flowering season promoted greater seed set in *R. miliioides* than in the more dispersed *R. bauii* var. *platypetala*. Neither selfing nor apomixis appear to be operating in these species, even though little pollinator activity was observed. Thus, viable seed production across these species’ boundaries followed by vegetative reproduction enable the persistence of hybrids, which over time could become species.

doi:10.1016/j.sajb.2013.02.092

Smoke-derived compounds with germination activity: Towards understanding the mode of action by investigating structure-activity relationships of synthetic analogues

H.B. Papenfus*a, M. Pošt*a, M.E. Lighta, J.F. Finniea, L. Kohoutb, J. Van Stadena

aResearch Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

bInstitute of Organic Chemistry and Biochemistry AS CR, v.v.i., Flemingovo nám. “2”, 166 10, Prague 6, Czech Republic

Active compounds known to promote seed germination in many species. A butenolide compound, 3-methyl-2H-furo[2,3-c]pyran-2-one (karrikinolide; KAR1), is one such compound that can promote germination at concentrations as low as 10⁻¹⁰ M. Conversely, a structurally-related butenolide, 3,4,5-trimethylfuran-2(5H)-one (trimethylbutenolide; TMB), also present in smoke, has been shown to inhibit germination and reduce the promotory effect of KAR1 in a test system using lettuce seeds (achenes of *Lactuca sativa* L. cv. ‘Grand Rapids’). Little is known, however, regarding the mechanism by which TMB inhibits germination or interacts with KAR1. Thus, several synthetic derivatives of TMB were prepared to investigate the effect of related compounds on the germination of Grand Rapids lettuce seeds. A range of concentrations (from 10⁻³ M to 10⁻⁶ M) of these analogues of TMB were tested in combination with 10⁻⁶ M KAR1 to determine the relative activity of the synthesised compounds. Of the 11 analogues tested, only two compounds were found to reduce the promotory effect of 10⁻⁸ M KAR1 in a similar manner as observed with TMB, with activity from 10⁻³ M to 10⁻⁵ M. Four of the compounds were also found to have inhibitory activity at 10⁻⁵ M and 10⁻⁶ M. Since some of the synthetic compounds have exhibited inhibitory activity similar to TMB, this may be useful for the design of novel compounds with better activity.

doi:10.1016/j.sajb.2013.02.094

Desiccation stress and the Xerophyta metabolome

H.J. Dace, M.S. Rafudeen, J.M. Farrant

Department of Molecular and Cell Biology, University of Cape Town, Rondebosch 7700, South Africa

Resurrection plants are unique in the ability to survive near complete water loss in vegetative tissues without loss of viability. In order to do so, they employ multifaceted strategies which include structural adaptations, antioxidant and photoprotective mechanisms, and the accumulation of proteins and metabolites that stabilise macromolecules. A full understanding of the phenomenon of vegetative desiccation tolerance will require a view of these adaptations at the levels of the genome, the control of gene expression, and the control of metabolic pathways. In this presentation, we report a high-throughput metabolomic analysis of the changes that occur in vegetative tissues of the resurrection plant *Xerophyta humilis* during dehydration. We have used a combination of chromatography, mass spectrometry and nuclear magnetic resonance, to discern numerous primary and secondary metabolites. Multivariate statistics have revealed a subset of metabolites that are significantly up- or down-regulated in response to water deficit stress. Mapping the identified metabolites onto known biochemical pathways lets us suggest possible regulatory schemes in the stress response, inviting deeper investigation in future.

doi:10.1016/j.sajb.2013.02.095

Determination of changes in membrane lipid composition during rehydration and dehydration of the resurrection plant *Xerophyta humilis* using multiple reaction monitoring mass spectrometry

F. Tshabusea, S. Rafudeenb, E. Ruellanda, D. Raineteb, J.M. Farrantb

aDepartment of Molecular and Cell Biology, Private Bag, Rondebosch 7701, South Africa

bUPMC Univ Paris 06, URS, Physiologie Cellulaire et Moléculaire des Plantes, Paris, France

Plants whose fully differentiated tissues are able to withstand desiccation to air-dryness for prolonged periods of time and able to resume their full metabolic functioning in existing tissues upon rewetting are termed resurrection plants. Considerable research has been conducted on the structural, physiological, biochemical and molecular changes accompanying dehydration and recovery of a number of resurrection plants in order to ascertain the protective
processes that enable survival of extreme water loss. However, to date there has been little published on the roles of lipids in desiccation tolerance. Lipids are a key component of membranes and maintenance of membrane integrity during abiotic stresses, such as water deficit, is likely to play an important role in survival. It has been shown that during the freeze-associated desiccation events, the nature and changes in lipid composition enable appropriate membrane re-arrangements, facilitating survival of this stress. In the current study LC-MS/MS in the MRM mode is being used to profile and quantify levels of membrane lipids in roots and leaves of the resurrection plant *Xerophyta humilis* during dehydration and rehydration cycles. For comparison purposes, lipid profiles and changes in the desiccation sensitive model organism, *Arabidopsis thaliana* is being followed. Preliminary results obtained thus far indicate considerable differences in lipid composition between *Xerophyta humilis* and *A. thaliana* tissues. During dehydration of vegetative tissues of the resurrection plant, changes in four distinct classes of glycerophospholipids have been noted. The nature of these changes, and potential implication thereof, will be discussed.

doi:10.1016/j.sajb.2013.02.096

**Cysteine protease expression and activity in soybean nodules during development and stress**

M. Du Plessis, J. Vorster, K. Kunert

*Department of Plant Science, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa*

Premature legume root nodule senescence is a challenge for agricultural production around the world. Soybean (*Glycine max* L.) forms a symbiotic relationship with *Bradyrhizobium japonicum* which leads to the formation of nitrogen fixing root nodules. These nodules have the ability to fix nitrogen from the atmosphere into an accessible form for plants. Root nodules have a very short life span of only 10–12 weeks where after nitrogenase activity decreases as measured by acetylene reduction. Root nodules’ lifespan is also affected by stressful environments like drought. Protein remobilization during senescence leads to an increase in cysteine proteases. A DCG-04 tag which contains the E-64, a cysteine protease inhibitor, which binds irreversibly to cysteine proteases, was used to detect the presence of cysteine proteases in nodules. This showed that cysteine proteases increased during the development of root nodules between 16 and 18 weeks of age. A high abundance of cysteine proteases were also seen with 4 weeks old nodules compared to 6–12 week old nodules. Enzyme kinematic assays, using fluorogenic substrates to measure papain and legumain-like protease activity, correlated with the cysteine protease profile established with the DCG-04 tagging system. However, a semi-QPCR indicated that not all members of the cysteine protease gene family are expressed. It showed that papain-like cysteine proteases decreased in expression during development as well as after 10 days drought. Enzyme kinematic assays also indicated that there is a decrease in papain and legumain-like proteases after drought stress which is in contrast to the proteases expression during development in the root nodules.

doi:10.1016/j.sajb.2013.02.097

**Plant-derived aerosol-smoke and smoke solutions influence agronomic performances of the traditional cereal crop, Tef**

H.M. Ghebrehiwot, M.G. Kulkarni, M.W. Bairu, J. Van Staden

*Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*

The positive role of plant-derived smoke and smoke-isolated karrkininolide, (KAR1) on seed germination and post-germination processes is well documented. The present study examined if plant-derived smoke with various methods of application influence the agronomic performance of a traditional cereal crop, tef [*Eragrostis tef* (Zucc.) Trotter]. Comparisons were made in potted tef plants which germinated from seeds treated with smoke-water (SW, 1:500 dilution), KAR1 (10^{-8} M) solutions and seeds pre-treated with cool aerosol-smoke for 10 min (rinsed and unrisned afterwards). During and after the growing season (> 100 days), several growth and agronomic parameters were measured, and photosynthetic pigments were quantified using spectrophotometric methods. The smoke-related treatments modified a number of physiological, morphological and agronomic features of *Eragrostis tef*. In response to the smoke-related treatments, significant differences were observed in synthesis and accumulation of chlorophyll a and b. Compared to the control, KAR1 and aerosol-smoke treatment of the seeds significantly improved plant height. All the smoke-related treatments significantly-promoted stem-thickness. Aerosol-smoke and SW treatment of the tef seeds resulted in a significant increase in number of tillers and increased grain and dry biomass yield per plant. These findings indicate that plant-derived smoke has great potential to improve grain and dry biomass yield of tef. Moreover, due to its role in improving stem thickness, smoke treatment may assist in fighting loading problems in cereals such as rice, wheat and barley.

Caffeic acid-induced salt stress tolerance is mediated by nitric oxide and hydrogen peroxide scavenging enzymes in soybean root nodules

A. Klein, M. Keyster, N. Ludidi

*Plant Biotechnology Research Group, Department of Biotechnology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa*

Recent evidence suggests that exogenously applied caffeic acid is involved in decreasing hydrogen peroxide content in soybean. However, there is also evidence suggesting that hydrogen peroxide accumulation occurs in response to exogenously applied caffeic acid. Furthermore, it has been suggested that hydrogen peroxide content can be regulated by nitric oxide biosynthesis in plants. We thus investigated the effect of caffeic acid and salt stress on soybean growth and root nodule nitric oxide content. Furthermore, we examined the effect of caffeic acid and salt stress on soybean nodule hydrogen peroxide content, lipid peroxidation and the activity of two enzymes involved in the antioxidant system for hydrogen peroxide scavenging in the ascorbate-glutathione cycle (ascorbate peroxidase and glutathione reductase). Caffeic acid and salt stress altered the hydrogen peroxide and nitric oxide content, malondialdehyde levels, ascorbate peroxidase and glutathione reductase activities in root nodules. The negative effects of salt stress on soybean growth and biochemical activities were reversed by exogenously applied caffeic acid. We therefore propose that exogenously applied caffeic acid alleviates the detrimental effects of salt stress in soybean via a