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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Late embryogenesis-abundant (LEA) proteins are accumulated to a high level during late stages of seed development, and have been proposed to play a role as osmoprotectants. Although a correlation between protein accumulation and osmotic protection in various plant species has been demonstration, the functions and mechanisms of LEA proteins remains to be elucidated. Currently, 6 major groups of LEA proteins have been described. In the present study, we report the characterization of two members of soybean LEA 4 proteins, the basic GmPM1 and the acidic GmPM28, by circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy. The spectra and protein folding parameters of both proteins revealed no, or limited, defined secondary structure in the fully hydrated state. Therefore, the soybean LEA 4 proteins should be classified as 'natively unfolded proteins'. Conformational changes of GmPM1 or GmPM28 proteins could be induced under hydrophobic or dry conditions. In the present study, after fast or slow dry, the two LEA 4 proteins slightly increased the proportions of defined secondary structures (α-helix and β-sheet) from 30% to 49% for GmPM1, or from 34% to 42% for GmPm28. Nevertheless, the interaction between non-reducing sugars and both soybean LEA 4 proteins still occurred after this kind of conformational change. Also, the soybean LEA 4 proteins possessed the ability to affect the conformations of poly-l-lysine after slow drying, whereas aggregated soybean LEA 4 proteins, which were denatured at room temperature, lost the ability. Our work suggests that soybean LEA 4 proteins may be functional during dehydration, and may play a role as a molecular chaperone to prevent protein aggregation.

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## Responses to chilling of recalcitrant seeds of *Ekebergia capensis* from different provenances

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Recalcitrant seeds of tropical origin may be chilling-sensitive, and it is possible that this may be influenced by provenance. In this study, the effect of chilling on recalcitrant seeds of *Ekebergia capensis* from Port Elizabeth (33°30′ S, warm temperate) and Mtunzini (28°22′ S, subtropical) was studied. Seed shed in Port Elizabeth occurs during winter (June/July, mean minimum temperatures 8 °C), whereas seed shedding is a summer phenomenon for trees growing in Mtunzini (Jan/Feb, mean minimum temperature 18 °C). Seeds from both provenances were stored hydrated; those from Port Elizabeth at 1, 3 and 6 °C, while those from Mtunzini were stored at 3 and 6 °C. Initial water contents were 1.5 and 1.8 g $g^{-1}$ for Port Elizabeth and Mtunzini seeds, respectively, and did not decline by more than 10% over the storage period. After 12 weeks seeds from Port Elizabeth stored at 6° showed 100% viability, and those stored at 1 and 3° retained 80% viability. Germinability of seeds from Mtunzini declined to 10% after only 20 days at 3 and 6°. The data strongly suggest an influence of provenance on chilling sensitivity of seeds of *E. capensis*. Molecular studies indicate that plants from the two populations are genetically distinct. Comparisons of biochemical and ultrastructural responses to chilling of seeds from the two provenances are currently underway.

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## Desiccation sensitivity of white and black (red) oak embryonic axes

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The genus *Quercus* constitutes the most important aggregation of hardwoods found on the North American continent and it is also one of the most valuable groupings growing in Europe. *Quercus* is generally divided into two categories black (red) and white oak groups though a third category of live oaks is sometimes recognized as well. Natural populations of oaks are at risk because of pollution, disease or loss of habitat. Populations of *Q. robur* (white) have been of particular concern in Europe since the 1970's and populations of *Q. kelloggii* (black) along the California coast were reported at risk in 1995. This study was undertaken to develop a feasibility study to preserve genetic resources of *Quercus ex situ*. Seeds of all *Quercus* species are considered recalcitrant. Viability decreases after seeds are stored for several months under moist conditions and immediately if seeds are dried to water contents less than 20 to 50% (fresh weight basis). We wished to compare desiccation sensitivity of embryonic axes isolated from acorns of white (*Q. macrocarpa*, *Q. robur*, *Q. virginiana*) and black (*Q. kelloggii*, *Q. shumardii*, *Q. velutina)* oak species. To do this, we isolated embryonic axes, dried them for different time intervals, and assessed viability using *in vitro* germination techniques. Axes from black oaks dried relatively quickly compared with those of white oaks. Black oak axes and *Q. virginiana* (sometimes classified to the live oak rather than white oak group) survived drying to water contents of 0.20–0.25 g water $g^{-1}$ dry mass and slight drying stimulated root and/or shoot growth. Axes of *Q. macrocarpa* and *Q. robur* (white oaks) survived drying only to water contents of about 0.40 g water $g^{-1}$ dry mass and recovery time increased progressively with drying. Drying and *in vitro* culture affected the timing of plumule and radicle development in growing axes of the different species. For example, root and shoot development was concurrent in *Q. shumardii* axes, but shoots formed before roots in *Q. robur* axes and roots formed before shoots in undried *Q. kelloggii* axes. When *Q. kelloggii* axes were partially dried shoots formed before the roots. Combined with cryopreservation studies, our results demonstrate the feasibility of genebanking *Quercus* germplasm.

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