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RESEARCH ARTICLE

In vitro study on salinity screening in rice

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SUMMARY

Two moderately resistant rice cultivars, White ponni and BPT – 52904 were used to selection of promising saline tolerant callus lines through screen for salinity tolerance under *in vitro* condition. Embryogenic calli was obtained when tissue culture basal media supplemented with 2 mg L^{-1} 2,4-D along with 0.5 mg L^{-1} kinetin and 1 mg L^{-1} NAA. A significant reduction in callus growth rate (Relative growth rate) and callus induction per cent was noticed in media supplemented with different concentration of saline treatment, from 0.5 to 1.0 and 1.5 per cent NaCl. But the time elapsed for callus induction was increased with higher concentration of salt in the medium. A significant number embrogenic calli was survived in lower concentration, 0.5 and 1.0 per cent of NaCl showed good regeneration capacity in regeneration media. Few promising saline tolerant plants were recovered and transferred to the rooting media before adapting to acclimatization. Hence, this *in vitro* technique with different NaCl stress could have been used effectively to screening for salt screening in rice, rather than the field screening.

Key Words : Relative growth rate, Embryogenic calli, Regeneration, Plantlets, Salinity screening

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Salinity is one of the ever unresolved abiotic factor limiting rice production. About 6.5 per cent (831 million ha) of the world's total area important cereal crops and a major crop consumed by (12.78 billion ha) is affected by salt in soils (FAO). In India saline and alkaline soil occupies more than 9 million ha (Raveendar *et al.*, 2008; Munns *et al.*, 1999 and Attia *et al.*, 2014). Breeding for salt tolerance through conventional method of breeding is highly expensive. To meet the increasing demand of food requirements of growing population, especially rice, conventional method is inadequate. Therefore, *in vitro* screening through tissue culture is a potential, rapid and reliable method. Adequate report has been furnished by various authors on the success of *in vitro* screening by tissue culture in rice. Among the tissue culture techniques mature embryo is an important one in rice to create additional variation and novel rice varieties (Lutts *et al.*, 1999 and Sathis *et al.*, 1995). The present investigation was aimed to screen for salinity tolerance through selection of callus derived tolerant lines cultured under *in vitro* saline condition in two moderately tolerant rice cultivars White ponni and BPT-5204.

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MATERIAL AND METHODS

Standardization of embrogenic callus induction media :

The experimental materials, White ponni and BPT -5204 (seeds) were obtained from Rice Research Station, Ambasamudram and from the Department of Rice, Tamil Nadu Agricultural University**,** Coimbatore. The mature embryos of two rice cultivars were soaked in water for 5-10 min separately and surface sterilized with 70 per cent ethanol for 39 sec. Surface sterilized seeds were washed 4-5 times with water before and after the embryos disinfected with 0.2 per cent $MgCl_2$ for 25 min, blotted with autoclave sterilized tissue paper and aseptically inoculated under laminar air flow chamber on to the callogenic media consisting of MS basal organic and inorganic components (Murashige and Skoog, 1962) containing 3.0 per cent sucrose, supplemented with various combinations plant growth promoting substances to induce embrogenic calli. The pH of the media was adjusted at 5.8 and gelled by adding 0.8 per cent agar-agar. The cultures were incubated at 22 ^{0}C – 25 ^{0}C temperature with 16/8 photoperiod. The four different media used for callus induction are; 1, MS + 1.5 mg L^{-1} 2, 4-D + 0.5 mg L^{-1} kinetin, 2, MS + 2 mg L^{-1} 2, 4 -D + 0.5 mg kinetin L^{-1} + 1 mg L^{-1} NAA, 3, MS + 2.5 mg L^{-1} 2, 4-D + 100 mg L^{-1} thiamine HCl, and 4, MS + 4 mg L^{-1} 2, 4-D + 0.5 mg L^{-1} kinetin + 1 mg L^{-1} NAA.

In vitro salinity screening :

Standardized media were supplemented with different concentration of NaCl, 0.5, 1.0 and 1.5 per cent. Sterilized matured embryos of two rice cultivars inoculated NaCl treated media. Four week old embryogenic calli sustained on different NaCl media were transferred to shoot differentiation media supplemented with 0.05 mg L^{-1} NAA and 5.0 mg L^{-1} BAP for shoot induction and proliferation.

Growth analysis :

Fresh weight of callus, seedlings and plant height were recorded at the beginning and at the end of the culture period. The different parameters were calculated as follows.

Relative growth rate :

For the callus growth analysis in salt stress, the fresh weights of callus were recorded at the beginning and the end of the culture period. The relative growth was calculated on the basis of the initial and final growths, as follows :

$$\text{Relative growth rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}$$

Callus induction frequency :

Callus induction frequency was calculated as follows :

$$\text{Callus induction frequency (CIF)} = \frac{\text{Number of calli}}{\text{Number of incubated seeds}} \times 100$$

Frequency of embryogenic calli :

Frequency of embryogenic calli was calculated as follows :

$$\text{Frequency of embryogenic calli} = \frac{\text{Number of embryogenic calli}}{\text{Number of incuabed seeds}} \times 100$$

Number of days to be taken for callus induction :

From the date of inoculation to the first day of visual appearance of callus on the surface of the germinating seeds was considered as the number of days for callus induction.

RESULTS AND DISCUSSION

Among the abiotic stresses, salinity is one of the major and ever-present threats to crop yields, in arid and semiarid regions of the world. Of which NaCl is the most abundant source of salinity in the soil (Flowers and Yeo, 1995). Rice is highly sensitive to salt stress (Bashir *et al.,* 2010). Attempts to improve the salt tolerance through conventional breeding programmes have met with very limited success (Bohnert and Jensen, 1996). Use of tissue culture techniques has the potential to increase the stress tolerance of plants because plant cells contain a complete species genome and are totipotent. Tissue culture is the one of the areas in which the *in vitro* selection approach has been used efficiently in plant breeding (Barakat and Abdel-Latif, 1996). The success of *in vitro* selection for tolerance to NaCl stress is dependent upon the development of efficient and reliable regeneration systems keeping in view the effects of individual components of salt stress (ionic or osmotic) on regeneration efficiency. *In vitro* screening starts with induction and screening of callus. The callus initiation was started at 7th to 8th day after inoculation. Both the varieties (BPT-5204 and White ponni), produced calli with a range of 56.28 to 90.33 per cent (Table 1), likewise the embryogenic callus induction was 40.42 to 68.66 per

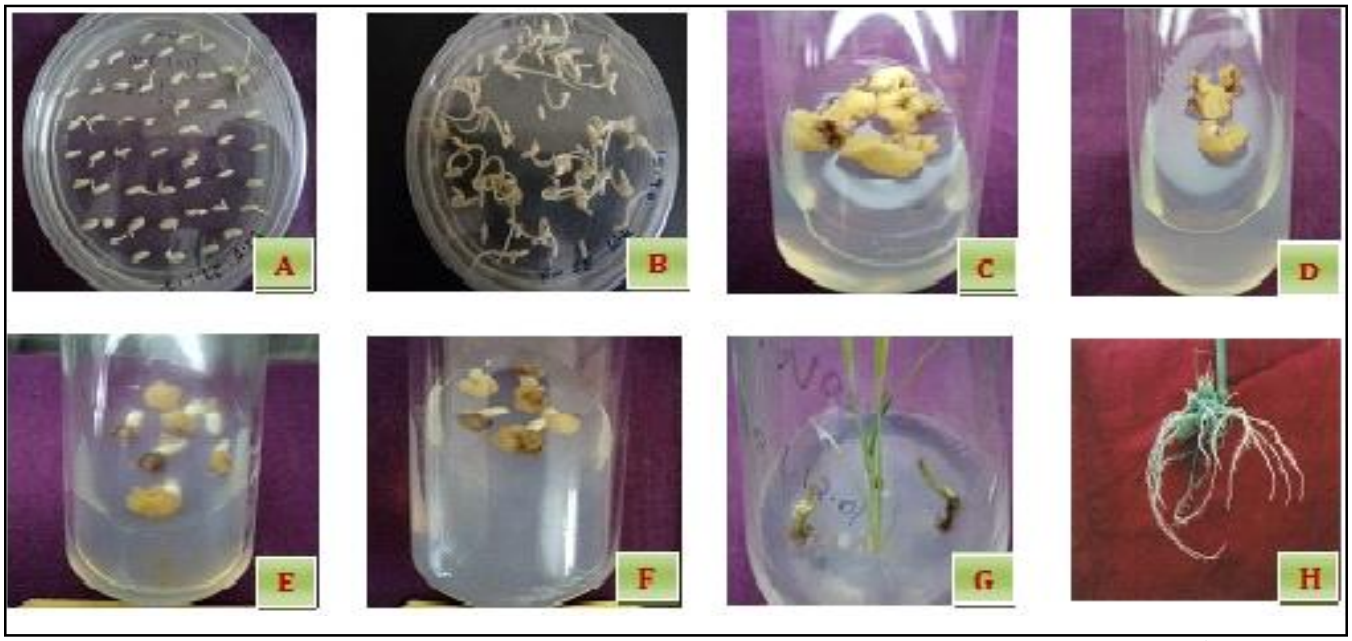

Fig. 1 : (a) Freshly inoculated seeds, (b) Callus induction, (c) Control, (d) 0.5 per cent NaCl, (e) 1 per cent NaCl, (f) 1.5 per cent NaCl, (g) Regeneration and (h) Rooting

Table 1 : Effect of different media composition on callus growth parameters

Parameters	Media composition			
	MS + 1.5 mg 2,4-D + 0.5 mg kinetin (%)	MS + 2 mg 2,4 -D + 0.5 mg kinetin + 1 mg NAA (%)	MS + 2.5 mg 2,4-D + 100 mg thiamine HCl (%)	MS + 4 mg 2,4-D + 0.5 mg kinetin + 1 mg NAA (%)
White ponni				
CIF	76.43 ± 1.9	74.30 ± 4.64	80.83 ±7.08	56.28 ± 8.16
ECIF	40.42 ± 0.11	59.39 ± 5.25	58.33 ± 2.27	46.51 ± 4.32
RGR	1.47 ± 0.12	2.66 ± 0.57	1.46 ± 0.24	1.68 ± 0.43
NDCI	6.99 ± 0.11	7.22 ± 0.42	6.76 ± 1.70	6.55 ± 1.41
BPT-5204				
CIF	72.33 ± 4.11	86.66 ± 9.02	90.33 ± 14.20	69.00 ± 10.65
ECIF	54.66 ± 6.98	68.66 ± 11.07	66.33 ± 9.63	65.33 ± 8.19
RGR	1.11 ± 0.64	1.46 ± 0.44	1.34 ± 0.30	0.96 ± 0.25
NDCI	7.43 ± 1.29	5.43 ± 1.07	5.76 ± 0.81	6.40 ± 1.06

*CIF: Callus induction frequency (%); ECIF: Embryogenic callus induction frequency (%); RGR: Relative growth rate; NDCI: Number of days for callus induction

Table 2 : Effect of different NaCl concentration in media on callus growth parameters

Parameters	NaCl concentration			
	Control	0.5	1	1.5
White ponni				
CIF	74.30 ± 6.41	83.00 ± 11.89	63.16 ± 5.77	46.00 ± 6.22
ECIF	59.39 ± 11.30	64.22 ± 9.11	50.33 ± 5.13	40.83 ± 3.73
RGR	2.66 ± 1.07	2.62 ± 0.65	2.28 ± 0.47	2.97 ± 0.76
NDCI	7.22 ± 1.36	7.36 ± 1.27	7.43 ± 1.23	8.16 ± 0.73
BPT-5204				
CIF	86.66 ± 8.98	85.66 ± 9.23	66.66 ± 9.28	47.66 ± 8.41
ECIF	63.33 ± 4.15	61.00 ± 2.86	57.33 ± 5.07	44.33 ± 3.50
RGR	1.46 ± 0.49	2.79 ± 0.29	2.31 ± 0.81	0.96 ± 0.47
NDCI	5.43 ± 1.25	5.80 ± 1.14	6.23 ± 1.01	6.40 ± 1.19

cent. The embryogenic calli contain globular cells, are nodular, shiny and watery in appearance are able to produce plantlets when cultured on suitable regeneration media. Among the two rice cultivars, the intensity of callogenesis and embryogenic callus was high in BPT-5204. In the present study, 2.5 mg L^{-1} 2, 4-D induced better callogenic response in both the cultivar, but higher callus induction was in BPT (90.33 %) than White ponni (80.83 %). Similar results were obtained by Pandey *et al.* (1994) at 2 mg L^{-1} 2, 4-D. Auxins, especially 2, 4-D, are essential for induction and proliferation of the callus. In most cases, 2,4-D as a strong synthetic auxin was sufficient to initiate and sustain embryogenic callus growth in rice and has been used as the only growth regulator in callus induction media (Lin and Zhang, 2005). However, a few reports indicated that the combination of 2,4-D with kinetin was more effective in producing embryogenic callus, while 2,4-D alone only produced a non-embryogenic calli (Al-Forkan *et al.*, 2005). Decrease in growth value and percentage of adapted callus through consecutive phases, is the result of media composition and growing condition. The highest growth rate were achieved in the media supplemented with 2 mg L^{-1} 2, 4-D for White pooni (2.66) was higher than BPT- 5204 (1.46). Similar results were disclosed by other authors.

Number of days for callus induction varied with genotypes and media used. The early callus induction was observed in BPT-5204 (5.43 days), which is responded well in media containing 2 mg L^{-1} 2,4-D along with 0.5 mg L^{-1} kinetin and 1 mg L^{-1} NAA, whereas White ponni took slightly longer duration for callus induction (6.55 days) in this media composition. The minimum number of days required for callus induction was more than six days for other three treatments. On the basis of growth parameters of initiate calli 2 mg L^{-1} 2, 4-D, proved to be beneficial for earlier callus induction in both the varieties. The callus induction is the primary step in the *in vitro* screening technique. Significant difference in callus induction frequency was observed between two genotypes under different concentrations of saline condition (Table 2 and Fig. 1). Callus growth parameters was rapidly reduced when the relative increase of NaCl concentration in the media. This result showed that NaCl had an inhibitory effect on the growth of callus (Shanthi *et al.*, 2010). Salt stress induced by NaCl caused a significant decrease in relative growth rate (Table 2). Treatment with 1-1.5 per cent NaCl significantly decreased the relative growth rate of calli compared to 0.5 per cent. A significant decrease in the number of somatic embryos, callus induction frequency (CIF) and increase in time elapsed for somatic embryos formation were observed at 1 and 1.5 per cent NaCl. However, it was also reported that the possibility for differentiation of salt susceptible lines were strongly inhibited in the presence of NaCl in the regeneration media (Aditya and Backer, 2006). The results of the study envisaged a significant reduction in relative growth rate is mainly as a result of decrease in callus fresh weight under salinity stress. However, the reverse was true for callus dry weight. The decrease in fresh weight and increase in callus dry weight under salinity stress has already been reported in a number of studies (Ahmad *et al.*, 2007). Treatment with 1.5 per cent NaCl tolerant calli obtained either by direct or indirect selection process did not sustained a regular growth on salt supplemented media. These calli were suffered with prolonged duration in NaCl medium. Similar to our findings Gulati and Jaiwal (2010), reported that NaCl tolerant lines maintained higher growth rate when compared to non-selected calli. Calli derived from lower concentration of NaCl showed good regeneration capacity when transferred to the regeneration media. Few promising saline tolerant plants were recovered and transferred to the rooting media before adapting it acclimatization. Hence, this *in vitro* technique with different NaCl stress can also be used as an effective screening technique for salt tolerance rather than the field screening, as field screening would take more duration.

REFERENCES

Aditya, T.L. and Backer, B.A. (2006). Selection of salt tolerant somaclones from indica rice through continuous *in vitro* and *ex vitro* sodium chloride stress. *Indian. J. Pl. Physiol.*, **11** : 349-357.

Ahmad, M.S.A., Javed, F. and Ashraf, M. (2007). Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (*Oryza sativa* L.) genotypes. *Pl. Growth Regul.*, **53** : 53-63.

Al-Forkan, M., Rahim, M.A., Chowdhury, T., Akter, P. and Khaleda, L. (2005). Development of highly callogenesis and regeneration system for some tolerant rice (*Oryza sativa* L.) cultivars of Bangladesh. *Biotechnol.*, **4** : 230-234.

Attia, S.A.A., Nadia Gad., Abdel-Rahman, H.M., Shenoda, J.E. and Aida, A. Rizkalla (2014). *In vitro* enhancement of salinity tolerance in rice using cobalt sulfate. *World Appl. Sci. J.*, **31**(7) : 1311-1320.

Barakat, M.N. and Abdel-Latif, T.H. (1996). *In vitro* selection of wheat callus tolerant to high levels of salt and plant regeneration. *Euphytica*, **91** : 127-140.

Bashir, M.U., Akbar, N., Iqbal, A. and Zaman, H. (2010). Effect of different sowing dates on yield and yield components of direct seeded coarse rice (*Oryza sativa* L.). *Pakisan J. Agri. Sci*., **47** : 361-365.

Bohnert, H.J. and Jensen, R.G. (1996). Strategies for engineering water-stress tolerance in plants. *Trends Biotechnol*., **14** : 89-97.

Cheema, A.A., Saleem, M.Y., and Awan, M.A. (2002). *In vitro* techniques for the selection of Basmati rice mutants better adapted to saline environments. In: *Mutation, in vitro and molecular techniques for environmentally sustainable crop improvement*. M. Mauszynski and K.J. Kasha (ed.) IAEA, Vienna. 161-168 pp.

Flowers, T.J. and Yeo, A.R. (1995). Breeding for salinity resistance in crop plants -Aust. *J. Pl. Physiol*., **22** : 875-884.

Gulati, A. and Jaiwal, P.K. (2010). *In citro* induction of multiple shoots and regeneration from shoot tips of mung bean (*Vigna radiata* L. Wilczke). *Plant Cell, Tiss. & Org. Cult*., **29** : 199-205.

Lee, I.S., Kim, D.S., Hyun, D.Y., Lee, S.J., Song, H.S., Lim, Y.P. and Lee, Y.I. (2003). Isolation of gamma-induced rice mutants with increased tolerance to salt by anther culture. *J. Pl. Biotechnol*., **5**(1) : 51-57.

Lin, Y.J. and Zhang, Q. (2005). Optimizing the tissue culture conditions for high efficiency transformation of indica rice. *Pl. Cell Rep*., **23** : 540-547.

Lutts, S., Bouharmont, J. and Kinet, J.M. (1999). Physiological characterization of salt resistant rice (*Oryza sativa* L.) somaclones. *Aust. J. Bot*., **47** : 835-849.

Munns, R., Hare, A., James, R.A. and Rebetzke, G.J. (1999). Genetic variation for improving the salt tolerance of duram wheat. *Aust. J. Agril. Res*., **51** : 69-74.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth bioassays with tobacco tissue culture. *Pl. Physiol*., **15** : 473-497.

Pandey, S.K., Ramesh, B. and Gupta, P.K.S. (1994). Callusing and plant regeneration in rice. *Indian. J. Genet*., **54**: 293.

Raveendar, S., Premkumar, A., Ignacimuthu, S. and Agastian, A. (2008). Effect of sea water on callus induction and regeneration of rice genotypes. *Internat. J. Integrative Biol*., **3**(2) : 92.

Sathish, P., Gamborg, O.L. and Nabors, N. W. (1995). Rice anther culture: callus initiation and somaclonal variation in progeny of regenerated plants. *Pl. Cell Rep*., **14** : 432-436.

Shanthi, P., Jebaraj, S. and Geeth, S. (2010). *In vitro* screening for salt tolerance in Rice (*Oryza sativa*). *Electronic J. Pl. Breed*., **1**(4) : 1208-1212.

Smith, M.K. and McComb, J.A. (1981b). Effect of NaCl on growth of whole plants and corresponding callus cultures. *Austr. J. Pl. Physiol.*, **8** : 267-275.

Zinnah, K.M.A., Nayem Zobayer, Saif, U., Sikdar., Lutfun Nahar Liza., Md. Al Nayem Chowdhury and M. Ashrafuzzaman (2013). *In vitro* regeneration and screening for salt tolerance in rice (*Oryza sativa* L.). *Internat. Res. J. Biological Sci.*, **2**(11) : 29-36.