Abiotic stress cross-tolerance in *Eucalyptus grandis*: Does pre-exposure to chilling stress induce cross-tolerance to cryopreparative drying of *in vitro E. grandis* shoots?

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ABSTRACT

In the forestry industry the requirement for the maintenance of a broad genetic base is integral to the success of breeding programmes such as those for *Eucalyptus grandis*, an important species to the South African forestry industry. Plant cryopreservation is an economical option to maintain such a genetic base, as it allows storage of vegetative materials at sub-zero temperatures, while maintaining juvenility. However, successful cryopreservation of this sub-tropical species has been restricted by its sensitivity to cryopreparative drying. As a consequence, the viability of material is reduced even before reaching the freezing stage. Despite this abiotic stress restriction, evidence of upstream 'cross-talk' implying downstream 'cross-tolerance' has suggested the possibility that cold acclimation may improve the tolerance responses towards dehydration stress by means of 'cross-acclimation'. It was therefore the aim of the study to understand some of the physiological and biochemical responses of *in vitro E. grandis* shoots to different non-freezing low (chilling) temperatures and exposure periods, and to establish an appropriate 'cross-acclimation' regime for the physical drying pre-treatment.

E. grandis shoot clusters (4-8 leaves and 2-5 axillary buds) were exposed to the chilling temperatures of 5°C, 10°C or 15°C for 1 or 3 days. The physiological and biochemical responses were evaluated, and thereafter the appropriate cold acclimation (or 'cross-acclimation') regime selected. The appropriate physical drying time was also selected for shoot clusters according to their physiological responses. When the appropriate regimes had been determined, the physiological and biochemical responses of shoot clusters treated consecutively to cold acclimation and then physical drying were evaluated. The physiological responses evaluated were water content, viability, and vigour (i.e. the number of visible axillary buds and shoots produced over 2 weeks). The biochemical responses measured were the concentrations of: 1) total soluble sugars, 2) starch, 3) phenolic acid, and 4) superoxide.

The data suggested that the appropriate cold acclimation regime was treatment at 10°C for 3 days. This was based on the accumulation of the high levels of phenolic acid (3.05 ± 0.09 mg GAE.g^{-1} FWS) and positive vigour responses (11.90 ± 0.60 visible axillary buds/week and 3.10 ± 0.20 visible shoots/week), compared with the other chilling temperature treatments. The appropriate drying time selected for shoot clusters was 80 min over activated silica gel to achieve a water content of 0.32 ± 0.04 g water.g^{-1} FWS. In the dried material there were high levels of soluble sugars (47.65 ± 1.90% of the fresh weight of shoots) and unknown components that accounted for 48.10 ± 1.86% of the fresh weight, followed by phenolic acid (3.09 ± 0.05%) and proline (0.490 ± 0.011%). Despite these measured responses, viability of the shoots was impacted by drying, dropping to 88.9 ± 3.9%. When shoot clusters were pre-treated at 10°C for 3 days and then physically dried, viability of all (100%) the material was retained and the water content did not drop as low as with physical drying alone, dropping to 0.52 ± 0.05 g water.g^{-1} FWS. The biochemical responses showed that tolerance was strongly dependent on a high proportion of soluble sugars (83.66 ± 1.48% of the fresh weight of shoots) and phenolic acid (3.77 ± 0.12%), followed by proline (0.406 ± 0.018%).

The study had confirmed that ‘cross-acclimation’ through means of cold acclimation (chilling pre-treatment at 10°C for 3 days) can induce ‘cross-tolerance’ towards physical drying, where osmotic adjustments and osmoprotection appeared to have been improved. It is therefore possible that this may have the potential to improve survival during the latter stages of the cryopreservation procedure, despite the higher retention of water in shoot clusters after drying.