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Abstracts

5th International Workshop on Desiccation Tolerance and Sensitivity of Seeds and Vegetative Plant Tissue held at the Nest Hotel, Drakensberg/Ukuhlamba, KwaZulu-Natal, South Africa from 14–21 January 2007

The format of the Workshop was such that it truly was a 'workshop'. There were 26 presentations concerning desiccation tolerance, 19 on sensitivity and 7 addressing intermediate or mild water stresses. The topics covered included desiccation damage, protection and/or repair processes, transcriptome/proteome studies, ecological considerations, and applied (biotechnological) aspects.

Presentations concerning desiccation damage covered reactive oxygen species (ROS) as agents of damage, and particularly the role of mitochondria in ROS generation, responses of membranes and the cytoskeleton to desiccation, and consideration of the mechanical implications of volume reduction associated with water loss. The aspects of protection covered included antioxidants (their stability, regeneration and the involvement of 'unusual' antioxidants), the involvement of sugars (especially sucrose), the role of LEAs (types and quantities) and of other compatible solutes. There was also some discussion as to whether programmed cell death could be involved in the response to desiccation. Repair processes were also covered; included in this topic were presentations that dealt with bryophytes (repair being less important in angiosperm resurrection plants) and recovery from mild stress, particularly with respect to recalcitrant seeds. There were several presentations covering transcriptomic and proteomic studies, particularly on resurrection plants (are the seed scientists lagging behind here, or has much of this been covered in previous developmental studies?).

It is pleasing to see that over the last few years there has been an increase in studies addressing the ecological consequences of desiccation tolerance and sensitivity, particularly in terms of recalcitrant seeds, and there were presentations on the importance (or lack thereof) of provenance, and on the cycling of desiccation tolerance in germinating seeds. The ecological considerations led on to the applied and biotechnology aspects. Most of the emphasis here was on cryopreservation of recalcitrant material for long-term conservation: aspects covered included the importance of extent of drying, the cooling rate, use of cryoprotectants, and problems associated with recovery from cryostorage.

Certain gaps in terms of deeper understanding of the phenomena of desiccation tolerance and sensitivity were highlighted. Much of our current understanding of the putative mechanisms of desiccation tolerance is based on correlative evidence. Future work should include metabolomic studies, but more than this, it should include functional studies. We need to understand not only which genes are up- or down regulated on drying, but what post-transcriptional control there is, and to what extent the gene products are actually accumulating, and what they are actually doing to protect against desiccation stress.

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Papers

Whole leaves to cellulose microfibrils: mechanical, molecular, and architectural approaches to study wall in-folding in vegetative tissues of desiccation tolerant plants

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Studies on the tensile properties of leaves have indicated that unlike desiccation-sensitive species, leaf tensile strength of the desiccation tolerant grass *Eragrostis nindensis* does not change with loss of tissue water content. Proton NMR studies indicate that *E. nindensis* retains mobile water while in the desiccated state whereas desiccation-sensitive species do not. Light microscopy, cryo-scanning electron microscopy, and transmission electron microscopy of leaves were performed to determine percent lignification and the nature of sub-cellular stabilization (wall in-folding, vacuolar fragmentation/packing, or both together) in the

dry state for five species of desiccation tolerant plants. Additionally, leaf tensile strength in the wet and dry state as well as for flash-dried leaves was determined for each species. There was a positive correlation between percent lignin/unit cross-sectional area and tensile strength of hydrated leaves. Tensile strength in wet vs. naturally dried or flash-dried tissues varied amongst the species tested, but was consistent within the three categories of sub-cellular stabilization. The desiccation-tolerant fern, *Polypodium polyploides*, is of the exclusively wall folding type of species during dehydration. One-dimensional SDS-PAGE revealed *de novo* expression of several polypeptides in fully dehydrated tissues. A 31 kD polypeptide was labeled via Western blotting using a polyclonal anti-dehydrin raised against the C-terminal consensus sequence. This putative dehydrin protein was present only during drying and in dried tissues and rapidly dissipated (within 24 h) upon tissue rehydration. Immunolocalization experiments tentatively place this protein in the cell walls of dried desiccation-tolerant tissues of *P. polyploides*. No labeling was observed in hydrated tissues of this species. Additionally no label was observed in Western blotting or immunolocalization in four other species of desiccation-sensitive ferns regardless of hydration state. For *P. polyploides*, preliminary results using atomic force microscopy indicate that microfibril arrangements are different for wet vs. fully dry mesophyll cell walls. These results suggest that the ability to avoid cell wall damage in some desiccation tolerant species by wall in-folding may be due to a combination of biochemical alterations as well as inherent flexibility in nanoscale architecture.

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Cell wall redox enzymes in lichens: A role in desiccation tolerance?

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Lichens are symbiotic organisms, comprising a fungus (the mycobiont) and either an alga or a cyanobacterium (the photobiont). They range in size from tiny crusts less than 1 mm^2 to pendulous forms that hang more than 2 m from tree branches. Lichenization is one of the most successful ways that fungi use to fulfill their need for carbohydrates, and about 20% of all fungi are lichenized. Although lichens represent a minor component of most terrestrial ecosystems, they form the dominant plant life on 8% of the world's land surface, mainly in Arctic and Antarctic. These habitats are characterized by severe abiotic stresses such as desiccation, temperature extremes and high light intensities. For this reason, lichens have been called "extremophiles", organisms that can thrive in conditions that would kill other, less specialized organisms. Lichens contain a rich diversity of cell wall enzymes, including phosphatases, cellulases, ureases, and also several redox enzymes. Redox enzymes are not present in all lichens, but occur mainly in species in Suborder Peltigerineae. The enzymes include laccases and tyrosinases, and, in a few species, peroxidases. The roles of redox enzymes in lichen biology remain unclear. Possibly tyrosinases are involved in melanization, while laccases could participate in reactions that delignify woody substrates on which lichens grow. However, it is also possible that these enzymes could have further roles in the removal of stress-induced harmful reactive oxygen species (ROS) and quinone radicals. Interestingly, desiccation stimulates the activity of these enzymes, consistent with a role in the removal of ROS. Furthermore, we recently found that although all the lichens that we tested could rapidly breakdown exogenously supplied H_2O_2, rates were twice as high in lichens in Suborder Peltigerineae. Although these lichens apparently lack extracellular peroxidases and catalases, their tyrosinases may breakdown H_2O_2 using a catalase-like mechanism. Tyrosinases may thus defend lichens against the harmful effects of desiccation-induced ROS. It seems likely that future research will discover additional roles for cell wall redox enzymes in desiccation tolerance in lichens.

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Is oxidative stress the cause of recalcitrant seed death in *Spartina alterniflora*?

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The causes of recalcitrant seed death during drying can be difficult to study; large seed size, high basal metabolism, and compromised seed quality during moist storage limit the number of experiments and replicates that can be analyzed. However, recalcitrant seeds of the salt-marsh grass *Spartina alterniflora* are unique because the seeds are dormant, small (0.003 g/seed), easily collected in large quantities and are of uniform size. In addition, *Spartina pectinata*, which produces orthodox seeds, can be used as a control. These two *Spartina* species constitute a good model system to study recalcitrance. This study examined the possible impact of oxidative stress on lipids, proteins, and total antioxidant capacity as *S. alterniflora* and *S. pectinata* seeds were dried. Lipid peroxidation was measured with the TBARS (thiobarbituric acid reactive substances) and FOX (ferric-xylenol orange) assays. When the seeds were freeze-clamped in liquid nitrogen prior to extraction, both species had low amounts of TBARS, and TBARS did not increase during drying. However, when extraction was performed at 4 °C, amounts of TBARS increased significantly during drying of both species, suggesting that the increase was a function of extraction temperature, rather than recalcitrance. Early products of lipid peroxidation were measured with the FOX assay. When dormant seeds were dried, there was no significant increase in FOX-positive products;