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Causes of death in Eucalyptus grandis partially dried in vitro axillary buds

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ABSTRACT

Eucalyptus grandis and its hybrids is the most important and widely planted eucalypt in South Africa. It has a wide range of uses including pulpwood, poles, firewood, charcoal, flooring, mining, furniture and general carpentry. Conservation of plant genetic resources including those used in agriculture, horticulture and forestry has become an issue of common global concern. Cryopreservation involves the storage of plant material at ultra low temperature (-196°C). The techniques for cryopreservation currently in use are varied and include the older classical techniques and the new vitrification-based techniques. Storage of biological material at -196°C causes metabolic functions to slow down considerably and minimize biological degradation, thus allowing for long-term preservation. However, there are particular stresses associated with the freezing process, e.g., ice crystal formation and cryo-dehydration, which may severely damage the material. Tolerance to drying is the key to successful cryopreservation and is commonly used in the preparation of *in vitro* material for cryostorage. However, drying may result in damages and a number of stresses that may activate caspase-like proteases and trigger cell death processes such as programmed cell death and necrosis. During the drying process, the physical and physiological characteristic of the cell changes because of the removal of water and damage is reflected by the lack of resumption of normal activity upon rehydration.

As part of a cryo-procedure, *Eucalyptus grandis* axillary buds isolated from *in vitro* shoots were dried over silica gel for 20 minutes. Pre-treatment of the shoots with 5mg.l^{-1} ABA for 5 days resulted in partial resistance of the isolated buds to water loss (76% to 45%) as compared with untreated buds (76% to 33%). Concomitantly, viability decreased from 100 to 70% for ABA treated buds and to 55% for the untreated buds. Ultrastructural examination showed cellular responses to drying, ranging from cell death, through partial disruption to organelles to apparently normal ultrastructure. The use of the vital stains, 4,6-diamidino-2-phenylindole and propidium iodide, showed that certain regions of the buds (e.g. the leaf primordia) were the most prone to drying damage. The meristem, however, appeared to survive drying and for up to 72 hours of rehydration.

High Reactive Oxygen Species (ROS) activity was associated with bud excision and the drying procedure. Caspase-3-like protease activity was detected after drying and rehydration in both nonviable treated and untreated buds, but not in the hydrated controls. The Caspase-3 inhibitors Ac-DEVD-CHO, pepstatin and leupeptin partially suppressed that activity. The ultrastructural studies and the use of the vital stains provided confirmation of the beneficial effects of ABA. The detection of a caspase-3-like protease has provided some evidence that the rehydrated buds, that had ultimately died, had undergone programmed cell death. The ROS production during bud isolation which was exacerbated by the drying procedure is considered to be the trigger for the programmed cell death. Data in the present study showed the role of both necrosis and PCD in the death of the tissues of the axillary buds of *E. grandis* axillary buds. The data also contributed to the better understanding of the impact of cryoprocedures on these clonal tissues which are ideal propagules for forestry germplasm conservation.