SUMMARY AND CONCLUSIONS

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World population by 2020 would touch 3,000 million and more than 90% would live in the developing country under conditions of extreme poverty and forced hunger. This population explosion is widening the gap between supply and demand of the agricultural produce. In order to narrow down this gap it is highly essential to bring the marginal lands including saline soils under agriculture. Salinity is one of the major problems limiting agricultural production in many areas of the world. According to a recent estimate more than 400 million hecters of land is salt affected at global level. India have 8.1% of the geographical area affected by salinity and Gujarat alone have 7 lakh uncultivable land due to salinity (Rao *et.al.*, 1999).

Rice is a staple food for a large group of population. It is a major food source for more than 65% of Indian population (Mathur *et.al.*, 1999). Since it is not possible to change the environment the crop has to be modified to suit the environment. The traditional crop improvement programmes are labour intensive and time consuming. Cell and tissue culture techniques help in plant improvement and selection of salt tolerant cell lines (Rains *et.al.*, 1986). The isolated cell lines can be subjected to go through somatic embryogenesis and the resultant embryoids can be encapsulated and can be used as synthetic seeds.

Somatic embryogenesis in cereals is reported by many workers (Lambe et.al., 1999; Vasil and Vasil 1994; George *et.al.*, 1989). Production of embryoids in rice is also reported by Ozawa *et.al.* (1996), Raval and Chatoo (1993). Eventhough there are such reports, the production of salt tolerant embryoids is still lacking. The present study was therefore, taken up for standardizing a protocol for

the production of salt tolerant embryoids in rice (*Oryza sativa* L. var. CSR-10). Attempt was also made to understand the physiological basis of salt tolerance, isolation of salt tolerant cell lines and their regeneration.

The induction and maintenance of callus cultures were carried out by using Linsmaeir and Skoog (L.S medium, 1965). The NaCl resistant cell lines have been isolated by culturing the callus on L.S. medium containing LD_{50} concentration of NaCl. Friable embryogenic callus were obtained by lowering the level of 2,4-D or its omission during subculture. This callus served as the source of suspension cultures. For induction of somatic embryoids different growth regulators were tested. In order to enhance the percentage of embryogenesis and their germination different aminoacids such as proline, hydroxyproine, polyamines like putrescine and inhibitor of polyamines like MGBG were incorporated into the embryogenic medium. To reduce the problem of rhizogenesis, NaCl (0-250 mM) and activated charcoal (0-8%) were incorporated in the medium. Embryoids were subjected to encapsulation and storage. The levels of proline, protein, amylase and IAA oxidase were determinated in embryogenic and non-embryogenic callus cultures during different growth intervals (at the end of 0, 10, 20, 30 and 40 days).

It was noticed that growth of the callus followed a typical pattern attaining maximum growth at the end of fourth week. Incorporation of NaCl (0-300 mM) led to the reduction in growth and growth inhibition was concentration dependent. The callus showed a 51.2% reduction in dry weight (250 mM NaCl) at the end of fourth week compared to the control.

Regeneration of the adapted callus was obtained in MS medium supplemented with IBA (1.5 mg/l) and KN (0.5 mg/l). A combination of auxin and

cytokinin was effective in achieving a high percentage of regeneration in adapted callus.

By lowering the level of 2,4-D or its omission during subculture resulted in the production of loose, friable callus with many green spots termed as embryogenic callus. A fine suspension was raised from these cultures in L.S. basal medium within 18-20 days. Induction of somatic embryoids was achieved in L.S. medium supplemented with IAA (0.1 mg/l) and BAP (0.5 mg/l). Almost 61% of NaCl adapted cultures produced embryoids with less rhizogenesis while 48% of non-adapted cultures produced embryoids with high percentage of rhizogenesis.

Incorporation of proline (8 mM) into the embryogenic medium brought about the maximum response with respect to number of embryoids per culture and percentage of embryogenesis. Hydroxyproline at 4 mM concentration was effective in enhancing embryogenesis. Addition of putrescine reduced the percentage of embryogenesis and enhanced the rhizogenesis in cultures. An inhibitor of polyamine MGBG at 0.5 mM concentration improved the percentage of germination of embryoids and reduced the rhizogenesis.

Under the influence of 50 and 100 mM concentrations of NaCl and 8% activated charcoal a reduction in rhizogenesis was observed in embryoids derived from non-NaCl adapted cultures. Among different additives tried 4% sucrose was found to the best for the maturation of embryoids. Matured embryoids germinated when placed on 1/2 strength L.S. medium supplemented with 5% sucrose and 8 mM proline. The encapsulated embryoids showed viability upto 10 days on storage at 4°C.

The highest level of proline in both the calli was observed at the end of fourty days. Salanization of the medium led to an increased accumulation of

proline in the embryogenic calli as compared to the non embryogenic ones. In contrast to the content of proline the level of total proteins showed a reduction under the influence of salt. However, the non-embryogenic callus accumulated more protein under the influence of salt.

The activity of amylase rose sharply in cultures during the growth period and showed a decrease under saline stress. The embryogenic calli exhibited a low activity of amylase compared to that of non-embryogenic calli. The activity of IAA-oxidase of calli under different treatments registered the peak on day 20 and declined thereafter. A higher activity of IAA-oxidase was observed in embryogenic callus as compared to that of non-embryogenic calli.

It is concluded from the present studies:

1) Callus induction in rice was observed on L.S. medium supplemented with 2% sucrose and 2.5 mg/l of 2,4-D within 5 days of inoculation.

2) NaCl at 0.25 M reduced the callus growth by 50%.

3) Lowering the level of 2,4-D or its omission has been found to be very critical in the production of embryogenic callus.

4) L.S. medium supplemented with 0.1 mg/l IAA and 0.5 mg/l BAP was the best for the induction of somatic embryogenesis.

5) Putrescine (0.4 and 0.8mM) did not promote embryogenesis but it promoted rhizogenesis while MGBG (an inhibitor of polyamine biosynthesis) exhibited an opposite effect; it promoted embryogenesis and reduced rhizogenesis.

6) The problem of rhizogenesis was overcome by using 50 and 100 mM NaCl as well as 8% activated charcoal.

7) Maturation of embryoids can be achieved by the incorporation of 4% sucrose in the maturation medium.

8) Incorporation of sucrose at 5% level and proline at 8mM into 1/2 strength L.S. medium can be employed to achieve the highest percentage of germination of embryoids.

9) Encapsulated embryoids can be stored at 4°C for a period of 10 days without loosing their viability.

10) Embryogenic nature of calli can be attributed to their high level of proline, low level of protein, a high activity of IAA-oxidase and a low activity of amylase. The high activity of IAA-oxidase and the requirement of an extremely low level of auxin or no auxin clearly indicate that auxin does not play a crucial role in somatic embryogenesis.