

contrasting rates. Evidence from a broad range of recalcitrant seeds of temperate species cryopreserved using different conditions suggests that axis size, water content (intracellular viscosity) and survival are intrinsically linked. The work presented will draw from calorimetric, microscopical and *in vitro* studies and proposes possible long-term cryopreservation strategies with broad applicability across recalcitrant species.

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An assessment of intermediate storage behaviour in Neem (*Azadirachta indica* A. Juss) seeds

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Mature seeds of neem (*Azadirachta indica* A. Juss.), shed at 42.2% water content (wc), fresh weight basis exhibited 100% germination up to 15 days after harvest, and deteriorated on natural desiccation below 10.9% wc at ambient conditions. The seeds showed complete loss of viability after 20 weeks (5.9% wc) in storage. Seeds dried rapidly over silica gel to 7.1% wc could not only be cryopreserved but also showed high survival after one year of storage in liquid nitrogen. Our results suggest that neem seeds are desiccation-tolerant to this water content, but once further dehydrated, exhibit desiccation-sensitivity. An effort to understand the mechanism of desiccation-sensitivity below this water content revealed that loss of viability was closely associated with the over-accumulation of ROS and lipid peroxidation products (LPP) both in the embryonic axes and cotyledons. The antioxidant enzymes showed a differential expression in the embryonic axes of the desiccating seeds. Activities of catalase, ascorbate peroxidase and guaiacol peroxidase exhibited substantially higher levels in the 100% viable seeds dehydrated up to LSWC. Their activities declined sharply in the embryonic axis of seeds dried below LSWC. On the contrary a high level of superoxide dismutase was discernible in highly desiccated and low viability seeds as well. Impairment of catalase and peroxidase activity probably led to high accumulation of ROS. Significant role of drying in loss of viability and vigour and role of ROS and antioxidant enzymes is discussed to explain the intermediate storage physiology of these seeds.

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Posters

Characterisation of 'seed' LEA1-Em genes in vegetative tissues of the resurrection plant *Xerophyta humilis*

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The presence and expression patterns of orthologues of LEA group 1 genes has been characterised in the resurrection plant, *Xerophyta humilis*. The group I LEAs (Em1 and Em6) were first identified as proteins that were abundantly and specifically expressed during the desiccation and germination phase of angiosperm seed development. The group I LEA genes are characterised by the presence of one or more tandemly repeated 20-amino acid motifs that are particularly rich in Gly residues. In *Arabidopsis*, the group I LEA genes (AtEM1 and AtEM6) have been used as a model to study the regulation of gene expression by ABA (abscisic acid) during seed development. Em1 is preferentially expressed in the pro-vascular tissues and in meristems in the embryo, whereas, Em6 is expressed throughout the embryo. Phenotypic analysis of AtEM6 T-DNA insertion mutants has shown that AtEM6 has been shown to play a role in buffering the rate of dehydration during the later stages of seed maturation, but does not otherwise affect plant development. Since the LEA1 genes are specifically associated with seed maturation and not abiotic stress responses in desiccation sensitive plants, we have targeted the characterisation of *X. humilis* LEA1 genes, to test the hypothesis that evolution of desiccation tolerance in *X. humilis* is a consequence of activation of seed specific genes in vegetative tissue. Degenerate PCR primers designed to conserved regions of LEA1 genes were used to amplify three LEA1 orthologues from cDNA prepared from *X. humilis* desiccated seed, root and leaves. The full-length cDNAs of these orthologues was cloned by 5' and 3' RACE PCR. These three *X. humilis* LEA1 orthologues XhLEA1-1, XhLEA1-2 and XhLEA1-4 respectively have one, two and four of the 20 amino acid motif repeats. A fourth LEA1 orthologue was identified in a microarray screen for mRNA transcripts that are up-regulated during desiccation in *X. humilis* leaves. The expression of these seed-specific LEA1 genes in desiccated vegetative tissues is consistent with our hypothesis that desiccation tolerance in *X. humilis* has evolved from the activation of seed-specific genes in vegetative tissues.

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Characterisation of two soybean (*Glycine max* L.) LEA 4 proteins — circular dichroism and Fourier transform infrared studies

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