Identification of Novel Membrane Transporters Associated with Potassium Acquisition and Homeostasis

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Abstract

Potassium has long been associated with many different cellular processes such as enzyme activation and osmoregulation. Because of its important role in cellular metabolism, K^+ is maintained at high and stable concentrations in the cytoplasm in relation to varying K^+ external conditions. This is achieved through synergistic low- and high-affinity mechanisms, which incorporate different ion channels/carriers and pumps located across different membranes within and across different tissues.

As potassium homeostasis is a complex process, analysis at the single-gene level would make it virtually impossible to obtain a complete view of membrane transporter processes. This is why a microarray approach was adopted, in order to obtain a broader picture of K^+ regulatory pathways. Whilst full genome chips are effective at gaining global expression profiles, we have narrowed the field to exclusively look at Arabidopsis Membrane Transporters through a customised AMT chip that contains probes for 1250 known and putative transporters.

Expression changes of membrane transporters were investigated in response to abiotic stress, mimicked by addition of ABA to 2-week-old *A.thaliana* seedlings grown in potassium-rich or potassium-deplete conditions. Our preliminary results indicate that at least three types of transporters showed consistent but different expression patterns. These included members of the MATE family, a novel putative transporter with 7 predicted transmembrane spanning domains (7TMS) as well as the high-affinity transporter HAK5.

Acknowledgements

A number of people greatly supported me during this year, and I would therefore like to express my gratitude to all members of the group. In particular to Patrick Armengaud, Richard Pattison and Anna Amtmann.

Cheers,

Caroline

Abbreviations

ABA Abscisic acid

ABRE Abscisic acid responsive element

AMT Arabidopsis membrane transporter

ATP Adenosine triphosphate

cDNA Complementary DNA

CTP Cytosine triphosphate

Cy3 Cytosine 3'

Cy5 Cytosine 5'

DNA Deoxyribonucleic Acid

GTP Guanine triphosphate

NTP Nucleotide triphosphate

PCR Polymerase chain reaction

RNA Ribonucleic acid

TE Buffer Tris EDTA buffer

TMS Trans Membrane Segment

TTP Thymine triphosphate

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APPENDIX

1.0 Introduction

In order for plants to grow and survive in their respective environments, it is necessary for them to adapt and compete in their surroundings. Considering that plants are anchored and relatively immobile, the way that they utilise their natural resources is of extreme importance. Essentially, it is their root system that is responsible for scavenging water and essential inorganic nutrients necessary for growth and biochemical synthesis. Once inside the plant, these ions are distributed between different organs through the co-operative action of a network of integral-membrane proteins that facilitate the transport of ions across membranes. Of all plant nutrients required for survival, the three most important in the field are nitrogen (N), phosphorus (P) and potassium (K).

1.1 Role of K^+ as an Essential Plant Nutrient

Analysis of whole plants across different species reveals that K^+ is in relatively high abundance amongst all tissue types which demonstrates that K^+ is both universally important and highly mobile, capable of regulating osmotic potential that will drive both long-distance transport as well as intracellular processes (reviewed by Marschner, 1995). For instance, potassium can have both direct and indirect effects upon cellular protein form and function, mainly because it is readily exchangeable between different proteins due to it only forming weak complexes and its inability to be metabolised (Wyn Jones *et al*, 1979). This

means that it does not strongly compete for binding sites (as opposed to divalent cations) allowing it to accumulate within the cytosol, at typical [K^+] levels of 100mM (Maathuis and Sanders, 1994). This makes it the dominant cation that counteracts and neutralises both organic and inorganic anions that contribute to the Donnan Potential.

Despite only being able to form weak complexes, K^+ is capable of inducing conformational changes amongst proteins, due to distortion of their hydration shells. This is found to be particularly true at 50-100mM [K^+], which is the concentration typically found within cells and can increase V_{max} of catalytic reactions. Such enzymes include kinases and membrane bound proton-pumping ATPases, where potassium again aids catalysis through maintenance of cytosolic pH. Potassium is also essential for protein synthesis where typically higher levels of K^+ are required (typically >130mM [K+] – Wyn Jones *et al*, 1979). Experimental evidence indicates that RuBP carboxylase is largely dependent upon K^+ abundance, as is ribosomal translation (Peoples and Koch, 1979). Thus photosynthesis, and general protein turnover are largely dependent upon K^+ status.

1.2 Role of K^+ in Osmoregulation

Potassium's most important role however is the way that it functions in plant water relations and turgor-driven processes. Examination of the stele for instance indicates a high water potential that is responsible for turgor-pressure-

driven solute transport into the xylem and phloem (Hsiao and Läuchli, 1986). Once inside the cell, K^+ is largely stored within the central vacuole that typically occupies 80-90% of total cell volume. Together with reducing sugars, turgor pressure is generated and drives cell extension.

Guard cells have long been used as a model for investigating K^+ regulatory mechanisms as they depend upon K^+ for turgor pressure (MacRobbie, 1987). They can incorporate both environmental and endogenous signals to bring about changes in transpiration, through stomatal pore aperture (Blatt, 2000). Potassium is largely responsible for generating the turgor pressure required, as there is a large influx into guard cells when the stoma open. Chloride ions will also follow this unilateral flow, and counteract the positive charge generated by potassium. In response to darkness or water deficiency (where the stress hormone ABA is released) the stoma will close due to a rapid efflux of both K^+ and Cl^- into the guard cell apoplasm.

1.2.1 Abscisic Acid (ABA) can Regulate K^+ Mediated Processes

Using the guard cell as a model to study K^+ dependent turgor processes, Blatt & Armstrong (1993) demonstrated that the plant hormone ABA, normally associated with seed germination and dormancy, was directly involved with initiating stomatal closure through the different activation states of the K^+ rectifying channels.

ABA is a signal molecule capable of both short and long-distance chemical signalling. Reduced soil water availability decreases osmotic pressure, allowing

ABA to accumulate in the xylem sap, and subsequently within leaf compartments (Zhang & Davies, 1989). Analysis of epidermal strips (Daeter & Hartung, 1995) demonstrated that symplasts were an ABA sink and proposed that cross-talk between the symplastic reservoir and the xylem could be places for ABA stress signal modulation. Furthermore, plants seem to operate a feedback mechanism originating within roots that could control the amount of ABA reaching guard cells, as K^+ loading from xylem parenchyma cells (XPC) into the xylem is inhibited by ABA (Roberts & Snowman, 2000; Drew *et al*, 1990). K^+ release into the apoplast of the stele is mediated by outward rectifying channels, which are regulated by ABA (Roberts & Tester, 1995).

The gene for one of these channels was identified as SKOR as it was found that mRNA levels were ABA-dependent. This provided further evidence that increased ABA levels in response to osmotic stress are a key signalling component in both long-distance K^+ transport and guard cell regulation. In addition to its role in K^+ channel regulation, an endogenous ABA signal is the basis for transcriptional activation of many genes involved in adaptive responses to stress. Promoter studies have identified ABA Responsive Elements (ABRE) such as G-Box proteins and upstream signalling elements (reviewed by Leung and Giraudat, 1998). Hence, the generation of endogenous ABA is the basis for transcriptional activation of certain genes to produce a defensive response to stress.

1.2.2 Osmotic Stress Responses might depend on K^+ Availability

Osmotic stress will activate a series of internal responses that will facilitate turgor-driven processes and hence rely upon K^+ availability. Whether it is in response to a lack of water, exposure to excess NaCl or cold temperatures, both ABA-dependent and –independent pathways can be activated. Accumulation of ABA in response to these stresses has been demonstrated and is the result of both increased synthesis and inhibition of degradation (reviewed by Zhu, 2002). However, the ability of ABA-deficient and insensitive mutants *aba1*, *aba2*, *abi1*, *abi2* (Koornneef *et al*, 1998) to produce osmotic stress responses suggests that an ABA–independent pathway is also present. Ultimately, the ability for plants to integrate a network of osmotic-related responses shows a complexity that can be predicted to depend on availability, uptake and distribution of osmotically active ions and compounds. The level of involvement of K^+ in stress perception and cross talk between signalling pathways is completely unknown.

1.3 Molecular Physiology of K^+ Acquisition

1.3.1 Mechanisms of K^+ Acquisition

Plants can grow in a wide range of varying $[K^+]$ levels from as low as 10μM to 10mM (Maathuis and Sanders, 1996). By contrast, typical cytosolic levels of K^+ within plants are maintained at a stabilised concentration of around 100mM K^+. Therefore, plants have developed mechanisms for cytoplasmic K^+ homeostasis that can perceive differences in external ionic content and keep internal levels relatively concentrated. This is achieved through an array of different membrane

proteins including channels, carriers and proton pumps that are required to mediate and regulate currents across the plasma membrane. Studies in the 1960s by Epstein (Epstein *et al*, 1963) elucidated that K^+ uptake was a biphasic process where there were at least two different uptake mechanisms present (Figure 1.1).

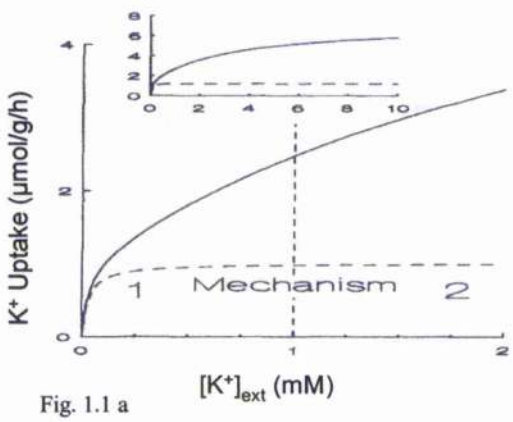


Fig. 1.1 a

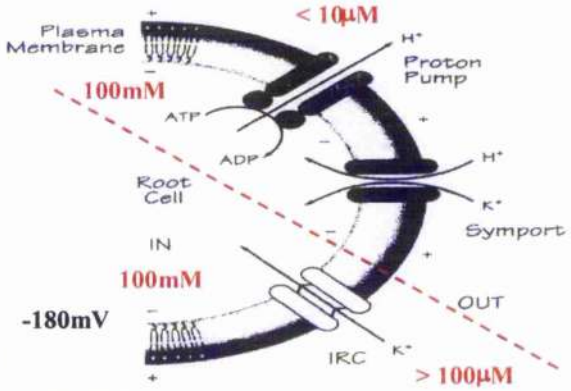


Fig. 1.1 b

Figure 1.1: Overview of Potassium Acquisition (1.1a) Potassium acquisition is via a biphasic mechanism in which high- and low-affinity transporters can mediate K^+ flux into cells (1.1b). Low-affinity channels will mediate the passive flow of K^+ into the cell in both high and low external $[K^+]$. If external $[K^+]$ concentrations drop to very low µM concentrations, high-affinity carriers operate to maintain cytosolic values of 100mM $[K^+]$. (Diagrams adapted from Maathuis & Sanders, 1996).

It is now known that Mechanism 1 is mediated by carriers and conforms to Michaelis-Menten kinetics, where the integral membrane protein functions similarly to an enzyme and reaches saturation, with K^+ as the substrate. This is an active process and is also known as the high-affinity system as it operates in the micromolar range of $[K^+]_{ext}$, when potassium is scarce. In contrast, Mechanism 2 functions in potassium abundant conditions (typically millimolar ranges) and is more linear in uptake kinetics.

Advances in molecular biology and electrophysiology are providing evidence that the dual-affinity process of K^+ acquisition is a lot more complex. Functional analysis of the role of AKT1 for instance (a K^+ selective inward rectifying channel KIRC) using a null mutant (*akt1-1*) and a K^+ analogue (Rb^+) has suggested that (in the presence of ammonium) AKT1 is capable of high-affinity K^+ uptake from very low concentrations (Hirsch *et al*, 1998). In addition, it is unlikely that it is the only channel responsible for low-affinity K^+ acquisition as at 1mM $[Rb^+]_{ext}$ concentrations, it showed a 70% similarity to the wild type. Therefore, there are arrays of integral membrane proteins that can transport K^+ into plant cells.

Absorption of all nutrients is largely dependent upon the electrochemical difference across the plasma membrane, which is regulated by members of P-type ATPases, a gene family of 45 members within Arabidopsis (reviewed by Axelsen & Palmgren, 2001). Activity of the proton pump depends on plant species, tissue and physiological conditions. In an average plant cell, proton pumps generate a membrane potential of minus 150mV (cytosol/apoplast) and a pH difference of around 2 units, making the cytosol basic (Maathuis & Sanders, 1994; Schroeder *et al*, 1994; Hirsch *et al*, 1998). Any further increases or decreases in membrane potential (hyper- or depolarisation respectively) can activate or deactivate voltage-gated channels which will promote influx or efflux of ions in a unilateral manner, and are therefore said to rectify inwardly or outwardly.

1.3.2 Overview of Cloned K^+ Transporters

Improvements in cloning and characterisation of membrane transporters inheterologous complementation systems has provided conclusive evidence of potassium transporter function, such as the identification of the first plant K^+ channels: AKT1 (Sentenac *et al,* 1992) and KAT1 (Anderson *et al,* 1992) They belong to the Shaker family of channels with their basic structure consisting of 1 pore loop and 6 transmembrane domains (TMD). AKT1 and KAT1 are both inward rectifying whereas two other members of the *A.thaliana* Shaker family, SKOR and GORK, are outward rectifying (Mäser *et al,* 2001 - figure 1.2). Another type of outward rectifying K^+ channels in plants was later identified (Czempinski *et al,* 1997, 1999) as members of the so-called KCO family, containing 1 or 2 pores, with 2TMD/4TMD respectively. HKT1 from wheat became the first K^+ transporter to be cloned and characterised (Schachtman and Schroeder, 1994; Rubio *et al.*, 1995) and represented the Trk/HKT family of transporters (8TMD and 4 pore regions). HKT1 is a K^+/Na^+ symporter in wheat, but acts as a Na^+ transporter in *A.thaliana* (Rubio *et al.* 1995; Uozumi *et al.*, 2000). AtKUP1 and HAK transporters (Fu & Luan, 1998; Quintero & Blatt, 1997) have also been cloned and represent a family of high-affinity transporters known as the Major Family of Superfacilitators (MFS) containing 12 TMD. Other putative cation channels and K^+ transporters include: cyclic-nucleotide-gated-channels (CNGCs); putative glutatmate receptors; LCT1 (low-affinity-cation-transporter); the CPA family (cation proton antiporters) and the CCC family (cation chloride cotransporters-reviewed by Mäser *et al.* 2001).

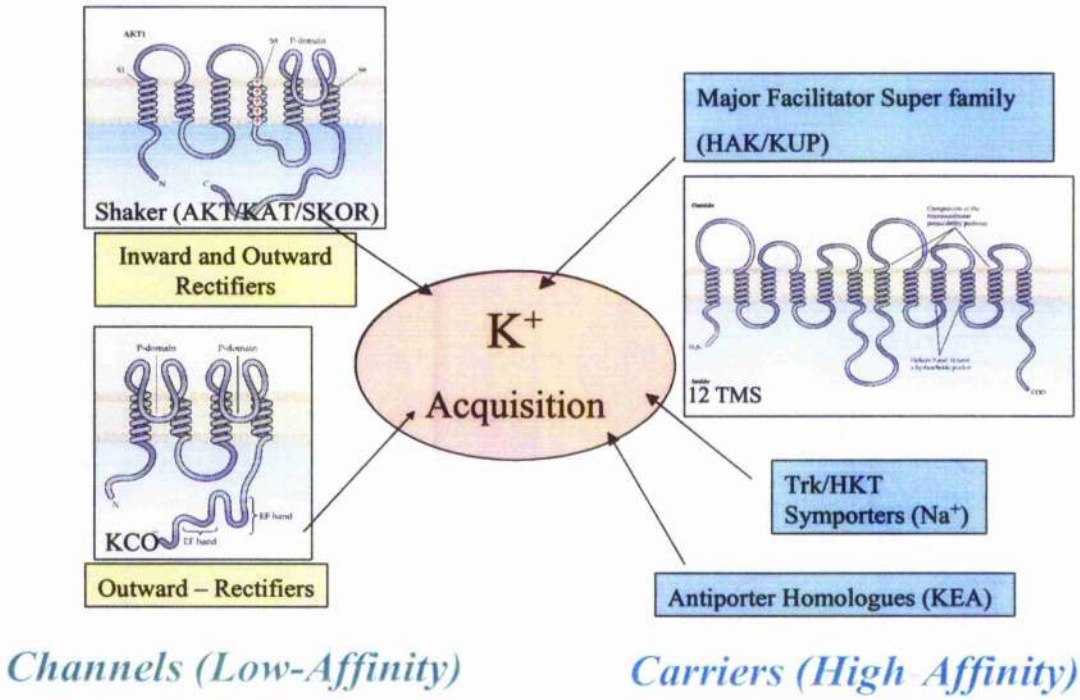


Figure 1.2: Five gene families for K^+ transport: Channels tend to be structurally less complex than transporters due to the presence of less membrane spanning domains. Many membrane transporters remain uncharacterised. (Diagram adapted from Buchanan *et al* 2000).

To summarise, hydrophobicity plots and functional characterisation of membrane proteins have suggested that transporters vary greatly in size, shape and function (Figure 1.2). The phylogeny of potassium transporters (Mäser *et al*, 2001) reveals five different families within four distinct categories, distinguished by their structure and function. These are: - two distinct K^+ channel families (15 genes in *A.thaliana*); the Trk/HKT transporters (one gene); KUP/HAK/KT transporters (13 genes) and K^+/H^+ antiporter homologues (six genes).

1.3.3 Evidence for K^+ Homeostasis

The vast array of different membrane channels and transporters indicates that K^+ regulation is far more complex than originally thought. This would suggest a sophisticated system of integration between the external environment and intracellular processes, but the mode of perception and response still remains unclear.

There is evidence however that plants are able to perceive changes in K^+ levels. Studies in *Hordeum vulgare* roots (Walker *et al*, 1996) have shown that endogenous potassium levels are regulated at the whole plant and cellular level, with the vacuole acting as a flexible store. Using triple-barrelled electrodes to differentiate compartments according to pH (coupled with $[K^+]$ and changes in membrane potential), Walker deduced a coordinated response as the vacuole acted as a pool for $[K^+]$ storage. The vacuolar $[K^+]$ declined or increased proportionately to tissue K^+ concentration. However, the cytosolic $[K^+]$ remained at a constant level unless under severe K^+ starvation. Below a "critical threshold" of 20mM, the vacuole would no longer release its K^+ supply, and thus further decreases in tissue K^+ would affect cytosolic K^+ levels.

1.4 Molecular Basis of K^+ Transport Regulation

1.4.1 Transcriptional Regulation of K^+ Transport

Members of the KUP/HAK/KT and HKT families have been shown to increase their transcript levels in response to K^+ shortage, hence providing evidence for

their roles in high-affinity uptake (reviewed by Véry and Sentenac, 2003). Furthermore, certain members show transcriptional responses upon exposure to NaCl stress with downregulation of HKT transcripts in rice roots (Assmann, 2002) and upregulation of HAK transcripts in the ice plant (Su *et al*, 2001). Both of these indicate a K^+ related response in the presence of NaCl stress, which could be subject to endogenous hormonal changes. As mentioned previously (section 1.2.1), SKOR has been shown to be transcriptionally regulated by ABA; this provides further evidence that K^+ related processes are governed to some extent at the genetic level.

However there is also evidence that regulatory proteins, heteromerization, voltage, pH, Ca^{2+} and cyclic nucleotides have effects upon K^+ transport (reviewed by Véry and Sentenac, 2003). Thus K^+ transport is a complex process that could be the result of both transcriptional and post-translational responses to external triggers. The fundamental basis for the cross talk between different types of regulation and the ways of perception are still unknown.

1.5 Project in Context

1.5.1 Agricultural Significance of Studying Potassium

The importance of potassium can be seen when looking at the physiological differences between healthy plants and those that have been reared when external potassium levels are low. First and foremost, regulatory enzyme activity is reduced and can result in an accumulation of soluble carbohydrates and a decrease in starch. The reduced export rates to storage organs together with an

impaired lignification of vascular tissues means that K^+ deficient plants will become more susceptible to lodging. Plants are also less likely to cope with drought stress, and typical symptoms will include wilting, and reduced cell expansion (reviewed by Mengel and Arneke, 1982). This typically leads to less growth and increased susceptibility to fungal attack or frost damage (Shinozaki *et al,* 2003).

Studying potassium and plant nutrition is becoming increasingly important: we are faced with a rising world population, changing climate conditions and plants that have been bred to achieve their maximum genotypic potential (Hay, 1995). This means that urgent attention is needed in global agriculture as understanding plant nutrition better could increase crop production and limit the use of fertilisers, thus decreasing the contamination of ground and surface water and eventual eutrophication.

To improve plant mineral nutrition further requires a more efficient mode of mineral uptake from the soil, and more effective allocation of nutrients to different plant tissues. It is therefore imperative that the underlying mechanisms of ion homeostasis are addressed in more detail to obtain optimal conditions for agricultural sustainability. Investigations into root plasticity and root to shoot signals could be a good basis for assessing mechanisms of mineral nutrition. This would provide knowledge of signal transduction cascades and the proteins involved in the influx and efflux of ions, both within the cell and across different tissues. To enable these improvements however requires more knowledge of cellular and whole plant responses (reviewed by Hell and Hillebrand, 2001), as

well as more knowledge on the molecular basis of these responses. It is therefore necessary to externally manipulate nutrient fluxes and developmental responses, and evaluate the molecular mechanisms of nutrient homeostasis through the application of suitable analysis tools. Such tools in the past have included the profiling of metabolites (Fiehn *et al*, 2000) as well as mRNA profiling (Wang *et al*, 2000) in various conditions and genotypes.

1.5.2 Experimental Approaches

The complexity of plasma membrane transport indicates that members of many different gene families are co-operating to produce an integrated and regulated response to external ionic levels. Novel computational techniques have elucidated that approximately 5% of the *A.thaliana* genome encodes membrane transport proteins, comprising 46 different families containing approximately 880 different members (Mäser *et al*, 2001). Since membrane transport is complex, adopting a genomics approach can take advantage of the full genome data readily available for *A.thaliana* (www.tigr.org), by using microarray technology to monitor genome-wide gene expression (Somerville and Sommerville 1999). This has become an increasingly popular method through the quick and reliable production of arrays containing thousands of cDNA or oligonucleotide probes (Schaffer *et al*, 2000; Lockhart & Winzeler, 2000). Microarray technology allows for the simultaneous profiling of potentially thousands of transcripts in response to a particular treatment, and can quickly identify genes of interest. The method is relatively simple and can demonstrate

both global and simultaneous responses occurring within or across different tissues.

1.5.3 A.thaliana and Microarrays: - a Model Approach to Genomic Expresion

We will use *A.thaliana* as a model system (Meinke *et al*, 1998) to assess changes in gene expression in response to ABA. Results from these experiments will then be compared with gene expression patterns observed in plants subjected to changes in external K^+ supply. Seedlings will be used as the young, growing tissue will largely be dependent on turgor-driven processes for growth and expansion, hence more likely to exhibit K^+ dependent gene regulation.

ABA is clearly an important signalling molecule and has been shown to activate turgor-driven responses, however the level of its involvement in communicating information about external K^+ levels to different tissues and cellular compartments remains unknown. Initially, the hormone ABA will be added exogenously to trigger stress responses and the time taken for optimum transcriptional activity evaluated. Once an optimal timer period for activity is elucidated, the plants will be starved and re-supplied with K^+, to study the effect of K^+ pre-treatments on the ABA response. The cDNA from these procedures will be hybridised to an Arabidopsis Membrane Transporter (AMT) chip (Maathuis *et al,* 2003). The AMT chip has been specifically designed so that probes for all putative membrane transporters are present; it will allow for the investigation of in depth responses that are specifically targeting membrane

transporters in relation to a stress response, thus taking away the complexity of other cellular processes that could otherwise disguise relatively small gene changes. The AMT chip consists of 1250 oligonucleotide probes, which are 50-mers representing 1153 genes, of which 1096 represent transporters and 57 are controls. The transcriptional profile of transporter genes during stress responses can hence be elucidated through the comparison of specific gene expression patterns with specialist software. This will help to identify specific gene families, or individual genes showing stress-induced responses in relation to K^+ supply.

2.0 Methods

(All chemical are from Sigma Chemical Company, unless otherwise stated).

2.1 Growth of Experimental Plants

2.1.1 Seed Surface Sterilisation and Sowing

Fifteen mg of *A.thaliana* cv. Col-0 seeds were immersed into 95% ethanol, vortexed for 1 minute, left to settle and the liquid removed by pipette, including any floating seeds. A 2.5% Sodium Hypochlorite (BDH chemicals)/0.1% Tween 20 solution was then added at 0.1ml/mg of seeds, shaken thoroughly for 10 minutes and removed by pipette, as before. Seeds were rinsed five times in sterile, deionised water (0.1ml/mg of seeds) before adding a final volume of 1ml of sterile, deionised water. Seeds were sown directly onto pre-prepared media (section 2.1.2) using a sterile, cut pipette tip.

2.1.2 Growth of Seedlings

Either K^+-present media (0.625mM KNO_3, 0.25mM $Ca(NO_3)_2.4H_20$, 0.25mM $MgSO_4$, 21.25μM FeNaEDTA, 0.3125mM KH_2PO_4, 1mM NaCl, pH5.6) or K^+-absent media (1mM $Ca(NO_3)_2.4H_20$, 0.5mM $MgSO_4$, 42.5μM FeNaEDTA, 0.625mM NaH_2PO_4, 1.375mM NaCl, pH5.6) was mixed with 1% Agar type "A", 3% sucrose and 1x micronutrients (ready-made from Sigma). The medium was

autoclaved and when hand-hot, 70ml of media medium per plate was poured into square, 14cm^2 petri dishes (Greiner Bio-One). Once set, 20 sterilised seeds (section 2.1.1) per plate were sown in a horizontal line, 2cm from the top. The plates were then sealed using Parafilm and vernalised in the dark for 4 days at 4°C. After this, the plants were placed vertically and grown in a controlled growth room set at the following conditions: 16h days with a light intensity of 80μMol/M^2/s and a temperature of 20°C.

2.2. Experimental Conditions

All plants were treated 14 days after the vernalisation period (when first introduced to vertical growth). The plates were opened under sterile conditions and any condensation residue drained off.

2.2.1 Treatments

A stock solution of ABA was dissolved in ethanol and prepared according to manufacturer's instructions, before storage at -20°C for up to 1 month. Prior to treatment, the stock solution was thoroughly defrosted and heated up to 65°C if precipitation had occurred. Once at room temperature, a standard treatment solution of 50μM of ABA in 0.1% ethanol was used and diluted in either K^+-present or K^+-absent media, according to conditions that seedlings had been exposed to (section 2.1.2). To account for the ethanol presence in ABA stock

solution, the control solution (non-ABA) would be spiked with 0.1% ethanol. Five ml of either control or test solution (+ABA) was added directly to roots before resealing with Parafilm. Plants were then returned to vertical growth conditions, placing vertically for harvest at designated time points of 2h, 3h, 5h, and 7h. For potassium re-supply experiments, 10mM or 50mM KCl was added to the K^+-absent or K^+-present solutions respectively.

2.2.2 Tissue Harvesting

Following treatments, the shoots were cut using a sterile blade and immediately frozen in liquid nitrogen. The roots were removed from the nutrient base and rinsed briefly in deionised water before blotting dry. These were flash-frozen separately from shoots. Both roots and shoots were later ground to a very fine powder using a pestle and mortar before storage at -80°C.

2.3 RNA Isolation

2.3.1 RNA Extraction

For optimum RNA extraction, 450-500 mg of frozen root powder was used and 350-400 mg for shoots. Trizol solution (0.8M Guanidium thiocynate, 0.4M Ammonium thiocynate, 0.1M Sodium Acetate pH 5, 5% Glycerol, 38% phenol complete with water) was added in the ratio of 1.5 ml per 100 mg of frozen tissue

and left for 5 mins at room temperature, vortexing occasionally, before spinning at 4000 rpm (swinging-bucket rotor - *Sorvall SUPER T21*) for 5 mins at 4 °C to remove excess debris. The supernatant was poured into a fresh, sterile tube (not previously weakened by liquid nitrogen). One and a half ml of chloroform was added and shaken for 30 secs before leaving at room temperature, inverting occasionally before spinning with the above conditions for 20 mins. The upper phase was carefully removed, ensuring that the protein layer was not disturbed, and placed into an equal volume of isopropanol (5x 0.75ml aqueous phase in 5x 1.5 ml eppendorfs containing 0.75 ml isopropanol (shoots); 10x 0.5ml aqueous phase/isopropanol for roots) and spun for 20 minutes, 4°C at 13000 rpm (*eppendorf CENTRIFUGE 5415R*). The supernatant was removed and the pellet washed in 1 ml 70% ethanol before centrifugation for 5 mins, 4°C, 13000 rpm. The supernatant was removed and the pellet air-dried by exposing to a 65°C heating block for 1 min before leaving at room temperature for 10 minutes.

2.3.2 Pellet Resuspension

RNase free water (Sigma) was added in the following quantities: 75µl for shoots (x5); 50µl for roots (x10). This was heated for 5 mins at 65°C then chilled immediately on ice for 2 mins to aid re-suspension before collecting into a single tube. The heat/chill process was then repeated for empty tubes, with 25µl of RNase-free water per tube. Again, the resuspended RNA was decanted into the collection tube.

2.4 RNA Quantification

Quantity and quality of RNA was determined using a *Perkin Elmer PTP-6 Peltier System* spectrophotometer and accompanying *FL Winlab Software (PE Instruments)*. Measurements of absorbance (A) were made at wavelengths (λ) of 320, 280, 260 and 230nm, using RNase free water originally used for pellet resuspension as the blank. The absorptions were calibrated by subtracting $A_{(\lambda 320)}$ from A measured at other wavelengths and RNA quantity and quality calculated using formulas (i) and (ii):

(i) Purity of RNA = $A_{\lambda 260}/A_{\lambda 280}$ (calibrated values)

(ii) Concentration of RNA = $A_{\lambda 260}$ (calibrated value) x 40 x Dilution Factor

RNA was stored at -80°C.

2.5 AMT Hybridisation

The AMT chips used had previously been designed by the AMT consortium (Maathuis *et al*, 2003) and spotted by MWG (Ebersberg, Germany). Only RNA of high purity (>1.8 - ratio calculated as in section 2.4 (i)) was used for hybridisation. Root and shoot RNA were hybridised separately using 150μg of RNA for shoots and 90-100μg for roots. Cy3 dye($A_{\lambda 550}$) was always used for the control and Cy5 dye ($A_{\lambda 650}$) for treatments.

2.5.1 Reverse Transcription

The required RNA was concentrated using Microcon columns (according to manufacturer's instructions) and the eluted volume adjusted to a standard volume of 20µl with sterile, RNase free water. 1µg of Oligo dT_{20} primer was added (0.5µl from 2µg/µl -MWG, Ebersberg, Germany) before annealing at the following conditions: 65°C for 10 mins, room temperature for 10 mins and ice for 2 mins. Reverse transcription was carried out using Superscript II Reverse Transcriptase (Invitrogen) reagents. To the annealed RNA mix (20µl total volume), 2x Buffer (Invitrogen), 1x dNTP mastermix (500µM dA-,dG-, dTTP/200µM dCTP (Sigma), 1x DTT (10mM) and 5% of either 2mM Cy3 or Cy5 dye. The reaction mix was pre-warmed for 2 mins at 42°C before addition of 300 units Superscript II (Invitrogen). This was incubated for 2 hours at 42°C in the dark in a circulating water bath. Another 200 units of Superscript II were added after 1h. To terminate the reaction, 10µl NaOH (1M) was added, heated for 10 mins at 65°C and neutralised with 10µl HCl (1M), before addition of 200µl of TE buffer pH7.2.

2.5.2 cDNA Cleaning and Quantification

The cDNA was cleaned using a Qiaquick PCR Purification Kit (Qiagen), according to manufacturer's instructions. cDNA quantity and label quality was checked using the same procedure outlined in section 2.4, however the elution buffer used during the final process of cDNA cleaning was used as the blank (Buffer EB – Qiagen). As an indicator of dye incorporation, absorbence was

measured at $A_{\lambda 260}$, $A_{\lambda 280}$, $A_{\lambda 320}$, $A_{\lambda 550}$ (Cy3) and $A_{\lambda 650}$ (Cy5). The absorptions were calibrated by subtracting $A_{\lambda 320}$ from all other A values and cDNA quantity and quality calculated using formula (iii):

(iii) cDNA concentration: $(A_{\lambda 260})$*(35)*(dilution factor)

Only cDNA that showed distinctive peaks at λ_{260} (cDNA), λ_{550} (Cy3) and λ_{650} (Cy5) were considered for microarray analysis (Figure 2.1). cDNA was then placed in a speed-vac for 30 minutes, until dried to less than 2µl volume.

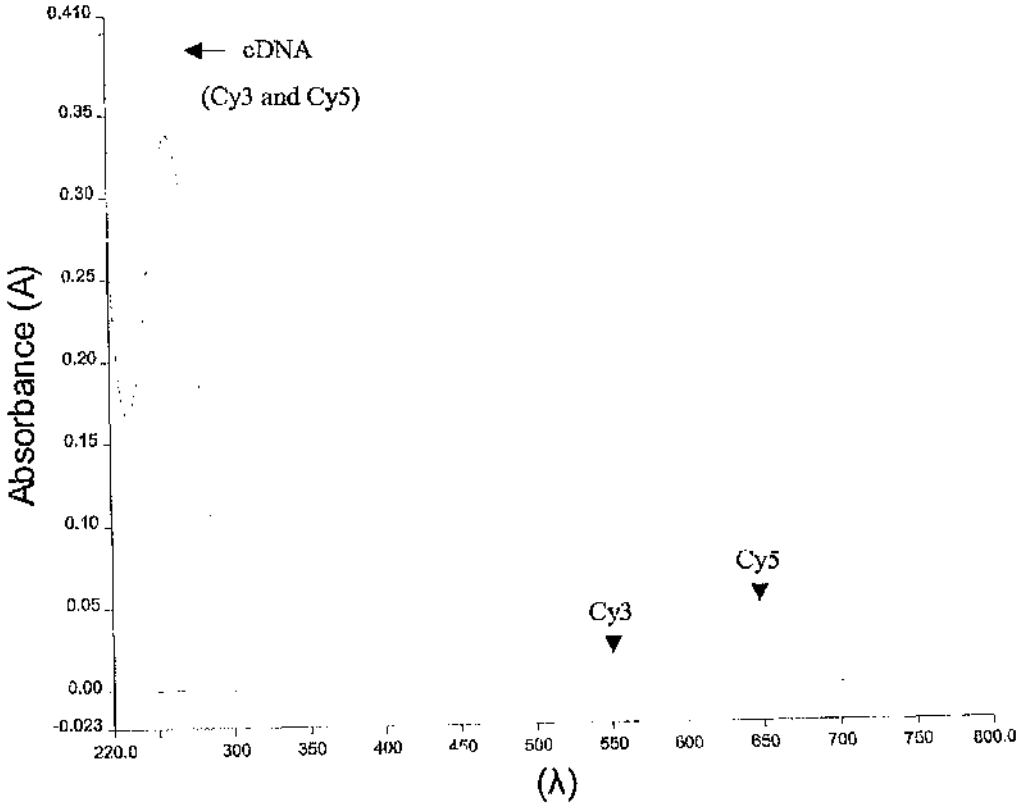


Figure 2.1: cDNA Absorbance Profile of Cy3 and Cy5 Incorporation: After elution, the level of incorporation was observed by checking the absorbance at the appropriate wavelength. This was a suitable checking method for subsequent microarray hybridisation.

2.5.3 Array Blocking

Fifty millilitres of preheated (42°C) blocking solution (5x SSC; 0.1x SDS; 1g BSA in sterile water) was placed into a 50ml Falcon tube for each microarray. The unmarked* array was then carefully immersed and the tube incubated for 45 mins at 42°C in a circulating water-bath. After incubation, the array was rinsed 5 times in sterile, deionised water (room temperature) and carefully dry-spun for 5 mins, 3000 rpm at room temperature (swinging-bucket rotor - *Sorvall SUPER T21*).

2.5.4 Hybridisation

Following speed-vac evaporation (section 2.5.2), Cy3 labelled cDNA was re-dissolved with 12µl of MWG-provided hybridisation buffer (preheated and well dissolved at 42°C). The mix was carefully transferred to the Cy5 tube and again thoroughly mixed before heating to 95°C for 3 mins, cooling on ice for 30 secs, and incubating at room temperature for 2 mins. All liquid (12µl) was applied to the spotted area of the slide. A hydroscopic cover slip (Sigma) was carefully applied and any air bubbles gently removed by pressing. The array was placed into a customised hybridisation chamber (MWG), and kept moist by adding 50µl of deionised water to corners. After sealing, it was made light tight by wrapping in tin foil and left to incubate for 18 hours in a circulating water bath at 42°C.

* It is imperative that nothing other than the manufactured label is placed onto the microarray, as certain ink dyes from pens will smear during hybridisation.

2.5.5 AMT chip washing

Hybridised chips were washed by successively immersing for 5 mins at 30°C into three pre-warmed washing buffers: Buffer 1: 2x SSC, 0.1% SDS; Buffer 2: 1x SSC; Buffer 3: 0.5x SSC. After the final wash, the array was spun-dry as before (section 2.5.3). Hybridised arrays were kept in a cool, dark and dry place.

2.6 AMT analysis

2.6.1 Quantifying Transcriptional Changes

Fluorescence from both dyes was measured using a *Packard Lite 4000 Scanner* (Packard Biochip Technologies, Pagbourne, UK) and associated software. Lasers were adjusted so that equal average fluorescence intensities were shown in both Cy3 and Cy5 scans. The scanned data was then transferred to Quantarray (Packard Lite) software for quantification of signal and background in each spot (probe) area. The numerical data generated from Quantarray were standardised by subtracting background and removing low intensity spots. Transcript abundance was then quantified by using GeneSpring (Silicon Genetics) software.

To be considered for further analysis, both duplicate probes of a particular gene had to produce a significant signal in at least one of the samples. The cut-off for

"significant signals" was set at 3x times the background signal. This was in order to reduce the number of false-positives. The expression of a particular gene in the control sample (Cy3) would then be compared to its expression in the treated sample (Cy5) to establish differential gene expression in response to the treatment. This was done by calculating the ratio of treatment/control signal, and normalising it according to the 50th percentile of all measurements, and the residual of the Lowess fit of the intensity curve (GeneSpring, Silicon Genetics). If the average ratio of treatment to control signals (R_1) was greater than 1.7 or less than 0.6, it was considered to be a change.

GeneSpring software allowed us to compare between different experiments through the use of Venn diagrams (see results). However, the Venn diagram is not always a good indicator of the dependence of a transcriptional response between experiments; it only shows the number of genes that either do or do not share a response and therefore relies on an artificial cut-off. To give a better indication of dependence of gene expression on a particular parameter (for example K^+ supply or tissue type), the ratio of fold change was calculated using the following procedure: - We first converted all ratios into absolute fold changes:

$$e^{\left|\ln(\text{average ratio of treatment to control signal})\right|}$$

The fold changes were then divided between experiments using different K^+ pre-treatments or tissues (R_2). A ratio of 1 meant that there were no marked differences of gene regulation between the experiments whereas R_2>1.5 or

R_2<0.6 meant a greater dependence of the expression response for the respective parameter.

Figures 2.2 and 2.3 show an overview of a typical microarray experiment.

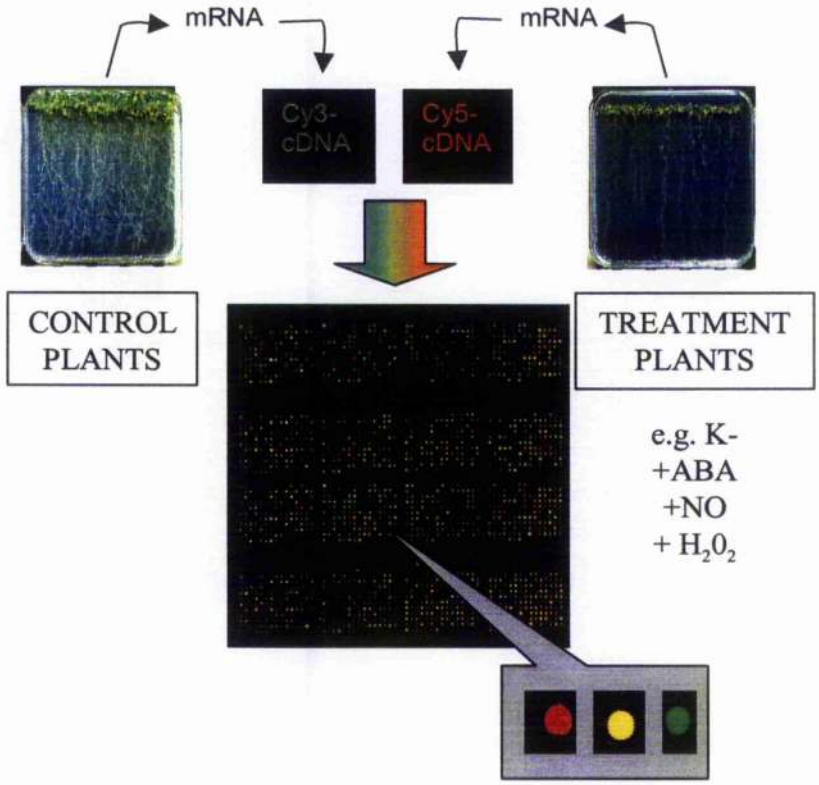


Figure 2.2: Overview of Microarray Experiment: 2-week-old *A.thaliana* seedlings were used for all experiments. After incorporation of the fluorescent dyes, the cDNA is hybridised onto the array. Red spots indicate hybridisation of more Cy5 (treatment) transcripts and hence up-regulation of the genes represented by the spot, whereas the green spots are the result of increased Cy3 (control) hybridisations i.e. down-regulation. Yellow spots indicate an equal number of transcripts between control and treated plants. Intensities of individual dyes in each spot are quantitatively detected on a scanner (Packard Lite) and analysed with imaging software (Quantarray – Packard Lite).

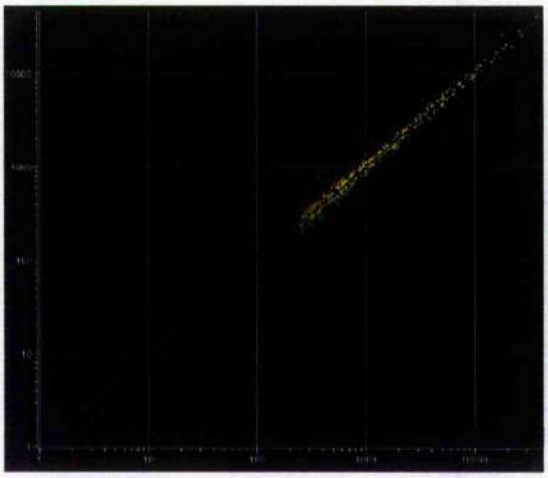


Figure 2.3a: 3hour ABA Shoots/K^+ replicate 1

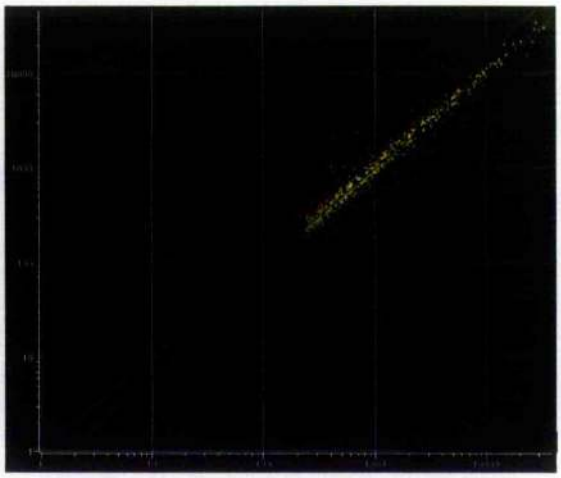


Figure 2.3b: 3hour ABA Shoots/K^+ replicate 2

Figure 2.3: Scatterplot of 3 hour ABA Shoot K^+ (control) AMT gene data, as generated by GeneSpring. Figures 2.3a and 2.3b show the data for each of the two replicate probe sets on the chip. Control signal is shown on the *x*-axis, treatment signal on the *y*-axis (arbitrary units).

3.0 Results

3.1 Overview

For clarity, the experiments carried out are listed below. They were designed to induce a stress response through exogenously applied ABA or through extreme changes in K^+_{ext} conditions:

A. Effect of ABA-induced response in K^+-sufficient plants.

B. Effect of ABA signal in K^+-deficient plants

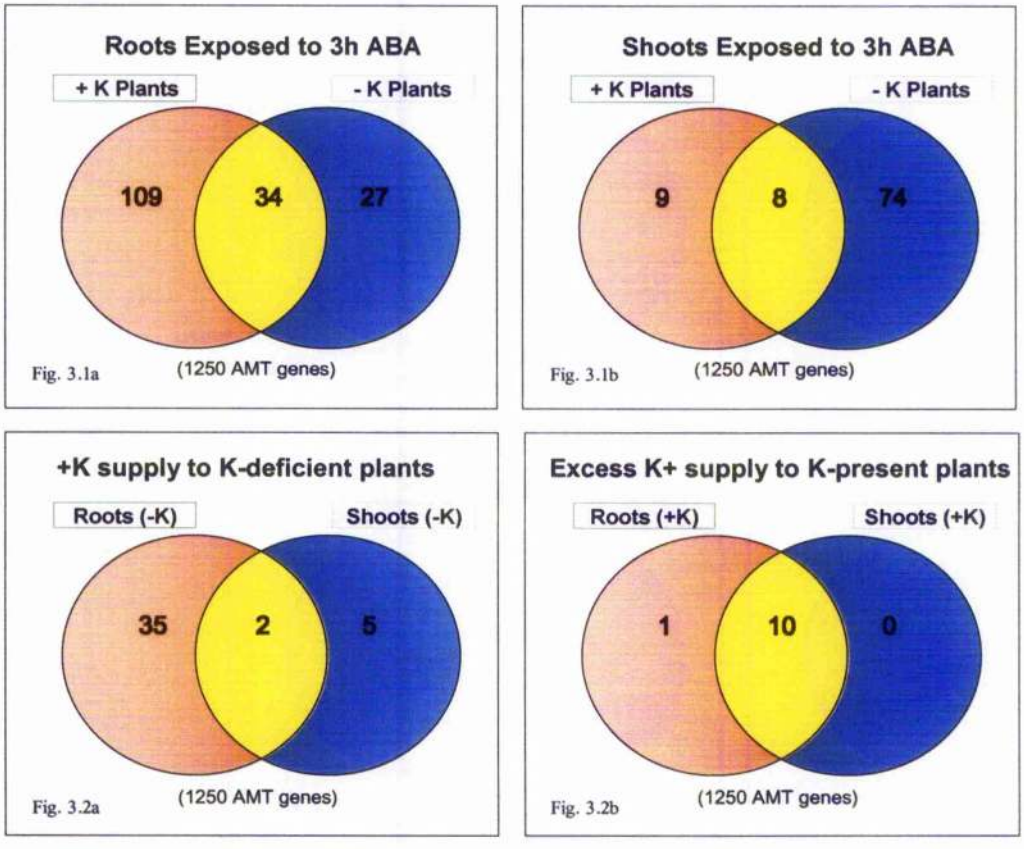
C. Effect of short-term supply of K^+ to K^+-deficient plants

D. Effect of excess K^+ supply to K^+-sufficient plants

All experiments were carried out on 2-week-old *A.thaliana* plants grown in different K^+_{ext} environments i.e. with 1.875mM K^+ or without. For all subsequent analysis, the data have been split according to tissue type and K^+_{ext} environment that the plants were exposed to. This was in order to establish any differential responses amongst gene families within different tissues (roots or shoots); also to establish the existence of any K^+ –dependent or –independent responses.

The main objective of the experiments was to identify gene candidates for subsequent and functional characterisation, through suitable comparisons and

analysis of the *strongest and most consistently* regulated genes. As this is essentially a preliminary screen to identify genes of interest, a strictly quantitative approach has not been used. Figures 3.1 and 3.2 show Venn diagrams of all transcripts changed (for definition of "changed" see section 2.6.1) in experiments A and B (Figure 3.1) and experiments C and D (Figure 3.2). In the following paragraphs, the data underlying these Venn diagrams will be presented in greater detail.



Figures 3.1 and 3.2: Overview of experiments and comparisons made using GeneSpring to locate and quantify genes of interest. The Venn diagram allows us to see the number of commonly or independently regulated genes within particular treatments. Figures 3.1a & b displays a comparison of the root responses triggered with an exogenous ABA signal in K^+ sufficient and K^+ deficient plants. Figures 3.2a and b show a comparison between tissue types of K+-starved and K+-rich plants that are supplied with either 10mM [K^+] (a), or 50mM[K^+] (b).

3.2 Effect of ABA in plants exposed to K^+-present environments

In order to establish "trademark" expression profiles from the AMT chip, the optimum time for 50μM ABA to produce a response in a K^+-present environment was determined using the following time points: 2h, 3h, 5h and 7h (data not shown). After comparisons of the strength of response amongst all time-points, the 3h ABA time-point was found to produce the most responses (Figure 3.2, 3.3 & 3.4) and so was used as a suitable time-point for future ABA-related experiments. This was also consistent with past findings (Goh *et al*, 2003). Notably, there was consistent up-regulation of control genes that contain ABA-responsive elements COR78, KIN2, P5CS1 and ADH1 (all raw data included in appendix). Despite not being membrane proteins, these genes were included upon the chip to measure for reliability of stress-induced responses. COR78 (or RD29A) is responsive to desiccation and is known to respond to cold, drought and ABA (Horvath *et al*, 1993) and is similar to KIN2, a gene that responds to cold, ABA, salinity and drought (Kreps *et al*, 2002; Kurkela & Borg-Franck, 1999). In addition, they are known to contain ABA-responsive elements and thus another indicator that the AMT chip was producing a reliable response.

Comparisons between root and shoot responses of the 3h ABA in K^+ (Figures 3.2 and 3.3) showed greater transcript abundance in roots (143 genes from 32 gene families) than in shoots (17 genes from 8 gene families). The strength of the response from the stress control genes was similar with both COR78 and KIN2 being 4-5fold increased. However, within roots transcript abundance of APX1

was observed (R_1=2.14). APX1 is a good indicator of oxidative stress as it encodes ascorbate peroxidase, which scavenges H_20_2 within the cytosol. In addition, it has been linked to dehydration (Mittler & Zilinkas, 1994) where it was up regulated. This provides further evidence that application of external ABA is producing a reliable and effective response.

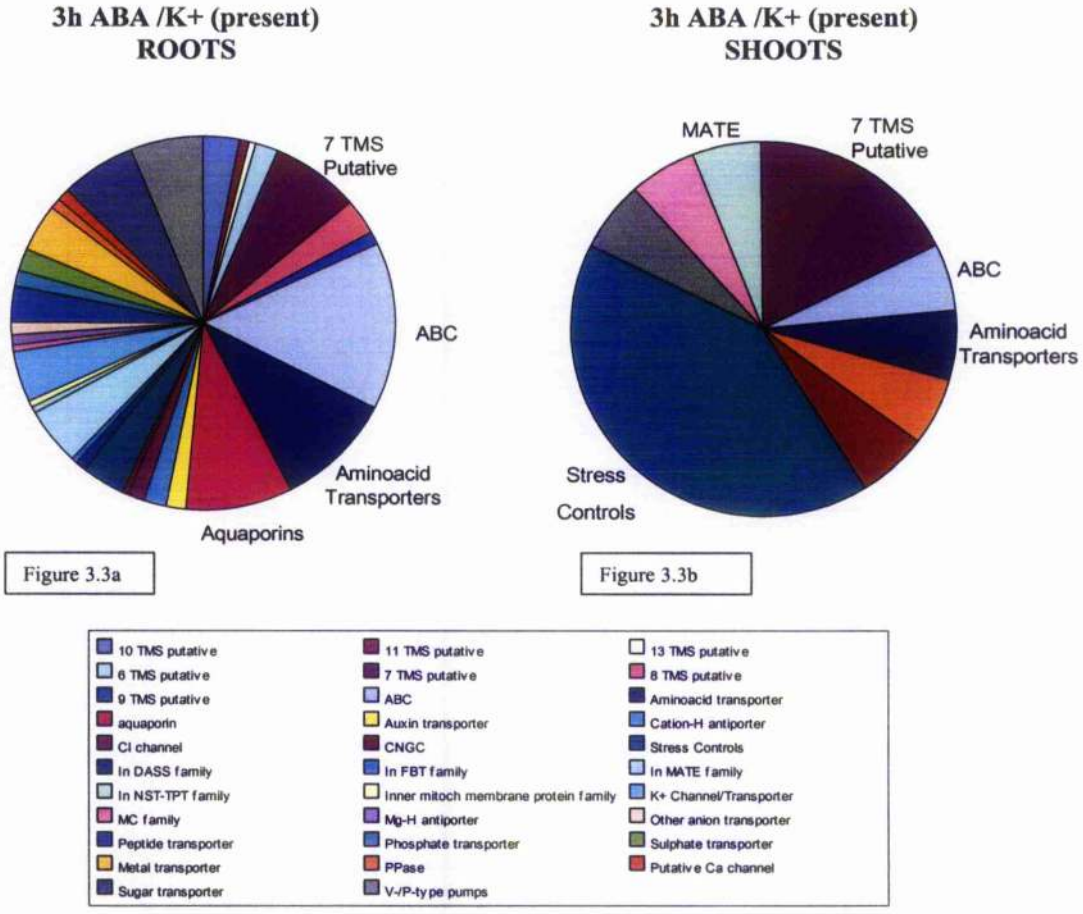


Figure 3.3a

Figure 3.3b

Figure 3.3: Comparison of transcriptional regulation of gene families between tissue types of seedlings exposed to 3 hour ABA in a K^+-sufficient environment. Figure 3.3a shows an abundance of gene transcripts within roots, where a larger number of different gene families responded . Figure 3.3b shows transcript abundance within shoots, of which the stress controls constituted most of the responses.

Within shoots, it was mainly stress controls that were up-regulated, contributing to almost 50% of the total number of the most strongly regulated genes. The 7TMS family of transporters showed comparatively higher transcript abundance also. Within both roots and shoots, a MATE transporter (At1g12950 – MATE*) and 7TMS transporter (At4g20100 – 7TMS*) were the strongest regulated genes displaying R_1=12.33,6.45 (MATE*) in roots and shoots respectively, and 7TMS* showing R_1=0.16, 0.395.

3.3 Effect of ABA in plants exposed to K^+-deficient environments

Using plants grown in a K^+-deficient environment, 50μM ABA for 3h was added and again the roots and shoots analysed on separate chips (Figure 3.4). The reverse of what happened in the presence of K^+ was observed; there was greater transcript abundance in shoots than roots of K^+-deficient plants. In shoots, there were 82 genes regulated from 25 gene families whereas roots showed regulation of 61 genes from 21 gene families. Again, up-regulation of ABA-induced stress controls was apparent, with COR78 showing stronger regulation in roots than shoots (R_1= 4.99 and 2.88 respectively). In shoots, the cold-responsive genes LT16A and LT16B were also up-regulated by ABA, whereas ABA-regulation of LTI6B was only apparent in roots.

K^+-deficient roots displayed responsive activity in potassium transporters with up-regulation of GORK (R_1=2.612) and down-regulation of HAK5 (R_1=0.495) by ABA.

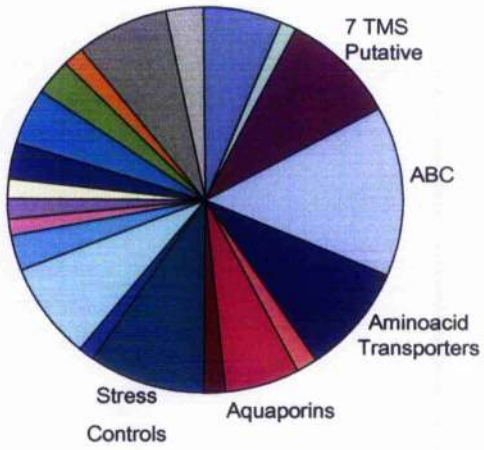


Figure 3.4a

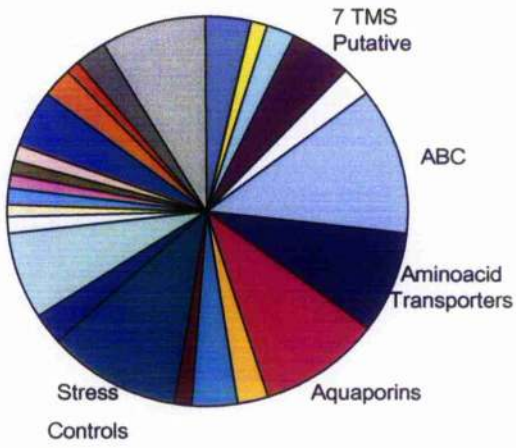


Figure 3.4b

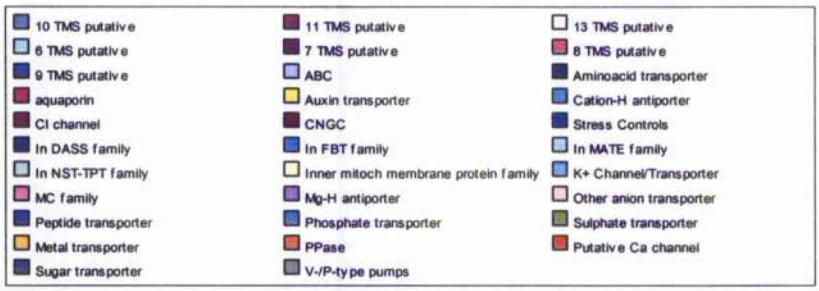


Figure 3.4: Comparison of transcriptional regulation of gene families between tissue type of seedlings exposed to 3h ABA in a K^+-deficient environment. Figure 3.4a shows root transcript abundance whereas Figure 3.4b shows shoot transcript abundance. Unlike the K^+-present data, there was more transcript abundance within shoots.

GORK (Ache *et al*, 2000) is similar in nature to SKOR (Roberts & Tester, 1995), a K^+ rectifying channel that has been shown to be responsive to ABA (Gaymard *et al*, 1998). The down-regulation of the high-affinity carrier HAK5 (Rubio *et al*, 2000) within K^+-deficient roots could suggest that other high-affinity transporters are more active, and that this could also work in a supporting or secondary function in ABA-induced responses. The only K^+-transporter present within shoots was the K^+/H^+ antiporter KEA3 (At4g04850) which showed up-regulation by ABA (R_1=1.8).

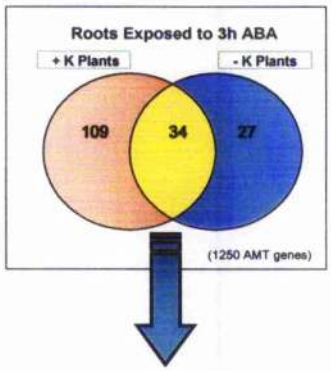
Again MATE* (AT1g12950) was the most up-regulated gene in both tissue types, showing double the increase in expression upon ABA treatment than the next nearest up-regulated gene (COR78), with R_1=8.09 in shoots and R_1=4.01 in roots. For down-regulated genes, 7TMS* was again displaying the largest change with R_1=0.16 (shoots) and R_1= 0.197 (roots).

3.3.1 Dependency of K^+ for ABA experiments

The Venn diagram analysis of ABA-regulated transporter transcripts demonstrated that a large number of genes responded to ABA either only in K^+-deficient or only in K^+-sufficient plants (Figure 3.2a and b). This indicates that the response to ABA is strongly modulated by the concentration of K^+ in the external medium and/or by the plant K^+ status. Since the Venn diagram is based on an artificial cut-off for what is "regulated" or "not regulated", it does not

provide a measure for K^+-dependence within genes that display ABA-induced transcript changes in both K^+ conditions. To evaluate K^+-dependence of "commonly ABA-regulated" genes (i.e. genes placed in the centre of the Venn diagram) the magnitude of change was compared for both K^+ conditions with the formula outlined in section 3.1.2. For root tissues there were 34 commonly ABA-regulated genes (Table 3.1) compared to 8 in shoots (Table 3.2).

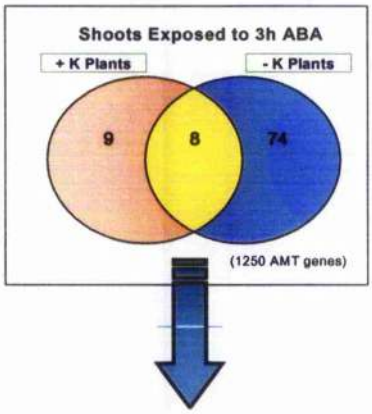
The analysis showed that for several "commonly ABA-regulated" genes the amount of ABA-induced transcript change depended on the K^+-treatment (indicated in pink and blue in the last column of tables 3.1 and 3.2). For most genes the response to ABA was stronger in K^+-sufficient conditions than in K^+-deficient conditions (pink). Within roots, MATE* and 7TMS* showed a stronger response in the presence of K^+. In shoots, MATE* was again more responsive in K^+ but 7TMS* was more responsive in the absence of K^+. P5CS1, a control for salt stress, showed a higher response in K^+-sufficient conditions in both tissues.



AMT Regulated Gene			K Present		K Absent		K+/K-
Common Name	AGI Code	Description	ave ratio Signal	Fold Change	ave ratio Signal	Fold Change	Magnitude of Change
	At2g17500	10 TMS putative	2.804	2.804	1.755	1.755	1.598
	AT4g15430	10 TMS putative	2.641	2.641	1.863	1.863	1.417
	AT3g01100	10 TMS putative	1.767	1.767	1.912	1.912	0.924
	At1g12730	7 TMS putative	2.412	2.412	2.280	2.280	1.058
	AT5g20270	7 TMS putative	3.100	3.100	2.306	2.306	1.344
7 TMS*	AT4g20100	7 TMS putative	0.124	8.065	0.197	5.076	1.589
	At1g78610	7 TMS putative	2.254	2.254	1.897	1.897	1.188
	AT3g14810	7 TMS putative	2.484	2.484	1.982	1.982	1.253
ATWBC24	At5g19410	ABC	2.625	2.625	1.883	1.883	1.394
ATPDR7	At1g15210	ABC	2.925	2.232	0.389	2.574	0.867
ATMRP9	At3g60160	ABC	0.458	2.183	0.389	2.132	1.024
	At3g62150	ABC	4.016	4.016	2.129	2.129	1.886
	At5g65990	Aminoacid transporter	4.836	4.836	2.442	2.442	1.981
NIP1,1	At4g19030	aquaporin	0.265	3.781	0.381	2.625	1.440
CNGC8	At1g19780	CNGC	0.281	3.565	0.467	2.141	1.665
KIN2	At5g15970	Condition, cold stress	4.572	4.572	2.195	2.195	2.083
COR78	At5g52310	Condition, dessication	5.688	5.688	4.992	4.992	1.140
P5CS1	At2g39800	Condition, salt stress,	4.457	4.457	2.434	2.434	1.831
ADH1	At1g77120	Condition,anaerobiosis	3.570	3.570	3.196	3.196	1.117
	At2g38330	In MATE family	1.919	1.919	2.034	2.034	0.943
	At1g15150	In MATE family	2.343	2.343	2.430	2.430	0.964
MATE*	At1g12950	In MATE family	12.330	12.330	8.091	8.091	1.524
GORK	At5g37500	K channel	2.918	2.918	2.612	2.612	1.117
	AT5g26200	MC family	2.681	2.681	4.089	4.089	0.656
UREA ACTIVE	AT5g45380	Other anion transporter	0.426	2.347	0.504	1.986	1.182
PTR35	At1g59740	Peptide transporter	2.363	2.363	1.939	1.939	1.219
NTP2=PTR10	At2g26690	Peptide transporter	4.917	4.917	3.158	3.158	1.557
MITO 3	At2g17270	Phosphate transporter	4.196	4.196	2.270	2.270	1.849
EF-HAND 1	At5g49900	Putative Ca channel	3.747	3.747	3.014	3.014	1.243
STP 44	At1g30220	Sugar transporter	0.372	2.688	0.486	2.153	1.249
SUGTL2	At1g08920	Sugar transporter	9.156	9.156	2.363	2.363	3.876
STP13	AT5g26340	Sugar transporter	2.925	2.925	2.075	2.075	1.410
	At1g73220	Sugar transporter	2.925	2.947	6.215	6.215	0.474
SULTR3.1=AST	At3g51900	Sulphate transporter	2.637	2.637	2.117	2.117	1.245

Colour	Signal Strength (average)		Strength of Transcript Change
	T/C Ratio Below:	0.5	strongly down-regulated
		0.6	medium down-regulated
	T/C Ratio Above:	2	strongly up-regulated
		1.7	medium up-regulated
	Magnitude of Change		
	Above:	1.5	More Responsive in + K^+ conditions
	Below:	0.67	More Responsive in – K^+ conditions

Table 3.1: Comparison of roots of 3h ABA treated plants in relation to changing $[K^+]_{ext}$. The average fold change (R_1) is divided between K^+ and K- conditions to generate the magnitude of K^+-dependent regulation (R_2). This enables us to quantify the dependence of K^+ that gene regulation has within the treatments. In total, there were 34 commonly regulated genes within roots of which a majority of them were up-regulated.



AMT Regulated Gene			K Present		K Absent		K+/K-
Common Name	AGI Code	Description	Average Signal	Fold Change	Average Signal	Fold Change	Magnitude of Change
P5CS1		Condition, salt stress	3.073	3.073	2.039	2.039	1.507
COR78		Condition, dessication	3.073	5.563	2.876	2.876	1.934
	AT5g26200	MC family	3.073	3.056	1.785	1.785	1.713
MATE*	At1g12950	In MATE family	3.073	6.446	4.012	4.012	1.607
7TMS*	AT4g20100	7 TMS putative	0.397	2.522	0.162	6.192	0.407
	AT3g14810	7 TMS putative	1.861	1.861	2.006	2.006	0.927
LTI6B	At3g05890	Stress induced	2.753	2.753	2.541	2.541	1.083
LTI6A	At3g05880	Stress induced	2.966	2.966	2.423	2.423	1.224

Colour	Signal Strength (average)		Strength of Transcript Change
	T/C Ratio Below:	0.5	strongly down-regulated
		0.6	medium down-regulated
	T/C Ratio Above:	2	strongly up-regulated
		1.7	medium up-regulated
	Magnitude of Change		
	Above:	1.5	More Responsive in + K^+ conditions
	Below:	0.67	More Responsive in – K^+ conditions

Table 3.2: Comparison of shoots of 3h ABA treated plants in relation to changing K^+_{ext}. The average ratio (R_1) is divided between K^+-sufficient and K^+-deficient conditions to generate the magnitude of change (R_2). This enables us to quantify the dependence of K^+ that an active gene has within the treatments. In total, there were 8 commonly regulated genes within treated shoots, of which a majority of them were up-regulated.

3.4 Effect of short-term supply of K^+ to K^+-deficient plants

There were fewer genes regulated by short-term re-supply of K^+ to K^+-deficient plants than in ABA experiments (Figure 3.2). Furthermore, the control genes that were so strongly regulated by ABA did not feature among K^+-regulated genes. This suggests that an endogenous ABA signal was not being generated by changes in K^+ supply, at least not to the extent that they had been when used as an exogenous signal generator. Figure 3.5 shows the representation of different transporter families within K^+ regulated genes.

There was strong tissue specificity in the response of K^+-deficient plants to K^+ re-supply. Only 2 genes responded in both roots and shoots (7TMS* and TIP5,1). There were more responsive genes in K^+-deficient roots (37 genes, 6 gene families) than in shoots (7 genes, 6 gene families). Again, aquaporins were more responsive in shoots than in roots, which was similar to the ABA data when K^+ was absent. Within roots, many members of the ABC family of transporters responded to K^+ supply. The most up-regulated gene was a sugar transporter STP33 (At4g 36670) with R_1=2.98. In roots, HAK5 was down-regulated (R_1= 0.182) as was 7TMS*(R_1=0.15), which is similar to the ABA/K^+-deficient data. A lack of K^+ seems to induce the high-affinity HAK5 system which is subsequently down-regulated after K^+ re-supply. For shoots, TIP5, 1 was the strongest up-regulated gene (R_1=2.67) and 7TMS* the most down-regulated (R_1=0.37).

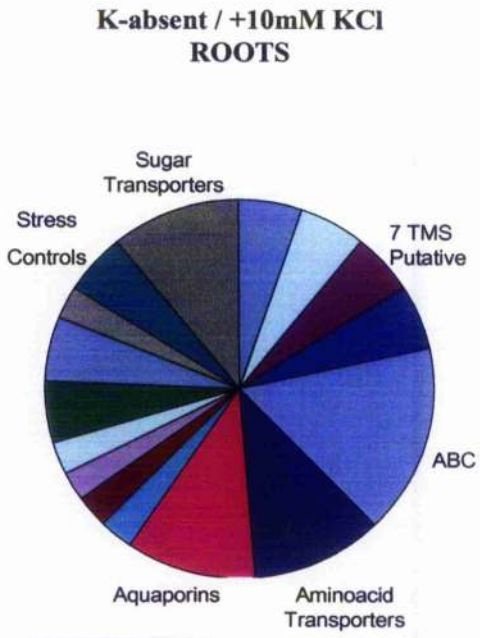


Figure 3.5a

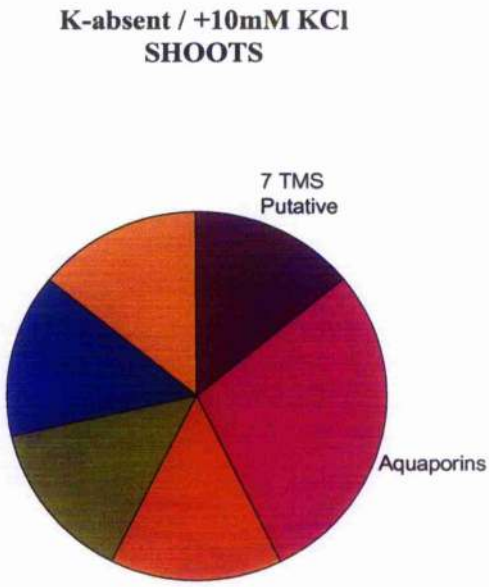
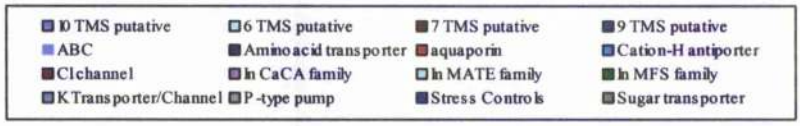


Figure 3.5b



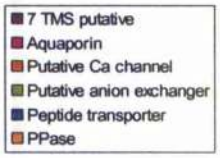
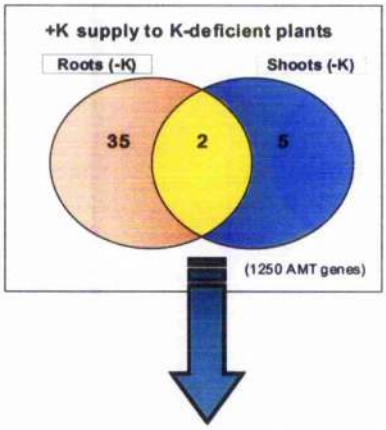


Figure 3.5: Comparison of gene family transcript abundance between roots and shoots of K^+-deficient plants in response to K^+ supply. Figure 3.5a shows root genes whereas Figure 3.5b shows shoot genes. There was more regulation within roots than shoots (compared to Fig 3.2c), with aquaporins constituting most of the transcriptional changes.

Comparison of K^+-regulated genes in different tissues (Table 3.3) shows that there are 2 commonly regulated genes of which 7TMS* is more responsive within roots. The response of aquaporin TIP5,1 to K^+ re-supply does not differ significantly between tissue types.

3.5 Effect of excess K^+ supply to K^+-present plants

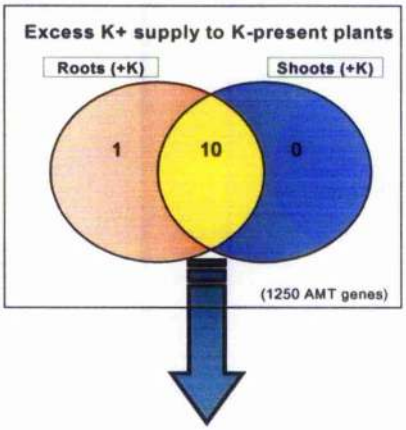
To evaluate whether the transcriptional regulation of transporters by K^+ supply was specific for K^+-deficient ("hungry") plants, K^+ was also supplied to K^+-sufficient plants. Indeed, far fewer genes responded to K^+-supply in these plants with all but one gene responding to K^+ within both tissue types. In total there were 7 gene families regulated, with roots showing 11 responsive genes and the shoots 10 (Table 3.4). The most down-regulated gene was 7TMS* (R_1=0.23) in roots and shoots and FRUCT3 (At1g62660) was up-regulated (R_1=2.16 – roots; R_1=2.00 – shoots). This gene encodes invertase, an enzyme that is crucial for converting starch to sucrose and may give an indication that sugar metabolites are being generated to establish turgor pressure. This shows that plants don't respond to external K^+ supply if they don't require it, thus strengthening the results obtained from K^+-deficient plants.



			K Absent ; short-term re-supply of 10mM KCl				
			Roots		Shoots		R & S
Common Name	AGI Code	Description	Average Signal	Fold Change	Average Signal	Fold Change	Magnitude of Change
7TMS*	AT4g20100	7 TMS putative	0.150	6.667	0.373	2.685	2.483
TIP5,1	At3g47440	aquaporin	2.132	2.132	2.677	2.677	0.797

Colour	Signal Strength (average)		Strength of Transcript Change
	T/C Ratio Below:	0.5	strongly down-regulated
		0.6	medium down-regulated
	T/C Ratio Above:	2	strongly up-regulated
		1.7	medium up-regulated
	Magnitude of Change		
	Above:	1.5	More Responsive in Shoots
	Below:	0.67	More Responsive in Roots

Table 3.3: Comparison of commonly regulated genes between roots and shoots of K^+-deprived plants in response to K^+ supply. The average ratio (R_1) is divided between roots and shoots to generate the magnitude of change (R_2). This enables us to quantify the dependence that at active gene has between tissues. In total, there were 2 commonly regulated genes of which 7TMS* showed more response in shoots.



			K Present ; re-supply of 50mM KCl				
			Roots		Shoots		R & S
Common Name	AGI Code	Description	Average Signal	Fold Change	Average Signal	Fold Change	Magnitude of Change
	AT4g20100	7 TMS putative	0.233	4.310	0.233	4.292	1.004
ATMDR6	At2g39480	ABC	0.572	1.750	0.571	1.751	0.999
	At2g33260	Aminoacid transporter	0.546	1.832	0.512	1.953	0.938
ATPROT2	At3g55740	Aminoacid transporter	0.442	2.265	0.324	3.086	0.734
TIP1,3	At4g01470	aquaporin	1.833	1.833	none	n/a	1.833
TIP1,2	At3g26520	aquaporin	1.849	1.849	1.835	1.835	1.008
NLM3	At2g29870	aquaporin	0.555	1.802	0.556	1.799	1.002
CHX15	At2g13620	Cation-H antiporter	0.471	2.123	0.486	2.060	1.031
FRUCT3	At1g62660	Condition, Invertase	2.157	2.157	2.017	2.017	1.069
ADH1		Condition,anaerobiosis	0.549	1.821	0.552	1.813	1.005
PTR7	At1g72140	Peptide transporter	0.379	2.639	0.376	2.663	0.991

Colour	Signal Strength (average)		Strength of Transcript Change
	T/C Ratio Below:	0.5	strongly down-regulated
		0.6	medium down-regulated
	T/C Ratio Above:	2	strongly up-regulated
		1.7	medium up-regulated
	Magnitude of Change		
	Above:	1.5	More Responsive in Shoots
	Below:	0.67	More Responsive in Roots

Table 3.4: Comparison of commonly regulated genes between roots and shoots of K^+-sufficient plants in response to excess K^+ supply. The average (R_1) is divided between roots and shoots to generate the magnitude of change (R_2). The data generated was very similar in both tissues. In total, there were 10 commonly regulated genes of which 7TMS* showed the strongest response in both tissues. *(TIP1,3 was not regulated in shoots).*

3.6 Gene candidates for cloning

The main purpose of this study was to identify suitable gene candidates for cloning and functional characterisation. This would essentially be based upon the strength of transcriptional response and the level of consistency that they displayed. According to these criteria, I have identified MATE* and 7TMS* as suitable gene candidates. In addition, the down-regulation of HAK5 is also very interesting, and has shown similar behaviour in other in-house experiments (P.Armengaud – personal communication).

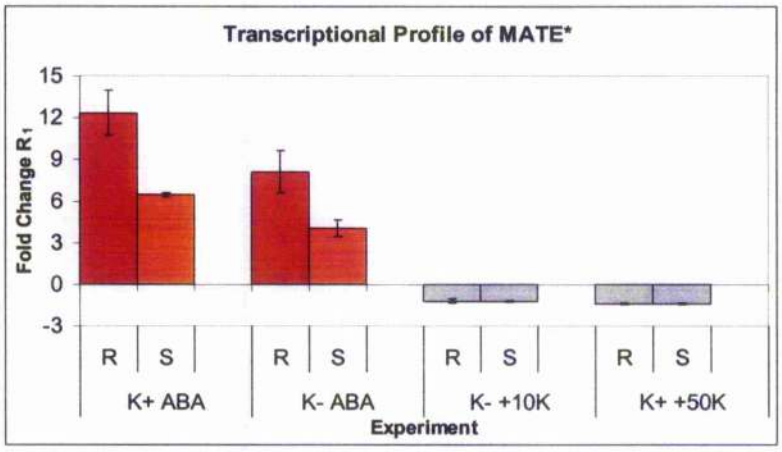


Figure 3.6a: Transcriptional profile of MATE* across different experimental treatments

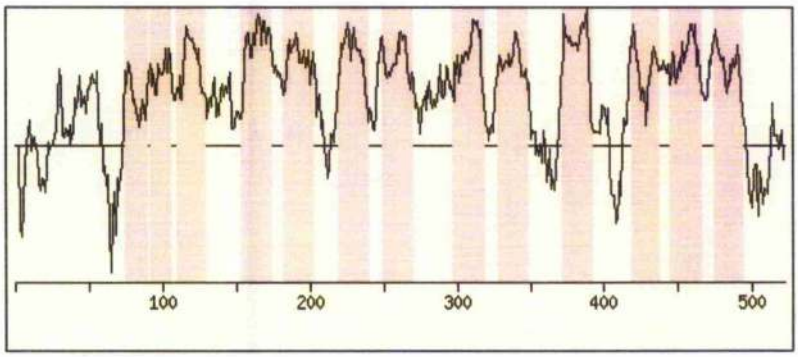


Figure 3.6b: Hydropathy plot of MATE* transporter (length of amino acids on *x*-axis; hydrophobicity on *y*-axis.

Figure 3.6: Overview of potential gene for cloning – MATE*. Figure 3.6a demonstrates the transcriptional response across all sets of experiments whereas Figure 3.6b gives an idea of its morphology (from Aramennon web-site).

3.6.1 MATE (At1g12950)*

The MATE* transporter is of great interest as the strength of its response was greater than any other gene, including many of the controls. These controls (COR78, P5CS1, KIN2 and ADH1) are known to be ABA responsive and as they and MATE* did not appear in the other experiments, it suggests that MATE* is specifically and strongly responsive to ABA, showing a higher level of response when K^+ is present (Figure 3.6).

At1g12950 belongs to the MATE family of transporters (multidrug and toxin extrusion) and is thought to have 12 TMS (Aramemnon web-site), with a structure similar to the ripening regulated protein DDTFR18 from *Lycopersicon esculentum* (TAIR). Most MATE investigation has been carried out using yeast or bacteria, where some members have been shown to pump antimicrobial agents out of bacterial cells in exchange for Na^+ (Morita *et al*, 2000). Within plants, MATE transporters are thought to be involved in the transport of many different organic molecules (Nawrath *et al*, 2002), which could be the case in these experiments. However, its level of responsiveness to ABA is of particular importance, particularly as the existence of an ABA receptor within plants has been assumed but never been identified. MATE* is particularly unusual as the 5' sequence shows no known ABA-responsive elements (compared by using TAIR web-site).

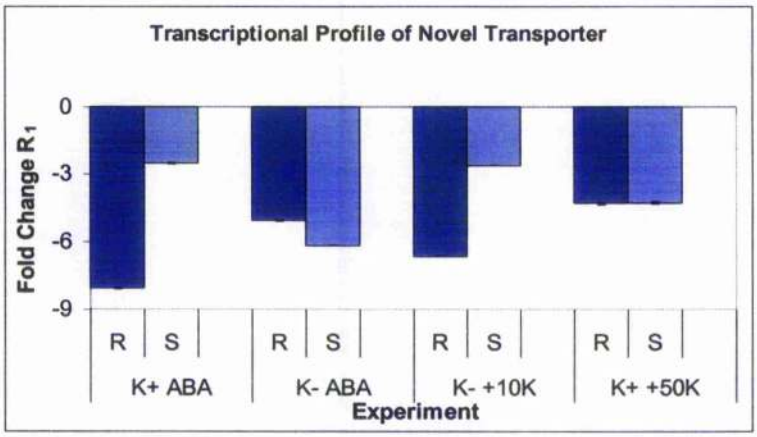


Figure 3.7a: Transcriptional profile of putative 7 TMS* transporter across different experimental treatments

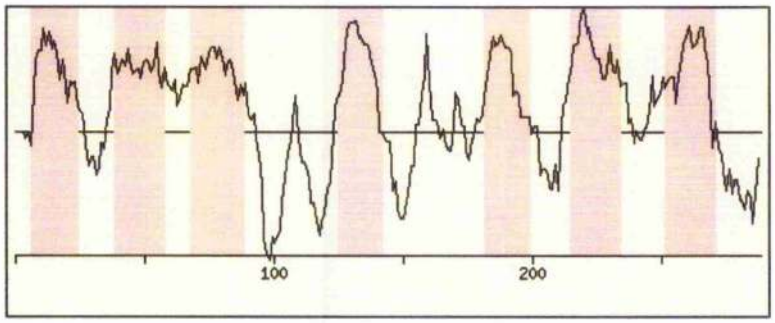


Figure 3.7b: Hydropathy plot of putative 7 TMS* transporter. (Length of amino acids on *x*-axis; hydrophobicity on *y*-axis.

Figure 3.7: Overview of potential gene for cloning – 7 TMS*: Figure 3.8a demonstrates the transcriptional response across all sets of experiments whereas Figure 3.8b gives an idea of its morphology (from Aramennon web-site).

3.6.2 7TMS (At4g20100)*

The 7TMS* transporter was the only transporter to show regulation across all treatments and tissue types (Figure 3.7). In all experiments, it was strongly down-regulated, though to different magnitudes, depending upon the tissue type.

At4g20100 is a putative membrane transporter that is thought to have 7 transmembrane spanning domains (Aramennon). Its N' terminus is predicted to be on the cytoplasmic side of the membrane with a PQ loop. (PQ peptides contain repeat sequences in TMS 1&2 and TMS 5&6 forming a loop between them (Zhai *et al*, 2001). This is a novel transporter with no known homologues to any other known organisms. It is therefore an interesting gene candidate for cloning and characterisation purposes.

3.6.3 HAK5 (At4g13420)

HAK5 (At4g13420) only displayed regulation in K^+-deficient conditions, where it was down-regulated after K^+ re-supply (Figure 3.8). It therefore appears that HAK5 expression is induced by K^+ starvation. As similar work within our research group has also identified HAK5 as a gene of interest, we will use this data to try and characterise its function.

At4g13420 has previously been cloned (Rubio *et al*, 2000) however its function has not been characterised, though it is thought to be involved with high-affinity K^+ uptake. It belongs to the HAK/KUP/KT' family of K^+ transporters with 13 predicted TMS. It is known to be both permeable to K^+ and Na^+ and similar to these findings, has been shown to change its regulation in K^+-deprived conditions. As these transcripts were only present in K^+-deprived conditions, the function of this transporter in high-affinity K^+-uptake will be of particular interest.

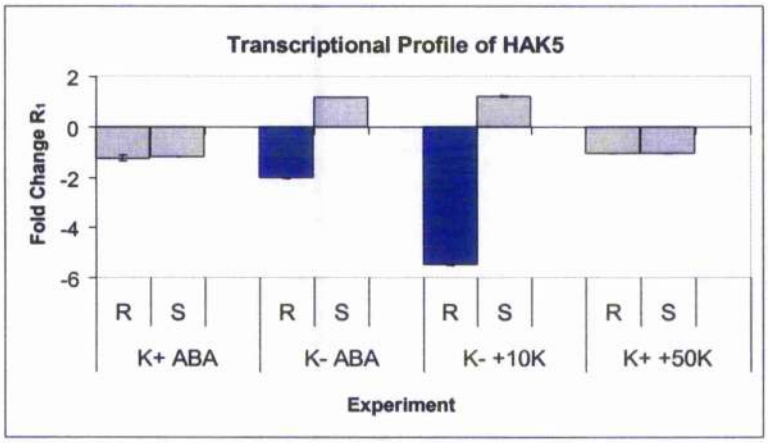


Figure 3.8a: Transcriptional profile of HAK5* across different experimental treatments

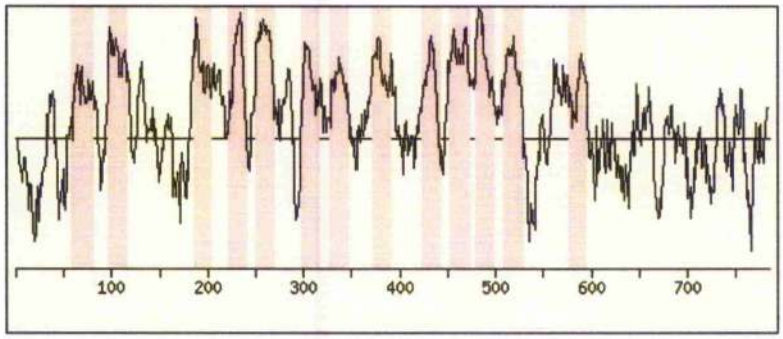


Figure 3.8b: Hydropathy plot of HAK5* transporter. (Length of amino acids on *x*-axis; hydrophobicity on *y*-axis).

Figure 3.8: Overview of potential gene for cloning – HAK5*: Figure 3.9a demonstrates the transcriptional response across all sets of experiments whereas Figure 3.9b gives an idea of its morphology (from Aramennon web-site).

3.8 Comparisons of Aquaporin Activity

Out of all gene families represented upon the AMT chip, the aquaporins showed the most interesting response to ABA and K^+. Aquaporins, also known as water channels, facilitate the movement of water across either the plasma (PIP) or tonoplast (TIP) membranes. They are members of the MIP (Major Intrinsic Protein) family and are characterised by 6 TMS and a signature sequence motif Asn-Pro-Ala (NPA) between TMS 2&3 and TMS 5&6 and is thought to represent the aqueous pore (Chrispeels *et al*, 1999).

Amongst gene families, the differential regulation of the aquaporins was the most prominent amongst the different experiments and between tissue types (table 3.5). In terms of treatment, the application of ABA on K^+-sufficient roots showed the highest number of regulated aquaporins with TIP1,1 (R_1=7.75) and PIP2,2 (R_1=7.76) showing very strong up-regulation , and TIP3,2 (R_1=0.49) and NIP1,1 (R_1=0.26) very strong down-regulation. However, there was no regulation in the corresponding shoots. In the absence of potassium however, the ABA data showed the opposite with more aquaporins regulated in shoots than roots. Furthermore, there was no up-regulation of genes but a far stronger down-regulation. In particular, TIP1,1 was severely down-regulated (R_1=0.16) but showed the opposite in roots.

There were fewer aquaporins regulated in the experiments where K^+ was in plentiful supply, which may suggest that ABA has a large capacity in generating

a response. In addition, it was only up-regulation of gene expression that was observed. It was essentially the re-supply of K^+ to K-deprived plants that showed the highest level of response. Across all different treatments, TIP1,3 and TIP1,2 were the most consistently regulated, though TIP1,1 was displaying the most differential behaviour between tissue types.

Aquaporins are thought to be regulated by phosphorylation (Maurel *et al*, 1997) as they contain conserved phosphorylation motifs and characterisation of isolated aquaporins in heterologous systems (Johansson *et al*, 1998). In addition promoter-fusion studies have indicated that some aquaporins are strongly transcription-regulated in meristems and tissues that can experience high water or metabolite flux (reviewed by Chrispeels *et al*, 1999). As the plants used were 2-week-old seedlings, it is likely that we are seeing so much aquaporin regulation due to this.

Whilst TIP1,3; TIP1,2 and TIP1,1 could be of definite interest for cloning (as depicted from the original aims of the AMT experiments), the strength and variation of the aquaporin responses requires far more in-depth investigation, and could be possible grounds for long-term research. In addition, the age of plants used could be imperative in establishing any effects upon the aquaporins.

AGI Code	Description	K+ / +ABA		K- / +ABA		K- / +10mM KCl		K+ / +50mM KCl	
		R	S	R	S	R	S	R	S
At3g47440	TIP5,1					1.97	2.67		
At2g25810	TIP4,1	0.56							
At1g17810	TIP3,2	0.49			0.49				
At4g17340	TIP2,2				0.35				
At3g16240	TIP2,1				0.22	2.01			
At4g01470	TIP1,3	0.51		0.51	0.16	1.85		1.83	
At3g26520	TIP1,2	0.56		0.51	0.12			1.85	1.83
At2g36830	TIP1,1	7.75			0.16	1.97			
At4g35100	PIP2,7	1.94							
At2g39010	PIP2,6				0.23				
At2g37170	PIP2,2	7.76				1.92			
At3g53420	PIP2,1				0.33				
At4g00430	PIP1,4	2.86							
At1g01620	PIP1,3	5.13							
At3g61430	PIP1,1	0.48			0.31				
At4g19030	NIP1,1	0.26		0.36					

Colour	Signal Strength (average)		Strength of Transcript Change
	T/C Ratio Below:	0.5	strongly down-regulated
		0.6	medium down-regulated
	T/C Ratio Above:	2	strongly up-regulated
		1.7	medium up-regulated

Table 3.5: Overview of transcript abundance of the aquaporins. There is a lot of differential regulation occurring across the different experiments and between tissue types.

4.0 Discussion

It is clear from the results that I have fulfilled our experimental aims and identified the strongest regulated genes (7TMS* and MATE*) as well as identified the differential behaviour of aquaporin activity. In addition, HAK5 has also produced interesting results in light of current in-house experiments. Multi-gene expression profiling has therefore allowed me to narrow down and identify new areas for future research. However, there are certain areas that need to be addressed.

4.1 Identification of novel genes by extreme response

Firstly, I have narrowed down our genes of interest purely by looking at the strength of response and those genes that displayed the most extreme responses. This approach may not capture the fundamental basis of transcriptional control in terms of homeostasis, as it is possible that many fundamental regulatory gene activities that don't exert extreme effects have been overlooked. However, the dependency of MATE* upon ABA led us to believe that this could be a very important basis for understanding stress responses, particularly as the stress controls were also active within these treatments. Secondly, whilst 7TMS* was consistently the most down-regulated gene between all treatments, this level of

consistency led us to believe that this putative transporter may have a very important role in signalling or general homeostasis.

HAK5 was a gene that showed comparatively "medium" abundance in relation to the two previously discussed genes and is a good example of how we may have overlooked novel transporters that could have a crucial role in nutrient transport. We have decided to continue analysis with this gene however due to recent findings within our research group that verifies the AMT data generated here.

As discussed previously, the data generated by aquaporin activity is very encouraging and would require far more in-depth analysis. In addition, the activity of V- and P-type pumps should also be assessed to establish relationships between ion compartmentation and changing osmotic potential. These pump genes however tended to show a more "medium" level of regulation, which is why I did not analyse them in more detail.

4.2 Experimental restrictions

There weren't enough biological replicates used within this data for suitable statistical analysis and ideally, at least 2 more replicates for experiments A, B, C and D should have been carried out. However, the presence of the internal

control upon the AMT data has significantly narrowed down the number of false-positives seen. It was not possible to complete the replicates within this project, due to cost, time constraints and the number of AMT chips that we had access to. Another suitable measure therefore could involve Real-Time-PCR; this will be discussed in more detail later. It is also important to bear in mind that the original aim was to simply provide a *preliminary screen to identify novel gene transporters*.

It would have been interesting to build up a profile of the effect of different plant ages within the agar-plate sealed system, and compare their transcriptional responses to young plants. Whilst older *A.thaliana* plants had previously been investigated using a hydroponics approach (in-house experiments, data not shown), it was found that there was too much external environmental change that created "noise", and did not produce consistently reliable microarray results

4.3 Improvement of Analysis Methods

A major problem of analysing microarray data is trying to establish whether or not the transcriptional changes seen are significant, particularly when there is a lack of biological replicates. As we were unable to use appropriate statistical analysis[3] we compared fold changes between arrays. This in itself is not the best

3 Methods are now available to statistically analyse functional "groups" of genes within gene lists, ranked by fold change and can be used for single replicates (Breitling *et al*, 2004b). However at the time this method had not been developed for me to incorporate into my analysis.

method, as each array is different and it is difficult to suitably compare the transcript changes when there are so many genes involved. Therefore, what was termed significant on one chip may be insignificant to another and hence the fold change is implying a false inference.

Typically, microarrays can be assessed through use of the Rank Product technique, which essentially calculates the level of fold change and comparing it to random variation (Breitling *et al*, 2004). Alternatively, it is possible to use an ANOVA analysis. However, the lack of biological replicates has meant that it has not been possible for me to suitably quantify or validate the changes.

4.4 Interaction Between ABA and K^+

The ABA data in K^+-sufficient and K^+-deficient environments (A&B) are comparable as the only changing variable was the presence of K^+. In addition, the presence of up-regulated controls further provided evidence that the AMT chip was producing a reliable response. The most important result of the ABA experiments is that the transcriptional response of transporter genes is modulated by the external K^+ supply (and/or plant K^+ status). As K^+ is the most important osmoticum and counteracts Na^+ toxicity, these findings might have important implications for the adaptation of salt and drought-stress responses (both involving ABA) to the supply of K^+. The K^+ re-supply experiments (C&D) did not produce a classical stress or dehydration response as completely different

gene profiles were produced, and there was a lack of regulation of ABA stress controls. It appears that these experiments were not inducing a stress response that involved an ABA signal. In addition, experiment D (addition of surplus K^+) produced a comparatively weak response both within the experiment (roots and shoots), as well as compared with other treatments. This indicates that plants "sense" their requirement for K^+ and thus only respond to K^+ supply when starved.

4.5 Suggestions for Future Work

4.5.1 Biological Replicates

All of the experiments will need to be repeated in order to reduce experimental error and provide statistical evidence to suggest that these findings are true. However, as stated earlier, the initial purpose of the experiment was to provide a preliminary screen, which is why we were only looking for the most reactive genes.

4.5.2 Quantify transcript levels through Real-Time-PCR and Northern Blots

In addition to the biological replicates or in place of them, the transcriptional activity can be quantified and measured accurately through the use of Real-Time-PCR and Northern blotting techniques. Due to the restraint of materials (AMT

chips), this could be a very real solution as the preliminary screen has identified the genes of interest. Alternatively, a Semi-Quantitative RT-PCR approach could be used to further validate the findings.

4.5.3 Cloning and functional characterisation

Having identified our three genes of interest, it is imperative that we clone and characterise them in order to investigate their function. Functional characterisation is facilitated by the use of mutant knockout lines and heterologous expression systems. Within the research group, there is advanced knowledge of oocyte and yeast heterologous systems, which could be used to characterise these transporters. Neither 7TMS* or MATE* have been cloned but HAK5 has and is currently under investigation in a number of research groups.

5.0 Conclusion

In conclusion, we have demonstrated that the AMT array can be used to identify genes of interest that could play a major part in plant K^+ transport. These are: two novel membrane transporters (MATE* - At1g12950; 7TMS* - At4g2100), and one previously cloned transporter (HAK5 – At4g13420). .

We have shown that the agar-plate sealed system, using 2-week-old *A.thaliana* seedlings, produced a reliable response to externally applied ABA. This was based on the strength of the known ABA stress controls that were up-regulated, and was similar to past findings (Goh *et al*, 2003). It appears that K^+ starvation does not induce an ABA signal, since ABA controls were not affected by this treatment.

Whilst we cannot quantify or compare the results statistically (due to lack of suitable analysis tools, as discussed in section 4.3) we have shown that many genes have differing levels of dependency of K^+_{ext}, tissue type or ABA. For example, the MATE* transporter was solely responsive to ABA regardless of the K^+_{ext}. The 7TMS* transporter was down-regulated in all treatments independent of tissue type or K^+_{ext}, whereas HAK5 only showed transcript abundance in K^+-deficient conditions.

We can therefore conclude that despite the experimental and statistical restraints of the project, the use of microarrays as global-expression tools is a reliable and quick way of screening and identifying genes of interest in various environmental conditions.

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K+Present Plants exposed to 3h ABA (50uM) (R<0.6;>1.67)						
ROOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
	At1g12950	In MATE family	13.9440	10.7160	12.3300	12.3300
SUGTL2	At1g08920	Sugar transporter	10.9210	7.3910	9.1560	9.1560
	AT3g29060	In DASS family	13.6290	3.8210	8.7250	8.7250
PIP2,2	At2g37170	aquaporin	8.2720	7.2530	7.7625	7.7625
TIP1,1	At2g36830	aquaporin	8.7120	6.2020	7.4570	7.4570
ATMDR13A	At3g28345	ABC	9.6480	4.6900	7.1690	7.1690
PIP2,1	At3g53420	aquaporin	8.8790	4.4840	6.6815	6.6815
TIP2,2	At4g17340	aquaporin	8.3860	3.7460	6.0670	6.0670
COR78		Condition, cor78 protein	9.1260	2.2500	5.6880	5.6880
PIP1,3	At1g01620	aquaporin	6.8570	3.4000	5.1285	5.1285
NTP2=PTR10	At2g26690	Peptide transporter	6.6400	3.1930	4.9165	4.9165
	At5g65990	Aminoacid transporter	7.2620	2.4090	4.8355	4.8355
KIN2		Condition, cold stress, kin2	6.0500	3.0940	4.5720	4.5720
P5CS1		Condition, salt stress,	4.8530	4.0610	4.4570	4.4570
MITO 3	At2g17270	Phosphate transporter	2.9740	5.4180	4.1960	4.1960
ACA8	At5g57110	P-type pump	4.8800	3.3930	4.1365	4.1365
PIP1,1	At3g61430	aquaporin	4.6370	3.5320	4.0845	4.0845
ATMDR17	At3g62150	ABC	2.9370	5.0950	4.0160	4.0160
EF-HAND 1	At5g49900	Putative Ca channel	4.3660	3.1270	3.7465	3.7465
	At1g01740	9 TMS putative	4.0240	3.4040	3.7140	3.7140
V-ATPASE6	At4g38920	V-type pump (VHA-c3)	3.8270	3.3410	3.5840	3.5840
ADH1		Condition,anaerobiosis,	4.1540	2.9860	3.5700	3.5700
SUC2	At1g22710	Sugar transporter	3.3600	3.7550	3.5575	3.5575
	AT4g37660	7 TMS putative	3.8720	3.3990	3.5355	3.5355
CHX17	At4g23700	Cation-H antiporter	3.6240	3.0820	3.3530	3.3530
V-ATPASE82	At1g20260	V-type pump (VHA-B3)	4.0450	2.5610	3.3030	3.3030
SULTR3.4	AT3g15990	Sulphate transporter	4.0270	2.6450	3.2860	3.2860
LTI6B	At3g05890	Stress induced	3.2550	3.2840	3.2695	3.2695
	AT3g20300	6 TMS putative	2.7860	3.6660	3.2260	3.2260
	At1g53470	7 TMS putative	3.2110	3.1860	3.1985	3.1985
	At1g73650	7 TMS putative	3.7750	2.5440	3.1595	3.1595
	AT5g20270	7 TMS putative	4.0150	2.1850	3.1000	3.1000
NA-SULFATE	At5g47560	Other anion transporter	4.1350	2.0390	3.0870	3.0870
	At1g72750	Inner mitoch membrane protein family	3.6500	2.5180	3.0340	3.0340
	At1g73220	Sugar transporter	3.4180	2.4760	2.9470	2.9470
STP13	AT5g26340	Sugar transporter	3.1770	2.6720	2.9245	2.9245
GORK	At5g37500	K channel	2.5770	3.2680	2.9175	2.9175
	AT3g04800		3.3080	2.5220	2.9150	2.9150
PIP1,4	At4g00430	aquaporin	2.9570	2.8380	2.8625	2.8625
ATAAP1	At1g58360	Aminoacid transporter	2.8410	2.8820	2.8615	2.8615
	At2g17500	10 TMS putative	2.8990	2.7090	2.8040	2.8040
SUGAR2	AT4g35300	Sugar transporter	2.9990	2.6710	2.7850	2.7850
ATWBC6	At5g13580	ABC	2.9640	2.5460	2.7550	2.7550
AVAP2	At1g19910	V-type pump (VHA-c2)	2.9360	2.5620	2.7490	2.7490
ATMTP1	At2g46800	Metal transporter	2.9750	2.4770	2.7260	2.7260
	AT4g21570	7 TMS putative	2.9910	2.4070	2.6990	2.6990
	AT6g26200	MC family	2.4630	2.8990	2.6810	2.6810
	AT4g15430	10 TMS putative	3.3270	1.9540	2.6405	2.6405
SULTR3.1=AST12	At3g51900	Sulphate transporter	3.4490	1.8240	2.6365	2.6365
ATWBC24	At5g19410	ABC	2.5960	2.6530	2.6245	2.6245
AVP2	At1g78920	PPase	2.6100	2.6160	2.6130	2.6130
V-ATPASE SUB1	At2g21410	V-type pump (VHA-a2)	2.8180	2.3130	2.5655	2.5655
COPT5	At5g20650	Metal transporter	2.7290	2.2640	2.4965	2.4965
	AT3g14810	7 TMS putative	2.4410	2.5260	2.4835	2.4835
ATWBC2	At2g37360	ABC	2.9709	1.9900	2.4800	2.4800
ATMRP10	At3g62700	ABC	2.4640	2.4870	2.4755	2.4755
	AT5g47530	6 TMS putative	2.7840	2.0910	2.4375	2.4375
ECA4&ECA1/ACA1	At1g07670&	P-type pump	2.6150	2.2360	2.4255	2.4255
	At1g12730	7 TMS putative	2.5280	2.2950	2.4115	2.4115
ATWBC20	At3g53510	ABC	2.3630	2.4320	2.3975	2.3975
PTR35	At1g59740	Peptide transporter	2.0100	2.7160	2.3630	2.3630
	At1g15150	In MATE family	2.2590	2.4270	2.3430	2.3430
ATMTPA2	At3g61940	Metal transporter	2.3710	2.3100	2.3405	2.3405
ATAUXR2	At2g21050	Auxin transporter	2.3960	2.2550	2.3255	2.3255
CLC-A	At5g40890	Cl channel	2.2320	2.3290	2.2805	2.2805
HMG1		Constitutive, HMGcoA reductase	2.5160	2.0150	2.2655	2.2655
	At1g76610	7 TMS putative	2.2320	2.2750	2.2535	2.2535
ATPDR7	At1g15210	ABC	2.2310	2.2320	2.2315	2.2315
	At1g64890	In FBT family	2.4530	1.9060	2.1795	2.1795

K+Present Plants exposed to 3h ABA (50uM) (R<0.6;>1.67)						
ROOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
CAX3	At3g51860	Ca-H antiporter	2.5150	1.8300	2.1725	2.1725
SUGTL4	At1g08890	Sugar transporter	2.0890	2.2510	2.1700	2.1700
APX1		Condition, oxidative stress,	2.3450	1.9440	2.1445	2.1445
KEA3	At4g04850	K-efflux system	2.1700	2.0280	2.0990	2.0990
	AT3g03700	7 TMS putative	2.2660	1.9160	2.0910	2.0910
STP 30	At2g48020	Sugar transporter	2.2850	1.8450	2.0650	2.0650
	AT5g18520	8 TMS putative	2.1610	1.9680	2.0645	2.0645
ATPDR12	At1g15520	ABC	2.2170	1.9080	2.0625	2.0625
	At1g55240	7 TMS putative	2.0440	2.0610	2.0525	2.0525
	At2g21120	9 TMS putative	1.9790	2.0570	2.0180	2.0180
	At1g04570	In FBT family	2.2000	1.7530	1.9765	1.9765
PIP2,7	At4g35100		2.1200	1.7600	1.9445	1.9445
	AT3g21620	10 TMS putative	2.1000	1.7790	1.9395	1.9395
	At1g66760	In MATE family	1.8740	1.9750	1.9245	1.9245
	At2g38330	In MATE family	2.0370	1.8000	1.9185	1.9185
MTG2	At1g16010	Mg-transporter	2.0950	1.7240	1.9095	1.9095
V-ATPASE SUB1 2	At2g28520	V-type pump (VHA-e1)	1.7220	2.0750	1.8985	1.8985
ATCAT7	At5g36940	Aminoacid transporter	1.7100	2.0720	1.8910	1.8910
PHT1;4/ATPT2	At2g38940	Phosphate transporter	1.9110	1.8510	1.8810	1.8810
KUP/HAK/KT8	At5g14880	K-transporter	1.8590	1.8880	1.8735	1.8735
LAX1	At5g01240	Auxin transporter	1.8520	1.8530	1.8525	1.8525
	At4g10770	Aminoacid transporter	1.8350	1.8460	1.8405	1.8405
MHX1	At2g47600	Mg-H antiporter	1.8580	1.8220	1.8400	1.8400
ATAAP2	AT5g09220	Aminoacid transporter	1.9130	1.7630	1.8380	1.8380
HMA5	At1g63440	P-type pump	1.8830	1.7650	1.8240	1.8240
ATAUX1	At2g38120	Auxin transporter	1.7290	1.8560	1.7925	1.7925
	AT3g01100	10 TMS putative	1.8230	1.7110	1.7670	1.7670
	At1g03040	In MFS family	1.7580	1.7640	1.7610	1.7610
CL-CHANNEL	At5g33260	Cl channel	0.5710	0.5880	0.5795	1.7256
ATAAP6	At5g49630	Aminoacid transporter	0.5920	0.5540	0.5730	1.7452
	At2g33260	Aminoacid transporter	0.5560	0.5890	0.5725	1.7467
PUP14	At1g57990	Aminoacid transporter	0.5870	0.5490	0.5680	1.7606
NAP8	At5g02270	ABC	0.5660	0.5670	0.5665	1.7652
KEA2	At4g00630	K-efflux system	0.5270	0.5960	0.5615	1.7809
TIP4,1	At2g25810	aquaporin	0.5960	0.5240	0.5610	1.7825
ACA1/PEA1	At1g27770	P-type pump	0.5390	0.5810	0.5600	1.7857
ZIP6	At1g31260	Metal transporter	0.5760	0.5420	0.5590	1.7889
EIN2	At5g03280	Metal transporter	0.5140	0.5990	0.5565	1.7968
ATPDR8	At1g59870	ABC	0.5280	0.5810	0.5545	1.8034
	AT5g47580	8 TMS putative	0.5680	0.5100	0.5490	1.8215
	At2g37360	8 TMS putative	0.5520	0.5420	0.5470	1.8282
ATATM3=STA1	At5g58270	ABC	0.4990	0.5930	0.5460	1.8315
ATSMC3	At5g48600	ABC	0.5640	0.5250	0.5445	1.8365
	AT4g09010	In NST-TPT family	0.5420	0.5410	0.5415	1.8467
	AT5g65380	In MATE family	0.5090	0.5710	0.5400	1.8519
KCP	At1g04690	K channel	0.5430	0.5260	0.5345	1.8709
FPS1		Constitutive	0.5430	0.5400	0.5335	1.8744
SULF	AT5g15600	Sulphate transporter	0.4580	0.5970	0.5275	1.8957
	AT4g27720	11 TMS putative	0.5350	0.5110	0.5230	1.9120
	At4g16370	Aminoacid transporter	0.5330	0.5130	0.5230	1.9120
PTR23	At1g18880	Peptide transporter	0.5200	0.5250	0.5225	1.9139
CHX15	At2g13820	Cation-H antiporter	0.5300	0.5120	0.5210	1.9194
ATPDR6	At1g59870	ABC	0.5290	0.4980	0.5135	1.9474
PTR26	AT3g16180	Peptide transporter	0.5380	0.4860	0.5120	1.9531
TIP1,2	At3g26520	aquaporin	0.5600	0.4630	0.5115	1.9550
	AT5g19520	7 TMS putative	0.5200	0.4980	0.5090	1.9685
TIP1,3	At4g01470	aquaporin	0.5260	0.4880	0.5070	1.9724
ATWBC14	At1g31770	ABC	0.5180	0.4830	0.5006	1.9980
HAK5	At4g13420	K-transporter	0.5140	0.4760	0.4950	2.0202
FRUCT1	At3g13790	Condition, Sugar, Invertase	0.4660	0.5190	0.4925	2.0305
SP2		Spiking, Myosin heavy chain	0.4850	0.4870	0.4860	2.0576
TIP3,2	At1g17810	aquaporin	0.5380	0.4340	0.4860	2.0576
ATPDR9	At3g53480	ABC	0.4880	0.4800	0.4840	2.0661
ATPDR9	At3g53480	ABC	0.4900	0.4760	0.4830	2.0704
	AT4g21910	In MATE family	0.5470	0.4100	0.4785	2.0899
STP4	At3g19930	Sugar transporter	0.4430	0.5130	0.4780	2.0921
	AT5g52540	13 TMS putative	0.4550	0.4890	0.4720	2.1186
STP1	At1g11260	Sugar transporter	0.4030	0.5380	0.4660	2.1368
GUS		Transgene, Beta-glucuronidase (uidA)	0.4930	0.4410	0.4670	2.1413

K+Present Plants exposed to 3h ABA (50uM) (R<0.6;>1.67)						
ROOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
ATPROT2	At3g65740	Aminoacid transporter	0.4840	0.4480	0.4660	2.1459
AMT1-2	At1g64780	Ammonium transporter	0.4100	0.5090	0.4595	2.1763
ATMRP9	At3g60160	ABC	0.4120	0.5040	0.4580	2.1834
	AT3g05460	8 TMS putative	0.5020	0.4020	0.4520	2.2124
	AT3g08040	In MATE family	0.4750	0.4280	0.4515	2.2148
ATPDR9	At3g53480	ABC	0.4690	0.4320	0.4505	2.2198
	At1g31820	Aminoacid transporter	0.4790	0.4210	0.4500	2.2222
KAT3, AKT4, ATH	At4g32650	K channel	0.4750	0.4220	0.4485	2.2297
	At1g23330	6 TMS putative	0.5040	0.3850	0.4445	2.2497
PHT1.1/ATPT1&PH	AT5g43350		0.3970	0.4900	0.4435	2.2548
ATMDR1	At2g36910	ABC	0.4530	0.4340	0.4435	2.2548
	At3g28960	Aminoacid transporter	0.4080	0.4550	0.4315	2.3175
UREA ACTIVE	AT5g45380	Other anion transporter	0.4310	0.4210	0.4260	2.3474
	At5g64410	Aminoacid transporter	0.4670	0.3820	0.4245	2.3557
	At4g26690	Aminoacid transporter	0.4930	0.3410	0.4170	2.3981
ATPDR7	At1g15210	ABC	0.4420	0.3870	0.4145	2.4125
ATCCH1	At4g03560	Putative Ca channel	0.3680	0.4560	0.4120	2.4272
NLM9	At2g21020	aquaporin	0.3660	0.4550	0.4105	2.4361
STP 44	At1g30220	Sugar transporter	0.4140	0.3300	0.3720	2.6882
NAP3	At1g67940	ABC	0.3720	0.3190	0.3455	2.8944
IRT1	At4g19690	Metal transporter	0.3630	0.3170	0.3350	2.9851
ACA11	At3g57330	P-type pump	0.3510	0.3080	0.3295	3.0349
PTR48	AT3g45690	Peptide transporter	0.3040	0.3260	0.3145	3.1797
ATLHT1	At5g40780	Aminoacid transporter	0.2910	0.2970	0.2940	3.4014
CNGC8	At1g19780	CNGC	0.2660	0.2950	0.2805	3.5651
ATNRAMP1	At1g80830	Metal transporter	0.3080	0.2500	0.2790	3.5842
NIP1,1	At4g19030	aquaporin	0.2690	0.2600	0.2645	3.7807
	AT4g23010	10 TMS putative	0.2280	0.2850	0.2565	3.8986
	AT5g45470	6 TMS putative	0.2500	0.2610	0.2555	3.9139
ATPDR8	At1g59870	ABC	0.2610	0.2380	0.2495	4.0080
	AT4g20100	7 TMS putative	0.0880	0.1600	0.1240	8.0645

K+Present Plants exposed to 3h ABA (50uM) (R<0.6;>1.67)						
SHOOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
COR78		Condition, cor78 protein	5.5960	5.4300	5.5630	5.5630
P5CS1		Condition, salt stress,	2.9560	3.1900	3.0730	3.0730
	AT5g26200	MC family	3.1090	3.0030	3.0560	3.0560
LTI6A	At3g05880	Stress induced	3.0790	2.8520	2.9655	2.9655
LTI6B	At3g05890	Stress induced	3.0360	2.4700	2.7530	2.7530
KIN2		Condition, cold stress, kin2	2.9610	2.4890	2.7250	2.7250
STRESS	At4g30650	Stress induced	2.5550	2.5420	2.5485	2.5485
ATAAP1	At1g58360	Aminoacid transporter	2.0340	1.9300	1.9320	1.9320
SUGTL2	At1g08920	Sugar transporter	2.0560	1.7840	1.9200	1.9200
	At1g55240	7 TMS putative	1.9150	1.9730	1.8940	1.8940
EF-HAND 4	At5g61810	Putative Ca channel	1.9510	1.8990	1.8800	1.8800
	AT3g14810	7 TMS putative	1.7390	1.9820	1.8605	1.8605
FRUCT3	At1g62660	Condition, Sugar, Invertase	1.8020	1.8830	1.8425	1.8425
ATGCN5	At5g64840	ABC	1.7080	1.9600	1.8340	1.8340
CNGC8	At1g19780	CNGC	0.5590	0.5160	0.5375	1.8605
	AT4g20100	7 TMS putative	0.3530	0.4400	0.3965	2.5221

K-Deficient Plants exposed to 3h ABA (50uM) (R<0.6;>1.67)						
ROOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
	At1g12950	In MATE family	9.6290	6.5530	8.0910	8.0910
	At1g73220	Sugar transporter	5.1540	7.2750	6.2145	6.2145
COR78	At5g52310	Condition, dessication, cor78 protein	6.2230	3.7600	4.9915	4.9915
	AT5g26200	MC family	4.7860	3.3910	4.0885	4.0885
	AT3g29060	In DASS family	4.0930	2.6160	3.3545	3.3545
LTI60	At3g05890	Stress induced	3.2550	3.2840	3.2695	3.2695
	At2g18690	6 TMS putative	2.7860	3.6660	3.2260	3.2260
ADH1	At1g77120	Condition,enaerobiosis, alcohol dehydrogen	3.1570	3.2350	3.1960	3.1960
NTP2=PTR10	At2g26690	Peptide transporter	2.7460	3.5700	3.1580	3.1580
EF-HAND 1	At5g49900	Putative Ca channel	3.0690	2.9580	3.0135	3.0135
ATAAP1	At1g58360	Aminoacid transporter	2.8410	2.8820	2.8615	2.8615
ATWBC6	At5g13580	ABC	2.9640	2.5460	2.7550	2.7550
	AT4g21570	7 TMS putative	2.9810	2.4070	2.6990	2.6990
GORK	At5g37500	K channel	1.6950	3.3290	2.5120	2.5120
	At5g65990	Aminoacid transporter	2.7640	2.1190	2.4415	2.4415
	At5g65890	Aminoacid transporter	2.7640	2.1190	2.4415	2.4415
P5CS1	At2g39800	Condition, salt stress, pyrroline-5-carboxylat	2.4810	2.3770	2.4340	2.4340
	At1g15160	In MATE family	2.6370	2.2220	2.4295	2.4295
SUGTL2	At1g09920	Sugar transporter	2.0570	2.6680	2.3625	2.3625
	AT5g20270	7 TMS putative	2.2220	2.3900	2.3060	2.3060
	At1g12730	7 TMS putative	2.0950	2.4640	2.2795	2.2795
MITO 3	At2g17270	Phosphate transporter	1.9960	2.5410	2.2685	2.2685
KIN2	At5g15970	Condition, cold stress, kin2	2.5280	1.8610	2.1945	2.1945
SUGTL4	At1g08890	Sugar transporter	2.0690	2.2510	2.1700	2.1700
At3g62150		ABC	1.9810	2.2770	2.1290	2.1290
SULTR3.1=AST12	At3g51900	Sulphate transporter	2.0710	2.1630	2.1170	2.1170
STP13	AT5g26340	Sugar transporter	1.9080	2.2410	2.0745	2.0745
	At2g38330	In MATE family	1.8570	2.2110	2.0340	2.0340
	AT3g14810	7 TMS putative	1.7690	2.1950	1.9820	1.9820
	AT3g21620	10 TMS putative	2.1000	1.7790	1.9395	1.9395
PTR35	At1g59740	Peptide transporter	2.0750	1.8030	1.9390	1.9390
	At1g56760	In MATE family	1.8740	1.9750	1.9245	1.9245
	AT3g01100	10 TMS putative	2.0720	1.7510	1.9115	1.9115
	At1g78610	7 TMS putative	1.9040	1.8890	1.8965	1.8965
ATWBC24	At5g19410	ABC	1.8670	1.8980	1.8825	1.8825
PHT1.4/ATPT2	At2g38940	Phosphate transporter	1.9110	1.8510	1.8810	1.8810
	AT4g15430	10 TMS putative	1.7600	1.9660	1.8630	1.8630
MHX1	At2g47600	Mg-H antiporter	1.8580	1.9220	1.8400	1.8400
HMA5	At1g63440	P-type pump	1.8830	1.7650	1.8240	1.8240
	At2g17500	10 TMS putative	1.8030	1.7060	1.7545	1.7545
	At2g33260	Aminoacid transporter	0.5560	0.5890	0.5725	1.7467
ACA1/PEA1	At1g27770	P-type pump	0.5390	0.5810	0.5600	1.7857
ATPDR8	At1g59870	ABC	0.5260	0.5810	0.5545	1.8034
SULF	AT5g19600	Sulphate transporter	0.4590	0.5970	0.5275	1.8957
	At4g16370	Aminoacid transporter	0.5330	0.5130	0.5230	1.9120
ATPDR8	At1g59870	ABC	0.5290	0.4980	0.5135	1.9474
TIP1,2	At3g26520	aquaporin	0.5600	0.4630	0.5115	1.9550
TIP1,3	At4g01470	aquaporin	0.5260	0.4880	0.5070	1.9724
UREA ACTIVE	AT5g45380	Other anion transporter	0.4200	0.5870	0.5035	1.9861
HAK5	At4g13420	K-transporter	0.5140	0.4760	0.4950	2.0202
TIP3,2	At1g17810	aquaporin	0.5380	0.4340	0.4860	2.0576
ATMRP9	At3g60160	ABC	0.4140	0.5240	0.4690	2.1322
GUS		Transgene, Beta-glucuronidase (uidA)	0.4930	0.4410	0.4670	2.1413
CNGC8	At1g19780	CNGC	0.5240	0.4100	0.4670	2.1413
STP 44	At1g30220	Sugar transporter	0.4180	0.5110	0.4645	2.1529
AMT1-2	At1g64780	Ammonium transporter	0.4100	0.5090	0.4595	2.1763
	AT3g06040	In MATE family	0.4750	0.4280	0.4515	2.2148
PHT1.1/ATPT1&P1	AT5g43350&At5g4	Phosphate transporter	0.3970	0.4900	0.4435	2.2548
	At4g26690	Aminoacid transporter	0.4930	0.3410	0.4170	2.3981
ATPDR7	At1g15210	ABC	0.3910	0.3860	0.3885	2.5740
ATPDR7	At1g15210	ABC	0.3910	0.3860	0.3885	2.5740
NIP1,1	At4g19030	aquaporin	0.4210	0.3410	0.3810	2.6247
	AT4g20100	7 TMS putative	0.2360	0.1580	0.1970	5.0761

K+Deficient Plants exposed to 3h ABA (50uM) (R<0.6;>1.67)						
SHOOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
	At1g12950	In MATE family	4.6130	3.4110	4.0120	4.0120
COR78		Condition, cor78 protein	3.5970	2.2150	2.8760	2.8760
LTI6B	At3g05890	Stress induced	2.4080	2.6740	2.5410	2.5410
LTI6A	At3g05880	Stress induced	2.7870	2.0890	2.4230	2.4230
	AT3g29060	In DASS family	2.4880	2.2310	2.3595	2.3595
ATWBC28	At3g52310	ABC	2.3080	1.9750	2.1415	2.1415
ADH1		Condition,anaerobiosis,	1.8320	2.3310	2.0815	2.0815
P5CS1		Condition, salt stress,	1.9680	2.1090	2.0385	2.0385
	AT3g14810	7 TMS putative	1.7420	2.2700	2.0060	2.0060
	AT3g20900	6 TMS putative	1.7570	2.2200	1.9885	1.9885
NTP2=PTR10	At2g26690	Peptide transporter	1.8280	2.1160	1.9720	1.9720
	AT3g43840	7 TMS putative	1.9290	1.9410	1.9350	1.9350
	At1g15150	In MATE family	1.8310	1.9770	1.9040	1.9040
	AT4g21570	7 TMS putative	1.7590	2.0440	1.9015	1.9015
	At1g71870	In MATE family	1.8010	1.7970	1.7990	1.7990
KEA3	At4g04850	K-efflux system	1.7470	1.8440	1.7955	1.7955
	At1g12600	10 TMS putative	1.7290	1.8410	1.7850	1.7850
	AT5g26200	MC family	1.8460	1.7230	1.7845	1.7845
	At1g29020	19 TMS putative	1.8580	1.7050	1.7805	1.7805
	AT3g01100	10 TMS putative	1.8310	1.7110	1.7710	1.7710
CHX22	At2g31910	Cation-H antiporter	1.7620	1.7160	1.7390	1.7390
CHX10	At3g44930	Cation-H antiporter	0.5610	0.5910	0.5760	1.7361
CNGC11	At2g46440	CNGC	0.5810	0.5710	0.5760	1.7361
ATMDR6	At2g39460	ABC	0.5560	0.5720	0.5640	1.7730
	At2g03240	In DASS family	0.5340	0.5830	0.5585	1.7905
ATLHT3	At1g61270	Aminoacid transporter	0.5390	0.5740	0.5565	1.7969
ATMRP9	At3g60160	ABC	0.5160	0.5860	0.5510	1.8149
B-TUB		Constitutive, Beta-8- tubulin	0.5920	0.5050	0.5485	1.8232
PTR34	AT3g53960	Peptide transporter	0.5550	0.5380	0.5465	1.8298
PUP6	At4g18200	Aminoacid transporter	0.5250	0.5500	0.5375	1.8605
ATMDR2	At4g25960	ABC	0.5170	0.5500	0.5335	1.8744
AMT1-1	AT4g13510	Ammonium transporter	0.5420	0.5220	0.5320	1.8797
ALA6	At1g54280	P-type pump	0.5510	0.5080	0.5295	1.8886
AVAP2	At1g19910	V-type pump (VHA-c2)	0.5000	0.5460	0.5230	1.9120
	At5g53520	Aminoacid transporter	0.5620	0.4540	0.5080	1.9685
NAP12	At2g37010	ABC	0.4230	0.5920	0.5075	1.9704
ACA1/PEA1	At1g27770	P-type pump	0.5640	0.4380	0.5010	1.9960
ATAUXR2	At2g21050	Auxin transporter	0.4730	0.5180	0.4980	2.0080
PTR4	At1g22550	Peptide transporter	0.4660	0.5280	0.4970	2.0121
ATATH3	At3g47750	ABC	0.4540	0.5340	0.4940	2.0243
V-ATPASE SUBD	At3g28710/15	V-type pump (VHA-d1)	0.4660	0.5190	0.4925	2.0305
	At1g61890	In MATE family	0.5630	0.4050	0.4840	2.0661
ACA12	At3g63380	P-type pump	0.3970	0.5680	0.4825	2.0725
ATMTPC3	At3g58060	Metal transporter	0.4240	0.5240	0.4740	2.1097
PIN3	At1g70940	Auxin transporter	0.3990	0.5320	0.4655	2.1482
ZIP10	At5g62160	Metal transporter	0.4980	0.4280	0.4630	2.1598
CHX12	At3g44910	Cation-H antiporter	0.5580	0.3410	0.4495	2.2247
	AT5g01460	9 TMS putative	0.5180	0.3640	0.4410	2.2676
V-ATPASE5	At4g38920	V-type pump (VHA-c3)	0.4040	0.4520	0.4280	2.3364
NRT2.7	At1g12940	Nitrate tansporter	0.3980	0.4550	0.4265	2.3447
STP 30	At2g48020	Sugar transporter	0.3580	0.4810	0.4195	2.3838
PTR7	At1g72140	Peptide transporter	0.3560	0.4760	0.4170	2.3981
	AT5g11690	In MPT family	0.4930	0.3990	0.4160	2.4038
AMT1-3	AT3g24300	Ammonium transporter	0.4740	0.3470	0.4105	2.4361
	AT3g23560	In MATE family	0.4820	0.3320	0.4070	2.4570
AVP2	At1g78920	PPase	0.4180	0.3880	0.4030	2.4814
	At1g72750	Inner mitoch membrane protein family	0.4450	0.3550	0.4000	2.5000
	At2g04050	In MATE family	0.3860	0.4110	0.3985	2.5094
ATMDR22		ABC	0.4490	0.3390	0.3940	2.5381
ANR1	At2g14220	Condition,nitrate, MADS Box	0.3930	0.3910	0.3920	2.5510
ATPDR8	At1g59870	ABC	0.2760	0.5060	0.3915	2.5543
	At1g14350	9 TMS putative	0.3220	0.4570	0.3895	2.5674
ATMRP3	At3g13080	ABC	0.4790	0.2930	0.3860	2.5907
MALATE	At5g64280	Other anion transporter	0.3120	0.4490	0.3805	2.6281
NAP2=POP1	At5g44110	ABC	0.4850	0.2500	0.3675	2.7211
STP 33	AT4g36670	Sugar transporter	0.3560	0.3570	0.3565	2.8050
	AT3g07390	6 TMS putative	0.4250	0.2680	0.3465	2.8860
TIP2;2	At4g17340	aquaporin	0.4010	0.2910	0.3460	2.8902

K+Deficient Plants exposed to 3h ABA (50uM) (R<0.6;>1.67)						
SHOOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
PIP2,1	At3g53420	aquaporin	0.4080	0.2460	0.3280	3.0488
PIP1,1	At3g61430	aquaporin	0.3070	0.3130	0.3100	3.2258
PUP14	At1g57990	Aminoacid transporter	0.3130	0.2970	0.3050	3.2787
V-ATPASEB2	At1g20260	V-type pump (VHA-B3)	0.3290	0.2390	0.2840	3.5211
ATENT3	At4g05110	Aminoacid transporter	0.3090	0.2080	0.2585	3.8685
PGK		Constitutive, Phosphoglycerate kinase	0.2600	0.2260	0.2430	4.1152
PIP2,6	At2g39010	aquaporin	0.2720	0.1830	0.2275	4.3956
TIP2,1	At3g16240	aquaporin	0.2340	0.1950	0.2160	4.6512
	AT3g54610	10 TMS putative	0.1170	0.2550	0.1860	5.3763
	AT4g20100	7 TMS putative	0.1360	0.1870	0.1615	6.1920
TIP1,1	At2g36830	aquaporin	0.1610	0.1570	0.1590	6.2893
TIP1,3	At4g01470	aquaporin	0.1510	0.1610	0.1560	6.4103
TIP1,2	At3g26520	aquaporin	0.1410	0.0820	0.1115	8.9686
CAB		Constitutive, Chlorophyll binding Prot	0.0800	0.1020	0.0810	12.3457

K-Deficient Plants exposed to 6h 10mM KCl (R<0.6;>1.67)						
ROOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
STP 33	AT4g36670	Sugar transporter	2.9830	2.4980	2.7405	2.7405
FRUCT3	At1g62660	Condition, Sugar, Invertase	2.5050	2.7500	2.6275	2.6275
	At1g53470	7 TMS putative	2.2710	2.4100	2.3405	2.3405
ATMRP10	At3g62700	ABC	2.8140	1.7550	2.2845	2.2845
KEA1	At1g01790	K-efflux system	2.5510	1.8380	2.1945	2.1945
TIP5,1	At3g47440	aquaporin	2.3010	1.9630	2.1320	2.1320
CLC-A	At5g40890	Cl channel	2.0090	1.9470	1.9780	1.9780
TIP1,1	At2g36830	aquaporin	1.8940	2.0500	1.9720	1.9720
PIP2,2	At2g37170	aquaporin	1.9890	1.8650	1.9270	1.9270
	At1g69450	10 TMS putative	1.9510	1.8740	1.9125	1.9125
SP4		Spiking, Insulin-like growth factor	2.0890	1.7230	1.9060	1.9060
STP 37	AT4g16480	Sugar transporter	1.8560	1.9460	1.9010	1.9010
	At2g18590	In MFS family	1.9170	1.8630	1.8900	1.8900
STP 29	At1g75220	Sugar transporter	1.8080	1.9360	1.8720	1.8720
TIP1,3	At4g01470	aquaporin	1.9180	1.7810	1.8495	1.8495
	AT5g01460	9 TMS putative	1.8410	1.7570	1.7990	1.7990
	AT4g16070	In CaCA family	1.7470	1.8410	1.7940	1.7940
	At5g03555	Aminoacid transporter	1.7140	1.7140	1.7140	1.7140
	At1g33110	In MATE family	0.5910	0.5820	0.5865	1.7050
ATGCN5	At5g64840	ABC	0.5830	0.01*	0.5830	1.7153
ATMRP5	At3g13090	ABC	0.5610	0.5980	0.5795	1.7256
	At1g32090	10 TMS putative	0.5960	0.5580	0.5770	1.7331
CHX17	At4g23700	Cation-H antiporter	0.5340	0.5940	0.5640	1.7730
	At2g33260	Aminoacid transporter	0.5820	0.5390	0.5605	1.7841
ATWBC9	At5g13580	ABC	0.5430	0.5580	0.5505	1.8165
	At4g16370	Aminoacid transporter	0.5370	0.4970	0.5170	1.9342
	AT5g47530	6 TMS putative	0.6840	0.4660	0.5145	1.9436
	At2g03520	9 TMS putative	0.5540	0.4670	0.5105	1.9589
ATATM3=STA1	At5g58270	ABC	0.4430	0.5340	0.4885	2.0471
STP 20	AT3g20460	Sugar transporter	0.5180	0.4250	0.4715	2.1209
ACA12	At3g63380	P-type pump	0.4870	0.4410	0.4690	2.1322
	At2g22730	In MFS family	0.5810	0.3510	0.4660	2.1459
ATMRP11	At2g07680	ABC	0.4030	0.5070	0.4550	2.1978
PUP12	At5g41160	Aminoacid transporter	0.4560	0.4380	0.4470	2.2371
	At2g18690	6 TMS putative	0.5010	0.3600	0.4305	2.2701
HAK5	At4g13420	K-transporter	0.1700	0.1940	0.1820	5.4945
	AT4g20100	7 TMS putative	0.1640	0.1360	0.1500	6.6667

K-Deficient Plants exposed to 6h 10mM KCl (R<0.6;>1.67)						
SHOOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
TIP5,1	At3g47440	aquaporin	2.4590	2.8940	2.6765	2.6765
PTR29	AT4g21680	Peptide transporter	2.3570	1.7620	2.0595	2.0595
TIP2,1	At3g16240	aquaporin	2.1760	1.8460	2.0110	2.0110
AVP1	At1g15690	PPase	1.8960	1.8140	1.8550	1.8550
PUT ANION XC	At1g15460	Putative anion exchanger	0.5070	0.5510	0.5290	1.8904
ATCCH1	At4g03560	Putative Ca channel	0.5010	0.4920	0.4965	2.0141
	AT4g20100	7 TMS putative	0.3540	0.3810	0.3725	2.6846

K-Present Plants exposed to 6h 50mM KCl (R<0.6;>1.67)						
ROOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
FRUCT3	At1g62660	Condition, Sugar, Invertase	2.0270	2.2870	2.1570	2.1570
GUS		Transgene, Beta-glucuronidase (uidA)	1.9050	1.9460	1.9255	1.9255
TIP1,2	At3g26520	aquaporin	1.9400	1.7580	1.8490	1.8490
TIP1,3	At4g01470	aquaporin	1.8830	1.7830	1.8330	1.8330
ATMDR6	At2g39480	ABC	0.5830	0.5800	0.5715	1.7488
NLM3	At2g29870	aquaporin	0.5330	0.5770	0.5550	1.8018
ADH1		Condition,anaerobiosis	0.5200	0.5760	0.5490	1.8215
	At2g33260	Aminoacid transporter	0.5730	0.5190	0.5460	1.8315
CHX15	At2g13620	Cation-H antiporter	0.4740	0.4680	0.4710	2.1231
ATPROT2	At3g55740	Aminoacid transporter	0.3330	0.5500	0.4415	2.2650
PTR7	At1g72140	Peptide transporter	0.3590	0.3990	0.3790	2.6385
	AT4g20100	7 TMS putative	0.1750	0.2890	0.2320	4.3103

K-Present Plants exposed to 6h 50mM KCl (R<0.6;>1.67)						
SHOOTS						
Systematic		Genbank	replica a R	replica b R	AVE. AB R1	Fold Change R2
FRUCT3	At1g62660	Condition, Sugar, Invertase	1.9550	2.0790	2.0170	2.0170
GUS		Transgene, Beta-glucuronidase (uidA)	1.9020	2.0040	1.9530	1.9530
TIP1,2	At3g26520	aquaporin	1.9380	1.7310	1.8345	1.8345
ATMDR6	At2g39480	ABC	0.5710	0.5710	0.5710	1.7513
NLM3	At2g29870	aquaporin	0.5320	0.5800	0.5560	1.7986
ADH1		Condition,anaerobiosis	0.5200	0.5830	0.5515	1.8132
	At2g33260	Aminoacid transporter	0.5720	0.5200	0.5460	1.8315
CHX15	At2g13620	Cation-H antiporter	0.6120	0.4590	0.4855	2.0597
ATPROT2	At3g55740	Aminoacid transporter	0.3350	0.5470	0.4415	2.2650
PTR7	At1g72140	Peptide transporter	0.3590	0.3920	0.3755	2.6631
	AT4g20100	7 TMS putative	0.1740	0.2920	0.2330	4.2918