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## POS-THU-233

## ANALYSIS OF PHOSPHOPROTEIN RESPONSES TO ABSICISIC ACID IN RICE

Ramesh S., Doblin M., Ford K., Patterson J. and Bacic A.  
Australian Centre for Plant Functional Genomics, School of Botany,  
The University of Melbourne, Victoria, 3010, Australia.

Abscisic acid (ABA) is a lipophilic phytohormone that modulates plant development, seed dormancy, germination, cell division and cellular responses to environmental stresses such as drought, cold, salt and UV radiation. The biosynthetic and catabolic pathways of ABA have been elucidated and phosphorylation events are central mediators of ABA signaling – indeed protein phosphorylation is a key regulatory factor in plant molecular biology. The identification of the actual site(s) of phosphorylation, however, is necessary for functional characterization and this represents a major bottleneck for the analysis of individual proteins. We are using a rice (*Oryza sativa cv. japonica*) suspension cell culture system to monitor the effects of ABA exposure on protein phosphorylation. The identification of phosphorylation sites by mass spectrometry requires an enrichment method for phosphopeptides due to ionization suppression effects which prevent the detection of phosphopeptides in complex samples. We are using immobilized metal-ion affinity chromatography (IMAC) to enrich for phosphopeptides specifically. The identification of phosphorylation sites is then achieved by MS/MS spectral interpretation. In combination with techniques to examine changes in total protein profiles under ABA exposure (ITraq™ Applied Biosystems), these studies are providing a deeper understanding of the proteins and events involved in ABA signaling.

## POS-THU-235

## DIFFERENTIAL GENE EXPRESSION OF WHEAT PROGENY CONTRASTING FOR TRANSPIRATION EFFICIENCY IDENTIFIES CANDIDATE GENE MARKERS

Xue G.P., McIntyre C.L., Chapman S.C. and Shorter R.  
CSIRO Plant Industry, QBP, 306 Carmody Rd, St Lucia, QLD 4067,  
Australia.

High water use efficiency or transpiration efficiency (TE) in wheat is a desirable physiological trait for increasing grain yield under water-limited environments. Genes associated with this trait could be used as molecular markers to facilitate selection for genotypes with higher TE. Expression profiling (microarray) analysis of approximately 16,000 unique wheat ESTs was undertaken using field grown material to identify genes that were differentially expressed between wheat progeny lines with contrasting levels of TE from a cross between the parents, Quarrion (high TE) and Genaro 81 (low TE). Ninety-three genes that were differentially expressed between high and low TE progeny lines were identified of which approximately 20% were found to be markedly responsive to drought stress. A subset of the TE differentially expressed genes were further analysed using quantitative RT-PCR on a separate set of field grown progeny plant samples from those used for microarray analysis. The expression levels of 11 out of the 18 genes were positively correlated with the high TE trait, measured as carbon isotope discrimination ( $\Delta^{13}C$ ). In addition, several of the TE differentially expressed genes have been mapped to QTLs for TE. These data indicate that some of these TE differentially expressed genes are candidates for investigating processes that underlie the high TE trait and can be used as expression and/or DNA markers for selection for increased TE.

## POS-THU-234

## TARGETED APPROACHES TO INCREASING THE NITROGEN USE EFFICIENCY OF MAIZE

Garnett T.P.<sup>1</sup>, Conn V.M.<sup>1</sup>, Conn S.J.<sup>1</sup>, Dhugga K.<sup>2</sup>, Rafalksi J.A.<sup>3</sup>, Tester M.<sup>1,4</sup>, Tingey S.V.<sup>3</sup> and Kaiser B.N.<sup>4</sup>

<sup>1</sup>Australian Centre for Plant Functional Genomics, The University of Adelaide, Waite Campus, Urrbrae, South Australia, Australia. <sup>2</sup>Yield Enhancement, Dupont Pioneer, Johnston, Iowa, USA. <sup>3</sup>Crop Genetics, Dupont Pioneer, Wilmington, Delaware, USA. <sup>4</sup>School of Agriculture Food and Wine, The University of Adelaide, Waite Campus, Urrbrae, South Australia, Australia.

Agricultural crop production involves the annual application of approximately 90 M tonne of nitrogen (N) fertilisers worldwide. Over half of this N fertiliser is used in the production of cereals including wheat, maize and rice. Unfortunately, nitrogen use efficiency (NUE) in cereals is poor where on average only 30% of supplied N will end up in the final harvested grain, while the remaining is lost to the environment. Improving NUE in cereals will help reduce N escape through reduced application and consequently lessen the negative environmental impacts associated with N leaching and water pollution. Our current research is focussed on identifying important N-linked traits that influence NUE in seed producing maize. We believe, nitrogen transport, storage and redistribution are key control points where gains in NUE can be readily achieved. We have initiated a NUE profiling study in Maize where diverse inbred lines are being compared for differences in growth, <sup>15</sup>N transport, N assimilatory processes and N-linked transcriptomics at varied external N concentrations and developmental growth stages. Complimentary to this we have generated gateway-enabled maize cDNA library collections that will be used for functional analysis of nitrate (NRT, NAR), and ammonium (AMTs) transporters in heterologous expression systems including *Xenopus laevis* oocytes and yeast (*S. cerevisiae*), respectively. Preliminary results on the maize N flux characterisation and N-linked transcriptomics analysis will be presented.

## POS-THU-236

## CANDIDATE GENES IN THE PUP1 LOCUS FOR TOLERANCE OF PHOSPHORUS DEFICIENCY

Gatdula K.A.<sup>1</sup>, Lu X.<sup>1</sup>, Yano M.<sup>3</sup>, Wissuwa M.<sup>2</sup>, Chua T.<sup>1</sup>, Ismail A.M.<sup>1</sup> and Heuer S.<sup>6</sup>

<sup>1</sup>International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines. <sup>2</sup>JIRCAS, Japan. <sup>3</sup>NIAS, Japan.

Phosphorous deficiency is a major abiotic stress limiting crop productivity worldwide. We have mapped a major QTL for tolerance of P-deficiency (Pup1) on chromosome 12 in a Nipponbare (intolerant) x Kasalath (tolerant) mapping population. The Pup1 locus was fine-mapped to a 195-kb region and the Pup1 genes were initially annotated based on the Nipponbare genomic sequence predicting 34 putative genes. Subsequent sequencing of the Kasalath Pup1 locus showed that it encompasses about 350 kb and 69 putative genes. Of these, about 50% are putative transposable elements likely to be responsible for the observed major structural differences between the Nipponbare and Kasalath Pup1 locus. Four genes seem to be absent from the Nipponbare and the 93-11 genome. Many genes show limited sequence similarity to Nipponbare genes and some are present on different chromosomes. This demonstrates that it is essential to sequence a given QTL locus in the respective donor parent. A preliminary annotation of the Kasalath Pup1 genes revealed similarities to Leucine-Zipper proteins (gene #65), Zn-finger transcription factors (gene #51) and kinases (leucine-rich repeat kinase, gene #38; transmembrane receptor serine/threonine kinase, gene #43; wall-associated kinase gene #67). Genes #26 and #50 show some similarity to phospholipases, the latter gene is absent from the Nipponbare and 93-11 genome. Kinases and phospholipases may play a major role in processes triggered by Pi-starvation. A detailed analysis of all Kasalath annotated genes is ongoing. In parallel, PCR based markers specific to the Kasalath Pup1 locus are currently tested in a range of tolerant and intolerant germplasm to validate the potential of the Pup1 QTL for improving P-efficiency of widely grown varieties in Asia.