Salt and Water Stress in Plants

TRAVEL REPORT

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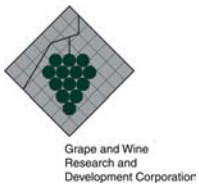
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Purpose

To attend the Gordon Conference on 'Cellular basis of adaptation to salt and water stress in plants' and to visit the Department of Plant Sciences, Cambridge University, UK.

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SUMMARY

Travel was conducted between 14-24 July 2002. The primary objective was to attend the Gordon Conference on 'Cellular basis of adaptation to salt and water stress in plants'. A secondary objective was to discuss potential for interaction in the area of regulation of cellular control of chloride levels in plants.

An overall impression gained from attendance at the Gordon Conference was that there have been substantial advances, particularly in US and Europe, in molecular approaches to improving adaptation of plants to abiotic stresses. During the last few years in particular many laboratories in several countries have embarked on genomics approaches to identify key genes involved in plant response to abiotic stresses. Substantial new information is now becoming available and the challenge from here will be to identify and utilize specific key genes for improving plant tolerance. Demonstration that this can be achieved was clearly shown by the elegant studies of Blumwald and colleagues in isolating the Na^+/H^+ transporter gene and through transformation, substantially increasing the tolerance of tomato and Brassica to salinity.

Several papers dealt with abscisic acid and calcium signaling in plants. The abscisic acid work focused heavily on ABA signal transduction mechanisms which are linked to calcium ion channels in membranes and how these regulate cytosolic calcium oscillations which are in turn linked to guard cell potassium efflux and stomatal closure.

There were also some interesting presentations dealing with regulation of long distance sodium transport in plants. This included observations that potassium ion channels in membranes are linked to sucrose loading in the phloem and therefore sugar transport processes in the plant.

In terms of potential interactions, several contacts were made, the key ones being with researchers at the Universities of Cambridge and Sussex (UK) and Nevada (USA). There is a strong possibility that key people from each of these Universities with interests in ion uptake and transport processes and plant tolerance to abiotic stresses will visit Australia in 2003-2004. The visits will include seminars at key centres in Australia, and consideration of the potential for joint work in the area of cellular regulation of chloride levels in plants.

DETAILED OVERVIEW

GORDON CONFERENCE ON CELLULAR BASIS OF ADAPTATION TO SALT AND WATER STRESS IN PLANTS.

The Conference was held at Queens College, Oxford, from 14-19 July 2002. It was attended by 139 invited delegates from 29 countries. Poster and oral sessions were involved, but there was no formal publication of presented material. This is a feature of Gordon Conferences, as the aim is to encourage participants to present the latest results and engage in open discussion.

There were seven sessions, as follows:-

1. Salt and water stress

2. Ion Homeostasis

3. Ion Homeostasis and Signaling

4. Ca^{2+} signaling in the plant stress response

5. Plant osmotic stress responses

6. Crop production and QTL's

7. Plant and cellular responses to stress

Highlights of the specific sessions are as follows:-

Salt and water stress

There were three speakers, Emanual Epstein (USA), Hans Bohnert (USA) and Bob Sharp (USA). Their task was to set the scene for the sessions to follow.

Epstein noted the success of conventional breeding in producing a highly salt tolerant barley genotype and a tomato genotype that could tolerate 50% seawater. He contrasted this with the newer gene transformation approaches, in particular the Eduardo Blumwald breakthrough in isolating the tonoplast Na^+/H^+ transporter gene and through transformation producing a tomato genotype that could tolerate 40% seawater. Epstein drew attention to the element silicon

which is mostly present in cell walls and suggested it provides a protective effect against various stresses.

Bohnert spoke about salinity and drought stress genomics. He commented on the "substantial amounts of data" that are being generated and suggested there were relatively few new insights so far. He spoke about the aquaporin gene family (AQP genes) and suggested that by putting sequences into evolutionary trees we should be able to determine functions of specific genes. He spoke about development of mutant populations to help elucidate gene functionality. He also noted that micro-array approaches indicated specific gene responses to certain stresses but also some overlap in expression patterns between salt, waterlogging and drought stress. He questioned the value of research on abiotic stress using Arabidopsis in terms of whether the results of such work would be transferable to the field. Field stresses are distinct from the constant, controlled and replicated conditions of Arabidopsis experiments. He noted also that in many cases, controlled experiments have involved application of 'shock' levels of stress and commented this does not represent reality. He reminded participants that the research would always have to be carried through to the field for validation.

Sharp spoke about the influences of ABA and ethylene in regulation of root and shoot growth under water stress. He cited the example of maize where root elongation increased as ethylene production decreased (Sharp et al 2000, Plant Cell and Environment 25, 211-222 and Spollen et al Plant Physiology 122, 967-976). He also noted evidence that ABA accumulation is required for maintenance of maize primary root elongation (Wright, Planta 148, 381-388) and discussed the possibility of ABA having a more direct role in maintaining shoot growth of tolerant plants under water stress, citing several references (Sharp et al 2000, Journal of Experimental Botany 51, 1575-84; Saab et al, Plant Physiology 93, 1329-1336).

Ion Homeostasis

Speakers in this session were Schactman (USA), Apse (USA) and Takabe (Japan). Schachtman spoke about mechanisms controlling chloride exclusion in grapevines, possibilities being reduced chloride uptake, reduced chloride transport and increased chloride efflux. Root chloride concentrations were higher for excluders than non-excluders and there was no relationship between chloride and sodium accumulation in grapevines. One of the best chloride excluders had the highest transpiration rate indicating that chloride exclusion was not necessarily linked to transpiration rate differences between genotypes. Evidence of a relationship between concentrations of ABA and chloride in xylem

sap was noted, leading to the suggestion that ABA may be involved in regulating ion transport processes in roots.

Apse spoke about the role of vacuolar Na^+/H^+ antiports in salt tolerance and ion homeostasis (Apse et al 1999, Science 285, 1256-1258). The AtNHX gene family involves at least 5 genes (AtNHX 1-5) differentially expressed in various tissues. AtNHX1 is the principal cation/H^+ antiporter in leaves. Over-expression of AtNHX1 leads to high accumulation of Na^+ in leaves (vacuoles) and low accumulation in fruit. In Brassicas grown under saline conditions, leaf sodium can be up to 6% of dry weight. In tomato grown under saline conditions, sodium concentrations in fruit are low, whereas fruit potassium and proline concentrations are significantly increased and soluble sugar concentrations are high. Plans are underway to field test tomatoes transformed for over-expression of AtNHX1.

Takabe extended the discussion on Na^+/H^+ antiporters dealing specifically with the Na^+/H^+ antiporter in Cyanobacterium. The gene controlling the Na^+/H^+ antiporter in Cyanobacterium belongs to the NhaP family. Over-expression of the ApNhaP gene in Cyanobacterium enables fresh water Cyanobacterium to tolerate seawater conditions.

Uozumi transferred the discussion to the Arabidopsis Na^+ translocating AtHKT1 protein and presented results on structure/function characterisation of the AtHKT1 gene.

Cellier then outlined her work on identification and characterisation of Arabidopsis mutants disrupted in putative Na^+/H^+ antiporter genes. She outlined what is known about the AtCHX 13 and AtCHX 17 genes. AtCHX 13 is known to be expressed in the pollen grain and pollen tube and may have a role in pollen germination. AtCHX 17 is known to be expressed in roots, specifically in root cortical cells. AtCHX 17 activity is influenced by ABA and is known to be induced by salt stress, K^+ deprivation and acidic pH.

Pardo concluded the session with a discussion on sodium in salinised plants – "from foe to friend". This relates to the fact that unregulated partitioning of Na^+ in plants and excessive accumulation in cytoplasm is a key factor in salt sensitivity, whereas through manipulation of the AtNHX1 gene, Na^+ could effectively be compartmentalised in the vacuole. Together with accumulation of compatible solutes in the cytoplasm, this enables salt tolerance as distinct from salt sensitivity. Pardo noted that the NHX1 gene was not expressed in the root tip but was expressed widely in the root behind the root tip. He referred also to the SOS gene family, with SOS1 a plasma membrane Na^+ transporter expressed in the root and potentially involved in release of Na^+ from the symplast to the xylem. He talked also about Na^+/K^+ exchange at the xylem/symplast boundary

(Lacon and Durand 1996, Plant Physiology 110, 705-711) and involvement in regulation of long distance Na^+ transport in plants.

Ion Homeostasis and Signaling

The four speakers in this session were Mark Tester (UK), Sylvie Coursol (USA), Kendal Hirschi (USA) and Yu Kanesaki (Japan).

Mark Tester began his presentation with a reminder to participants that Ca^{2+} activity (as distinct from concentration) decreases with increasing Na^+ concentrations and that in experiments involving different Ca^{2+} and Na^+ concentrations in the medium, it is important to adjust concentrations to ensure the level of available Ca^{2+} is maintained. He outlined three components of Na^+ efflux from plant tissues. The first is Ca^{2+} and DEPC sensitive Na^+ efflux, the second is Ca^{2+} insensitive and DEPC sensitive Na^+ efflux and the third is Ca^{2+} and DEPC insensitive Na^+ efflux. It is important to realise that while Ca^{2+} does not alleviate Na^+ toxicity, it does reduce it (Zhu et al 1998, Plant Cell 10, 1181-1191). Sodium influx through non-selective cation channels appears to be significant (Demidchik and Tester 2002, Plant Physiology 128, 379-387; Davenport and Tester 2000, Plant Physiology 122, 823-834) and Ca^{2+} and Mg^{2+} partially inhibit Na^+ influx with similar affinity. The molecular identity of non-selective cation channels has been addressed by Demidchik et al 2002, Annual Review of Plant Biology 53:67-107. Sodium influx into Arabidopsis roots is not affected by SOS3 or HKT1. However the role of HKT1 in plant response to high Na^+ is not known. Possible roles for HTK1 include catalysis of Na^+ efflux (Elphick et al 2001, Plant Cell and Environment 24; 733-740) or set point sensing e.g. for Na^+ or for turgor.

The title of Sylvie Coursol's presentation was "Guard cell abscisic acid signaling involves sphingosine-l-phosphate and heterotrimeric G proteins". She began by listing the range of factors that influence opening/closing of stomates, including CO_2, light, ozone, temperature and stresses (drought, salt, pathogens etc.). ABA inhibition of stomatal opening is due to inhibition of K^+ uptake and activation of anion and K^+ efflux from guard cells. At least two signaling pathways are involved in this process. The presentation focused particularly on sphingosine-1-phosphate which is an important signal molecule (Ng et al, Nature 410: 596-99). DMS is known to attenuate both regulation by ABA of stomatal aperture and regulation by ABA of ion channels. It is also known to reduce activity of sphingosine kinase in Arabidopsis leaf lysates. Sphingosine-1-phosphate (SIP) regulates stomatal aperture and ion channels. Coursal's work involved use of the Arabidopsis gpc1 mutant in which SIP inhibition of stomatal opening and the inward K^+ channel in guard cells is impaired. The presentation included various hypotheses on how the signaling processes operate to control stomatal opening.

Kendal Hirschi moved the discussion to "regulation of plant H^+/Ca^{2+} transporters". His primary research interest is manipulation of plant Ca^{2+} through genetic engineering approaches. He spoke about the calcium transporter gene family (CAX genes). Arabidopsis contains many CAX-like genes. CAX1 also has a role in extending postharvest life of specific fruits and vegetables, (Hirschi et al 1996, Proceedings National Academy of Sciences of the USA 93: 8782-8786 and Pittman and Hirschi 2001, Plant Physiology 127: 1020-1029). The full length CAX1 gene is expressed in the vacuolar membrane (K. Hirschi, Journal of Biological Chemistry, in press). A 9 amino acid domain in CAX1 confers calcium transport properties to the protein. CAX1 is highly expressed in response to Ca^{2+} and knockouts have reduced Ca/H transport. CAX1 and CAX2 may be regulated by N-terminal inhibition. In the discussion, Hirschi presented a series of schematics on Ca^{2+} regulation in plants and explained the roles of calmodulin and calcineurin in this process.

To conclude the session, Kanesaki spoke about the "Regulation of salt inducible and hyperosmolarity-inducible genes in Synechocytis". He opened the discussion by outlining the difference between stress-inducible genes (which lead to proteins such as proteases) and stress-repressible genes (which lead to proteins such as desaturases). Salt stress induces expression of at least 150 genes whereas repression (of salt stress) induces expression of at least 230 genes. Osmotic stress induces expression of at least 110 genes whereas repression (of osmotic stress) induces expression of at least 160 genes. Salt stress and osmotic stress clearly regulate different sets of genes. Histidine kinases are regulators of salt inducible genes.

Calcium signaling in the plant stress response

Speakers in this session were John Cushman (USA), Frans Maathius (UK), Tina Romeis (Germany), Julian Schroeder (USA) and Jeff Harper (USA).

John Cushman opened with an overview of "substrates and interactions of calcium-dependent protein kinases". Basically, calcium-dependent protein kinases are primary sensors of Ca^{2+} signals in plant cells. They function in diverse locations throughout the plant and relatively little is known about their functional roles. The presentation highlighted the scale of genetic screens being conducted in world leading laboratories. In this case, a substantial number (millions) of bacterial cDNA clones were screened using robotic instrumentation. Further detail on changes in sub-cellular markers during stress is given in a paper by Partharker and Cushman, Plant Journal, In press.

Frans Maathius moved the discussion towards "Transport pathways involved in salinity stress". He began by noting that various groups are using micro-arrays to research genes involved in plant membrane transport. He noted that cyclic nucleotides reduce Na^+ influx (Leng et al 1999, Plant Physiology 121, 753-761) and that little is known about genes involved in chloride channels. A small family of CLC genes has been identified in Arabidopsis (CLCabcd), one of which has been shown to have a role in NO_3^- transport. His work has also extended to studying the response of aquaporins to salinity. He has observed early down-regulation of aquaporin genes in response to salt stress, but a later up-regulation. Various genes shown to be up-regulated or down-regulated in response to salt stress were listed.

Schroeder's specific interest is regulation of Na^+ distribution in plants and in this case spoke on "A calcium module in stress signaling and a mechanism regulating root/shoot Na^+ distribution". He began by talking about the AthKt1- gene which is expressed in the root stele and vasculature and is involved is Na^+ removal from the xylem and release into the phloem. He then went on to talk about ABA signal translocation mechanisms which are linked to Ca^{2+} channels and cytosolic Ca^{2+}oscillations which are linked to stomatal closure.

To conclude the session, Jeff Harper introduced the meeting to "Genomics of Ca^{2+} signaling'. He began by noting the stimuli that increase cytosolic Ca^{2+} concentrations. These include red light, ABA, GA, as well as stresses such as salinity and drought (Allen et al 2000, Science 289: 2338-2342; Allen et al 2001, Nature 411: 1053-1057). It was noted that external Ca^{2+} shock fails to trigger Ca^{2+} oscillations for triggering stomatal closure. Further it was noted that (cytoplasmic) Ca^{2+} spikes with 5 minute durations are optimal for triggering stomatal closure. Calcium transporters have been identified in Arabidopsis. Disruption of the calcium pump aca9-1 results in poor seed set in the distal part of Arabidopsis siliques, the hypothesis being that the pollen tube growth is defective due to disruption of calcium gradients at the tip. On expression profiling, some 2409 changes have been shown to be induced by cold, salt and osmotic stress in Arabidopsis. Less than 5% of the total changes are shared by all three stresses (cold, salt and osmotic). Harper then went on to talk about mineral nutrient profiling (ionomics).

Model genetic systems for plant stress research

The three speakers in this session were Alonso Rodriquez-Navarro (Spain), Ray Bressan (USA) and Haruo Saito (Japan).

Rodriquez-Navarro covered potassium influx and sodium efflux in model systems, fungi, rice and Arabidopsis. His presentation centered on the role of HKT genes in these various systems.

Ray Bressan moved the discussion to a consideration of "Genomic scale evaluation of Arabidopsis T-DNA insertion mutants for altered salinity responses". He has 15,000 insertion mutants and is looking for instances where a change has occurred from salt tolerant to salt sensitive.

Saito's topic was "Osmoregulatory HOG MAP kinase pathway in yeast". He focused on membrane-bound osmosensing proteins and MAP kinase pathways.

Plant osmotic stress responses

The speakers in this session were Kazuko Yamaguchi-Shinozaki (Japan), Dorothea Bartels (Germany), Miguel Botella (Spain), Jerome Giraudat (France) and Marc Knight (UK).

Bartel's presentation was on novel gene products and signaling molecules in resurrection plants. Resurrection plants are able to tolerate dessication by accumulating high concentrations of octulose, a C8 sugar. The hypothesis that she presented was that dessication tolerance is determined by coordinated expression of protective proteins. Her presentation focused on genes that may be involved in dessication tolerance e.g. the Arabidopsis CDT1 gene.

Yamaguchi-Shinozaki discussed Arabidopsis stress proteins noting that DREB1A, DREB2A and rd29A were each induced by salinity. He noted that DREB/CBF genes are master switches of ABA independent gene expression in response to drought, cold and salinity.

Botella's topic was "Identification of genes essential for drought tolerance". He has identified a range of tomato mutants which show varying responses to salt and osmotic stress. The TSS2 line is hypersensitive to Na^+ and Li^+, to osmotic stress and to external ABA. The TDS1 line is hypersensitive to ionic stress, has reduced growth at low humidity and shows a wilty phenotype at mid-day. He is using the mutants in an attempt to identify the specific genes involved.

Giraudat moved the discussion to "Abscisic acid signaling in Arabidopsis". The presentation centered on two Arabidopsis ABA insensitive mutants, abi3 and abi5 and the use of infrared thermal imaging to screen for mutants with open stomata (OST) under drought. A range of OST mutants were identified through this process.

Marc Knight concluded the session with a discussion of "signaling during osmotic stress in *Arabidopsis thaliana*". He opened with a discussion on Ca^{2+} signals in plants (Knight, 1997, The Plant Journal 12, 1067-1018) and went on to discuss the timing, magnitude and spatial localisation of Ca^{2+} signatures.

Crop Production and QTL's

The three speakers in this session were Mike Kearsey (UK), Brian Forster (UK) and Majid Foolad (USA).

Mike Kearsey opened the session with an introduction to methodology and limitations involving QTL's (Quantitative Trait Loci). These are the genes that underlie variation in a quantitative trait, also known as polygenes. The problems of defining a quantitative trait were outlined. For example, in some instances they can be confused with a single gene which has a lot of "environmental noise". The confidence intervals on QTL position within a chromosome are often large. For example, they are seldom less than 5 centimorgans and often greater then 30 centimorgans. For comparison a typical chromosome is 100 centimorgans. It is also difficult to distinguish QTL's that are less then 20 centimorgans apart (note: 1 centimorgan = 10^6 base pairs). Of the QTL's that have been identified, many have regulatory functions.

Brian Forster spoke about development of molecular marker maps that show the location of specific traits.

Majid Foolad spoke about QTL's linked to salt tolerance in the germination and vegetative growth phases of plant development. He has found that different genes contribute to tolerance during seed germination relative to tolerance during the vegetative growth phase.

Stress Signaling

Speakers in this session were Zhu (USA), Pages (Spain), Yun (Korea), Seki (Japan) and Hirt (Austria).

Zhu's topic was "Salt stress signaling and ion homeostasis". He dealt mainly with the SOS gene family, noting that overexpression of SOS1 improves salt tolerance of Arabidopsis and that plasma membrane Na/K exchange is reduced in SOS mutants.

Page extended the discussion on signaling with his presentation titled "Function of ABA induced proteins is modulated by multiple kinases during osmotic stress".

He noted that the protein kinase CK2 gene is ubiquitous and highly conserved, and that the protein is present in both the nucleus and cytoplasm. Overexpression of maize CK2β subunits increases salt tolerance in yeast. Further it is known that the protein Rab17 interacts with CK2 β1 and CK2β3 and Rab17 plays a role in growth inhibition. The Rab gene family is associated with ABA.

Yun's presentation was centered around cell death (apoptosis) and anti-apoptosis and pro-apoptosis genes.

Seki's presentation was in the area of Arabidopsis functional genomics and the mapping of expressed genes and mutant genes (Seki et al 2002, Science 296: 141-145). He has monitored the expression pattern of Arabidopsis genes under drought, cold and salt stress and documented 299 drought inducible genes, 54 cold inducible genes, 213 salinity inducible genes and 245 ABA inducible genes (Seki et al 2002, Plant Journal, in press). Current work in Dr. Seki's laboratory involves construction of a micro-array containing 15,000 full length c.DNA clones for studies on stress response genes.

The final presentation in this session by Heribert Hirt was titled "Functional analysis of salt-stress induced MAP Kinase (MAPK) pathway reveals roles in stress and development". He began by noting that different stresses, including salt stress, activate the SIMK gene and that salt activates the MAPK gene. He commented also that inhibition of MAPK activation affects root hair growth and morphology and also actin organisation. The SIMKK protein (an activator of SIMK) is known to be localised in the epidermis and root hairs.

Plant and cellular responses to stress

The three speakers in this session were Hirofumi Uchimiya (Japan), Gerry Berkowitz (USA) and Rainer Hedrich (Germany).

Uchimiya continued the discussion on cell death inducers and repressors while Berkowitz talked about the CNGC gene family which are linked to ion channels. The final presentation of the Conference was by Rainer Hedrich who spoke about "Mineral nutrition – regulation of root ion channels by salt and water stress". He began by talking about the AtKC1 protein which is present in the plasma membrane and involved in ion channel functionality. He commented also that K^+ ion channels (Roberts and Snowman, Journal of Experimental Botany 51, 1585-1594) are linked with sucrose loading in the phloem and therefore sugar transport processes in the plant.

General

Tim Flowers presented some data from an internet data base search noting the relatively few new cultivar registrations involving salinity or drought tolerant plants. From the patents data base, there was only one new salinity tolerant cultivar since 1994 and no drought tolerant cultivars. However, the Journal of Crop Science listed 7 new salinity tolerant cultivars and 5 new drought tolerant cultivars since 1994. The comment was made that the substantial gene-based approaches as outlined in the conference should (in theory) deliver a marked increase in stress tolerant cultivar registrations over the next 1-2 decades.

On the poster sessions, a poster by Bageshwar et al (Texas A&M University) noted that salinity resulted in an increase in proton pumping activity in leaves and roots and that there was a moderate but consistent enhancement in ATP hydrolysis as a result of salinity stress. It was noted also that salinity stress may alter the composition of VATPase.

Of interest to viticulture was the poster by Tattersal and Kramer (Nevada, USA). They have initiated an expressed sequence tag based gene discovery program focused solely on stressed grapevine plants. This work has links with Mark Matthews at Davis (Matthews and Anderson, American Journal of Enology and Viticulture 39, 313-320).

The poster by Apse listed several references on the AtNHX1 gene (Ruiz and Blumwald 2002 Planta 214, 965-969); Zhang et al 2001, Proceedings of the National Academy of Sciences of the USA 98, 12832-12836; Zhang and Blumwald 2001, Nature Biotechnology 19, 765-768). It was noted that reductions in AtNHX1 activity (which mediates K^+ and pH homeostasis in cells) may have an impact on the ability of cells to expand and therefore have an effect on (e.g. leaf) cell size. It was noted that NHX genes are likely to be present as multiple isoforms in many species.

In a separate poster, salt tolerance in rice was shown to be increased by insertion of an Na^+/H^+ antiporter gene from Atriplex. Activity of the Na^+/H^+ antiporter was 8 times that in the rice wild type, and the resultant plant was able to survive 300 mM NaCl.

It was noted that DNA micro-array based analysis that measures steady state mRNA abundance is currently the technology of choice to identify and study the complete repertroire of plant genes triggered by a variety of environmental stresses. Nevertheless, fragment PCR-based technologies such as cDNA AFLP's coupled to RT-PCR expression analysis may still be useful.

Finally, a key impression was the obvious rapid advancement in molecular (especially micro-array) approaches to understanding and manipulating tolerance to abiotic stresses. The strength of work in the US and Europe in this area is outstanding. There was a strong focus on sodium (relative to chloride) as a key factor in salt tolerance of many species, and advances with the AtNHX gene (Na^+/H^+) antiporter were a highlight. The strength of work in the Ca^{2+} signaling area was impressive, as were approaches with the molecular physiology of ABA.