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C3–SALINITY

Organised by Timothy Flowers (University of Sussex) and Jose Pardo (CISC, Seville)

C3.1

World salinisation with emphasis on Australia

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Salinisation is the accumulation of water-soluble salts in the soil solum or regolith to a level that impacts on agricultural production, environmental health and economic welfare. Salt-affected soils occur in more than a hundred countries of the world with a variety of extents, nature and properties. No climatic zone in the world is free from salinisation, although the general perception is focussed on arid and semiarid regions. Salinisation is a complex process involving seasonal dynamics of salts, water movement in a soil profile and hydrological interactions of groundwater. While rainfall, aeolian deposits, mineral weathering and stored salts are the sources of salts, surface and ground waters can redistribute the accumulated salts. Sodium salts dominate in many saline soils of the world. But salts of other cations such as calcium, magnesium and iron are also found in specific locations.

Different types of salinisation with a prevalence of sodium salts affect about 30% of the land area in Australia. While more attention is given to groundwater associated salinity and irrigation salinity, which affect about 16% of the agricultural area, recent investigations suggest that 67% of the agricultural area has a potential for 'transient salinity', a type of non-groundwater associated salinity. Agricultural soils in Australia, being predominantly sodic, accumulate salts under seasonal fluctuations and have multiple subsoil constraints such as alkalinity, acidity, sodicity and toxic ions.

This paper also examines soil processes that dictate the exact edaphic environment upon which root functions depend and can help in research on plant improvement.

C3.2

Increasing salt tolerance in monocotyledonous plants, with emphasis on wheat

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Sodium exclusion from leaves is the most common and important mechanism of salt tolerance in monocotyledonous plants. Na⁺ is

largely excluded from the transpiration stream in all plants, but some plants do it more effectively than others and maintain low Na⁺ concentrations in leaves. Na⁺ exclusion is a result of restricted Na⁺ uptake by roots and low rates of transport in the xylem from root to shoot. The latter can be achieved by restricted loading of the xylem, or efficient removal of Na⁺ from the upper part of the root system and the base of the shoot. A durum wheat landrace has been identified with major genes for both these traits and a molecular marker found for one. X-ray microanalysis of roots of the Na⁺-excluding durum wheat indicates that epidermal cells, and the outermost cells in the stele, may provide the major control points in limiting salt entry to the xylem. The endodermis is not the barrier to radial Na⁺ transport. A second important mechanism of salt tolerance is the sequestration of Na⁺ and Cl⁻ in the vacuoles of root and leaf cells, so that high tissue concentrations can be tolerated. Barley has the capacity to tolerate concentrations as high as 500 mM in the vacuoles of leaf cells and probably accounts for its superior salt tolerance amongst glycophytes. Recent work with wheat and barley in selecting for Na⁺ exclusion, or tolerance of high tissue concentrations will be presented, and breeding for Na⁺ exclusion in durum wheat.

C3.3

Increasing salt tolerance in dicotyledonous plants with an emphasis on the tomato

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In this paper, we will review a number of strategies to overcome the deleterious effects of salinity including molecular markers and genetic transformation as tools to develop salinity-tolerant genotypes.

Increasing salt tolerance is a major goal in plant breeding. QTL analysis has been attempted for more than 12 years to aid understanding the genetics of salt tolerance traits and to deal with those traits in breeding programmes. Despite innovations like

C3.34 Proteomic analysis of plasma membrane aquaporins in salt-treated roots

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In *Arabidopsis thaliana*, the hydraulic conductivity (L_p) of whole root systems decreases by 70% in the 2 h following a salt treatment (100 mM NaCl). This decrease is thought to be mediated through inhibition of aquaporins. One important class comprises the plasma membrane intrinsic proteins (PIPs), which are located in the plasma membrane (PM). We previously showed that the early inhibition of L_p by salt cannot be accounted for by a decrease in the abundance of aquaporin transcripts (Boursiac et al., submitted). In the present work, we have used a proteomic approach coupled to stopped-flow water transport assays in PM vesicles to investigate the mechanisms involved in the early down-regulation of PIP aquaporins. In agreement with the whole root response, the water permeability of purified PM vesicles was decreased by 60% in the 2 h following a salt treatment. By contrast, the abundance of PIPs in these vesicles, as quantified by western blot and ELISA assays, was not changed. Using a mass spectrometry-based analysis, we previously identified 6 PIP isoforms in the root PM (Santoni et al., 2003, *Biochem. J.* 373, 289). A more recent study revealed that these PIPs undergo N-terminal maturation and are methylated and phosphorylated on their N- and C-terminal tails, respectively. A semi-quantitative characterisation of these modifications in membranes from roots of control or salt-treated plants is under development.

Keywords: Aquaporin, Salinity, Post-translational modification

C3.35 Unusual element accumulation in activation tagged *Arabidopsis* mutants, investigated using an ICP-MS screen

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The aim of this screening program is to identify genes controlling the accumulation of nutrients and inorganic toxins in the shoots of higher plants. The plant lines investigated are the activation tagged lines of *Arabidopsis* generated in the Weigel laboratory.¹ These lines have a Columbia-7 background and contain four 35S enhancer elements (inserted randomly into the genome) that increase the rate of transcription of nearby genes. Inductively coupled plasma mass spectroscopy (ICP-MS) is used to analyse leaf content for 23 elements including some of interest to plant and human health and phytoremediation. Lines that are found to have statistically different shoot nutrient concentrations are selfed and re-screened.

To date, we have analysed 5842 Weigel lines and identified 512 putative mutants with increased and/or decreased elemental

accumulation. We have found 30% to 50% of the putative mutants have unusual accumulation of more than one element. Furthermore, 29% of identified mutants have statistically altered accumulation of sodium. Preliminary second screen results indicate that we have so far identified four mutants with heritable and statistically significantly altered elemental accumulation. One of these lines has low K^+ accumulation in the shoots, which may be interesting as Na^+ transporters have also been found to alter K^+ balance in plants. These mutants are currently undergoing molecular analysis to determine the gene(s) involved in this phenotype.

Keywords: Activation tag mutants, ICP-MS, Screen, Nutrients

[1] Weigel, D. et al. (2000) Activation tagging in *Arabidopsis*, *Plant Physiol* 122: 1003–1014.

C3.36 High affinity sodium transport in epidermal root cells of barley

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Sodium transport has been investigated in barley epidermal root cells using electrophysiological techniques. In sodium and potassium starved seedlings, the addition of micromolar concentrations of sodium (0.1 μ M–1000 μ M of sodium chloride) to the assay medium (2.5 mM of calcium chloride at pH 6) evoked rapid membrane depolarisations. These depolarisations were lower than the depolarisations induced by the same potassium concentrations. Preliminary data showed that sodium-induced depolarisations were partially inhibited in media containing micromolar (20 μ M) potassium concentrations. Furthermore, the presence of millimolar concentrations (4 mM) of barium chloride in the assay medium inhibited sodium-induced depolarisations, but not potassium-induced depolarisations. On the other hand, sodium-induced depolarisations were higher at pH 9 than at pH 6, whereas potassium-induced depolarisations were lower in alkaline than in acid conditions. These results point out that sodium and potassium are transported by different high affinity transport systems, as it has been recently described in rice roots (1). It has been also proposed that OsHKT1 mediates sodium uptake in roots when plants are potassium deficient (1). This transport system is expressed in epidermal and cortical root cells of rice (2), it exhibits high affinity for sodium and it is blocked by potassium and inhibited by barium (1). These characteristics are in agreement with the results presented here, suggesting that high affinity sodium uptake in barley epidermal root cells could also occur through HKT-like transporters.

Keywords: Barley, High affinity transport, Sodium

Garcia deblás et al. (2003) *The Plant Journal* 34, 788–801.

Golldack et al. (2002) *The Plant Journal* 31, 529–542.

C3.37 Partial cloning and expression analysis under salt stress of *MTTRE1*, a *Medicago truncatula* gene coding for trehalase

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Trehalose, a disaccharide widespread among microbes and lower invertebrates, was generally believed to be nonexistent in higher plants. However, it was described that trehalose may occur in plants with diseases or colonized by microorganisms, for example in mycorrhizal roots, nitrogen-fixing nodules and actinorhizal nodules. It is a well-known osmoprotectant in many organisms and is one of the most abundant non-structural carbohydrates in legume nodules. Trehalose in these plant organs is believed to be of microbial origin, whereas the plant-derived trehalase, the trehalose catabolising enzymatic activity, is unusually induced in these symbiotic organs. Despite the biological importance of trehalose and the ubiquitous nature of trehalase few sources have reported about what are trehalases implications on several aspects of the plant physiology. The main goal of this work is to uncover the role of trehalase in the adaptation of nitrogen-fixing legumes to salt stress conditions.

We are using the model legume *Medicago truncatula* to carry out our experiments. We are interested on trehalase gene expression in nodules. For such purpose, we have cloned the cDNA coding for trehalase from *Medicago* root nodules in order to perform RT-PCR experiments. In silico analyses have shown that cDNA coding for *M. truncatula* trehalase presents high identity (up to 89 %) with *GMTRE1*, the corresponding trehalase gene in *Glycine max*, and therefore we have renamed the cDNA as *MTTRE1*, similarly to that of soybean. The gene is expressed at low levels roots and leaves and its expression is induced in nodules (where biological nitrogen fixation takes place) formed in symbiosis with *Sinorhizobium meliloti*. Therefore, we have chosen nodules to continue with the corresponding expression analysis under salt stress. Results will shed lights of how these nitrogen-fixing organs can help to the general mechanisms of the plant for adaptation to salt stress. Furthermore, our results will significantly increase the current scarce knowledge of trehalose in the plant kingdom.

Keywords: *Medicago truncatula*, RT-PCR, Trehalase, *Sinorhizobium meliloti*

C3.38

Responses of different species of turfgrass to salinity

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Water stress due to drought and salinity is probably the most significant abiotic factor limiting plant (crop) growth and development. As well as an increase in this stress due to climate change, areas such as the middle East, Australia and the southern United States are likely to see an increase in the use of drainage/effluent water for irrigation. This water has a higher salt content than normal and provides another source of salt stress. Sports turf varieties may be subject to this source of salinity to an even greater extent than agricultural crops; this is likely to become a significant factor in the UK, which will require the development of more salt-tolerant species as the quality of water supplies for sportsturf irrigation diminishes.

We have carried out experiments in vitro and in vivo with a number of commercial varieties of rye grass (*Lolium perenne*), and have assessed growth, photosynthetic activity and internal salt concentration. Seedlings of four different commercial varieties were grown in petri dishes with different concentrations of salt and, after measurements of root growth, cell contents were extracted and analysed to determine whether there were varietal differences in uptake/exclusion. Pot experiments were used to determine overall growth and photosynthetic responses. In addition, we report some preliminary results of experiments with warm season C4 seashore paspalum (*Paspalum vaginatum*), which is gaining wider usage in the U.S. as it has increased salt tolerance compared with the traditionally used Bermuda grasses (*Cynodon*, *Dactylon*).

Keywords: Salinity, *Lolium perenne*, Warm-season grasses, Growth, Tolerance