Summary and Conclusions

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Environmental stress is a major constraint in crop production and this calls for evolving varieties with improved stress resistance. Rice is an important cereal crop grown in India where soil salinity is one of the major stress factors. Saline affected area around the globe is estimated to be 1 billion hectares. In India, about 8.1% of the total geographical area is affected by salinity (Rao, 1990) with Gujarat alone having 7 lac hectares of saline land.

An increase in population demands an increase in agricultural production. The world population which was 5.3 thousand billion in 1990 is expected to reach 6.3 thousand billion in 2000. Thus, the need to produce more food grains, mainly due to population explosion, is continuously pushing agriculture farther into marginal lands characterized by soils and waters with a high degree of salinity. In the recent years the development of crops with genetic resistance to salt has been proposed as a means of expanding agriculture into regions affected by excess salt. Whole plant breeding system have met with limited success in improving the response of crops to saline stress (Epstein, 1980; Norlyn, 1980). At the same it is now well established that plant tissue and cell culture techniques can be successfully employed to develop salt tolerance in plants (Rains *et al.*, 1986).

Proline accumulation has often been shown to occur in plants as a consequence of salinity stress (Li, 1990; Prakash and Sarin, 1993). The physiological significance of this accumulation is assumed to be associated with its ability to act as a osmoregulator, a protective agent for membranes and cytoplasmic enzymes or as a storage compound for post-stress growth (Stewart and Lee, 1974; Pollard and Wyn Jones, 1979; Jolivet *et al.*, 1982; Aspinall and Paleg, 1981). Cell lines overproducing proline could be a powerful tool in evolving salt tolerant varieties (Ricardi *et al.*, 1983; Van Swaaij *et al.*, 1986; Chen and Wang, 1991). Although cell lines with increased tolerance to NaCl have been isolated from rice, very little is known about the mechanisms of salt tolerance. The present study was, therefore, taken up with a view to isolating hydroxyproline resistant cell lines of rice for increased salt tolerance. Attempt is also made to understand the physiological basis of salt tolerance rendered by hydroxyproline to the stressed systems. Two varieties of rice (*Oryza sativa* L.) differing in salt tolerance (var.Bhoora rata - salt tolerant and GR_{11}-salt susceptible) were selected to understand the mechanism of salt tolerance in them. An attempt is also made to develop a protocol for complete plantlet regeneration of hydroxyproline resistant calli.

The induction and maintenance of callus cultures of the two rice varieties were carried out by employing Linsmaeir and Skoog's medium (L.S.medium, 1965). The hydroxyproline resistant cell lines have been isolated by culturing the calli of both cultivars on L.S.medium containing LD_{50} concentration of hydroxyproline (10 mM). The hydroxyproline resistant cell lines were then incubated on L.S.medium containing LD_{50} concentration of NaCl (200 mM) for assessment of salinity tolerance.

The levels of various ions (Na^+, K^+, Cl^-, Mg^{2+} and Ca^{2+}), total protein, proline, polyamines and the activity of cellulase, invertase, amylase, IAA oxidase and proline oxidase were determined in callus cultures of both rice varieties during different growth intervals (at the end of 0,2,4 and 6 weeks of incubation).

It was observed that callus growth of both varieties followed a typical growth curve registering the maximum growth by the end of fourth week. Incorporation of NaCl at 200 mM concentration resulted in significant reduction in the dry weight of calli of both cultivars. BR callus exhibited 42% reduction while GR_{11} callus exhibited 50% reduction in its dry weight as compared to control at the end of fourth week. However, hydroxyproline exposed cells when grown on medium containing both NaCl and hydroxyproline showed better dry weight accumulation. An increase of 33% and 40% respectively in the dry weight of BR and GR_{11} callus over the salt control was observed.

The levels of Na^+ and K^+ increased as the growth progressed. Under NaCl stress as compared to the control, BR callus showed 20 fold accumulation of Na^+ at the end of the fourth week while GR_{11} cultivar showed 23 fold accumulation. In contrast to Na^+ ions, there was decrease in K^+ content of both rice cultivars under NaCl stress. At the end of fourth week, salinized calli of BR and GR_{11} exhibited respectively 1.4 and 1.9 fold less K^+ than that of their controls. Thus, a comparison of two varieties revealed that under NaCl stress tolerant variety accumulates more K^+ and less Na^+ compared to the susceptible one. It is interesting to note that prior exposure of the cells to hydroxyproline helped them in accumulating less Na^+ and more K^+ ions. Hydroxyproline

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treated BR salinized callus growing on medium containing both hydroxyproline and NaCl accumulated 20% less Na^+ and 38% more K^+ content, while GR_{11} callus showed 25% less accumulation of Na^+ and an increase of 33% in its K^+ content (as compared to their salt control values). There was a significant increase in the level of Cl^- ions in the tissues of both rice cultivars growing on NaCl containing medium. As compared to the control, BR and GR_{11} calli showed a 4 and 5 fold increase respectively in their levels of Cl^- ions at the end of fourth week. In this case also hydroxyproline treated BR salinized callus growing on hydroxyproline containing saline medium accumulated 16% less Cl^- as compared to its salt control while GR_{11} salinized callus showed 18% less accumulation of Cl^-. The levels of Mg^{2+} and Ca^{2+} were highest during the period of maximum growth (i.e. at the end of fourth week). It is observed that under all treatments, the levels of Mg^{2+} and Ca^{2+} were higher in the salt tolerant variety as compared to the susceptible variety. The levels of Mg^{2+} and Ca^{2+} increased in both cultivars upon salinization. Their levels were further enhanced by treatment with hydroxyproline.

The free proline content of both rice cultivars rose steadily from the second week, reaching the maximum level at the end of sixth week. The tolerant variety exhibited comparatively more increase in its proline content than susceptible variety, under NaCl stress. Treatment of tissues with hydroxyproline significantly increased the proline level of salinized tissues growing on medium containing NaCl and hydroxyproline by 5 fold in BR and 6 fold in GR_{11}.

The total protein content of callus decreased as a result of salinization.

However, the tolerant BR variety showed less reduction (35%) in its protein content as compared to the susceptible GR_{11} (75%) at the end of sixth week. A significant rise in the protein content was brought about by treatment with hydroxyproline. Hydroxyproline exposed BR salinized cells growing on medium containing both NaCl and hydroxyproline showed nearly 45% increase in its protein content (over salt control value), while hydroxyproline exposed GR_{11} salinized cells recorded an increase of 58%.

The titers of polyamines were found to be affected by NaCl in both rice cultivars. The level of putrescine rose in calli of both varieties upon salinization. GR_{11} showed more accumulation of putrescine as compared to BR. Unlike putrescine, the levels of spermidine and spermine showed a marked decline under NaCl stress. As compared to BR salinized callus, the salinized callus of GR_{11} exhibited a more marked reduction in its level of spermidine and spermine. However, it is interesting to note that hydroxyproline treatment helped the stressed cells in maintaining low ratio of putrescine to spermidine and spermine. Hydroxyproline treatment reduced putrescine accumulation and increased the levels of spermidine (by 58% and 48%) and spermine (by 98% and 56%) in salinized BR and GR_{11} callus respectively (at the end of the fourth week).

The activity of amylase, cellulase and invertase was stimulated in both the varieties by hydroxyproline, each of the enzymes registering peak activity at the end of fourth week. Incorporation of NaCl into the medium significantly reduced the activity of these enzymes in both rice varieties. However, the reduction in the tolerant BR variety was comparatively less. It was observed that hydroxyproline treatment markedly enhanced the activity of amylase, cellulase and invertase in salinized tissues. The activity of IAA oxidase increased as a result of salinization in both rice cultivars. The salinized GR_{11} callus showed more activity of IAA oxidase as compared to BR. Treatment of tissues with hydroxyproline resulted in a reduction in IAA oxidase activity. Under NaCl stress, proline oxidase activity decreased in both rice varieties. A further decrease in the activity of proline oxidase was brought about by treatment with hydroxyproline.

It is inferred from the present studies that the rice variety BR tolerates NaCl stress by

i) accumulating less Na^+ and Cl^- ions and by maintaining higher levels of K^+, Ca^{2+} and Mg^{2+} ;

ii) maintaining higher levels of proline, and protein ;

iii) maintaining higher activity of amylase, invertase, cellulase and lower activity of IAA oxidase and proline oxidase ;

iv) accumulating less of putrescine and maintaining higher levels of spermidine and spermine as compared to the susceptible variety GR_{11}.

It is also concluded from the present studies that hydroxyproline renders tolerance to NaCl stressed cells by :

i) maintaining improved ionic balance (hydroxyproline decreased the net accumulation of Na^+ and Cl^- ions and enhanced the contents of K^+, Mg^{2+} and Ca^{2+} ions) ;

ii) increasing the contents of free proline and total protein ;

iii) maintaining low ratio of putrescine to polyamines (spermidine and spermine) ;

iv) lowering the activity of IAA oxidase and proline oxidase ;

v) enhancing the activity of amylase, cellulase and invertase.

The said conditions could be responsible for improved growth.

Salt tolerant rice plantlets can be generated from hydroxyproline resistant calli by culturing them on MS medium supplemented with 13.32 μM BA and 2.46 μM IBA. Omission of hydroxyproline from the medium results in a poor growth of the regenerants under NaCl stress.