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In vitro screening for salt tolerance in aromatic rice genotypes

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Abstract

Dehusked seeds of ten aromatic rice varieties along with a control variety Pokkali were placed on MS media supplemented with NaCl, Na_2SO_4 and KCl salts at four different levels (0%, 0.2%, 0.4% and 0.6%) to observe the callus induction and plant regeneration responses. For callus induction MS medium was supplemented with 2 mg/L 2,4 D while for plant regeneration MS medium was supplemented with 1 mg/L NAA and BAP. All the genotypes showed significant variation in callus induction and plant regeneration responses. Callus induction and plant regeneration percentage decreased with the increase of salt doses. Shakkhorkhora appeared as the best variety in terms of plant regeneration in most of the cases at different levels of the salts (0-0.6%) while Basmati showed its inferiority for callus induction and plant regeneration. It was concluded that salt tolerant aromatic rice varieties can be obtained employing salt stress in *in vitro* condition.

Keywords

Aromatic Rice, Salt Stress, Somaclonal Variation

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops, which supplies food for more than half of the world's population (Tyagi et al., 2004). The demand for rice is continuously growing with the increasing population, thus genetic improvement of important rice varieties have been targeted.

But rice production is very much suffering from severe damage of biotic and abiotic stresses. Biotic stress and abiotic stress breeding are essential ways to combat yield reduction. Scientists around the world are putting in their best efforts to produce varieties under stress affected environments (Takehisa et al. 2004).

Attempts to improve the salt tolerance of rice through conventional breeding programs have met with very limited success. At present *in vitro* technique of plant from cell or tissue is an important and essential component of biotechnology, which is required for the genetic manipulation of crops. Soil salinity is one of the major environmental stresses affecting plant growth adversely. Cell and callus cultures are the ideal systems in assessing the physiological effects of salts at the cellular level (Blumwald and Grover, 2006).

Efficient plant regeneration through in vitro micropropagation is very essential for the successful utilization of biotechnology in rice crop improvement (Hoque et al., 2007). The identification and screening of useful cultivars for embryogenic callus formation and subsequent plant regeneration through in vitro system is a vital step in rice genetic improvement programme (Hoque and Mansfield, 2004; Islam et al., 2005). In rice, the use of mature seed embryos has distinct advantage over other explants as staring material for in vitro regeneration. Successful application of *in vitro* techniques of somatic tissue offers a wide scope of achieving somaclonal variants in regenerated plants from the rice calli.

Initial salt stress in the medium has also been reported to

improve the frequency of somatic embryogenesis and plantlet regeneration (Ping *et al.* 2006 ,Tariq *et al.* 2008). It is possible that such plantlets can be adapted to a saline environment resulting in enhanced crop production (Rafique et al., 2011).

Several local aromatic rice varieties are present in Bangladesh but their *in vitro* evaluation at different salinity levels has not yet been studied. Henceforth, the present study was undertaken to find out the *in vitro* response of different aromatic rice varieties under different salt stress.

2. Materials and Methods

Ten Bangladeshi aromatic rice varieties along with a salt tolerant variety Pokkali as control were used in the experiment (Table 1).

Table 1. *List of materials used in the experiment*

Sl. No.	Varieties
1	Pokkali
2	Shakkhorkhora (SK)
3	Kataribhog (KB)
4	Basmati
5	Kalijira (KJ)
6	Chinishakkhor (CS)
7	Uknimodhu (UM)
8	Jirabhog (JB)
9	Badshahbhog (BB)
10	Rajbhog (RB)
11	BR5

2.1. Surface Sterilization and Callus Initiation

Mature dehusked seeds were surface sterilized with 0.1% $HgCl_2$ for 20 minutes followed by 4-5 rinse in autoclaved distilled water to remove traces of $HgCl_2$. The seeds were then cultured on MS (Murashige and Skoog 1962) medium supplemented with 2 mg/L 2,4 D, 30 g/L sucrose, 10% agar and different levels of NaCl salt (0, 0.2, 0.4 and 0.6%) to initiate callus.

2.2. Plant Regeneration

After 2 to 3 weeks of inoculation, calli were developed and transferred to MS regeneration medium supplemented with 1 mg/L NAA, 1 mg/L BAP, 30 g/L sucrose, 10% agar and above mentioned different levels of salts. Cultures were maintained in a growth chamber under 16h photoperiod with a light intensity of 2000 lux under fluorescent tubes and proliferation of callus as well as frequency of plant regeneration were studied.

2.3. Establishment of Plantlets

After 4 weeks of growth, the regenerated plantlets were washed under running tap water to remove agar and were transplanted to pots containing soil and cowdung in 2:1 ratio. Plantlets were covered with polythene bags. Inner side of these bags was moistened with water to prevent desiccation. After two days polythene cover was perforated to expose the plants under natural environment. Finally, the polythene bags were completely removed after 4-5 days. When the regenerated plants were fully established in the small pots, they were then transferred to larger pots for further growth and to get seeds from those regenerated plants.

2.4. Statistical Analysis

The Analyses of Variance for different characters such as callus induction and plant regeneration were performed and means were compared by the Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

In the present study, *in vitro* technique for callus induction and plant regeneration on NaCl, Na_2SO_4 and KCl salt supplemented media was established using seed of different indigenous Bangladeshi aromatic rice varieties as explants. Effect of the salts on callus induction and plant regeneration was also investigated. The results are elaborated based on the nature of morphogenetic response of genotypes, salt and salt concentrations and their interaction.

The percentage callus induction and plant regeneration was found to decline with the increase of salt concentrations (Fig.1). This finding agrees with that of Dang and Lang (2003) who reported that an increase in salt concentration resulted decreased shoot and root formation, which establishes the deleterious effect of salt on plant growth.

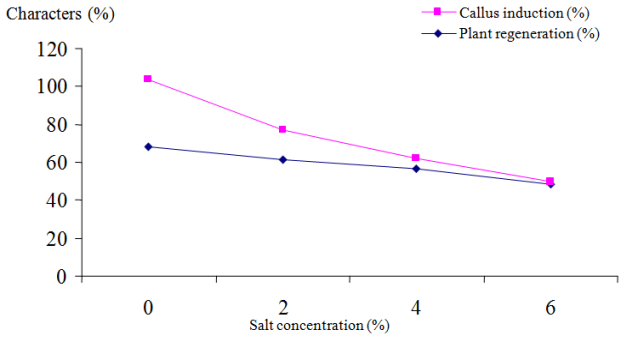

Fig. 1. *Mean effect of different salt concentration on callus induction (%) and plant regeneration (%) in test variety*

3.1. Varietal / Genotypic Difference

The experiment was conducted to find out whether the genotypes show any differences in terms of callus induction and plant regeneration on different salts supplemented media. The mean performances of the varieties are summarized in Fig. 2.

Highly significant variations were observed among the varieties. The highest callus induction was found in Zirabhog (79.13%) which was statistically similar to Pokkali (78.79%) followed by Shakkhorkhora (77.78%). The lowest response was observed in Bashmoti (19.96%).

In terms of plant regeneration, the best performer was Pokkali (25.83%) which was used as control. Shakkhorkhora (19.15%) had the second highest response in terms of plant

regeneration while the lowest response was observed in Basmati (5.778%).

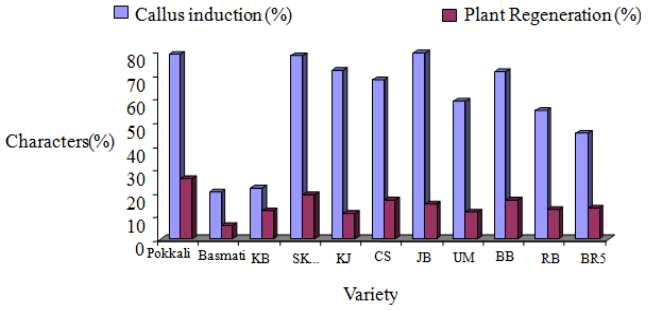

Fig. 2. *Combined mean effect of variety on callus induction (%) and plant regeneration (%)*

So, Shakkhorkhora appeared as a very good variety (Fig 2.) in terms of callus induction and plant regeneration response.

Based on the above findings, it was clear that all the genotypes showed significant variation in callus induction and plant regeneration responses under salt stress. Aditya and Baker (2005) found significant variation among four indigenous Bangladeshi aromatic rice varieties under NaCl stress.

3.2. Effect of Salt Concentrations on Different Genotypes

The interaction between the variety and salts were found statistically significant for callus induction and plant regeneration which is presented in Table 2.

Table 2. *Callus induction and plant regeneration frequency of rice genotypes at different salt concentrations*

Varieties	Callus induction (%)				Plant regeneration (%)			
	Salt concentration (%)				Salt concentration (%)			
	0	0.2	0.4	0.6	0	0.2	0.4	0.6
Pokkali	87.50 a	81.89 b	74.94 de	70.83 f	46.67 a	26.67 g	16.67 j	13.33 m
Basmati	29.86 s	23.61 t	19.44 u	6.941 w	16.67 j	5.333 q	1.111 t	0.00 u
Kataribhog	31.25 s	23.61 t	19.44 u	13.89 v	30.00 f	15.56 k	2.222 s	0.00 u
Shakkhorkhora	87.50 a	83.33 b	76.39 d	63.89 h	43.28 b	24.44 h	8.889 o	0.00 u
Kalijira	79.17 c	73.61 e	69.44 f	64.00 h	26.67 g	11.11 n	5.556 q	0.00 u
Chinishakkhar	79.17 c	73.61 e	69.44 f	64.00 h	40.00 c	18.89 i	8.889 o	0.00 u
Jirabhog	87.50 a	83.33 b	79.00 c	66.67 g	40.00 c	18.89 i	2.222 s	0.00 u
Uknimodhu	66.67 g	61.11 ij	56.94 l	50.00 n	36.67 d	6.667 p	2.222 s	0.00 u
Badshahbhog	87.50 a	70.83 f	66.67 g	59.72 jk	40.00 c	18.94 i	8.889 o	0.00 u
Rajbhog	62.50 hi	58.33 kl	54.17 m	44.44 p	40.00 c	8.889 o	1.111 t	0.00 u
BR5	54.17 m	47.22 o	41.67 q	37.50 r	33.33 e	14.44 l	3.333 r	1.111 t

Table followed by the same letter in a column do not differ significantly by DMRT.

The highest callus induction was recorded in Pokkali, Shakkhorkhora, Jirabhog and Badshahbhog on the control media (salt-free media). The lowest callus induction was recorded in Basmati (6.941%) on 0.6% salt supplemented media. It should be mentioned here that with the increase of salt doses, the callus induction showed decreasing trend for the tested varieties.

According to the Table 2, the highest plant regeneration was observed in Pokkali (46.67%) on the control media. No plant regeneration was observed in Basmati, Kataribhog, Shakkhorkhora, Kalijira, Chinishakkhor, Jirabhog, Uknimodhu, Badshahbhog and Rajbhog at 0.6% salt concentration.

The results of Table 2 have the similar findings with the experiment of Pushpam R and Rangasamy (2000). They found that in a low concentration of salt-supplemented media, all tested cultivars developed normally, but the cultivars which responded poorly were from the media supplemented with high levels of salt. This obviously suggests that salt concentration inhibits callus initiation and plant regeneration as well.

3.3. Interaction among the Varieties, Salt and Salt Concentrations

The interaction among the varieties, salt and salt concentrations were found highly significant for callus induction and plant regeneration. Responses to variety x salt x salt concentration interaction for the character, such as callus induction (%) is presented in Fig. 3, 4 and 5 and plant regeneration (%) in Table 3.

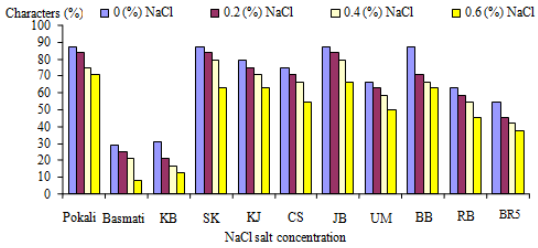

Fig. 3. *Combined effect of variety x NaCl salt x salt concentration on % callus induction in test variety*

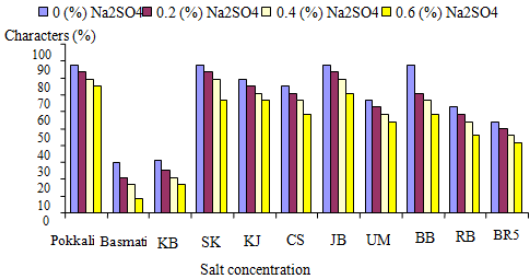

Fig. 4. *Combined effect of variety x Na_2SO_4 x salt concentration on % callus induction in test variety*

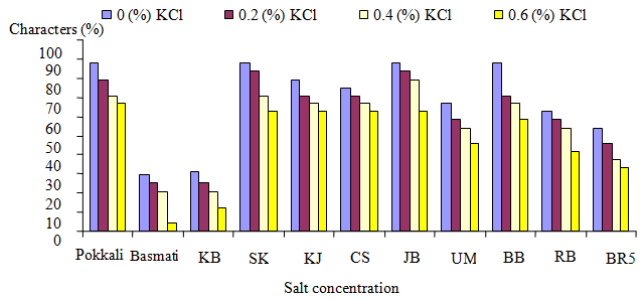

Fig. 5. *Combined effect of variety x KCl salt x salt concentration on % callus induction in test variety*

Highest callus induction (87.50%) was recorded in Pokkali which is statistically similar to the varieties Shakkhorkhora, Jirabhog and Badshahbhog at control treatment while the lowest response was observed in Basmati (4.16%) at 0.6% KCl (Fig. 5.)

Plant regeneration was highest (46.67%) with the interaction of Pokkali on medium without salts. Shakkhorkhora (43.33%) gave second highest response followed by Chinishakkhor (40.00%), Jirabhog (40.00%), Badshabhog (40.00%) and Rajbhog (40.00%) varieties with control treatment (without salt). No plant regeneration was observed with most of the varieties at 0.6% salt concentration of NaCl, Na2SO4 and KCl except Pokkali and BR5. Similar response was found in Basmati and Rajbhog at 0.4% of NaCl; Kataribhog and Uknimodhu at 0.4% of Na2SO4; Basmati, Jirabhog, Uknimodhu, Rajbhog and BR5 at 0.4% of KCl salt.

From the above result it may be concluded that Shakkhorkhora had very good response while checking the interaction of variety x salt x salt concentration.

Table 3. *Plant regeneration (%) frequency of test variety with different salt concentration*

Variety	Without salt	NaCl			Na_2SO_4			KCl		
		2.0 g/L	4.0 g/L	6.0 g/L	2.0 g/L	4.0 g/L	6.0 g/L	2.0 g/L	4.0 g/L	6.0 g/L
Pokkali	46.67a	26.67g	16.67 j	13.33k	30.00 f	16.67 j	13.33 K	23.33 h	16.67 j	13.33k
Basmati	16.67j	6.333m	0.00 o	0.00o	6.333 m	3.333 n	0.00o	3.333 n	0.00o	0.00o
Kataribhog	30.00f	16.67j	3.333n	0.00o	20.00 i	0.00o	0.00o	10.00 l	3.333 n	0.00o
Shakkhorkhora	43.33b	23.33h	10.00 l	0.00o	30.00 f	6.667m	0.00o	20.00 i	10.00 l	0.00o
Kalijira	26.67g	10.00l	3.333 n	0.00o	6.667 m	3.333 n	0.00o	16.67 j	10.00 l	0.00o
Chinishakkhor	40.00c	26.67 g	10.00 l	0.00o	20.00 i	10.00l	0.00o	10.00 l	6.667 m	0.00o
Jirabhog	40.00c	20.00 i	3.333 n	0.00o	10.00 l	3.333 n	0.00o	26.67 g	0.00o	0.00o
Uknimodhu	36.67d	10.00 l	6.667 m	0.00o	6.667 m	0.00o	0.00o	3.333 n	0.00o	0.00o
Badshahbhog	40.00c	20.00i	10.00 l	0.00o	26.83g	10.00 l	0.00o	10.00l	6.667 m	0.00o
Rajbhog	40.00c	10.00 l	0.00o	0.00o	13.33 k	3.333 n	0.0000 o	3.333 n	0.00o	0.00o
BR5	33.33e	13.33 k	3.333n	0.00o	23.33 h	6.667 m	3.333n	6.667 m	0.00 o	0.00o

4. Conclusion

It is evident that different salt concentrations play a vital role in callus induction and plant regeneration as well. On an average, all the parameters were arrested at high level of salt concentration (0.6%) and the magnitude of the parameters callus induction and plant regeneration was maximum at 0% salt supplemented media. The results are in agreement with the experiment of Zinnah et al., (2013) where they found that the value of parameters declined with the increase of salinity levels. In conclusion, it can be said that salt-tolerant somaclonal variants can be obtained in rice and the progenies of some of the Bangladeshi aromatic rice varieties can also be utilized in developing salt tolerant rice varieties.

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