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Seed Culture of Aromatic Rice Varieties Under Salt Stress

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Abstract

Dehusked seeds of five aromatic rice varieties were placed on MS medium supplemented with NaCl and Na_2SO_4 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 presented significant variation in callus induction and plant regeneration responses. Callus induction and plant regeneration percentage decreased with the increase of salt doses. Among the tested varieties, in most of the cases, Kalijira performed the best both for callus induction and plant regeneration at different levels of salts (0 - 0.6%). It was concluded that salt tolerant aromatic rice varieties can be evolved applying salt stress in *in vitro* condition.

Keywords

Callus Induction, Plant Regeneration, Salinity, Somaclonal Variation

1. Introduction

Saline soils are one of the major biotic stresses that adversely affect the overall metabolic activities and cause plant demise (Blumwald and Grover, 2006). Production capabilities of certain crops are reported to be reduced in saline conditions (Anbazhagan *et al.,* 2009). Agricultural land use in these areas is very poor. Whereas the world's population continues to rise, the total land area under irrigation appears to have leveled off. The need for increased food production therefore needs to be met by increase in yield per unit area. To reach this goal, application of biotechnology into the crop plants for enhanced salt tolerance will be an important approach. However, saline soils represent a massive and increasing challenge to the plant breeder and agronomist in many parts of the world. Crop plants selected for increased tolerance to saline environments should permit the use of those salt affected soils. Traditional breeding strategies might be supplemented by the production of plants with improved salt tolerance through selection of the salt tolerance by in vitro culture and subsequent regeneration of such cells in the presence of salt to drive plants with improved salt tolerance (Ping *et al.,* 2006, Tariq *et al.,* 2008).

Rice seed culture is an important tool for crop improvement program, which is widely used in variety development programs. It offers many advantages to rice breeders because of their shortened breeding periods and high efficiency in selecting useful recessive agronomic traits. 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 salts stress.

2. Materials and Methods

Five Bangladeshi aromatic rice varieties namely Lalsoru, Kalobhog, Kaminisoru, Kalijira and Jirabhog were used in the experiment.

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 medium (Murashige and Skoog 1962) supplemented with 2mg/L 2, 4 D, 30 g/L sucrose, 10% agar and different levels of NaCl and Na_2SO_4 salts (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 polyethylene bags. Inner sides of these bags were moistened with water to prevent desiccation. After two days polythene cover was perforated to expose the plants under natural condition. 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 techniques for callus induction and plant regeneration have been established very carefully using seeds as explants of five Bangladeshi varieties of aromatic rice. The effect of two different salts (NaCl and Na_2S0_4) on callus induction and plant regeneration of the variety was also investigated. The results are elaborated based on the nature of morphogenic response of varieties, salts and salt concentrations and their interaction.

3.1. Effect of Different Salts

The results on the effect of different salts are presented in Table 1.

The highest callus induction (50.17%) and plant regeneration (30.83%) were observed in the medium containing Na_2S0_4 and the lowest callus induction (45%) and plant regeneration (27%) was observed in the NaCl containing medium.

Table 1. *Mean effect of different salts on callus induction (%) and plant regeneration (%) in aromatic rice genotypes.*

Treatment		Callusinduction (%)	Plant regeneration (%)
Salt	NaCl	45.00b	27.00b
	Na_2SO_4	50.17a	30.83a

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

Callus induction and plant regeneration were least affected in the Na_2SO_4- stressed medium while comparing with NaCl containing medium. Among the two salts, Na^+ is common in NaCl and Na_2S0_4. It thus appears that CI^- containing salts are inhibitorier in in vitroresponse. According to the Table 1, NaCl causes more damage to callus induction and plant regeneration. The results have the similar findings with the experiment of Adil *et al.,* (2009). They found that while comparing with the Na_2S0_4 supplemented media the tested parameters were worse affected when the medium was supplemented with NaCl salt.

3.2. Effect of Salt Concentrations on Different Genotypes

Different concentrations of salts showed significant variations for all the characters studied. Combined effect of both the NaCl and Na_2SO_4 salt concentrations are summarized in Fig. 1.

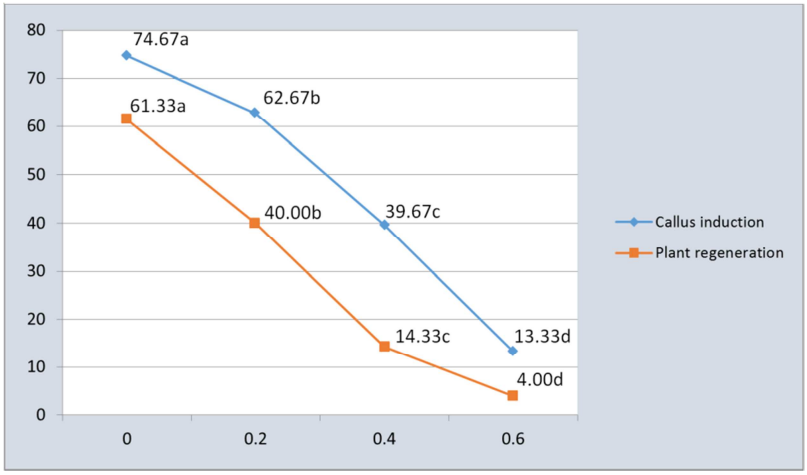

Fig. 1. *Mean effect of different salt concentrations on callus induction (%) and plant regeneration (%) in aromatic rice genotypes.*

The percentage of callus induction (74.67%) and plant regeneration (61.33%) was the highest in MS medium supplemented with no salt and it was the lowest (13.33%, 4.00% respectively) in 0.6% salt concentration. So, it is clear that the percentage of callus induction and plant regeneration was found to decline with the increase of salt concentrations. The decreasing trend with the increase of salt concentrations was also reported by Hasan and Sarker (2013) and Aditya *et al.,* (2005).

3.3. Differential Genotypic Responses

The experiment was conducted to find out whether the genotypes show any differences in terms of callus induction and plant regeneration on both the NaCl and Na_2SO_4 salts supplemented media. The mean performances of the genotypes are summarized in Table 2.

Table 2. *Mean effect of different aromatic rice genotype on callus induction (%) and plant regeneration (%).*

Genotype	Callus induction (%)	Plant regeneration (%)
Lalsoru	40.42d	32.92a
Kalobhog	44.59c	29.17b
Kaminisoru	31.25e	29.39 b
Kalijira	69.17a	30.83ab
Jirabhog	52.50b	22.50c

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

Highly significant variations were observed among the genotypes. The highest callus induction response was found in Kalijira (69.17%) followed by Jirabhog (52.50%). The lowest callus induction was observed by Kaminisoru (31.25%). Shavindra and Amitabh *et al.,* (2005) reported that callus induction frequency varied depending on the cultivar, which is in agreement with the above findings.

The highest plant regeneration was observed in Lalsoru (32.92%) which was at par with Kalijira (30.83%), while Jirabhog (22.50%) showed the lowest plant regeneration response. Zahid *et al.,* (2014) also found genotypic variations and effects in terms of in vitro plant regeneration.

3.4. Effect of Salt on Different Aromatic Rice Genotypes

Effect of genotype and salt interaction combinedly for 0%, 0.2%, 0.4% and 0.6% NaCl and Na_2SO_4 supplemented media was analyzed both for callus induction and plant regeneration.

Effects of genotype x salt interaction on different characters are presented in Fig. 2.

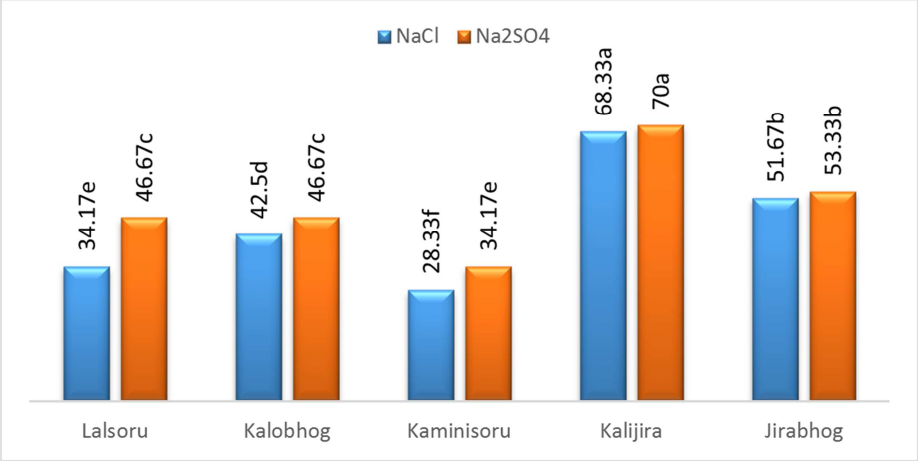

Fig. 2. *Combined effect of different aromatic rice genotypes and salts on callus induction (%).*

The highest callus induction (70%) was recorded in Kalijira with Na_2S0_4 salt which was statistically similar to Kalijira (68.33%) with NaCl salt. Lalsoru (46.67%) and Kalobhog (46.67%) gave medium response with Na_2S0_4. The lowest callus induction response (28.33%) was observed in Kaminisoru with NaCl. Genotype x salt interaction was observed by S*arker* (2010) where different rice cultivars responded differently to various levels of NaCl and Na_2S0_4salt.

The highest plant regeneration (36.67%) was recorded in Lalsoru with Na_2S0_4salt which is significantly higher than other genotypes (Fig. 3). Kalobhog (26.67%) gave medium response with NaCl salt which was statistically similar to Kaminisoru (26.37%) with NaCl salt. The lowest plant regeneration response (21.67%) was observed in Jirabhog with NaCl. Adil *et al.,* (2009) found similar results where the best performer variety in terms of callus induction and plant regeneration was found on Na_2S0_4 supplemented media.

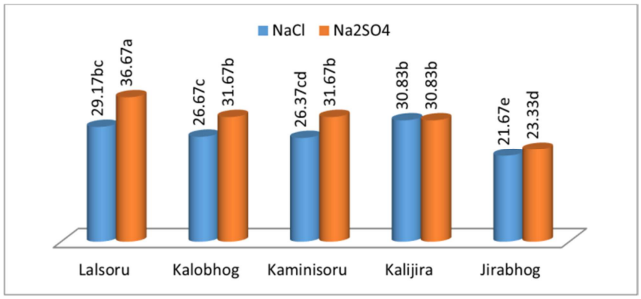

Fig. 3. *Combined effect of different aromatic rice genotype and salt on plant regeneration (%).*

3.5. Effect of Salt Concentrations on Different Aromatic Rice Genotypes

The interaction of genotype x salt concentration on the test parameters are presented in Fig. 4 and Fig. 5.

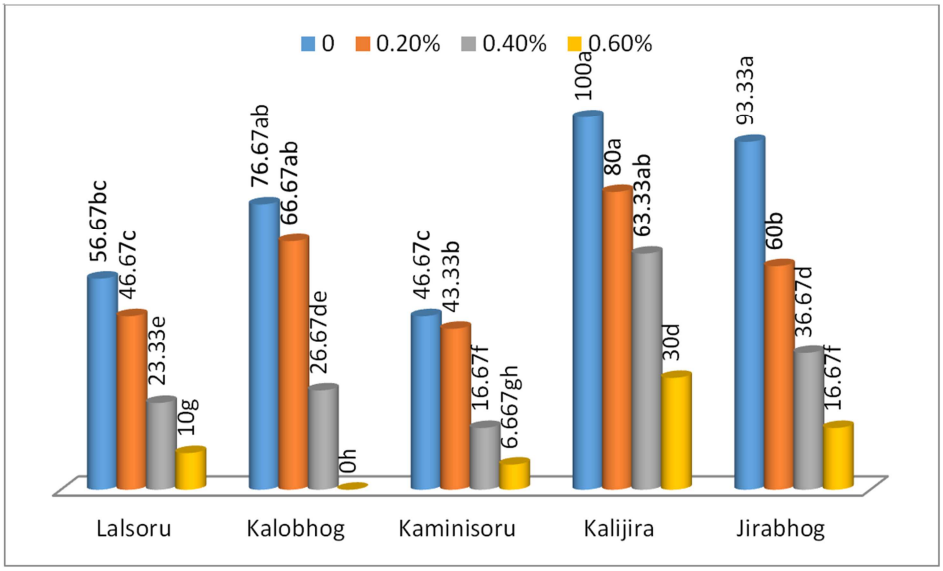

Fig. 4. Combined effect of different genotypes and salt concentrations on callus induction (%) in NaCl salt.

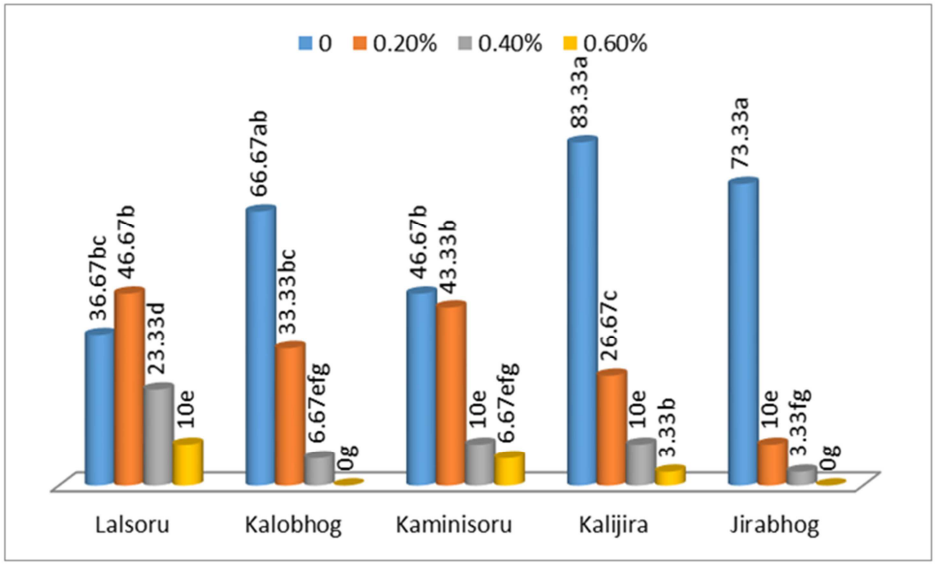

Fig. 5. Combined effect of different genotypes and salt concentrations on plant regeneration (%) in NaCl salt.

The highest callus induction (100%) was recorded in Kalijira with 0% salt supplemented medium which is statistically similar with Jirabhog (93.33%) at 0% salt and Kalijira (80%) with 0.2% salt (Fig. 4). Kalobhog (76.67%) with 0% salt, Kalobhog (66.67%) with 0.2% salt, Kalijira (63.33%) with 0.40% salt gave similar response. The lowest callus induction was observed in Kalobhog (0%) with 0.6% salt, which is statistically similar with Kaminisoru (6.67%) with 0.6% salt.

The highest plant regeneration (73.33%) was recorded in Jirabhog with 0% salt supplemented medium which is statistically similar with Kalijira (83.33%) at 0% salt and Kalobhog (66.67%) with 0% salt (Fig. 5). The lowest plant regeneration was observed in Kalobhog (0%) with 0.6% salt, which is statistically similar with Kaminisoru (6.67%) with 0.6% salt, Kalijira (3.333%) with 0.6% salt, Jirabhog (0%) with 0.6% salt.

The results of Fig. 4 and Fig. 5 have the similar findings with the experiment of Dang and Lang (2003) and Sarker (2010). They found that in a low concentration of salt supplemented media all tested cultivars developed normally, but the cultivars which responded poorly, they were from the media supplemented with high levels of salt. This obviously suggests that salt concentration inhibits callus initiation and plant regeneration as well.

4. Conclusion

The results obtained in the present study confirm that salt has a profound effect on callus cultures and shoot regeneration. The percentage of survival calli and shoot regeneration decreased with increasing degrees of salt stress. On an average, all the parameters were arrested at high level of salt concentration and the magnitude of the parameters was maximum at 0% salt supplemented medium. The results are in agreement with the experiment of Kumari *et al.,* (2015) where they found that the value of parameters declined with the increase of salinity levels. While comparing both the NaCl and Na_2SO_4 salts, it was clear that NaCl causes more damage to callus induction and plant regeneration. In conclusion, it can be said that salt - tolerant somaclonal variants can be obtained in aromatic rice in *in vitro* condition.

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