

P1–THE GENETICS OF MINERAL NUTRITION IN PLANTS

Organised by P.J. White and M.R. Broadley for the Plant Transport Group and sponsored by the Journal of Experimental Botany

P1.1–Regulation of K⁺ channel activities: molecular aspects

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Plant channels of the Shaker family (9 members in *Arabidopsis*) display a common structure with a transmembrane domain including the pore, a short N-terminus and a long intracytoplasmic C-terminal tail. They are selective for K⁺ and involved in many essential functions, such as potassium uptake and translocation throughout the plant or control of cell turgor (crucial for cell growth/elongation and cell movements: stomatal opening and closing, nastic movements). Electrophysiological and biochemical analyses indicate that these channels are regulated by phosphorylation/dephosphorylation and by interactions with partner proteins. We developed a systematic search for proteins interacting with the C-terminal tail of Shaker channels by screening an *Arabidopsis* cDNA library using the yeast two hybrid system. This allowed us to identify in particular two proteins reacting strongly with the AKT2 channel which is mostly expressed in phloem tissues: a protein phosphatase 2C (AtPP2CA) involved in abscisic acid signalling, and a protein of unknown function, displaying similarities with some cytoskeleton-associated proteins. Characterisation of the interactions, including localisation of gene expressions and electrophysiological analyses following co-expressions of the channel proteins and putative partners in heterologous systems, will be presented. The analysis of T-DNA mutants and other genetically modified plants has also been undertaken to give an insight into the role of the interactions.

P1.2–Improving crop salt tolerance

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Salinity is an ever-present threat to crop yields, especially in countries where irrigation is an essential aid to agriculture. Although the tolerance of saline conditions by plants is variable, crop species are generally intolerant of one third the concentration of salts found in seawater. Attempts to improve the salt tolerance of crops through conventional breeding programmes have met with very limited success, due apparently to the complexity of the trait: salt tolerance is complex physiologically and genetically. Tolerance often shows the characteristics of a multigenic trait, with quantitative trait loci (QTLs) associated with tolerance identified in

barley, citrus, rice and tomato and with ion transport under saline conditions in barley, citrus and rice. However, in spite of the complexity of salt tolerance, the transfer of single genes can increase the tolerance of plants to saline conditions, although in many cases the enhanced tolerance has only been demonstrated in very particular circumstances. Whether enhanced tolerance, where properly established, is due to the chance alteration of a factor that is limiting in a complex chain or an effect on signalling remains to be elucidated. After ten years of research using transgenic plants to alter salt tolerance, the value of this approach has yet to be established in the field.

P1.3–The genetics of heavy metal tolerance and hyperaccumulation

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Heavy metals are toxic to most plants when present at greater than trace amounts. A number of species have evolved tolerance to levels of heavy metals that are toxic to other species. A very few species have evolved the ability to accumulate exceptional amounts of metals in their aerial parts (hyperaccumulation). This paper will review our knowledge of the genetics of both phenomena. Tolerance is frequently determined by major genes, though modifiers of the degree of tolerance have also been described. The molecular genetics of tolerance is still largely unknown. Hyperaccumulation is even less well characterised, though there is some evidence that variation in metal transporters is associated with this phenomenon.

P1.4–Breeding for micronutrient density in staple food crops

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Globally, over 3 billion people are micronutrient malnourished. Most of those afflicted are dependent on staple crops for their sustenance. Importantly, these crops can be enriched (i.e. 'biofortified') with micronutrients using plant breeding and/or transgenic strategies. Available research has demonstrated that micronutrient enrichment traits are available within the genomes of staple crops that could allow for substantial increases in micronutrients in their edible parts without negatively impacting crop productivity. Additionally, 'proof of concept' studies have been published using transgenic approaches to biofortify staple crops (e.g., high β -car-

otene 'golden rice' grain, high ferritin-Fe rice grain, etc.). Furthermore, micronutrient enrichment of seeds can increase crop yields when sowed to micronutrient-poor soils assuring their adoption by farmers. Bioavailability issues must be addressed when employing plant breeding or transgenic approaches as tools to reduce micronutrient malnutrition. Reducing antinutrient substances (e.g., phytate, polyphenolics, etc) that inhibit micronutrient bioavailability, or increasing substances (e.g., ascorbic acid, S-containing amino acids, etc.) that promote micronutrient bioavailability are both options that could be pursued, but these approaches should be used with caution. The world's agricultural community should adopt plant breeding and other genetic technologies to improve human health, and the world's nutrition and health communities should support these efforts. Sustainable solutions to this enormous global problem of 'hidden hunger' will not come without employing agricultural approaches.

P1.5—Transcriptional control of plant responses to phosphate starvation

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Plants have evolved a number of responses to adapt their growth to conditions of limited phosphate supply, involving biochemical and developmental changes. In this communication we report on the molecular and genetic studies in our laboratory showing the role of *PHR1* and cytokinins in the transcriptional control of these responses in *Arabidopsis thaliana*. *PHR1* encodes a transcriptional activator of the MYB superfamily which is conserved in the unicellular algae *Chlamydomonas reinhardtii*. *PHR1* is post-translationally controlled, either directly or indirectly, and exerts its role by directly interacting with a sequence, *PBS1*, present in the regulatory region of many Pi starvation responsive genes, indicating that *PHR1* acts downstream in the Pi starvation signalling pathway. Cytokinins negatively modulate the expression of Pi starvation responsive genes mostly in the root, and this effect involves the participation of *CRE1*. *CRE1* encodes a cytokinin receptor which is part of two-component signalling circuitry also mediating many other types of cytokinin response, indicating that the negative modulation of Pi starvation responses occurs through a general mechanism of cytokinin signalling. The great differences in the regulation of Pi starvation responses between plants and yeast, which lacks both *PHR1* and *CRE1*, underlines the importance of changes in transcriptional control in the evolution and diversification of eukaryotic organisms.

P1.6—The plant sulfate transporter family

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The co-ordinated expression of a gene family for sulfate transporters regulates and manages whole plant S-nutrition. Analysis of the *Arabidopsis* genome has indicated that the gene family may consist of up to 14 isoforms showing homology to one another. H⁺-sulfate co-transport has been demonstrated for some but not all of these isoforms. Occurrence of the sulfate transporter is ubiquitous and many examples have been cloned from a variety of plant species, as well as animals and yeast. This is a unique transporter family with no apparent homology to any other transporter type. Phylogenetic analysis of the plant gene or amino acid sequences indicates that there are 5 or more distinguishable clusters within the family of sulfate transporters. Analysis of functional characteristics and patterns of regulation together with localisation data, suggests that these groups have specific roles, such as high affinity uptake in the root, translocation in vascular tissues and cell to cell transfer in leaves and seeds. Additionally, some members of this sulfate transporter family may have discrete sub-cellular locations in plastid or tonoplast membranes. Roles for some isoforms, or for transporters in specific tissues remain controversial. This overview will summarise the current thinking as to how this complex gene family integrates sulfate acquisition and distribution *in planta*, distinguishing between specialisation and redundancy of isoforms. Aspects of the regulation of expression will also be questioned, particularly with regard to possible inconsistencies between mRNA abundance and transporter activity.

P1.7—Plants that make the most of phosphate

J.P. Hammond, P.J. White, M.R. Broadley, M.C. Meacham, HRI, Wellesbourne; M.J. Bennett and R. Swarup, Plant Sciences, Nottingham

The UK agricultural industries routinely apply excess P fertiliser to maintain crop yields and quality. This can be costly and may lead to unnecessary pollution. This situation arises because chemical assays of plant P status are unreliable. An alternative approach to monitor plant P status is to use technologies that exploit the changes in gene expression that occur under incipient P deficiency. Changes in the expression of P-sensitive genes indicate a physiological requirement for P fertilisation. Both transcript profiling and/or the use of sentinel plants, in which a P-sensitive promoter controls the expression of a marker-gene, would allow precise management of P fertilisation for sustainable crop production.

In parallel experiments, N, P and K were individually removed from hydroponically grown *Arabidopsis thaliana*. RNA was extracted from shoots of nutrient replete (control) and nutrient starved plants 4, 28 and 100 h after the withdrawal of a nutrient. Gene expression profiles were determined using Affymetrix *Arabidopsis* GeneChips. The expression of 115 genes changed in response to P withdrawal, and the promoters and transcripts of these genes are being used to develop novel sensor technologies for crop P status. The concept of sentinel plants has been proven with *Arabidopsis*. Transgenic *Arabidopsis* were generated in which the phosphate-sensitive promoter of SQD1 controlled the expression of β -glucuronidase (GUS) or green fluorescent protein (GFP). These plants responded to P withdrawal (and reduced shoot P concentrations) with increased marker-gene expression, demonstrating the feasibility of this approach to monitor plant P status.

P1.8—Structural and functional significance of chloride permeability in the TRK proteins

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The TRK-family proteins, responsible for moderate affinity potassium accumulation in plants, fungi, and bacteria, were initially described as 12-TM transporter proteins, based on hydropathy plots (Mol. Cell. Biol. 8: 2848, 1988); but were later modelled as potassium-channel analogues, based on sequence homology with bacterial channels (Biophys. J. 77:789, 1999). These would comprise 8 transmembrane α -helices sandwiching 4 pore loops, to make 4 MPM motifs in a single chain, folded like an assembled K^+ channel. Physiological evidence indicates that TRK proteins effect simultaneous transport of 2 ions, coupling inflow of Na^+ , K^+ , or H^+ , in different species, to uptake of a K^+ ion. Conventional patch-clamp measurements with high-salt electrodes have revealed that TRK proteins in the yeast, *Saccharomyces cerevisiae*, can also admit large chloride currents at low external pH (<pH 6.0) and large membrane voltages (negative to -120 mV), recalling the solid-state 'punch through' seen in giant algae (J. Membr. Biol. 89: 153, 1986). Studies of site-directed mutants of yeast TRK1 and TRK2 proteins, via voltage-clamping, ion-flux measurements, and fluorescent immunocytochemistry, are fully consistent with the channel-analogue (4-MPM) model, but the high-chloride effect is reminiscent of certain neurotransmitter transporters, and suggests that folding of TRK transporters is much looser than that of typical K^+ channels. This may have important implications for antimicrobial therapy in both medicine and agriculture.

P1.9—The genetics of nitrogen use efficiency in maize

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To study the genetic variability and genetic basis of nitrogen use efficiency in maize a set of recombinant inbred lines crossed with a tester was studied for grain yield and other traits, including protein content, at low input (N $-$) and high input (N $+$). Other physiological traits, such as nitrate content, nitrate reductase (NR) and glutamine synthetase (GS) activities were studied at the level of the lines. Genotype x nitrogen (G x N) interaction was significant for yield and correlated to that for kernel number. In N $-$, N uptake and nitrogen nutrition index at silking were positively correlated to grain yield whereas leaf senescence was negatively correlated. Nitrogen uptake efficiency was more variable in N $+$ than in N $-$ and the reverse for nitrogen utilisation efficiency. A positive correlation was observed between nitrate content and GS activity before flowering and yield mainly in N $-$. These results suggest that increased productivity in maize genotypes was due to their ability to accumulate nitrate in their leaves during vegetative growth and efficiently remobilise this stored nitrogen during grain filling. More QTLs were detected in N $+$ than in N $-$ for traits of vegetative development, grain yield and its components, whereas it was the reverse for grain protein content. As a whole, genetic variability was expressed differently in N $+$ and N $-$. Several coincidences between genes encoding for enzymes of N and C metabolism and QTLs for the traits studied were observed. In particular, coincidences of QTLs for yield and its components with genes encoding cytosolic GS and the corresponding enzyme activity were detected. It appears that the GS locus on chromosome 5 is a good candidate gene which can, at least partially, explain variation in yield and kernel weight. Experiments are in progress to clone and transfer the favourable allele.

P1.10—Quantitative genetic analysis in Brassica crops

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Brassica species represent a range of distinctive vegetable, salad, condiment, fodder and oilseed crops encompassing considerable genetic diversity and are closely related to the model plant *Arabidopsis*. For *Brassica oleracea* (kales, cabbage, broccoli, cauliflower, Brussels sprout, Kohl rabi) there is an increasing amount of information available at the genetic and genomic level which provides an excellent opportunity to understand constraints and scope for variation in gene expression. The *Brassica* genome has resulted from a series of segmental

chromosomal duplication events and contains a high proportion of retrotransposable elements, which make it attractive to study effects of gene duplication and silencing on developmental plasticity, and the ability to adapt to environmental changes. The BBSRC *Brassica* IGF programme has constructed physical map contigs anchored to the *Arabidopsis* genome. In addition to the large amounts of sequence data now available, this provides an exciting opportunity to capitalise on genetic information and resources accumulated over the past decade for a range of crop traits. We have characterised a wide range of quantitative trait loci (QTL) using reference doubled haploid populations and saturated linkage maps. These include seed and seedling vigour, developmental and morphological traits, water use efficiency, circadian rhythms, nutrient uptake, adventitious rooting and transformation competence, responsiveness to microspore culture, post harvest quality and senescence. By using a combination of segregating populations, substitution lines and comparative genomic data we are well placed to study genomic regulatory networks and interactions between genome structure and transcription both during development and as a result of environmental variation.

P1.11—Phylogenetic variation in the shoot mineral content of angiosperms

M.R. Broadley, H.C. Bowen, H.L. Cotterill, J.P. Hammond, M.C. Meacham, A. Mead and P.J. White, Horticulture Research International, Wellesbourne

In addition to carbon dioxide and water, flowering plants (angiosperms) require about 14 essential mineral elements to grow. When cultivated under identical conditions, the variation in the shoot mineral content ($[M]_{\text{shoot}}$) between different plant species is usually much greater than variation within any single plant species. By conducting both designed experiments, and also meta-analyses on literature data, the $[M]_{\text{shoot}}$ of angiosperms (predominantly for European herbaceous species) grown in comparable environments has been systematically described for numerous elements including essential minerals, non-essential heavy metals and radionuclides.

For several elements (e.g. Ca, Mg, Ni and Zn), a large proportion of the total variation in $[M]_{\text{shoot}}$ occurs between different orders of angiosperms. For example, within the monocotyledons, $[Ca]_{\text{shoot}}$ is lower in the order Poales (e.g. grasses, rushes and sedges) than it is in the order Asparagales (e.g. onions). This feature correlates with certain root properties, such as cell wall composition and cation exchange capacity. For other elements (e.g. N, P and K), more of the variation in $[M]_{\text{shoot}}$ occurs within orders.

The $[M]_{\text{shoot}}$ of certain elements are correlated. For example, when grown under identical conditions, $[Ca]_{\text{shoot}}$ and $[Mg]_{\text{shoot}}$ of species are highly correlated, with the exception of several species from the Caryophyllales, an order with other unusual $[M]_{\text{shoot}}$ features. The data from this study can be compared to the $[M]_{\text{shoot}}$ of UK herbaceous species grown in their natural habitats, published in ecological surveys, which support the conclusions.

P1.12—Gene therapy for salty environments

M. Tester, Cambridge

In this paper, I will briefly review the published successes of engineering salt tolerance (from work done by other groups!), and then present the strategy being employed in my own lab. In this, we are aiming to minimise both the initial entry of Na^+ into the plant and its transfer to the shoot. We are using two complementary strategies: (1) A cellular approach, by trying to characterise the mechanisms and control of Na^+ fluxes and the genes encoding the non-selective cation channels responsible for passive Na^+ fluxes, and (2) A genetic approach, by activating random genes in specific cell types within the root and measuring the effects of such overexpression by high throughput elemental analyses. Genes are currently being activated in two different cell types: epidermis/cortex and stele – when considering the control of solute accumulation in the shoot, this approach is considered better than simply activating gene expression constitutively. This genetic approach is currently being performed in *Arabidopsis*, but we are now gathering the tools to repeat these experiments in rice. In this talk, I will report on the current state-of-play for these projects in my group.

P1.13—Expression of selected genes involved in sucrose/starch metabolism as affected by phosphate deficiency

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The effects of inorganic phosphate (Pi) deficiency on expression of genes encoding ADP-glucose pyrophosphorylase small (*ApS*) and large subunits (*ApL1*, *ApL2*, *ApL3*), UDP-glucose pyrophosphorylase (*Ugp*), sucrose synthase (*Sus1*, *Sus2*) and hexokinase (*Hxk1*), all involved in carbohydrate metabolism, were investigated in *Arabidopsis thaliana* (L.) Heynh. leaves. The lack of phosphorus in the nutrient medium lowered Pi content in the tissues and inhibited shoot growth. A decrease in internal Pi status led to an increase in an overall content of glucose and starch, but had little effect on sucrose content. The expression of *ApS*, *ApL1*, *ApL3* and *Ugp* was upregulated in the leaves of P-deficient plants,

whereas that of *ApL2*, *Sus1*, *Sus2* and *Hxk1* was unaffected. The expression of most of the P-starvation-affected genes was also upregulated after feeding the excised leaves with D-mannose, which acts as a sink for cytosolic pool of Pi. The effects of Pi status on expression of studied genes were confirmed using leaves of both *pho1* and *pho2* mutants of *Arabidopsis* (P-deficient and P-accumulating, respectively). The data suggest that ADP-glucose pyrophosphorylase and UDP-glucose pyrophosphorylase might represent transcriptionally regulated steps in starch/sucrose metabolism, involved in homeostatic mechanisms acclimating nutritional status of a plant to the Pi-stress conditions.

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P1.14—Mechanisms for Caesium uptake by *Arabidopsis thaliana*

C.R. Hampton, K.A. Payne, H.C. Bowen, M.R. Broadley, P.J. White, HRI Wellesbourne; J. Pritchard, Biosciences, Birmingham

Eating food containing radioactive isotopes of caesium (^{137}Cs and ^{134}Cs) has damaging and, sometimes, fatal effects. Caesium enters the food chain through plants. Release of ^{137}Cs during weapons testing and industrial activity has contaminated thousands of hectares of land, restricting agricultural production worldwide. The generation of 'safe' crops that exclude Cs and can be cultivated on contaminated land requires knowledge about the mechanisms for Cs uptake. We are using *Arabidopsis thaliana* to gain this knowledge.

Caesium is chemically similar to potassium and might enter plants through K^+ transporters in the plasma membrane of root cells (White & Broadley 2000, New Phytologist 147: 241–256). To determine which transporters mediate Cs entry to plants we have compared the accumulation of Cs and K by wildtype *Arabidopsis* with mutants lacking specific K^+ transporters. Two mutants of particular interest were *akt1*, which lacks the highly-selective K^+ channel that contributes most to plant K^+ nutrition, and *cngc4*, which lacks a non-specific cyclic-nucleotide gated cation channel (CNGC), permeable to a wide range of monovalent and divalent cations. Preliminary results show that, although K accumulation was reduced, Cs accumulation was greater in *akt1* than wildtype plants. By contrast, Cs concentrations were reduced in shoots of *cngc4*, which survived higher Cs in the rhizosphere. Thus, Cs is likely to enter plants through non-specific cation channels rather than through highly selective K^+ channels. A reduction in CNGC activity could restrict Cs uptake into plants and enable 'safe' low radiocaesium crops to be grown on contaminated land.

P1.15—Identification and characterization of transport proteins of the ectomycorrhizal fungus *Hebeloma cylindrosporum*

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Most plants are tightly connected to their environment via symbiotic interactions, in particular with mycorrhizal fungi. Positive effects of ectomycorrhizal symbiosis on plant mineral nutrition have been clearly demonstrated by (eco)physiological approaches but the transporters involved in the corresponding processes are still unknown at the molecular level. We have chosen the fungus *Hebeloma cylindrosporum*, in association with *Pinus pinaster*, as a model for developing molecular analyses of solute transport in ectomycorrhizal symbiosis. This fungus can be transformed and grown in vitro from spore to spore. Systematic sequencing of expressed genes is currently in process and will offer a broad basis for molecular studies. So far, 3000 ESTs have been obtained. Half of them correspond to previously identified genes from different organisms and about 1% encodes proteins involved in membrane transport. A set of carriers and channels likely to be involved in transport of, e.g., sugars, amino acids, phosphate or K^+ , has been identified. We are interested in the characterization of systems involved in K^+ transport. Molecular and functional analyses of the identified transport systems (1 K^+ carrier of the TRK type, several K^+ channels of the Shaker type) are under progress. Several copies of the Shaker-like gene are present in one of the 2 compatible fungus strains studied in the laboratory while a single copy is present in the other strain. Characterization of fungal transporters will allow us to unravel the mechanisms by which ectomycorrhizal symbiosis improves mineral nutrition of the host plant as well as signalling mechanisms between both partners.

P1.16—CPx-ATPases in *Arabidopsis*—a role in heavy metal transport?

R.F. Mills, P.J. Baccarini, G.C. Krijger, R. Badyal, J.L. Hall and L.E. Williams, Southampton

Mechanisms are required by all organisms to maintain the concentration of essential heavy metals (eg. Zn and Cu) within physiological limits and to minimise the detrimental effects of non-essential metals (eg. Cd). CPx-ATPases or heavy metal P-type ATPases (HMAs) are a sub-group of the P-type ATPase superfamily that could contribute to metal homeostasis in plants. To investigate this we have cloned and characterized *AtHMA1* and

AtHMA4, two members of this family from *Arabidopsis*. Sequencing showed that these HMAs contain conserved motifs found in all P-type ATPases and also motifs that are characteristic of heavy-metal ATPases. *AtHMA1* and *AtHMA4* were heterologously expressed in *Saccharomyces cerevisiae* and the sensitivity to various heavy metals was investigated. Expression of *AtHMA1* made the yeast more sensitive to Cd while *AtHMA4* conferred resistance. Expression of these transporters in yeast did not alleviate Zn or Cu toxicity but these metals reduced the Cd resistance conferred by *AtHMA4* and the Cd sensitivity imparted by *AtHMA1*. RT-PCR showed that *AtHMA1* was expressed in a range of tissues while *AtHMA4* was expressed predominantly in the roots. *AtHMA4* was up-regulated on exposure to elevated levels of Zn and Mn. Possible physiological roles of these transporters in *Arabidopsis* are discussed.

P1.17—Genetics of caesium accumulation in *Arabidopsis thaliana*

K. Payne, H. Bowen, M. Broadley, J. Hammond, C. Hampton, A. Mead, P. White, HRI, Wellesbourne; M. Bennett and K. Swarup, Plant Sciences, Nottingham

Radiocaesium (Cs) is released to the environment by anthropogenic activities. Following deposition to soils, Cs enters the food chain through root uptake and subsequent accumulation in edible crop tissues. This poses a serious health risk to humans and animals consuming contaminated produce.

There is natural genetic variation in the accumulation of Cs in plant shoots. It may be possible to use this information to develop 'safe' crops that accumulate less Cs in their tissues, either by selecting existing crop varieties, or by identifying alleles and/or candidate genes that impact on the trait of Cs accumulation and developing subsequent breeding strategies. The feasibility of developing 'safe' crops can be determined within a short time scale using *Arabidopsis thaliana*.

Arabidopsis were grown in nutrient-enriched agar supplemented with 1 μM Cs. Variation in shoot Cs content and shoot Cs extraction occurred between *Arabidopsis* ecotypes, between parents of three existing mapping populations of *Arabidopsis* recombinant inbred lines (RILs), and within the RIL populations. In the Landsberg *erecta* (*Ler*) x Cape Verdi Island (*Cvi*) cross, Cs content varied three-fold and in the Columbia (*Col*) x *Ler* and Niedersenz (*Nd*) x *Col* crosses, Cs content varied two-fold. Variation in Cs extraction was greater. Quantitative genetical analyses were performed on the three RIL populations. The heritability of shoot Cs content and shoot Cs extraction traits ranged from 7–19%. Putative genetic loci impacting on the traits of shoot Cs content and shoot Cs extraction have been identified using a quantitative trait loci (QTL) approach.

P1.18—Selenium accumulation by *Arabidopsis thaliana*

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Animals require selenium (Se) in their diet, which is supplied primarily by plants. Little is known about how plants accumulate selenium, except that it is taken up primarily as selenate. Since selenium can be toxic to plants, growth in the presence of high selenate concentrations can be used to identify plants (mutants) with impaired selenate uptake and/or improved selenate tolerance, that survive these conditions. We have screened a collection of 'insertional' *Arabidopsis* mutants, in which mutagenised genes are tagged by the presence of heterologous DNA, for survival in the presence of high selenate concentrations. Sequencing the disrupted genes will allow us to identify those involved in selenium accumulation.

The *Arabidopsis* ecotypes Wassilewskija (*Ws2*) and Columbia (*Col-5*) were grown on agar containing a complete mineral nutrient supplement plus selenate. Increasing selenate concentration in the agar initially enhanced plant growth, but growth was inhibited at selenate concentrations above 10 micromolar. When plants were grown at a density of 10 000 seeds m^{-2} a selenate concentration of 1 mM was sufficient to prevent any wildtype seedling growing. Four plants out of 1000 EMS mutagenised *Col5* seed sown on agar containing 1 mM selenate survived and produced seed. Progeny from two of these plants also grew on agar containing 1 mM selenate. Thirty-nine out of the 6000 T-DNA mutants we screened grew on agar containing 1 mM selenate. We are currently screening the selenium-tolerance of the progeny from these mutants and identifying the mutated genes.

P1.19—Functional genomics: does the *Arabidopsis* gene *At2g31910* really encode a Na^+/H^+ antiporter?

D. Thomas, H.J. Newbury and J. Pritchard, Biosciences, Birmingham

Na^+/H^+ antiporters have been shown to play an important role in plant salt tolerance. We have investigated the function of the *Arabidopsis thaliana* gene *At2g31910* described as a 'putative *Arabidopsis* Na^+/H^+ antiporter', by examining the response of homozygous mutants at this locus to salt (sodium chloride) stress.

Under unstressed conditions the mutant exhibited quantitative trait differences from the wild type (*Col-0*), including delayed flowering time. The germination rates of mutant and wild type seeds did not differ in

unstressed conditions, but were significantly higher for mutant than wild type seeds when exposed to 50 mM NaCl. In contrast, root extension of the mutant was reduced in comparison to Col-0 in the presence of salt. Following salt stress, the bulk leaf sap of mutant plants contained significantly less Na⁺ than the wild type. Xylem sap was sampled from transpiring plants using the spittlebug *Philaeenus spumarius*. The flux of Na⁺ to the leaves in the xylem was lower in the mutant in both unstressed (20.3% of Col-0 flux) and salt-stressed (46.4% of Col-0 flux) treatments. Phloem sap sampled using aphid stylectomy showed elevated osmotic pressure in the mutant but no difference in Na⁺ concentration was observed.

These physiological data are consistent with a model in which the *Arabidopsis* gene At2g31910 encodes a Na⁺/H⁺ antiporter that plays a role in the loading of Na⁺ into the xylem in plant roots. Immunolocalisation of the antiporter protein is seeking to confirm this.

P1.20—The Nramp family of heavy metal transporters in *Arabidopsis*

R. Vaughan, T. Biggs, J.L. Hall and L.E. Williams, Southampton

Natural resistance-associated macrophage proteins (Nramps) appear to be a highly conserved family of proteins found in a wide range of organisms including bacteria, yeast, mammals and plants. They are thought to function in the transport of a number of divalent cations including Fe²⁺, Mn²⁺, Zn²⁺ and Cu²⁺. Six putative *Nramps* can be identified in the genome of *Arabidopsis thaliana* (*Nramp1–6*) and cDNAs for four of these (*AtNramp1–4*) have previously been cloned. *Nramp1*, 3 and 4 are thought to be involved in the transport of Fe²⁺ and Cd²⁺ (Curie et al. 2000 *Biochem. J.* 347: 749–755; Thomine et al. 2000 *PNAS* 97: 4991–4996). We have cloned a full-length cDNA for *AtNramp5* using RT-PCR. Two cDNAs (*AtNramp6a* and *b*) have been cloned for *AtNramp6*. *AtNramp6a* is the fully processed form whereas *AtNramp6b* contains an intron and if translated would give rise to a truncated protein. *AtNramp5* and *AtNramp6a* code for proteins with predicted molecular weights of 58.8 kDa and 57.2 kDa respectively and hydropathy analysis suggests 10–12 transmembrane domains. We will report our progress in the further characterization of Nramp family members. Semi-quantitative RT-PCR analysis and promoter-GUS

transformed plants are being used to determine the tissue-specific expression patterns of *AtNramp5*, *AtNramp6a* and *AtNramp6b*. GFP-*Nramp5* and GFP-*Nramp6a* constructs will be used to study the cellular location of Nramp5 and 6 in *Arabidopsis*. *AtNramp5* and *AtNramp6a* have been cloned into *pBecks* for over-expression studies in *Arabidopsis*. Functional complementation of yeast mutants is being used to assess the possible substrates for members of this family.

P1.21—Post-translational regulation of high-affinity NO₃⁻ transporters in barley

M.N. Murphy, M. Hansen and B. G. Forde, Biological Sciences, Lancaster

The *NRT2* genes in plants belong to a family of high-affinity NO₃⁻ transporters found also in fungi and algae (Forde, 2000). In barley there are an estimated seven to ten *NRT2* genes, which are mainly or exclusively expressed in roots, rapidly induced by NO₃⁻ and down-regulated by products of NO₃⁻ assimilation (Trueman et al., 1996; Vidmar et al., 2000). As well as being strongly regulated at the mRNA level, there is good evidence that the high-affinity nitrate transport system in plants is feedback regulated at a post-transcriptional level (Forde, 2000). We are investigating the possibility that post-translational modifications, such as phosphorylation/dephosphorylation, are involved in the regulation of *NRT2* transporters. Crude extracts from roots of 10-day-old barley seedlings have been electrophoresed on SDS-PAGE gels and western-blotted. An antiserum raised against the C-terminus of the HvNRT2.1 protein cross-reacted with a NO₃⁻-inducible band that has an electrophoretic mobility higher than predicted from the known polypeptide sequence, as is commonly observed for hydrophobic proteins. Results have shown that changes in the electrophoretic mobility of this NO₃⁻-inducible band occur rapidly (<15 min) after the roots have been treated with glutamine, consistent with some form of post-translational modification. Latest results of experiments aimed at identifying the nature of this post-translational modification will be reported.

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