B-40: Establishment of cell cultures of *Oryza sativa* (rice) for studies on salinity tolerance in two potential rice cultivars

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Rice is the staple food in many Asian countries including Sri Lanka. As a result of breeding programmes at Rice Research Institutes in Sri Lanka, high yielding varieties are available today. With increasing salinity levels in rice growing soils, emphasis has recently been given to improve the salinity tolerance, especially in high yielding varieties. Use of plant cell and tissue culture would shorten the long term traditional breeding programmes. Attempts have been made during the past 3 years to develop tissue culture techniques for plant regeneration from single cells and, to study the salinity tolerance of the cells in culture. The main objective of this study is to improve 2 high yielding varieties BW 351 and AT 354, for salinity tolerance. A study on screening for salinity tolerance in cell cultures is reported.

De-hulled seed of the varieties BW 351 and AT 354 were used. The following experiments were carried out:

(1) *Establishment of callus*
* Selection of the most suitable medium out of the media tested
* Effect of the position of the embryo on the medium
* Effect of light on callus production

(2) *Establishment of cell cultures*
* Selection of the most suitable media out of the media tested
* The effect of the duration of cells in culture

(3) *Screening for salinity tolerant cells in culture* NaCl (1N) was added to the IRRI medium to adjust the salinity level to 6, 8, 10 ms/cm. An equal amount of callus pieces (5 pieces each with a size of 0.5 x 0.5 mm^2) were transferred to the liquid IRRI basal medium supplemented with 0.5 mg/l 6-benzylamino purine (BAP) and was incubated on an orbital shaker under continuous shaking at 60 rpm. The flasks were kept in 16 h light period with white cool fluorescent light.

Cell culture development
(1) The number of cells (round, oval, elongated) were counted every 2 days,
(2) Cells in the division stage were also counted.
(3) The colour of the liquid medium (milky or not).

Salinity tolerance: Samples were observed every 2 days and the number of plasmolysed cells were counted using a haemocytometer. The no. of round cells and elongated cells were also counted.

The study reveals that of the 3 basal media tested (Murashige & Skoog, N-6 and Leishmeir & Skoog (LS)), on BW-351 and AT-353, the best callus production was on MS and N-6. However, on LS, callus was produced but the development was poor. Of the 3 positions of the embryo on the media, best callus development was seen when the embryo was above or on the surface of the culture media. No callus was observed in those seeds where the embryo was within the medium. This clearly shows that the callus is produced from the embryo. Light appears to enhance callus growth.

Cell cultures were established on MS basal with BAP (2.0 mg/l) and MS with BAP (2.0 mg/l) + 2,4-D (2.5 mg/l). Presence of BAP appears to reduce abnormal elongation of the single cells in culture, thus maintaining cells in their round/oval shape. The IRRI medium showed a salinity of 5.6 ms/cm and in that medium, without the addition of growth regulators (synthetic plant hormones), a 50% and 25% plasmolysis was shown by BW-351 and AT-354 respectively. When the salinity was increased to 6.0 ms/cm, there was no change in the % plasmolysis. However, at 8 ms/cm, BW-351 showed 75% while AT cultivar showed only 40% plasmolysis.

A better callus production could be obtained on MS and N-6 basal media. The embryo of the seed should be positioned on the surface or above the medium for better callus development. The callus of de-hulled seed could be used to develop cell cultures and presence of BAP cytokinin appears to maintain cells in culture, in round/oval shape state. Such cell cultures have the potential for plant regeneration.

The results confirmed that AT-354 has better salinity tolerance than the BW-351 variety. The former can tolerate upto 8 ms/cm. In general, in both varieties, 95% plasmolysis is observed at 10 ms/cm. However the 1% of non plasmolysed cells, if survived, can be utilized to regenerate plants and to test for salinity tolerance. The study reveals that there is a potential for *in vitro* development of salinity tolerance in rice.