THESIS TITLE : CELL SUSPENSION CULTURE OF SUGARCANE cv. F140 AND TESTING FOR SALT TOLERANCE OF CELLS AND PLANTLETS *IN VITRO*

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Abstract

The objectives of this study were to develop a technique for cell suspension culture of sugarcane (cv. F140) and to test obtained cells and plantlets *in vitro* for salt tolerance.

To develop a technique for cell suspension culture of sugarcane (cv. F140), Microcallus was produced from culture of sugarcane callus induced from young leaf in agar MS medium supplemented with 3 mg/l of 2,4-D and 15% of coconut water (v/v). Microcallus was further developed to produce plant top and root. The optimum age of callus to be cultured was 4 weeks. Sugarcane cells were also cultured in liquid food. Those cells were sieved to pass through 200 μm nylon mesh. The cells were then cultured in liquid MS medium supplemented with 0.5 mg/l of NAA, 0.1 mg/l BA and 10% of coconut water and liquid food was renewed every 5 days. The cells took approximately 15 weeks before developing to be 1.7 – 1.9 mm calli. Brown color

substance occurred on some of induced callus and could be prevented by reducing 50% of ammonium nitrate concentration in agar and kept culture in dark for approximately 7 days. Microcalli could be induced to form plantlets on MS medium supplemented with 0.1 mg/l NAA and BA and 15% of coconut water (v/v). However, 16% of total calli obtained were white, while there was no white callus developed from sieved cell. Sugarcane roots only developed when calluses were cultured in MS agar with NAA.

Sugarcane cells obtained from the above technique were tested for salt tolerance by culture in MS with 7 levels of salt (NaCl) added at 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% concentration(w/v). After 20 days, it was found that the higher salt concentration the lower viability of cells was. Sugarcane cells could not survive after being cultured in 1.5% to 3.0% of salt longer than 20 days. After cells from suspension culture were transferred to semi-solid MS with the same salt concentration for 6 weeks only cell culture with no salt could developed to 92 colonies. The colony size was 0.5 - 0.7 mm. in diameter. The cell culture on 0.5% of salt concentration could develop only 7 colonies and that on the MS medium with salt concentration at 1% could not develop any colony. Somaclones of sugarcane were also tested for salt tolerance by culture in MS with 6 levels of salt added at 0, 0.5,1.0, 1.5, 2.0 and 2.5% concentration. These somaclones were cultured for 6 weeks. After 4 – 5 days, the rolled leaf tip was generally observed. Increasing level of salt resulted in poorer growth (low dry matter, low shoot number). From this study, there were no evidences of differences in responses to salt tolerance among somaclones of sugarcane tested.