reduce the water content of the material prior to explant excision, are currently being investigated.

doi:10.1016/j.sajb.2007.04.027

The effect of desiccation on the oxidative burst from isolated embryonic axes of recalcitrant *Castanea sativa* seeds

T. Roach [a], M. Ivanova [b], R. Beckett [b], F. Minibayeva [c], I. Green [a], H.W. Pritchard [a], I. Kranner [a]
[a] *Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, West Sussex RH17 6TN, United Kingdom*
[b] *School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*
[c] *Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, PO Box 30, Kazan 420111, Russian Federation*

An estimated 90% of the world's seed-bearing plants have desiccation tolerant, orthodox seeds. In contrast, recalcitrant seeds, mainly found in trees, cannot withstand desiccation and need to germinate within a limited period of time after dispersal from the mother plant. Due to their intolerance of desiccation, recalcitrant seeds are not part of natural *in situ* soil seed banks and they cannot be stored *ex situ* in conventional gene banks. However, cryopreservation techniques exist that allow storage of embryonic axes of recalcitrant seeds in liquid nitrogen. Here we report that excision is accompanied by a burst of superoxide (O_2^-) and show how O_2^- production is affected by desiccation. Extracellular production of highly toxic reactive oxygen species (ROS) such as O_2^- is a typical wounding response used by organisms to deter microbial pathogens. Patterns of O_2^- production were studied in isolated embryonic axes of European Sweet Chestnut (*Castanea sativa*). Superoxide was detected colorimetrically after staining with nitroblue tetrazolium, and electron spin resonance with Tiron as a spin trap was used to confirm its chemical nature. Superoxide was immediately produced on the cut surface after isolation of the axis from the seed, with an initial 'burst' in the first 5 min. Isolated axes subjected to variable levels of desiccation stress showed a decrease in viability and vigour, as well as a loss of membrane integrity, indicative of intracellular damage by ROS. The pattern of O_2^- production showed a typical Gaussian pattern in response to stress. The initial burst of O_2^- production first increased from below 4 μmol g^{-1} h^{-1} in un-desiccated axes with a water content (WC) of about 60% (fresh weight basis) to more than 10 ìmol g^{-1} h^{-1} in desiccated axes that had lost 50% viability (WC = ~40%), with O_2^- production then falling to close to 4 μmol g^{-1} h^{-1} in axes that had lost viability (WC = ~25%). The results indicate a complex interaction between excision and subsequent drying that merits further investigation.

doi:10.1016/j.sajb.2007.04.028

Desiccation sensitivity of excised embryonic axes of selected Amaryllid species

S. Naidoo, N.W. Pammenter, P. Berjak
Plant Germplasm Conservation Research, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa

The study assessed the desiccation-sensitivity of the excised embryonic axes of the following Amaryllidaceae species: *Scadoxus puniceus*, *Amaryllis belladonna*, *Nerine huttoniae*, *N. humulus*, *N. bowdenii*, *N. filifolia*, *Haemanthus humulus humulus*, *H. coccineus*, *H. deformis*, *H. bakerae*, *Brunsvigia gregaria*, *Brunsvigia orientalis*, *Boophane disticha*, *Strumaria discifera*, *Crinum macowanii* and *C. bulbispermum*. Using SurGeo, a database integrating spatial and geographic parameters, the relationship between environmental characteristics associated with the populations sampled and seed characteristics/responses was also investigated. Excised embryonic axes were rapidly dehydrated (flash-dried). Curves of water content (g g^{-1}) and viability (%) versus drying time (min) were used to interpolate the water content (WC) and drying time (DT) values corresponding to 80% viability (WC/DT V = 80) for individual species, in a comparison of desiccation-sensitivity between developmental stages and among years, provenances, species and genera. The seeds of all sixteen species were shed with high axis water contents (2.60 ± 0.16 to 6.94 ± 0.93 g g^{-1}, dmb) and were sensitive to desiccation, with WC V = 80 ranging from 0.13 to 3.15 g g^{-1}. Germination in amaryllids is unusual in that part of the cotyledon, to which the embryonic axis is attached, grows right out of the seed, when axes were found to be more desiccation sensitive in six out of nine species investigated. Both axis shedding water content (SWC) and the degree of desiccation-sensitivity were species-characteristic. SWC was not significantly related to the time taken to flash-dry axes to water content at which viability was equivalent to 80%. Seed fresh mass (SFM) differed widely across species. Parent plants were not confined to any particular biome type and some occurred in areas characterised by seasonal dry spells. Seed and habitat characteristics were not useful in predicting the degree of desiccation-sensitivity across a number of species belonging to the same family.

doi:10.1016/j.sajb.2007.04.029

The effects of cryopreservation of recalcitrant excised *Amaryllis belladonna* embryonic axes on the vigour of recovered seedlings

S. Naidoo, N.W. Pammenter, P. Berjak
Plant Germplasm Conservation Research, School of Biological and Conservation Sciences University of KwaZulu-Natal, Durban, South Africa

Cryopreservation at liquid nitrogen temperatures is the most promising route for the long-term germplasm conservation of recalcitrant seeds. Considerable attention has been paid to