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Induced mutagenesis in rice (*Oryza sativa* L.) for improving salt tolerance

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ABSTRACT

The study was undertaken to induce variability, through gamma irradiation, in Pokkali and Improved White Ponni for seven yield and its component traits under salt stress. It was observed that mutagenic treatments generated substantial magnitude of genetic variability for all characters and influenced the mean, range and coefficient of variation independently in both the varieties. Coefficient of variation was high for spikelet sterility at 200Gy in Pokkali, while it was high for single plant yield and panicle weight at 300Gy. In Improved White Ponni, the coefficient of variation was high in 200Gy for single plant yield, while sterility was high at 300Gy. The coefficient of variation was equal in both the doses for panicle weight.

***Key words:** Rice, gamma rays, mutation, Pokkali, Improved white ponni, salt tolerance*

Rice, global grain, is consumed by more than half of the world population. In India rice supplies calorie requirement to more than 70 per cent of its population. Though India ranks top in rice acreage (43.61 mha), its productivity of 3.19 t/ha (rough rice) is far below world average of 4.12 t/ha and that of Asia's average of 4.19 t/ha (Shobharani *et al*. 2010). The low productivity is mainly due to some of the unfavourable ecologies of the varied ecosystems which, rice encounters during critical stages of its growth. Salinity, the second major abiotic stress, next to drought, is a global problem that affects approximately 20% of irrigated land and reduces crop yields significantly. In India nearly 8 mha are salt affected of which, 2 mha are coastal saline and 3.4 mha are sodic. However, rice is the predominant crop in these areas especially in the vast stretches of eastern and western coasts of India.

As genetic variability is pre-requisite for selection, creation and channelizing the variability form the central axis to any crop breeding programme. Mutagenesis is regarded as an important tool to create additional variability for agronomic traits in number of crops (Brock 1971). With the invention of mutagenic effect of X-rays on fruit fly by Muller in 1927 and barley by Stadler in 1928, a new field called as induced mutagenesis was born. Since then, it became an important tool in genetics to locate genes on chromosomes, studying gene structure, expression and regulation, and for exploring genomes (Ahloowalia *et al*. 2004). Ichijima was the first to induce mutations in rice as early as 1934 (Gustafsson and Gadd 1966). Around the same time, mutation research in rice was initiated in India by Ramiah and Parthasarathy in the year 1938 (Sharma 1986) and the practical utility of induced mutations for the improvement of quantitatively inherited characters in rice is well recognized (Oka *et al*. 1958). Induced mutations played greater role in evolving crop varieties with enhanced biotic and abiotic stress tolerance (Ahloowalia *et al*. 2004). Studies on improving salt tolerance, using induced mutagenesis in rice are, rather, limited. Hence, the present study was undertaken using two varieties namely, Pokkali and Improved White Ponni, differing in many characters viz., maturity, growth habit, grain size, grain colour, salt tolerance and yielding ability. Pokkali is highly salt-tolerant landrace but low yielding, poor tillering, tall, photoperiod-sensitive, and susceptible to lodging and possess red pericarp with poor cooking quality.

Improved White Ponni, is locally well adapted high yielding variety with good cooking qualities but highly susceptible to salinity and lodging. Using these varieties, the present investigation was carried out to study the mutagenic effects of gamma rays on induced variability for yield and its component traits in salt stressed environment in the M_2 and M_3 generations.

MATERIALS AND METHODS

The dry and healthy seeds (150g each) of Pokkali and Improved White Ponni were exposed to gamma rays at doses of 200 Gy, 300 Gy and 400 Gy at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Chennai. Radio sensitivity was measured using LD_{50} assay, for which 100 seeds of the two varieties in each dose were sown in petri dishes and germination percentage recorded. Probit analysis was done to calculate the LD_{50} values. These treated seeds along with seeds of parental varieties were sown in normal soil to raise M_1 generation at PAJANCOA & RI experimental farm and recommended package of practice were followed. All the surviving plants were advanced to M_2 by harvesting panicles in each plant separately. Seeds irradiated at 400 Gy did not germinate and, therefore, could not be forwarded further.

Generations were advanced by adopting panicle to row method and the surviving plants under salt stress, created through irrigation of poor quality water, were forwarded. Irrigation water (Iw) was sampled at critical stages of crop growth *viz.*, seedling, tillering and panicle initiation in both M_2 and M_3 generation. The characteristics of the Iw were Ec: 1.84-2.34 dS/m; p^H: 7.92-8.72; SAR: 11.00-18.30 and RSC: 4.00-9.10 meq/l. As per Gupta *et al*. (1994) classification, irrigation water used in the study was rated as saline-sodic. Wider spacing of 20 x 15 cm was adopted and normal package of practices followed in both the generations. Surviving plants under the salt stress were forwarded to next generations. As much as 6165 progeny rows (of 3m length comprising 20 plants) comprising of 4475 rows of Improved White Ponni and 1690 rows of Pokkali in M_2 were raised and a total of 1388 salt tolerant M_2 mutants (501 in Pokkali and 887 in Improved White Ponni) were selected and advanced to M_3 as progeny rows . Similarly, 389 M_3 mutants (187 in Pokkali and 202 in Improved White Ponni) that survived under salt stress were forwarded to M_4. Observations were recorded in all salt tolerant plants in M_2 and M_3 generations for seven biometrical traits *viz.*, days to 50 % flowering (DAF), plant height (PH), panicle length (PL), panicle weight (PW), 1000-grain weight (TW), spikelet sterility (SS) and single plant yield (SPY). The mean, range, and co-efficient of variation were calculated as per standard statistical procedures.

RESULTS AND DISCUSSION

Various mutants with a wide range of traits like yield, reduced plant height, disease resistance, and early maturation have been isolated and utilized in many breeding programmes. Since 1960 as many as 3000 mutant varieties have been officially released in over 60 countries (Jain 2010). Rice stands first, among crops, with 700 mutant varieties followed by barley, wheat and maize. Mutation induction with radiation has been the most frequently used method to develop direct mutant varieties, accounting for about 90% of varieties produced (1411), of which 64% by gamma rays (910) and 22 % by X-rays (Jain 2010). In the present investigation, Pokkali and Improved White Ponni were irradiated with γ rays to widen the variability for seven yield and its components besides retaining salt tolerance and improving agronomic traits in Pokkali and retaining grain quality and improving salt tolerance in Improved White Ponni. The dose of a mutagen that achieves the optimum mutation frequency with the least possible unintended damage is regarded as the optimal dose for induced mutagenesis (Mba *et al*. 2010). In the present study, optimal dose was estimated, using LD_{50} assay, to be 227.06 Gy for Improved White Ponni and 267.66 Gy for Pokkali indicating radio-sensitivity is related with grain boldness. Slender the grain, as in Improved White Ponni, more the sensitivity and bolder the grain, as in Pokkali, lesser the sensitivity.

For most of the quantitative characters, the range of variation induced by the mutagenic treatments was wider than control in many treatments. Induced mutagenesis, through γ-irradiation generated substantial magnitude of genetic variability for all the seven traits studied in M_2 and M_3 generations (Table 1).

For days to flowering, the general population mean in M_2 were higher in Pokkali mutants at 200 and 300 Gy when compared to untreated control indicating

Table 1. Effect of γ -irradiation on yield and its components in M_2 and M_3 generation of Pokkali and Imp. White Ponni

Sl. no.	Character	Variety	Parameter	Control		Doses of gamma rays			
						200Gy		300Gy	
				M_2	M_3	M_2	M_3	M^2	M_3
1	Days to flowering	*Pokkali*	Mean	85.20	84.30	88.74	83.25**	93.03**	81.33
			Range	84-89	80-83	70-98	77-90	82-108	78-83
			C.V	3.50	1.50	5.89	5.65	6.16	3.17
		Imp.-White-Ponni	Mean	113.80	103.80	104.15**	85.83**	109.80**	86.92
			Range	112-115	102.00-103.00	80.00-125.00	77-100	70.00-135.00	83-89
			C.V	1.14	1.14	10.76	4.65	8.56	2.79
2.	Plant height	Pokkali	Mean	153.10	149.20	128.60	96.92**	152.10	155
			Range	150.00-155.00	145.00-151.00	62.00-161.00	70-140	117.00-190.00	150-161
			C.V	1.41	0.99	18.65	30.35	7.88	2.97
		IWP	Mean	55.20	132.20	103.80	77.80**	124.40	107.50**
			Range	152.00-155.00	130.00-135.00	60.00-150.00	40-141	80.00-151.00	79-140
			C.V	1.49	1.79	17.38	27.00	13.19	17.12
3.	Panicle length	Pokkali	Mean	30.94	28.04	26.54	23.08**	24.23	23.22**
			Range	30.10-32.50	26.30-31.50	18.70-33.00	20.70-26.50	17.50-33.00	21.00-26.90
			C.V	3.00	2.76	12.29	8.78	15.95	9.69
		IWP	Mean	24.34	22.34	21.61	21.02**	21.82	21.67**
			Range	23.52-25.30	21.23-25.14	14.30-28.20	17.3-25.0	14.30- 26.30	14.0-26.3
			C.V	2.68	1.87	12.59	7.95	11.76	16.75
4.	Panicle weight	Pokkali	Mean	3.90	3.42	2.80	2.19*	2.39	2.04*
			Range	3.88-3.95	3.23-3.65	0.95-5.25	1.09-3.50	0.65-5.75	1.65-3.01
			C.V	0.71	0.71	38.47	42	49.12	24.51
		IWP	Mean	3.91	2.69	3.31	2.21*	1.98	1.89*
			Range	3.89-3.95	2.45-2.95	0.61-1.73	1.37-3.75	0.89-4.44	0.99-4.43
			C.V	0.58	0.58	41.95	26.00	41.80	51.99
5.	1000 grain weight	Pokkali	Mean	25.80	25.20	29.03	21.15**	32.31	23.13**
			Range	17.60-26.80	23.87-27.80	10.60-30.30	16.50-28.20	13.24-29.8	20.50-26.50
			C.V	1.63	1.08	19.16	20.94	19.12	8.39
		IWP	Mean	17.29	17.11	18.85	20.83**	17.74	17.96**
			Range	17-17.56	16.50- 17.56	10-29.20	3.0-29.20	13- 34.20	13.40-20.50
			C.V	0.92	1.23	17.64	22.73	17.06	11.09
6.	Spikelet sterility	Pokkali	Mean	5.86	5.86	12.09	24.13**	14.81	24.96**
			Range	5.49-6.69	5.49-6.69	0.00-35.18	7.19-37.30	2.14-33.33	16.00-30.60
			C.V	2.29	2.29	68.07	34.44	34.26	22.52
		IWP	Mean	54.82	54.82	28.56	24.02**	22.76	20.87**
			Range	53.2-54.6	53.2-54.6	4.16-60.52	5.50-53.90	5.81-73.16	2.23-35.48
			C.V	2.09	2.09	39.97	51.59	43.82	47.54
7.	Single plant yield	Pokkali	Mean	23.41	28.41	12.29	15.20*	10.83	26.93**
			Range	22.41-23.41	26.41-29.41	3.39-31.55	11.55-18.91	1.49-39.12	13.24-33.00
			C.V	0.74	0.74	48.82	12.97	62.23	27.63
		IWP	Mean	37.41	29.41	14.80	16.60**	9	17.63**
			Range	20.56-23.49	20.56-31.23	1.19-44.21	7.85-34.77	1.54-20.18	12.23-32.33
			C.V	3.12	1.43	58.9	38.23	45.05	29.38

*Significance at 5% level, ** Significance at 1% level

general lateness in flowering. However, in M_3 generation of Pokkali at 200 Gy and 300 Gy, the general population means were lower than the control indicating earliness in flowering. In Improved White Ponni population mean in M_3 at 200 Gy and 300 Gy was reduced, while in M_2 at both doses it was similar to control. The coefficient of variation was high at 200 Gy in both generations. As Improved White Ponni matures at 135-140 days, mutants maturing earlier at 105-110 days were indeed desirable. Few early mutants, identified in all treatments in both the generations, offer scope for selection. This is in contrast to Pokkali which is already an early maturing one and therefore, presents less scope for selection.

Plant height in all the treatments showed a negative shift from the control in both the varieties.

The coefficient of variation was higher in all the treatments. As Pokkali and Improved White Ponni are tall statured they are prone to lodging and therefore, semi-dwarf mutants in these varieties would be desirable. Observance of few mutants with semi-dwarf nature in both varieties offers scope for selection (Figure 1 and 2).

The mean values for panicle length showed negative directions in all the treatments in Pokkali as well as in Improved White Ponni. The coefficient of variation increased significantly over the control in all the treatments in both the varieties. There is shift in the mean values for panicle weight in the negative direction in all doses in both the varieties. Similarly, the coefficient of variation was maximum at 300 Gy and 200 Gy in Pokkali. The same trend was observed in Improved White Ponni too.

Fig. 1. Pokkali semi dwarf mutanta (compared with untreated partent)

Fig. 2. Improved white ponni mutants varying for height and maturity(compared with untreated parent)

While, Pokkali bears bold grain and red kernel colour, mutants with slender grain with white kernel colour is desirable. However, Improved White Ponni mutants with test weight of 17g (1000-grain weight of Improved White Ponni) or less, in the background of semi-dwarfism and earliness are desirable. Shifts in mean for 1000-grain weight were observed bi-directionally in both the varieties in all the mutagenic treatments. Observance of Pokkali mutants with reduced test weight and white kernel colour, though very few in number, offer scope for selection (Figure 3). In Improved White Ponni, wide variability for grain and kernel characteristics was observed (Figure 4). The coefficient of variation was highest over the controls in all the treatments. Spikelet sterility, a reliable measure of salt tolerance, was observed to be widely varying in different doses in both the varieties. Pokkali, a salt tolerant variety, exhibited positive shift than its control while, Improved White Ponni, a salt susceptible variety, showed negative shift than the control. It indicates salt tolerance has been improved in some of the mutants of Improved White Ponni. Similarly, wide coefficient of variation was observed in all doses in both varieties. There was a significant decrease in the mean value for yield per plant in all the treatments in both the two cultivars. The coefficient of variation also increased significantly over the control.

Similar results were observed by various workers in earlier studies for days to flowering and plant height (Oka *et al*. 1958 and Shaini and Sharma 1970), panicle length and panicle weight (Mallick *et al*. 1979), 1000-grain weight and grain yield (Amirthadevarathinam *et al*. 1970) and pericarp colour (Beachel 1957). The observed variance for various characters was far greater in magnitude than the respective control in most of the treatments. Increase in variance in quantitative characters was observed earlier following mutagenic treatments in rice (Oka *et al.* 1958 and Jana and Roy 1973). Further Sharma (1994) opined that one of the important aspects of changes in flowering due to mutagenesis is the correlated response for many traits with early flowering, such as yield (generally reduced), plant height (generally shortened), panicle length, test weight, tillers, straw-stiffness and protein content (all changed).

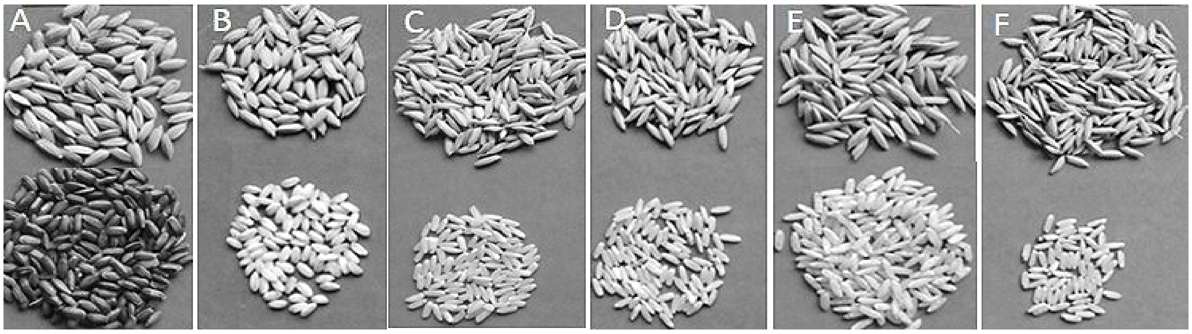

Fig. 3. Variability in *Pokkali* mutants for grain characterstics(A-*Pokkali*, B-F Mutants)

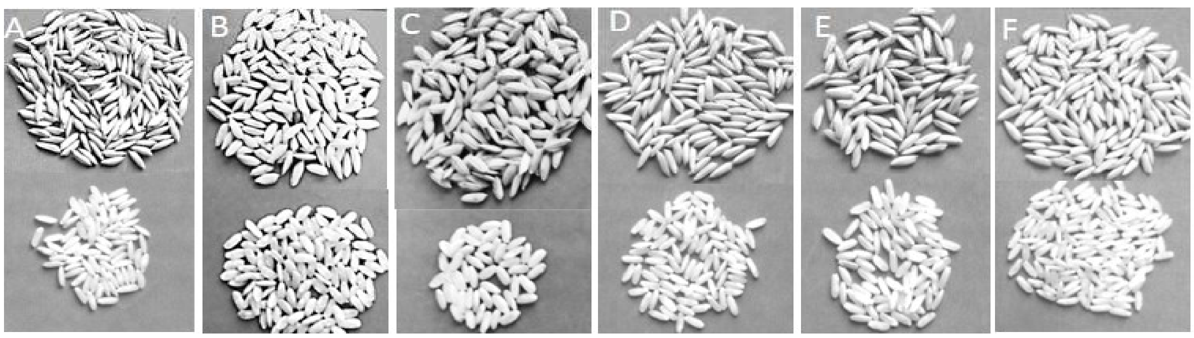

Fig. 4. Variability in *Improved White Ponni* mutants for grain characterstics (A-*IWP*, B-F Mutants)

Several mutants to abiotic stresses have been produced and are serving either directly or indirectly for crop improvement. Many of these mutants were developed using physical mutagens specifically γ - irradiation. Of 160 mutant varieties developed, for various abiotic stresses, till 2013, 36 were for salt tolerance and five were in rice (Suprasanna *et al*. 2015)

In the present study, all the mutagen treated populations produced mutants with extreme values in respect of all characters and such a widened variability would offer ample scope of selection for improvement of yield and its components under salinity. Observance of few numbers of Pokkali mutants with semi-dwarfism, slender grain, white kernel colour and Improved White Ponni mutants with semi-dwarfism, early maturity, slender grain, in the background of salt tolerance, proved the utility of induced mutagenesis in rice for these traits

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