

L3.1

High light exposure of leaves elicits rapid changes in hydrogen peroxide levels: new insights and limitations using HyPer

M. RODRIGUEZ EXPOSITO¹, P. LAISSUE¹, N. SMIRNOFF², P. MULLINEAUX¹

¹ School of Biological Sciences, University of Essex, United Kingdom

² College of Life and Environmental Sciences, University of Exeter, United Kingdom

Exposure of leaves to high light (HL) as been shown to elicit hydrogen peroxide (H₂O₂) in cells of various tissues. The H₂O₂ can elicit oxidative stress and irreversible photoinhibition. However, under moderate increases in light intensity, typically <10-fold above growth intensities, H₂O₂ has been proposed to be a signalling molecule. However, we still have no accurate determinations of the amounts, timing or subcellular distribution of H₂O₂. These data are needed to hypothesise how H₂O₂ may act as a signal transducer, especially from locations such as the chloroplast to the nucleus. In this presentation, I shall discuss progress and the limitations of using transiently expressed HyPer in *Nicotiana* epidermal cells of HL-exposed leaves. These cells do contain chloroplasts accessible for accurate detection of HyPer fluorescence. In this experimental system, changes in HyPer fluorescence will be described in the chloroplast stroma, on the outer surface the chloroplast, cytosol and nucleus. This will be related to changes in H₂O₂ concentrations, but also of pH observed using the GFP-based pHRed sensor. Our initial data suggest that our views on H₂O₂-mediated signalling in HL-exposed cells may need to be revised.

Oral presentations

O3.1

The CYSTEINE-RICH RECEPTOR-LIKE KINASE family in *Arabidopsis* – a phenotypic framework for stress responses and ROS signaling

G. BOURDAIS¹, P. BURDIAC², A. GAUTHIER³, L. NITSCH⁴, C. RAYAPURAM⁵, J. SALOJÄRVI³, D. TRI ANGGORO¹, D. GŁÓW², J. ZHOU¹, D. KAUFHOLDT³, K. ORACZ^{2,6}, N. IDÄNHEIMO³, J. WILLEM BORST⁴, D.B. COLLINGE⁵, S. KARPIŃSKI², M.F. LYGKJÆR⁵, S. ROBATZEK¹, J. KANGASJÄRVI³, M. WRZACZEK³

¹ The Sainsbury Laboratory, Norwich, United Kingdom

² Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

³ Plant Biology Division, Department of Biosciences, University of Helsinki, Helsinki, Finland

⁴ Laboratory of Biochemistry and Microspectroscopy Center, Wageningen University, Wageningen, The Netherlands

⁵ Department of Plant Biology and Biotechnology, University of Copenhagen, Frederiksberg, Denmark

⁶ Department of Plant Physiology, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

One of the largest groups of receptor-like protein kinases (RLKs) are the CYSTEINE-RICH RLKs (CRKs) with 44 members in *Arabidopsis*. CRK expression is regulated in response to various stresses that lead to specific alterations in ROS metabolism (Wrzaczek et al., 2010). CRKs are characterized by conserved cysteine motifs in the ecto-domain potentially involved in ROS signaling. To address this, we have characterized a mutant collection of the *Arabidopsis* CRK family in response to representative stresses that are closely linked to altered ROS production in apoplast, chloroplasts, mitochondria, and peroxisomes. In spite of their high level of conservation, phenotypical and in silico analysis of the *crk* mutants revealed specific deregulation of processes such as cell death in response to ozone, excess light or UV-B stress, stomatal closure, pathogen susceptibility, plant development, hormone signaling, seed germination, and photosynthetic processes. Altogether, our results provide a phenotypic framework of the CRK family and suggest a complex regulatory network of different CRKs in order to survive in a complex environment (Wrzaczek et al., BMC Plant Biology 2010; 10: 95).

03.2**Priming through H₂O₂-mediated signalling in *Arabidopsis thaliana*****S. BALAZADEH, S. SHAHNEJAT-BUSHEHRI, B. MÜLLER-RÖBER**

University of Potsdam and Max-Planck Institute of Molecular Plant Physiology, Germany

Plants can establish a molecular memory to a previous moderate stress that enables them to survive a subsequent harsher stress (priming, acclimation). An example for this is the response to high temperature in *Arabidopsis* (thermomemory). However, the molecular mechanisms underlying priming are largely unknown. Evidence suggests a role for H₂O₂ in establishing molecular memory and mediating priming towards stress conditions including heat. We have started to test the potential role of H₂O₂-responsive transcription factors (TFs) for thermomemory. Interestingly, the NAC TF JUNGBRUNNEN1 (JUB1), which is rapidly upregulated by H₂O₂ treatment, showed elevated expression even two days into the memory phase. JUB1 positively regulates longevity in *Arabidopsis* and enhances heat stress tolerance in primed and unprimed conditions, while in *jub1-1* knockdown plants thermomemory and tolerance are impaired compared to wild type. qRT-PCR-based transcriptome profiling of 1,880 TFs identified eight further TFs with altered expression during the thermomemory phase, two of which are H₂O₂-responsive, revealing them as new candidates for studies on molecular processes controlling thermomemory. Recent data will be presented.

03.3**Direct, calcium-independent, regulation of NADPH oxidase during plant innate immunity****Y. KADOTA¹, J. SKLENAR¹, P. DERBYSHIRE¹, L. STRANSFELD¹, S. ASAI¹,
V. NTOUKAKIS¹, K. SHIRASU², A. JONES¹, C. ZIPFEL¹**¹The Sainsbury Laboratory, United Kingdom²RIKEN Center for Sustainable Resource Science, Japan

In plant innate immunity, perception of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) leads to a ROS burst that is dependent on the NADPH oxidase RBOHD. Plant RBOHDs are mostly regulated through Ca²⁺ binding and Ca²⁺-dependent protein kinase (CDPK)-mediated phosphorylation to their N-termini. Here, we show that RBOHD forms a complex with the *Arabidopsis* receptor kinases EFR and FLS2, which perceive the bacterial EF-Tu and flagellin, respectively. RBOHD directly interacts with the cytoplasmic kinase BIK1, which is a direct substrate of the PRR complex. BIK1 phosphorylates the N-terminus of RBOHD upon PAMP perception. Importantly, both the interaction with and ligand-induced phosphorylation of RBOHD by BIK1 are Ca²⁺-independent. Using quantitative proteomics analysis, we identified PAMP-induced RBOHD phosphosites, which are BIK1-specific and distinct from CDPK-dependent phosphosites. Notably, phosphorylation of these residues is critical for PAMP-induced ROS burst, demonstrating the biological relevance of BIK1-mediated RBOHD phosphorylation. Our study reveals a novel rapid regulatory mechanism of plant RBOHDs, which occurs prior the paradigmatic Ca²⁺ based regulation.

03.4**What roles for hydrogen peroxide (H₂O₂) in the legume
– *Rhizobium* symbiosis?****E. ANDRIO, S. GUCCIARDO, K. MANDON, D. MARINO, E. OGER, A. PUPPO, N. PAULY**

Institut Sophia Agrobiotech, Université Nice Sophia Antipolis – INRA – CNRS, France

It is now well established that H₂O₂ is involved in the establishment and functioning of the legume – *Rhizobium* symbiosis which leads to the formation of new organs – called nodules – able to reduce atmospheric nitrogen into ammonia, directly assimilated by the plant. We aimed at identifying H₂O₂ molecular targets during the *Medicago truncatula* – *Sinorhizobium meliloti* symbiosis. We identified 301 and 98 genes regulated by H₂O₂ in *M. truncatula* and *S. meliloti*, respectively. These genes included a putative protein kinase (MtSpk1). MtSpk1 expression and H₂O₂ production were similarly distributed in the nodule. The establishment of symbiosis was impaired by Spk1 down regulation. Moreover, *ohr1*, a bacterial peroxiredoxin-encoding gene, was expressed during symbiosis and symbiotic abilities of the *ohr1/prxC* double mutant were greatly modified. We identified also 100 sulfenylated proteins (H₂O₂-driven post-translational modification) in both partners, including some directly involved in the nitrogen fixation process. This suggests that sulfenylation may regulate proteins playing major roles in the symbiotic interaction. Identification of H₂O₂ targets opens new prospects about its role during the rhizobial symbiosis.

03.5**Using high-resolution profiling of transcripts
to understand early drought stress signalling events****U. BECHTOLD¹, S. OTT², D. WILD², V. BUCHANAN-WOLLASTON³,
D. RAND², J. BEYNON³, N. SMIRNOFF⁴, P. MULLINEAUX¹**¹ School of Biological Sciences, University of Essex, United Kingdom² Warwick Systems Biology Centre, University of Warwick, United Kingdom³ School of Life Sciences and Systems Biology Centre, University of Warwick, United Kingdom⁴ Biosciences, University of Exeter, United Kingdom

Water availability is the biggest limitation on crop productivity worldwide. The mechanisms co-ordinating adjustments to drought stress are supported by changes in gene expression. Identification of genes that promote drought tolerance is important for the improvement of crops. We have produced a time series microarray of a slow drying experiment in *Arabidopsis* to understand the temporal coordination of drought stress at the transcriptional level. We also analysed metabolic and physiological changes over a 14-day period. A total of 2683 differentially expressed genes were identified and functional annotation clearly lacked Gene Ontology (GO) terms associated with water deprivation or abscisic acid. Conversely, GO terms related to carbohydrates and transport were significantly enriched, suggesting an early osmotic adjustment and sugar signalling in response to drying. Changes in gene expression were only observed markedly after physiological adjustments, and coincided with a drop in carbon assimilation and hormonal changes during the latter half of the experiment. This suggests that early physiological responses to a decline in soil water are not a stress response driven by major changes in gene expression or hormones.