

## DESICCATION AND CRYOPRESERVATION TOLERANCE OF BARE EMBRYONIC AXES FROM 'PINEAPPLE' SWEET ORANGE (*Citrus sinensis* [L.] OSB.) PRE-TREATED WITH SUCROSE

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The effect of dehydration to different water contents was studied in excised embryonic axes of 'Pineapple' sweet orange (*Citrus sinensis* [L.] Osb.). Total germination was obtained with control 'Pineapple' sweet orange axes that were not subjected to dehydration or freezing. As desiccation progressed a steady decline in seedling recovery was observed until total loss of viability was observed. Axes pre-cultured with sucrose retained regeneration potential even following dehydration to 0.12 mg H<sub>2</sub>O.mg<sup>-1</sup> dry mass. Embryonic axes were successfully cryopreserved, attaining up to 70% viability after their water content was reduced to approximately 0.20 mg H<sub>2</sub>O.mg<sup>-1</sup> dry mass. Dehydration of axes pre-cultured with sucrose occurred at a much slower rate as compared to controls. Carbohydrate analysis results indicate that embryonic axes did not take up sucrose from the pre-culture medium. However embryonic axes pre-cultured with sucrose showed increased sucrose and fructose levels when they were dehydrated, reaching maximum levels at 0.20 mg H<sub>2</sub>O.mg<sup>-1</sup> dry mass and decreasing with desiccation to lower water contents. Tissue glucose did not change significantly with desiccation. Raffinose and stachyose levels dropped as pre-cultured embryonic axes were dried. Tissue levels of glucose, in untreated embryonic axes increased with desiccation up to 0.20 mg H<sub>2</sub>O.mg<sup>-1</sup> dry mass, and decreased as the axes were desiccated to lower water contents. In contrast, fructose and sucrose levels did not show significant increases when untreated samples were desiccated for the same periods of time. Raffinose and stachyose levels decreased drastically as untreated embryonic axes were desiccated. DSC analysis revealed the presence of a broad melting peak was observed in fully hydrated embryonic axes; the size of the melting peak reduced as water was removed by desiccation. Minimum melting of water was observed at the point axes survived cryopreservation. Occurrence of a glass transition upon warming was not a condition for axes to survive liquid nitrogen exposure.

**Key words:** *Citrus*, cryopreservation, embryo, sugars