488 Abstracts

dependent components of photosystem recovery in resurrection plants.

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Reactive oxygen species production during imbibition of orthodox seeds and early seedling growth

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Desiccation-tolerant life forms, including orthodox seeds, can survive in the desiccated state. To revive from desiccation, they must be able to resume metabolism when water is available and environmental conditions are favourable. However, desiccation and imbibitional stress, as many abiotic and biotic stresses, increase intracellular production of potentially harmful Reactive Oxygen Species (ROS). By contrast, extracellular ROS production by organisms has been identified to play a "positive" role in stress response. Here we report on the characteristics of extracellular ROS release during seed germination and early seedling development in *Pisum sativum* seeds. We determined the time courses of extracellular superoxide production, hydrogen peroxide (H₂O₂) production and peroxidase activity. We found that the initial imbibition phase was accompanied by an "oxidative burst" of superoxide and H₂O₂. With further water uptake, rates of ROS formation dropped to almost zero. However, a second increase in superoxide, but not H₂O₂, production occurred after 40 h, a time associated with plumule emergence. Cell surface peroxidase activity was detected only 40 h after the initial imbibition of seeds. We also investigated the nature of the enzymes involved in superoxide production by testing the effects of inhibitors and reductants. The first "oxidative burst" was clearly enzymatic, showing a high Q10 (the reaction rate increased with temperature). However, the identity of the enzymes responsible is unclear. Superoxide production was not inhibited by the peroxidase inhibitor, cyanide, and by DPI, an inhibitor of NADPH oxidase. The enzymes responsible for the second increase of superoxide were probably peroxidases, because superoxide production was correlated with an increase in surface peroxidase activity, and superoxide production was inhibited by cyanide. Results from polyacrylamide gel electrophoresis suggested that the peroxidases were located in the radicle cell walls, but not in the seed coat or the embryo.

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Proteomics analysis of *Talauma ovata* seeds: Changes in protein expression during desiccation and after imbibition

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The development of proper strategies for conservation of seeds relies on the understanding of mechanisms involved in desiccation tolerance and sensitivity. Seeds of Talauma ovata, a tree widely distributed in swampy soils of riparian forests from Brazilian Atlantic Forest, have been reported to have limited desiccation tolerance. The effect of seed drying and imbibition after drying was studied by differential protein expression using two dimensional gel electrophoresis. After drying to a range of water contents, seeds were germinated to assess viability. Seeds of T. ovata did not withstand desiccation down to 0.10 g H₂O g⁻¹ dw. The critical water content below which desiccation sensitivity became apparent was around 0.18 g H₂O g⁻¹ dw (-26.5 MPa). Total protein was extracted and separated by 2D electrophoresis from fresh seeds (0.28 g H₂O g⁻¹ dw), mildly dried seeds (0.25 g $\mathrm{H_2O}$ g $^{-1}$ dw) and seeds at low water content $(0.10 \text{ g H}_2\text{O g}^{-1} \text{ dw})$ before and after imbibition for 10 days. The proteome profile revealed the presence of 588 spots on each silver stained gel. Analyzing silver stained gels of seeds from different conditions (different water content, before and after imbibition) enabled the identification of up to 21 differentially expressed protein spots that correlated with desiccation and germination. After MS/MS sequencing, 3 protein spots produced spectra that matched to a Magnolia salicifolia legumin precursor. Comparing the expression of these identified proteins with the germination data suggests a role for a legumin during both drying and imbibition of *T. ovata* seeds.

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Sugar protection of membranes: A trio of mechanisms for a range of water contents

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The accumulation of certain soluble sugars has long been associated with desiccation tolerance and a role for sugars in the stabilization of membranes *in vitro* has been thoroughly established. The mechanism by which sugars stabilize dehydrated membranes has, however, remained a subject of debate. The most frequently invoked model, the Water Replacement Hypothesis, proposes that sugars form hydrogen bonds with membrane lipids, replacing water molecules that bond with the membrane surface in a hydrated system, and thus maintaining the lateral spacing of

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