Research Article

In vitro screening for salt tolerance in Rice (*Oryza sativa*)

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Abstract

An experiment was conducted to study the performance of rice genotypes for salt tolerance under *in vitro* condition. Seven genotypes include salt tolerant (Pokkali, CSR 10, TRY 1 and TRY2), moderately tolerant to salt (White Ponni and BPT 5204) and susceptible (IR 29) were used for this study through embryo culture technique. Callus was initiated in MS medium + 2 mgl^{-1} 2,4D + 0.5 mgl^{-1} Kin and different concentration of Nacl *viz.,* 50mM, 100mM and 150mM were added with the medium to create salt stress. Statistical analysis revealed that all the genotypes and treatments and their interaction effect were significantly different from each other. Among the seven genotypes studied Pokkali was significantly superior for callus induction with 62 % followed by CSR 10, TRY(R) 2 and TRY1 with 58 %, 54.5 % and 53 % respectively. Pokkali registered the highest level of callus development (35%) even at high level of Nacl stress (150mM) followed by TRY 2 (25%) and CSR 10 (24%). When the callus for these genotypes were transferred to regeneration media in the same level of Nacl stress (50mm, 100mM and 150mM) highest level of regeneration of green islets was realized in Pokkali (37.5 %) followed by CSR 10 (31 %) and TRY (R)2 (25 %). From these observation, it was concluded that somaclones obtained form the varieties of Pokkali, CSR 10 and TRY(R) 2 could be evaluated further in the natural field condition to develop a high yielding salt tolerant varieties or could be used as a donor for the development of salt tolerant varieties. This *in vitro* screening technique with different concentration of Nacl stress could also be used as a goof screening technique for salt tolerances rather than the field screening because of less time, little space and less labour and the results also very accurate than the field screening.

Key words: *In vitro*, callus induction, regeneration, Nacl, salt screening.

Introduction

Salinity is widespread soil problem in rice growing countries (Senadhira 1987). The need for the improvement of salt tolerance in rice is well documented (Flower and Yeo 1995). Breeding for salt tolerance in rice is difficult due to the involvement of several genes and insufficient knowledge about mechanism(s) controlling the characters (Yeo et al. 1990). A considerable improvement has already been made by exploiting the natural variation through conventional breeding. Eventhough the success made in the last century, traditional breeding efforts alone can not meet the increasing demand of rice consumers in the 21st century. Therefore, plant cell and tissue culture techniques are being used for the genetic improvement and developing salt tolerant lines of rice plant throughout the world (Raina 1989).

Among the different tissue culture techniques the one of the important technique is mature embryo culture in rice to create additional variation and novel rice varieties (Lutts et al. 1999 and Sathish et al. 1995). The objective of this study was *in vitro* screening of callus under different concentration of NaCl, identification of salt tolerant callus, regeneration of the callus under different concentration of Nacl and development of somaclonal variant from the salt tolerant calli.

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Materials and Methods

The seven rice varieties with different adaptation to saline soil *viz.,* Pokkali, CSR10, TRY1, TRY(R) 2, BPT 5204, White Ponni and IR29 were used for the development of salt tolerant high yielding somaclones. The nutrient medium chosen for the study was Murashige and Skoog (MS) medium

(Murashige, and Skoog 1962) with 2 mgl^{-1} 2,4D and 0.5 mgl^{-1} Kin for callus induction and MS +Kin ($1mgl^{-1}$)+ BAP ($1mgl^{-1}$)+ NAA ($0.1mgl^{-1}$) for regeneration. The pH of the medium is 5.5 -5.8 and was maintained by 0.1N NaOH and finally 30 grams of sucrose (3%) was added per liter of medium as carbohydrate source and mixed well. Then 8 grams of agar (0.8%) was added per liter of medium to prepare semi solid medium and melted in the microwave oven for even spread of agar in the nutrient media. The medium was then transferred into the test tube (10 ml each) and plugged with nonabsorbent cotton. The cotton plugged test tube was autoclaved at 1.01kg/cm pressure at 121°C for 20 minutes. The medium was allowed to cool at room temperature and stored at 10°C.

The mature embryo was used as a explant. Seeds were dehusked and surface sterilized with 70% alcohol for 30sec followed by 15% of common bleach for 20 minutes then finally rinsed several times with the sterile distilled water before inoculation into the callus induction media. For *in vitro* salt screening the different concentrations of NaCl (0mM, 50mM, 100mM, 150 mM) were added with the callus induction medium. The regeneration was done with MS +KIN ($1mgl^{-1}$)+ BAP ($1mgl^{-1}$)+ NAA ($0.1mgl^{-1}$) along with the different concentrations of NaCl were used in the media for salt tolerant screening and development of salt tolerant somaclones.

The experiment was laid out in a Factorial CRD design (Gomez and Gomez 1984) with three replications. The data obtained with percent values were subjected to arc sin transformation and analysed using the AGRES (Agricultural statistics) computer package. Level of significance (P value) was determined using the standard Analysis of Variance (AVOVA) (Panse and Sukhatme, 1964). Differences among mean values were assessed by LSD (Least Significant Difference) test.

Results and Discussion

The callus induction was the primary step in any *in vitro* screening technique. The significant differences in callus induction frequency under different concentrations of saline condition among genotypes were noticed (Table 1). Similar trend of responses was observed in earlier studies also (Karim and Zapata 1994). The callus growth was rapidly reduced when the relative increase of NaCl concentration in the callus induction and subculture media. This showed that the NaCl had an inhibitory effect on the growth of callus. With increasing NaCl concentrations indicating that the inability of plant cells and tissues to adjust with incremental increases of salt over sufficient time periods might be due to osmotic or ionic shock. This was also supported by Senadhira (1987) and Aditya and Baker (2006).

The callus induction frequency (CIF) decreased with increasing NaCl concentration. At 50mM NaCl concentration Pokkali (75.96 per cent) and CSR10 (75.30 per cent) recorded better performance for CIF than the other genotypes. At 100 mM concentration TRY 1 (39.7 per cent) and CSR 10 (39.63 per cent) performed better and at the 150 mM concentration Pokkali (15.77 per cent) and CSR 10 (14.13 per cent) gave better performance than others. In all the NaCl concentration IR 29 was the lowest performing genotype followed by W.Ponni (Table 1).

The results revealed that the salt tolerant genotypes like Pokkali, CSR10 and TRY(R)2 recorded higher Callus Induction Frequency (CIF) at increasing levels of NaCl concentration than the salt susceptible genotypes like IR29 and W.Ponni. Out of these three genotypes Pokkali was highly tolerant to slat stress than the others. This conformed the results of Thach and Pant (1999). This finding revealed that this technique could be the one of the easiest technique to screen the salt tolerance in rice. This was also confirmed by Aditya and Baker (2006).

The Regeneration Frequency (RF) was higher in Pokkali in all the three NaCl stress condition followed by TRY(R) 2 whereas the genotype IR 29 showed lowest RF. At 50 mM NaCl concentration Pokkali showed 67.70 per cent of RF followed by TRY(R)2 recorded 56.41 per cent and the genotype IR 29 showed the lowest RF of 31.68 per cent. In the 100 mM NaCl concentration Pokkali showed maximum RF of 43.71 per cent and the IR 29 recorded the minimum of 9.31 per cent. While comparing the 150 mM Nacl concentration the genotype Pokkali recorded the maximum of 30.46 per cent and the genotype IR 29 was recorded almost nil RF and White Ponni recorded 1.8 per cent RF (Table 2).

All the genotypes performed better regeneration frequency in control regeneration media but the regeneration frequency decreased with increased salt concentration. In earlier reports it was found that salt pre-treatment had positive effect on plant regeneration (Yoshida et al 1983). But in the present study only 50mM stressed calli showed better regeneration frequency with good number of green islets. However, it was also reported that the possibility for differentiation of salt susceptible lines was strongly inhibited in the presence of NaCl in the regeneration media (Aditya and Backer 2006). In the present study also the embryogenic callus from salt tolerant genotypes showed the most tolerance than the susceptible genotypes. Hence, the salt tolerance can be considered as a characteristic of embryogneic callus of rice under *in vitro* culture conditions. This *in vitro* technique with different NaCl stress can also be used as a screening technique for salt tolerance rather than the field screening, as field screening would take more duration.

When regenerated plants are transferred to *ex vitro* condition under standardized environmental variables, they can exhibit non-genetic or epigenetic changes as well as heritable and genetic variation, heritable but reversible (Karp 1991). Theoretically, salt tolerance of individual plants could be correlated with that of its isolated cells and tissues under *in vitro* condition (Tal 1994) but this correlation is not always absolute (Casas et al. 1991). Because of the above reasons the *in vitro* selected materials are to be assessed at different growth stages by growing them under glass house with different levels of salt stresses. This step might help to understand the inheritance of salt tolerance from tissue level to whole plant level.

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Table 1. Callus Induction Frequency (CIF) on MS media supplemented with different concentration of Sodium Chloride

Genotypes	NaCl concentration				
	0mM	50mM	100mM	150mM	Mean
TRY 1	92.58 (74.35)a	71.09(57.51)de	39.7 (39.06)g	9.13 (17.57)	53.13 (47.12) bc
TRY(R)2	86.44 (68.45)bc	68.44(55.88)e	37.25 (37.57)g	12.13(20.38)j	51.07 (45.57) c
Pokkali	90.62 (72.32)ab	75.96(60.70)d	32.66 (43.95)f	15.77(23.39)ij	53.74 (50.09) a
BPT5204	74.69(59.81)d	35.71(36.68)g	23.17(28.76)h	3.74 (11.13)k	34.33 (34.10) e
CSR10	90.35 (71.92)a	75.30(60.28)d	39.63 (38.97)g	14.13 (22.06)j	54.10 (48.31) ab
W.PONNI	82.82 (65.57)bc	49.33(44.62)f	20.58(26.97)hi	3.37 (10.57)k	39.03 (36.93)d
IR29	67.52 (55.29)e	24.34(29.41)h	11.79 (20.01)j	0.67 (4.13)m	26.08 (27.21) f
Mean	83.57 (66.82)	57.17 (49.30)	29.25 (33.61)	8.42 (15.60)	44. 60 (41.33)

	SED	CD(0.05)	CD(0.01)
Genotypes	0.97	1.95	2.59
Treatment	0.73	1.47	1.96
GxE	1.94	3.89	5.18

* Values in parentheseis indicate the transformed arc sin values
* The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD)

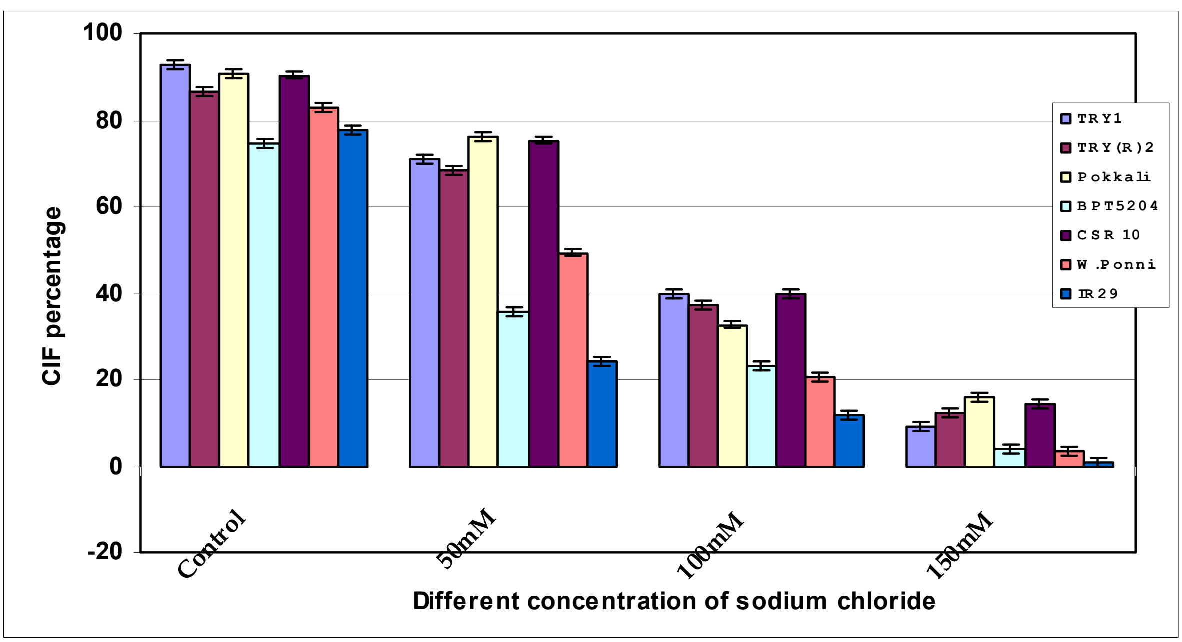

Table 2. Regeneration Frequency from the rice calli under different concentration of Sodium Chloride

Genotypes	NaCl concentration				
	0mM	50mM	100mM	150mM	Mean
TRY 1	49.02 (44.44)e	44.10 (41.61)g	23.63(29.08)k	15.51 (23.19)m	33.07 (34.28)
TRY(R)2	65.21 (53.87)c	56.41 (48.69)d	32.57(34.79)ij	24.69 (29.77)k	44.72 (42.37)
Pokkali	77.36 (61.59)a	67.70 (55.38)bc	43.71(41.38)g	30.46 (33.50)j	54.81(49.33)
BPT5204	64.72 (53.57)c	36.94 (37.43)h	18.51 (25.48)l	9.22 (17.66)n	32.35 (33.30)
CSR10	69.28 (56.36)b	52.39 (46.37)e	26.66(31.08)k	14.54 (22.41)m	40.72 (39.39)
W.PONNI	45.66 (42.51)fg	34.60 (36.03)hi	13.57(21.59)m	1.83 (7.72)o	23.92 (26.35)
IR29	43.47 (41.25)g	31.68 (34.26)ij	9.31 (17.71)n	0.33 (2.41)p	21.20 (23.37)
Mean	59.25 (50.51)	46.26 (42.82)	23.99 (28.73)	13.80 (19.52)	35.82 (35.48)

	SED	CD(0.05)	CD(0.01)
Genotypes	0.52	1.04	1.39
Treatment	0.39	0.79	1.05
GxE	1.04	2.08	2.77

* Values in parentheses indicate the transformed arc sin values
* The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD)