J. Bot., 41(5): 2487-2495, 2009.

IMPACT OF GAMMA IRRADIATION INDUCED CHANGES ON GROWTH AND PHYSIOLOGICAL RESPONSES OF RICE UNDER SALINE CONDITIONS

A. SHEREEN, R. ANSARI, S. MUMTAZ, H.R. BUGHIO, S.M. MUJTABA, M.U. SHIRAZI AND M.A. KHAN

Nuclear Institute of Agriculture, Tandojam, Sindh, Pakistan

Abstract

Seeds of 3 rice varieties viz., Shua-92, Sarshar and IR-8 were irradiated with gamma rays (150, 200 and 250 Gy of Cobalt-60) for determining the effectiveness of different doses of irradiation on growth behaviour and physiological responses (chlorophyll contents and ionic concentrations) of rice plants. Seeds of all these varieties from M_2 generation were collected along with their parents and were grown at different levels of salinity (0, 50 and 75mM NaCl) under water culture conditions for two weeks period. Significant reduction was observed in plant height, leaf area and fresh weight of all parent varieties. None of the parent variety was able to survive at 75mM NaCl salinity. Comparison among different doses of radiation have shown that 150 Gy was found comparatively more effective not only for survival under higher salinity level (75mM NaCl) but also exhibited an enhancement in biomass production and have pronounced effects on ionic responses of these plants. These studies have revealed that enhanced chlorophyll and K, and less Na uptake due to irradiation may be responsible for better growth under saline condition.

Introduction

Soil salinity is one of the major limiting factor that drastically affects crop productivity worldwide and the phenomena is of great importance in arid and semiarid areas. According to an estimate, approximately 10% of the world's arable land surface consists of saline or sodic soils and 50% of all irrigated lands are seriously affected by salinity and water logging (Francois & Maas, 1999). This problem is more serious in agriculture of South and South East Asia which account for more than 90% of the world rice production worstly affected by salinity. Of the 130 mha of land where rice is grown, about 30% contains high level of salts which hinders normal rice productivity (Mishra, 2004). According to an estimate, the number of rice consumers and rice requirement will increase by 50 and 70%, respectively during the next 25 years and there will be an additional rice requirement of 330 million tons by the year 2025 (Peng *et al*., 1999). To meet the ever-increasing demand of growing population, new rice varieties having potential of growing in saline soils through exploitation of genetic variability is the best option.

Considerable efforts have been made to overcome the problem of salinity through adoption of biological approaches. The existing data record showed intra and inter-specific variability for salinity tolerance among different crop species (Ashraf, 2004; Khan *et al*., 2003; Lacerda *et al*., 2003; Mishra & Gupta, 2005; Noori & McNeilly, 2000; Rashid *et al*., 2003, Shah *et al*., 2006 and Shah *et al*., 2008). All these studies revealed that genetic variability may be induced and exploited through hybridization/induced mutation followed by selection in order to improve salinity tolerance.

E-mail: niatjam@hyd.paknet.com.pk

In this way, unlimited combinations of new characters may be obtained through hybridization technique which can be selected in segregating population. In contrast, with induced mutations it is possible to improve a single trait without causing extensive disruption in the genome. The use of induced mutation in crop improvement has proven to be an effective approach to improve yield, quality and resistance to biotic and abiotic stresses (Bibi *et al*., 2009; Nichterlain *et al*., 2000). Thus, induced mutations can play a vital role in improving complex quantitative traits including tolerance to abiotic stresses.

This study was undertaken to explore the mutationally induced desirable physiological traits and their impact on growth of rice under saline environments.

Materials and Methods

Seeds of three non-aromatic, semi-dwarf rice (*Oryza sativa* L.) varieties viz., IR-8, Shua-92 and Sarshar were treated with different doses of gamma rays (150, 200 and 250 Gy from ^{60}Co radiation source) and grown. From M_2 population, desirable mutants were isolated on the basis of agronomic traits and their seeds grown in M_3 progeny for determining the growth and physiological responses. Seeds of all mutants along with their parent varieties were sterilized in 1% Sodium hypochloride for 15 minutes. These seeds were thoroughly washed with distilled water 3 to 4 times and after rinsing, the seeds were soaked overnight. Thereafter, the seeds were planted on nylon netted frame (12.5 x 17.5 cm) fitted in 2.5 L solution capacity plastic containers filled with culture solution (Yoshida *et al*., 1976). These boxes were placed in dark for germination at 30^{o}C in an incubator. Five days old seedlings were transplanted and salinized with 50 and 75mM NaCl at 10th day. The culture solution was renewed at weekly intervals. Electrical conductivity of treatment solution was maintained by topping up with Yoshida culture solution. After giving an exposure of salinity treatment for a period of two weeks, three plants from each treatment and each radiation dose were harvested. Observations on shoot root length, leaf area, fresh and dry mass were recorded; shoots were analyzed for ions and chlorophyll contents (Lichtenthaler, 1987).

The data were analyzed statistically by analysis of variance and comparison among treatment means was made by Duncan’s Multiple Range Test (DMRT) using computer software MSTAT-C.

Results and Discussion

The results revealed significant effects of radiation doses on all growth parameters studied at early seedling stage under different salinity treatments. Comparing the shoot and root length at different radiation doses under saline and non- saline conditions, it was observed that none of the parent variety was able to survive at 75 mM NaCl salinity after an exposure to salinity for two-weeks. The radiation dose of 150 Gy was found effective at which mutants of Shua-92 and IR-8 were not only alive at the higher level of salinity (75 mM) but also attained comparable height at this treatment, where the plants of parent varieties could not establish. Under non- saline conditions, the radiation doses of 150 and 200 Gy exhibited slight stimulatory effects on plant height of mutants of Sarshar and Shua-92 (Table 1). There was a general increase of 3 to 47% in leaf area of these mutants at 150 Gy as compared with their respective parents. No increase in leaf area was observed at 200 Gy and 250 Gy except in Sarshar, where 200 Gy was found equally effective in enhancing leaf area at 50 and 75mM NaCl (Table 2).

Table 1. Effect of different doses of radiation on root and shoot length (cm) under saline conditions.

Varieties	Shua-92			Sarshar			IR-8		
	Treatments (mM NaCl)								
Doses/ Parameters	**Control**	**50mM NaCl**	**75mM NaCl**	**Control**	**50mM NaCl**	**75mM NaCl**	**Control**	**50mM NaCl**	**75mM NaCl**
Parent									
Shoot length	32.7	29.6	-	32.3	30.9	-	35.7	24.0	-
Root length	13.9	14.4	-	14.5	14.6	-	16.1	13.9	-
150 Gy									
Shoot length	33.4	27.8	26.4	37.6	31.7	26.2	36.0	29.3	28.5
Root length	12.5	13.7	12.3	16.5	12.0	12.5	17.3	12.9	14.6
200 Gy									
Shoot length	35.1	25.2	-	39.7	32.1	29.5	33.0	26.9	-
Root length	16.0	13.0	-	16.0	12.8	15.0	15.0	14.0	-
250 Gy									
Shoot length	29.2	-	-	37.3	23.5	23.2	33.9	28.5	-
Root length	13.8	-	-	16.9	10.6	9.6	19.3	14.0	-

LSD values for varieties at α 0.05: 2.746 and 4.399 for root & shoot length, respectively
LSD values for treatments at α 0.05: 4.757 and 7.62 for root & shoot length, respectively

Table 2. Effect of different doses of radiation on shoot fresh weight and leaf area under saline conditions.

Varieties	Shua-92			Sarshar			IR-8		
	Treatments (mM NaCl)								
Doses/ Parameters	**Control**	**50mM NaCl**	**75mM NaCl**	**Control**	**50mM NaCl**	**75mM NaCl**	**Control**	**50mM NaCl**	**75mM NaCl**
Parent									
Fresh weight	1.56	0.65	-	1.32	0.93	-	1.61	0.40	-
Leaf area	51.0	17.8	-	40.7	36.5	-	50.59	11.0	-
150 Gy									
Fresh weight	1.60	0.87	1.03	1.38	1.25	0.59	1.65	0.91	0.67
Leaf area	60.8	27.8	23.9	53.8	46.4	15.4	52.08	26.34	17.7
200 Gy									
Fresh weight	1.71	0.57	-	1.6	1.26	1.26	0.89	0.56	-
Leaf area	53.3	14.0	-	53.1	39.6	31.4	26.7	16.5	-
250 Gy									
Fresh weight	0.76	-	-	1.27	0.67	0.83	1.42	0.77	-
Leaf area	22.2	-	-	46.9	19.83	22.4	41.08	13.8	-

LSD values for varieties at α 0.05: 6.30 and 0.09402 for shoot fresh weight and leaf area, respectively
LSD values for treatments at α 0.05: 0.1806 and 7.5 for shoot fresh weight and leaf area, respectively

The radiation dose of 150 Gy also exhibited similar enhancing effects on biomass production as was observed in leaf area. A slight stimulation in shoot fresh weights was observed at 150 and 200 Gy radiation dose in mutants of Shua and Sarshar under non-saline conditions. While at the higher level of salinity (75mM) where the parent varieties did not survive, the mutants of Shua-92, Sarshar and IR-8 (evolved by 150 Gy dose) have produced 66, 45 and 42% of the control fresh weights (fresh weight produced by parent varieties under non-saline condition), respectively. The variety Sarshar yielded comparatively more biomass at both levels of salinity with 200 Gy dose (Table 2).

Ion uptake responses of the parent varieties when compared on the basis of percent increase in Na in comparison to their respective controls, it was observed that under saline conditions (50mM), Na concentration increased with varying intensities in all parent varieties and the increase was more in Shua-92 (573%) and least in Sarshar (77%). While, in mutants of Shua-92 (150 Gy), Na concentration was reduced to 300% (Table 3). The mutant of Sarshar (obtained from 150 Gy radiation dose) was found remarkable in that there was very little (31%) increase in Na at lower level of salinity (50mM NaCl), while at the higher level (75mM), an increase of 277% was observed. Furthermore, the mutants of Sarshar obtained by 200 Gy radiation dose also exhibited comparatively lesser increase of 23% and 115% in their Na concentration at 50 and 75 mM NaCl salinity treatment, respectively. The response of these varieties for K concentration in shoot was also found variable under saline condition. The Shua mutants (150 Gy) were found comparatively better among all mutants as they exhibited least reduction (7 & 20%) at 50, 75 mM NaCl treatments, respectively (Table 3).

Chlorophyll contents of these mutants when compared under non-saline condition, showed that total chlorophyll was increased with the increase in radiation dose. Under saline conditions, a general reduction in chlorophyll, content was observed. When different pigments of chlorophyll were studied, it was observed that chlorophyll b was comparatively more in quantity than chlorophyll a. Comparison among different doses of radiation have shown that comparatively more chlorophyll contents were found in 150 Gy radiation dose at both levels of salinity (Table 4).

A negative relationship ($r= -0.72$) was observed between Na uptake and chlorophyll contents and the radiation dose of 150 Gy was found effective in reducing shoot Na concentration and maintaining chlorophyll pigment under salinity. This reduced Na concentration in shoot may be positively linked with better growth under salinity.

Reduction in plant growth due to salinity is a common feature as was observed in all parent varieties. This reduction in growth may be attributed to osmotic effects due to which water availability to plants is hindered. There may be salt specific effects. If absorption of excess quantity of toxic ions takes place, the concentration inside the plant may reach to toxic level and causes ionic imbalances at cellular and organ level. These imbalances may cause hindrances in various physiological functions and ultimately the growth and yield is affected (Hameed *et al*., 2008a). The magnitude of salt-induced yield losses is dependent on different physiological and biochemical factors governed at different developmental stages of plants. One of the most important factor under saline environment may be the overall control mechanism of salt uptake through root and its subsequent distribution to shoot. The other factor is the maintenance of mineral nutrients such as K and Ca which are essentially required for the activities of enzymes, proteins synthesis and integrity of cell wall and plasma membrane (Taiz & Zeiger, 2006). The results reported here for shoot ions concentration showed an increase in accumulation of Na coupled with decrease in K concentration in all parent varieties which are in agreement with the view that plants growing under saline conditions suffer from ionic imbalances, nutrient deficiency and specific ions toxicity (Ashraf, 1994; 2004; Munns, 2005).

Table 3. Effect of different doses of radiation on Na and K concentration (%) under saline conditions.

Varieties	Shua-92			Sarshar			IR-8		
	Treatments (mM NaCl)								
Doses/ Parameters	Control	50mM NaCl	75mM NaCl	Control	50mM NaCl	75mM NaCl	Control	50mM NaCl	75mM NaCl
Parent									
Na	0.11	0.74	-	0.13	0.23	-	0.12	0.42	-
	(0.00)	(573)			(77)			(250)	
K	1.28	1.08	-	1.61	1.08	-	1.48	0.86	-
	(0.00)	(-15.6)			(-32.9)			(-41.9)	
150 Gy									
Na	0.10	0.41	0.44	0.18	0.17	0.49	0.15	0.29	0.53
		(273)	(300)		(31)	(277)		(142)	(342)
K	1.56	1.19	1.02	1.51	1.23	0.71	1.50	1.09	0.70
	(21.88)	(-7.03)	(-20.3)	(-6.21)	(-23.6)	(-56)	(1.35)	(-26.4)	(-52.7)
200 Gy									
Na	0.13	0.83	-	0.12	0.16	0.28	0.22	0.31	-
		(655)			(23)	(115)		(158)	
K	1.45	1.01	-	1.40	1.25	0.86	1.46	0.8	-
	(13.28)	(-21.1)		(-13.04)	(-22.4)	(-46.6)	(-1.35)	(-45.9)	
250 Gy									
Na	0.19	-	-	0.15	0.29	0.59	0.13	0.98	-
					(123)	(354)		(717)	
K	1.48	-	-	1.42	0.94	0.72	1.45	0.58	-
	(15.63)			(-11.80)	(-41.6)	(-55.3)	(-2.03)	(-60.8)	

Figures given in parenthesis indicate % increase or decrease over control

LSD values for varieties at α 0.05: 0.1655 and 0.2038 for Na & K concentration, respectively

LSD values for treatments at α 0.05: 0.2867 and 0.3530 for Na & K concentration, respectively

Table 4. Effect of different doses of radiation on chlorophyll pigments (mg g^{-1} fresh weight) under saline conditions.

Varieties	Shua-92			Sarshar			IR-8		
	Treatments (mM NaCl)								
Doses/ Parameters	Control	50mM NaCl	75mM NaCl	Control	50mM NaCl	75mM NaCl	Control	50mM NaCl	75mM NaCl
Parent									
Chl. a	0.54	0.51	-	0.51	0.51	-	0.46	0.36	-
Chl. b	0.82	0.75	-	0.71	0.73	-	0.64	0.47	-
Total Chl.	1.36	1.26	-	1.22	1.24	-	1.11	0.84	-
150 Gy									
Chl. a	0.70	0.57	0.57	0.63	0.56	0.44	0.69	0.61	0.44
Chl. b	1.07	0.81	0.81	0.86	0.81	0.66	0.94	0.81	0.58
Total Chl.	1.77	1.37	1.39	1.48	1.37	1.10	1.62	1.42	1.01
200 Gy									
Chl. a	0.64	0.30	-	0.60	0.50	0.45	0.65	0.38	-
Chl. b	0.97	0.40	-	0.82	0.7	0.61	0.87	0.38	-
Total Chl.	1.61	0.40	-	1.42	1.19	1.06	1.52	1.06	-
250 Gy									
Chl. a	0.68	-	-	0.58	-	0.25	0.71	0.29	-
Chl. b	0.99	-	-	0.85	-	0.34	0.95	0.36	-
Total Chl.	1.67	-	-	1.43	-	0.59	1.65	0.65	-

LSD values for varieties at α 0.05: 0.09402, 0.1395 and 0.2341 for Chl a., Chl. b and total Chl., respectively

LSD values for treatments at α 0.05: 0.1628, 0.2415 and 0.4055 for Chl a., Chl. b and total Chl., respectively

Mutation induction technology has been used as a practical tool to develop new varieties through improving character of direct importance i.e., early maturity, tolerance to biotic and abiotic stresses. Gamma rays are used to obtain high frequency gene mutation and chromosomal alteration in different crops. According to data base of FAO, a number of mutant varieties have been evolved through induced mutation. This creates variation within the crop variety and it offers the possibility of inducing desired attribute to select improved cultivars and also for generating novel mutant traits like leaf colour mutants, low phytic acid mutants, giant embryo mutants and high resistant starch mutants. The abiotic stresses like salinity and drought are major threats to rice production. Using this technique, variability in different component traits involved in tolerance can be induced and exploited. Zhu *et al*., (2006) reported enhancement of drought tolerance in rice through induction of several mutants in which the water absorbing and water evaporating characters were changed.

Comparison among different doses have revealed that at lower dose (150 Gy), the effectiveness was higher in all three varieties. In the present study, the radiation dose of 150 Gy was found very effective for improving the ionic balance in shoots of all tested varieties, as significant reduction (26-40% reduction in comparison to their respective parents) in Na uptake was observed (Table 3). Similarly, an increase of 10-27% was observed in K uptake of mutants under saline treatment (50mM NaCl) in comparison to their respective parents. The reduction in the uptake of toxic ion like Na may have exerted positive role in enhancement of cellular functions like pigment production and other growth attributes, which collectively resulted in better growth of these mutants over the parent varieties under saline conditions. Higher efficiency at lower concentration of mutagen probably due to the fact that injury, mortality increase with the increase in mutagen concentration as was observed by Shah *et al*., (2008). Ashraf *et al*., (2003); reported drastic detrimental effects on panicle fertility with increasing radiation dose. Similarly Hameed *et al*., (2008b) reported for other physiological characters i.e., protein contents, peroxidase, protease and lipid peroxidation, which have an important role in abiotic stresses were found affected in chickpea with radiation dose rate, specially the lipid peroxidation activity decreased significantly from 100-200 Gy radiation dose. The mechanism of mutations in different varieties of crop plants is considered closely related to their radio sensitivity, and several physiological and genetical factors influence the radio sensitivity of plants (Baloch *et al*., 2004). In the future more attention needs to be given to other physiological characters/traits especially related to plant tolerance to adverse soil conditions.

References

Ashraf, M. 1994. Breeding for salinity tolerance in plants. *Crit. Rev. Plant Sci*., 13: 17-42.

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora*, 199: 361-376.

Ashraf, M., A.A. Cheema, M. Rashid and Z. Qamar. 2003. Effect of gamma rays on M_1 generation in Basmati rice. *Pak. J. Bot*., 35(5): 791-795.

Baloch, A.W., A.M. Soomro, H.R. Bughi, M.S. Bughio, T. Mohammad and N.N. Mastoi. 2004. Gamma irradiation induced chlorophyll mutations in rice (*Oryza sativa* L.). *SAARC J. Agric*., 2: 257-261.

Bibi, S., I.A. Khan, H.R. Bughio, I.A. Odhano, M.A. Asad and A. Khatri. 2009. Genetic differentiation of rice mutants based on morphological traits and molecular marker (RAPD). *Pak. J. Bot*., 41(2): 737-743.

Francois, L.E. and E.V. Maas. 1999. Crop response and management of salt affected soils. In: *Hand Book of Plant and Crop Stress*. (Ed.): M. Pessarakli. Marcel Dekker, Inc., New York, pp. 169-201.

Hameed, A., T.M. Shah, B.M. Atta, M.A. Haq and H. Sayed. 2008a. Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. *Pak. J. Bot*., 40(3): 1033-1041.

Hameed, A., S. Naseer, T. Iqbal, H. Syed and M. Ahsanul Haq. 2008b. Effect of NaCl salinity on seedling growth, senescence, catalase and protease activities in two wheat genotypes differing in salt tolerance. *Pak. J. Bot*., 40(3): 1043-1051.

Khan, A.A., S.A. Rao and T. McNeilly. 2003. Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). *Euphytica*, 131: 81-89.

Lacerda, C.F., J. Cambraia, M.A. Oliva, H.A. Ruiz and J.T. Prisco. 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ. Exp. Bot*., 49: 107-120.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol*., 148: 350-382.

Mishra, B. 2004. Present status issues and future strategies for increasing quality rice production and export. In: *National Symposium on Strategies for enhancing export of quality rice* held at NBPGR, New Delhi, pp. 1-16.

Mishra, N. and A.K. Gupta. 2005. Effect of salt stress on proline metabolism in two high yielding genotypes of green grain. *Plant Sci*., 169: 331-339.

Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytol*., 167: 645-663.

Nichterlain, K., H. Bohlman, S. Niclen and M. Maluszynski. 2000. Achievements and trends of using induced mutations in crop improvement. In: *DAEBRNS Symposium on the use of Nuclear and Molecular Techniques in Crop Improvement*. 2000. Mumbai, pp. 27-35.

Noori, S.A.S. and T. McNeilly. 2000. Assessment of variability in salt tolerance based on seedling growth in *Triticum durum Desf*. *Genetic Resources and Crop Evolution*, 47: 285-291.

Peng, S., K.G. Cassman, S.S. Virmani, J. Sheehy, G.S. Khush. 1999. Yield potential trends of tropical rice since the release of IR-8 and the challenge of increasing rice yield potential. *Crop Sci*., 39(6): 1552-1559.

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

Shah, T.M., J.I. Mirza, M.A. Haq and B.M. Atta. 2006. Induced genetic variability in chick pea (*Cicer arietinum* L.). I. Frequency and spectrum of chlorophyll mutations. *Pak. J. Bot*., 38(4): 1217-1226.

Shah, T.M., J.I. Mirza, M.A. Haq and B.M. Atta. 2008. Induced genetic variability in chick pea (*Cicer arietinum* L.). II. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. *Pak. J. Bot*., 40(2): 605-613.

Taiz, L. and E. Zeiger. 2006. *Plant Physiology* (4^{th} edn). Sinauer Associates, Inc., Sunderland, Massachusetts.

Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez. 1976. *Laboratory Manual for physiological studies of rice*. IRRI, Las Bano. Laguna., p.83.

Zhu, X.D., H.Q. Chen and J.X. Shan. 2006. Nuclear techniques for rice improvement and mutation induction in China National Rice Research Institute. *Plant Mutation Reports*, 1(1): 7-10.

(Received for publication 26 October 2008)