

understanding and optimising partial dehydration, freezing and thawing protocols, but since the long-term objective is the re-introduction of viable plants into the field, it is important to assess the vigour of plantlets derived from embryonic axes subjected to cryopreservation as in this study. Here, the excised embryonic axes of *Amaryllis belladonna* when subjected to partial dehydration (D) and the combination of partial dehydration and cooling (D+C) produced seedlings that were less vigorous than those from fresh axes. Prolonged exposure to relatively low leaf water potential, failure to equilibrate with soil water potential overnight, indications of permanent leaf wilting (i.e. persistent turgor loss), a decrease in potential photochemical efficiency and signs of metabolic disruption, as well as abnormal root growth in such seedlings, suggests that plantlets derived from cryopreserved *A. belladonna* axes may be more susceptible to water stress. Even though D-seedlings performed better than D+C-seedlings when water stressed, seedling mortality in stressed seedlings belonging to both these treatments was comparable. This suggests that partial dehydration of axes, even when not followed by cooling, can compromise vigour in recovered seedlings. Hence attention is now being directed to ameliorative post-treatment procedures.

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#### Identification of different temporal classes of gene expression during a cycle of desiccation in the resurrection plant, *Xerophyta humilis*

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Physiological studies have shown that desiccation tolerance in the vegetative tissues of angiosperm resurrection plants is a consequence of protective mechanisms being laid down during desiccation, and repair mechanisms being active during rehydration. We have completed a detailed analysis of the changes in mRNA transcript abundance over a cycle of desiccation in the indigenous Southern African resurrection plant, *Xerophyta humilis*, to match these physiological studies and to identify different temporal classes of genes that are activated in leaves during desiccation. We hypothesized that two distinct groups of genes are activated in response to desiccation: early desiccation-responsive genes overlapping with genes that are similarly activated in desiccation sensitive plants during mild water stress, and later, late-desiccation responsive genes overlapping with genes involved in conferring desiccation tolerance in seeds. mRNA transcript abundance of 3400 *X. humilis* cDNAs was measured by microarray analysis, in leaf tissue at six different stages of water loss during a cycle of desiccation. Following normalization of Microarray data, a total number of 2637 cDNAs were identified as differentially expressed across these conditions. The expression patterns of these differentially expressed genes were clustered using PAMSAM, to identify different temporal classes of genes that are activated or repressed during desiccation.

Our initial hypothesis was simplistic and clustering analysis identified a more complex pattern of gene expression patterns. Clustering of conditions done by SAMMON, PCA and DIANA revealed that fully hydrated leaves have a very distinct gene expression profile from the other desiccating leaf samples. These data suggest that the desiccation stress response is activated at very early stages of water loss in the leaves of *Xerophyta humilis*.

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#### Desiccation sensitivity of seeds and changes in respiratory rate and pathways of mitochondria

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*Artocarpus heterophyllus* and *Antiaris toxicaria* seeds are recalcitrant seeds, and *Zea mays* seeds are orthodox. *A. heterophyllus* seeds, *A. toxicaria* axes and *Z. mays* embryos, and their purified mitochondria were used as experimental materials in this work, where the relationship between desiccation sensitivity and changes in respiratory rates and pathways of mitochondria was studied. With dehydration, germination percentage of *A. heterophyllus* seeds, *A. toxicaria* axes and *Z. mays* embryos markedly decreased, water contents at which germination percentage decreased by 50% being about 0.675, 0.374 and 0.11 g H<sub>2</sub>O g<sup>-1</sup> DW, respectively. Activities and latency of cytochrome *c* oxidase (CCO) of mitochondria from *A. heterophyllus* seeds, *A. toxicaria* axes and *Z. mays* embryos decreased. Malate dehydrogenase (MDH) activity and respiratory rate (state III) of *A. heterophyllus* seeds increased at the early phase of dehydration, and then decreased; the MDH activities and respiratory rates of *A. toxicaria* axes and *Z. mays* embryos gradually decreased with dehydration. It was very interesting that respiratory rates inhibited by KCN (% of total respiratory rate) in mitochondria of *A. heterophyllus* seeds and *A. toxicaria* axes significantly decreased with dehydration, and of *Z. mays* embryos, and decreased at the early phase of dehydration and then increased; however, respiratory rates inhibited by salicyl-hydroxamic acid (SHAM) in mitochondria of *A. heterophyllus* seeds and *A. toxicaria* axes increased with dehydration, and of *Z. mays* embryos, and decreased with dehydration. In addition, respiratory rate uninhibited by KCN and SHAM also increased with dehydration in mitochondria mentioned above. It is concluded that desiccation sensitivity of seeds is closely related to changes in respiratory rates and pathway of their mitochondria.

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#### The development of genetically modified maize for abiotic stress tolerance