

## **Cryopreservation of *Capsicum annum* var. *grossum* using encapsulation/dehydration of apices produced *in vitro***

Senarath, WTPSK, Lee, Kui-Jae, Rehman, S and Lee, Wang Hyu  
Faculty of Bioresources Sciences, College of Agriculture,  
Chonbuk National University, Republic of Korea

Shoot tips of *in vitro* propagated plantlets were cryopreserved using encapsulation/dehydration procedures. Shoot tips were excised under filter sterilized antioxidants solution (0.2M phosphate buffer, pH 5.7 supplemented with 5g/l ascorbic acid and 15g/l sodium borate). They were drawn up into a sterile 10cm<sup>3</sup> disposable pipette and were dropped into the culture medium with 2.5w/v Na-alginate, then into 100mM CaCl<sub>2</sub>.2H<sub>2</sub>O. Encapsulated shoot tips were transferred into 10cm<sup>3</sup> of liquid culture medium with a range of sucrose concentrations (0.25-1.0M) and were incubated in dark for 24 hours in 18C at 40rpm. Beads were then dehydrated in silica gel for different time intervals (1-24 hours). Then they were freeze dried either rapidly (plunge directly into liquid N<sub>2</sub> or in two stages (samples were kept at 20C for 10 minutes, then reduced to 35C at 1C per minute. Then, plunge into liquid N<sub>2</sub>). The influence of sucrose and silica gel pre-treatment on pre- and post-freeze shoot growth was examined.

Shoot tips directly cryopreserved in liquid N<sub>2</sub> elongated into shoots in the culture medium after 28 days and managed to reproduce 100% roots. Encapsulated shoot tips showed slight shoot elongation and took 90 days to grow away from Ca-alginate. Sucrose pretreatment of encapsulated shoot tips significantly reduced the shoot growth at 0.75M and 1.0M concentration and produced the callus at the base. 0.5M sucrose has no effect on shoot elongation. Exposure to the silica gel for 2 hours after incubation at 0.5M sucrose has no effect on shoot growth but 3 hour exposure reduced the shoot elongation significantly. Two stage freezing and incubation of beads in 0.5M sucrose and 2 hour exposure to silica-gel causes callus production but no shoot regeneration. It is possible to recover the shoot tips from liquid N<sub>2</sub> after 24 hour pre-treatment in the medium containing 0.5M sucrose followed by 2 hour drying in silica-gel and rapid freezing.