Compartmentation and compatible solutes in abiotic stress resistance

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ABSTRACT

Many abiotic stress are linked by common effects on plant water relations. Salinity and water deficit directly affect the ability of plants to extract water from the soil by osmotic processes, while heat and cold affect membrane integrity and the relationship between hydraulic conductance and water relations. There are thus common responses to stress as well as effects that are specific to each stress. A major advance in understanding of stress responses followed from a model of cellular water relations that included intracellular compartmentation of solutes, and the effects of various solutes on cellular metabolism. The evidence for this model is reviewed, together with specific examples in cotton and related species. The intracellular model is also considered in relation to responses occurring at the tissue, organ and whole plant levels. Of particular interest are the compatible solutes, i.e. those that allow metabolism to function at high solute concentrations and/or protect metabolism from the perturbing effects of abiotic stress. In cotton, these are proline and glycinebetaine. Although both are compatible and protective solutes, there are many differences in their metabolism and probable roles in relation to abiotic stresses.

Introduction

Bulk analysis of plant material provides a basic level of information on nutrient status and solute concentrations. Solutes are not, however, distributed uniformly in different organs, cell types or sub-cellular compartments. These differences are not constant, but may change with developmental stage or changes in the environment. To really understand how a plants works requires a thorough understanding of what is happening at various levels of organization ranging from gene expression to whole-plant resource allocation. Part of that understanding involves an appreciation of the concentrations of ions and organic solutes present in every part of the plant and, in the present context, how these concentrations change when the plant is subjected to abiotic stress.

At its simplest, this means knowing the differences between roots, stems, petioles, leaves, flowers and seeds in terms of solute accumulation and retranslocation. These change with development and age. A young leaf has a different chemical composition from an old leaf. This applies both in uniform conditions, and where an abiotic stress is imposed after the development of the old leaf but before the young leaf emerges. Thus, there is frequently a gradient in solute concentrations from young to old leaves that reflects age-related differences in the degree of vacuolation or the delivery of solutes (mainly ions) with the transpiration stream. For example, old leaves tend to have more sodium and calcium (accumulation related to total transpiration?), but less potassium (retranslocated to developing tissues) than young leaves.

Concentration differences between the various tissues that make up an organ such as a leaf are more difficult to study. X-ray microanalysis of bulk frozen and fractured leaves and roots provided early evidence for differential distribution of elements between cell types, and recent developments in Single Cell Sampling and Analysis (SiCSA) using modified pressure probes (Tomos and Sharrock, 2001) are revealing differences in concentrations of organic solutes.

The idea of intracellular compartmentation of ions in higher plants subjected to salt stress was proposed by Greenway and Osmond (1972) and Flowers (1972) to account for the anomaly that enzymes extracted from the leaves of salt-accumulating halophytes were inhibited by NaCl concentrations well below those found in healthy leaves (Flowers and Dalmond, 1992; Flowers *et al*., 1975,1976,1978). Flowers *et al*. (1977) presented evidence that Na^+ and Cl^- are accumulated in the metabolically inert vacuole, while non-toxic organic solutes (compatible cytosolutes) accumulated in the cytoplasm. Details of organ, inter cellular and intracellular compartmentation will be discussed later with specific examples in cotton.

Experimental procedure

Experiment 1, Organ level

Plants of two *G. hirsutum* cultivars (Acala SJ2 from the USA and MNH-147 from Pakistan) were grown from seeds. Interspecific hybrids (see legend to Figure 1) were initially grown from seeds and then propagated as softwood cuttings. Plants of approximately the same size were grown singly in 1.5 l pots of John Innes compost No. 2. The pots were randomly arranged in a flood bench in which the pots were flooded to the level of the soil surface for 15 minutes once or twice per day. The treatment solution consisted of 'Phostrogen' nutrients (Phostrogen Ltd., Corwen, Wales, U.K.) at one g per litre, Hoagland and Arnon's micronutrients, NaCl at 250 mol m^{-3} and $CaCl_2$ at 12.5 mol m^{-3}. This solution was pumped from 200 liter reservoirs beneath the flood benches. At the start of the experiment the salt concentration was increased in daily steps of 50 mol m^{-3}. The experiment was conducted in a greenhouse with a minimum temperature of 25 °C, photoperiod of 16 hours and natural daylight supplemented with SON-T high pressure Na vapour lamps. Plant samples were taken from plants that had been growing for four weeks at the final salt concentration. Samples were frozen in 1.5 cm^3 microcentrifuge tubes and later thawed. One hole was made in the bottom of each tube and one in

the lid. Sap was extracted by centrifuging the sample tube contents into a second, intact tube. Ion concentrations were determined in diluted sap using a 'Dionex' ion chromatograph.

Experiment 2, Ion distribution in G. longicalyx shoots

Rooted softwood cuttings of *G. longicalyx* were grown as described above. The plants were grown for 12 weeks at 100 mol m^{-3} NaCl plus 10 mol m^{-3} $CaCl_2$. Fresh main-stem leaves were excised from different regions of the main shoot axis of the plants and extracted in appropriate amounts of water at 100 °C in sealed containers. Ion concentrations were determined by ion chromatography and calculated as mmol kg^{-1} fresh weight. Three shoots were analyzed and a typical example is shown in Figure 3 (sampling distances varied slightly between the three shoots).

After 12 weeks of treatment stem, leaf and petiole samples (from 100 mm behind the apex) for X-ray microanalysis were mounted in recessed copper blocks using Tissue-Tek embedding medium (Miles Inc., Elkhart, U.S.A.) and rapidly frozen in nitrogen slush at -210°C under a nitrogen atmosphere in an Emitech K1250 cryo-preparation unit (Emitech Ltd, Ashford, U.K.). All subsequent manipulations were performed under high vacuum. The bulk frozen material was fractured in the cryo-preparation unit at –173 °C, transferred to a cold stage mounted on an Hitachi S-520 scanning electron microscope and the surface water sublimed by heating until an etching pattern just became visible. The precise temperature and time required for this process depended on the contact between the copper block and the cold stage, but normally involved heating the stage to -70 °C for 2-5 minutes with a Sigma temperature controller on the K1250.

X-ray microanalysis was performed at -160 °C with a Link LZ-4 energy-dispersive detector in ultra-thin window mode (1000 Å Aluminium) and a Link QX 2000 analyser (Link, Analytical Ltd., High Wycombe, U.K.). Ultra-thin window mode was used to increase sensitivity to Na and Mg. Spectra were acquired for 60 s (live time) with the electron beam in reduced raster mode, accelerated at 10 kv and with an emission current of 100 μA. Standard solutions (250 mol m^{-3}) were applied to ashless filter paper and used to determine relative responses for the different elements. Since the background radiation spectrum (Bremsstrahlung) was almost flat in these conditions, peak areas were used for quantification. Spectra which did not have the expected background radiation spectrum, especially in the region of the Na and Mg K peaks, were rejected. Peak areas were integrated with the 'window integrals' facility of the QX 2000. The K peak for K (determined from the ratio of K to K determined in standards without Ca) was subtracted from the K peak for Ca (Harvey *et al*., 1985). After correction for relative elemental response factors, the results for each element were expressed as percentages of the total corrected net counts for Na+Mg+P+S+Cl+K+Ca. Absolute concentrations were not calculated because of different degrees of etching in different preparations and variations in take-off angles for the different cells examined. Except for the xylem parenchyma cells, all measurements were made from vacuoles, and at least 1 μm from the cell walls.

Results and Discussion

Compartmentation at the organ level

Concentrations of a number of cations and anions were measured in sap of leaves, petioles and stems of a number of cotton genotypes grown for four weeks at 250 mol m^{-3} (Experiment 1). Figure 1 shows the distribution of Na^+ and Ca^{2+}. Concentrations of Na^+ were highest in the stems, while Ca^{2+} concentrations were lowest in the stems and highest in the leaves. Mg^{2+} concentrations were slightly higher in the leaves than in the petioles or stems (which had similar concentrations), whereas K^+ concentrations were highest in the petioles and lowest in the leaves (data not shown). The differences between organs in individual cations were greater than the differences in total positive charge ($Na+K+Mg^2+Ca^2$). There were also differences between genotypes, with *G. stocksii x G. hirsutum* having lower Na^+ concentrations than other genotypes and higher Ca^{2+} in leaves and petioles. Differences in leaf water content made only small contributions to these differences (data not shown). Of the anions, chloride concentrations were similar in all three organs, although petiole concentrations were slightly higher, and stem concentrations slightly lower, than leaf concentrations (Figure 2). Nitrate concentrations were high in petioles, but low in leaves. In contrast, leaf sulphate concentrations were very much higher than those of petioles or stems. Malate concentrations were similar in all organs, but higher in *G. hirsutum* cv. Acala SJ2 than in the other genotypes (data not shown). In the second experiment Na^+ concentrations generally increased rapidly in leaves, stems and petioles with increasing distance from the apex (Figure 3). Na^+ concentrations were in most cases higher in the stems than in leaves or petioles. Although the youngest leaves of *G. longicalyx* had very low Na^+ concentrations, there were higher concentrations of this ion in the youngest parts of the stem and, particularly, the petiole than in the leaves or in slightly older tissues. Concentrations of K^+ showed the opposite trend, increasing rapidly immediately behind the apex before declining rapidly in older petiole and stem tissues (Figure 3). Leaf K^+ concentrations remained at about 200 mmol kg^{-1} fresh weight in all parts of the plant. Petiole K^+ concentrations were highest in the petioles just behind the shoot apex, and a similar pattern was observed in the stems. Ca^{2+} concentrations also showed a rapid increase in all tissues immediately behind the apex in *G. longicalyx* (data not shown). In the leaves the Ca^{2+} concentrations were maintained at a relatively constant value with

increasing distance from the apex, only increasing in the oldest leaf sampled. In the petioles the Ca^{2+} concentrations decreased before increasing sharply in older samples, but in the stems the Ca^{2+} concentrations continued to decline with increasing distance from the apex. Leaf and stem concentrations of Mg^{2+} decreased with increasing tissue age, but increased in the petioles of *G. longicalyx* (data not shown). Gradients of ion concentrations in leaves of different ages have been reported widely in cereals (Bogemans *et al.*, 1990; Boursier *et al*., 1987) and in *Suaeda maritima* (Gorham and Wyn Jones, 1983), as has compartmentation between leaf sheaths and blades (Bhatti and Wieneke, 1984; Bhatti *et al*., 1993; Boursier and Läuchli, 1989; Boursier *et al*., 1987; Klagges *et al*., 1993). In woody dicots there is also evidence for compartmentation between stems, petioles and leaves (Chavan and Karadge, 1986; Gorham *et al*., 1988).

Inter-cellular compartmentation

A detailed X-ray microanalysis study was performed on *G. longicalyx* grown at 200 mol m^{-3} NaCl. Data from the region approximately 100 mm from the shoot apex is shown in Figure 4. The percentages of Na (corrected counts for Na as a percentage of total corrected counts) were much higher (and K much lower) in the stems than in the leaves or petioles, and this agrees with the data from hot water extracts shown in Figure 3. The percentages of K (Figure 4) were higher in the veinal regions of both the upper and lower leaf epidermis, and the percentage of Ca was higher in the interveinal than the veinal epidermal cells of the upper epidermis, but not of the lower epidermis. Higher percentages of Ca were found in some epidermal cells than in other cell types. P constituted a larger proportion of the measured elements in the xylem parenchyma than in other leaf cells. The highest percentage of S was found in the interveinal region of the upper epidermis. In the petiole (data not shown) the percentage of K was highest in the epidermis and lowest in the large central pith cells. The opposite trend was exhibited by Ca, which was highest in the central pith and the xylem vessels and low in the epidermal, subepidermal and xylem parenchyma cells. A few crystals were found in the central pith region, and these were mainly composed of Ca. Na was again a minor component, except in some of the xylem parenchyma cells. All of the xylem parenchyma cells contained high percentages of P. As noted above, the percentages of Na were very much higher, and those of K lower, in the stem than in the petioles and leaves. The main exception to this was the subepidermal region of the stem, which had the lowest Na and highest K percentages.

Further X-ray microanalysis data were obtained from preparations both younger and older than that examined in Figure 4. Except in the veinal region of the upper epidermis, the percentage of K in epidermal cells was lower in the younger than in the older leaves, while that of Ca was generally higher in the younger leaves. Here higher percentages of K and lower percentages of Ca were again observed in the upper and lower veinal epidermal cells compared with epidermal cells in the interveinal regions. This difference was not so pronounced in the older upper epidermis, while in the older lower epidermis the number of spectra which were obtained was insufficient for reliable statistical analysis. The trend for Ca to accumulate in the epidermal cells rather than in the spongy mesophyll or palisade cells was strongest in the younger leaf. The percentage of Na was higher in the older than in the younger leaf for all except the palisade cells. In the young leaf palisade cells the percentage of Cl was low, and replaced by high percentages of P and S. A high percentage of P was also found in the spongy mesophyll of the young leaf. In the petioles there were no consistent differences between elemental percentages in young and old leaves, except that the Ca percentage was significantly higher in the cortical cells of the young leaf than in the same cells in the older leaf.

Almost no Na was found in any cell type in the younger stems, whereas in the older stems it accounted for between 20 and 44% of the measured elements. Percentages of K were concomitantly lower in the older stems. They were higher in the young epidermal cells and tended to decrease towards the central pith. Ca percentages were highest in the young stem cortex (as in the young petiole cortex), and in the central pith cell and the concentric layer. The percentage of P was highest, and that of Cl lowest, in the xylem parenchyma cells of the young stem.

There is increasing evidence for variation in vacuolar elemental and ionic compositions between different tissues within leaves of cereals (Leigh and Tomos, 1993). Several studies have revealed large differences between epidermal and mesophyll cells of barley (Dietz *et al.*, 1992; Fricke *et al*., 1994a; Huang and Van Steveninck, 1989; Leigh and Storey, 1993; Leigh *et al.*, 1986; Williams *et al*., 1993). Fricke *et al*. (1994c) have further shown differences in solute composition between the upper and lower epidermis of barley, while Fricke *et al*. (1994b,d) have reported spatial variations within the leaf epidermis.

Phosphate was found mainly in the mesophyll of barley, with only low concentrations in the epidermis, whereas Cl^- accumulated in the epidermis (Dietz *et al*., 1992; Huang and Van Steveninck, 1989; Leigh and Storey, 1993; Williams *et al*., 1993). Chloride also accumulated in epidermal layers of both sheaths and blades of sorghum leaves (Boursier and Läuchli, 1989). In barley calcium accumulated in the epidermis rather than in the mesophyll (Dietz *et al*., 1992; Fricke *et al*., 1994a; Leigh and Storey, 1993; Leigh *et al*., 1986; Williams *et al*., 1993). In *Vicia faba* (Outlaw *et al*., 1984) and *Lycopersicon esculentum* (V. Cruz and R.G. Wyn Jones, unpublished) calcium and magnesium accumulated preferentially in the mesophyll and palisade cells, whereas phosphorus was accumulated in the epi-

dermis. In *Lupinus luteus,* Van Steveninck *et al*. (1982) also found higher proportions of phosphorus in epidermal cells than in spongy mesophyll or palisade cells, and higher proportions of chloride in the spongy mesophyll than in the upper or lower epidermis.

Data on the distribution of cations in shoots of *G. longicalyx* shown in Figure 3 suggest that the youngest leaves are strongly protected from the influx of Na^+, which accumulated primarily in the petioles and, to a lesser extent, in the stem. In older tissues the stem was also the main site of Na^+ accumulation. Thus there is a change in ranking for Na^+ and other ion concentrations between leaves, petioles and stems of different ages. These results point to the need for great care in choosing which tissues to sample for ion contents, and suggest that a complete picture cannot be obtained by sampling leaves of only one age.

Within a particular tissue, elements (measured by X-ray microanalysis) are not evenly distributed between all cell types. In the present case, however, it is not the concentration of the element which is important, but those of its ionized forms. In the cases of K, Na and Cl these elements will almost entirely be present as the monovalent ions. For S there will be some which is organically bound, and some which may be insoluble, although most is thought to be in the form of soluble sulphate. For P there is less certainty about the chemical forms involved, especially where it is not clear that the X-ray spectrum comes entirely from the vacuoles (as in the case of the xylem parenchyma). Data from roots of wheat and sorghum (Hodson and Sangster, 1989) suggest, however, that much of the P in the pericycle of these plants is vacuolar. In the case of Ca, the major question is how much is present as soluble Ca^{2+} ions. In only one instance (a petiole of *G. longicalyx*) were insoluble Ca salt crystals observed, and analysis of expressed (and centrifuged) sap indicated high concentrations of soluble Ca. Leigh *et al*. (1986), comparing expressed sap and acid extracts of barley leaves, concluded that the majority of the Ca and Mg in this species were also soluble.

Fricke *et al*. (1994c) described differences between the upper and lower epidermis of barley, where in fully expanded leaves Ca^{2+} accumulated in the upper epidermis and K^+ in the lower epidermis. This was not generally true in *Gossypium*, and in some cases higher Ca percentages were found in the lower than the upper epidermis.

Although the evidence presented above is incomplete, the following hypothesis can be advanced, at least for *G. longicalyx*. The very youngest leaves are protected from Na accumulation by K/Na exchange in the young stems and petioles (or Na is used to 'spare' K for use in these very young leaves). As the leaf elongates the Na in the petioles is either diluted by growth or exchanged again for K, which accumulates to high concentrations. As the petiole ages this K is replaced by Na (and presumably retranslocated). In older tissues Ca also replaces some of the K in the petiole. Replacement starts at the proximal end of the petiole and continues towards the distal (leaf) end (data not shown). Na replaces K mainly in the outer tissues of the petiole, whereas K/Ca exchange occurs predominantly in the central pith. The idea of a trap effect in the stem protecting younger parts of cotton (*G. hirsutum*) cultivars has, however, been questioned by Slama (1991).

Intra-cellular compartmentation

Halophytes have high leaf Na/K ratios, and the total K^+ level often decreases with increasing external NaCl. In many species increasing external NaCl results in reduced leaf K concentrations and it has been suggested (Storey and Wyn Jones 1978a,b and 1979) that Na could induce K-deficiency in some species, an observation confirmed by more recent work (Bernstein *et al.*, 1995; Botella *et al.*, 1997). Marschner (1971) and others had shown that Na^+ could partly replace the requirement some plants for K^+. Vegetative growth, especially on a fresh weight basis, of a number of halophytes was promoted by high concentrations of sodium salts, in some cases greater than 250 mol m^{-3}. This phenomenon is usually associated with an increase in leaf succulence and is mainly characteristic of dicotyledonous halophytes. Collander (1941) showed that halophytic species had a greater capacity to grow with high leaf salt loads. Why then can many halophytes accommodate high leaf NaCl concentrations and high Na/K ratios whereas salt (Na^+ and Cl^-) exclusion is associated strongly with enhanced tolerance in most glycophytes? An explanation for these contradictory observations is that K and Na have distinct roles and that K is selectively accumulated in the cytoplasm while Na^+ and Cl^- are occluded in the vacuole. Studies of a range of terrestrial and marine eukaryotic organisms indicate a strong cytoplasmic selectivity for K^+. Na^+ salts are more likely to be present in bathing fluids in animals, e.g., serum or blood in animals, or in vacuoles in plants (Wyn Jones and Pollard, 1983). On this basis Wyn Jones *et al*. (1977) proposed a model for solute distribution at the sub-cellular level (Figure 5) in which:

1. Cytoplasm accumulates K^+ to concentrations in the range 100 to 150 mol m^{-3}, with Na^+ and Cl^- limited to 30 to 50 mol m^{-3} or lower.
2. Excess Cl^- and Na^+ accumulate in the vacuoles to achieve osmotic adjustment thus dominating most bulk tissue analyses.
3. Osmotic equilibrium across the tonoplast under hyperosmotic conditions is achieved by the accumulation of compatible cytosolutes, e.g. betaine, proline, sorbitol, quebrachitol, (usually characteristic of a particular taxonomic group) at cell osmotic potentials above about 350 to 400 mosmol kg^{-1}.

One deficiency of the model is that it considers only the cytosol, whereas a large part of the volume of the cytoplasm consists of organelles such as the nucleus,

chloroplasts and mitochondria. In the case of chloroplasts there is evidence for high K/Na ratios and accumulation of the compatible solute glycinebetaine (Schroppel-Meier and Kaiser, 1988; Robinson and Jones, 1986). It should also be noted that major solutes such as K^+ and, in cotton, glycinebetaine, cannot be accommodated exclusively within the cytoplasm, but must also be present at high concentrations in the vacuoles. Following the work of Oertli (1968) a further compartment must be considered at least for inorganic ions. If ions are delivered to a tissue (with the transpiration stream) faster than they can be used for cell expansion, they may accumulate in the cell wall and associated apoplasm. Here they reduce the difference in water potential between the symplasm and the apoplasm resulting in a reduction in turgor pressure. The two main compatible solutes in cotton are glycinebetaine and proline. (I do not think that the term 'osmolyte' is useful in this context - nothing about it implies the special functions of compatible and protective solutes. Compatible solutes do not inhibit metabolism at high concentrations, and may also have a protective effect, reducing the inhibition caused by salt, for example.) These two solutes share the property of compatibility with enzyme activity and protein synthesis, but are very different in many ways. Glycinebetaine concentrations are higher than those of proline (30-50 mol m^{-3} compared with <1 mol m^{-3}), and the concentration of glycinebetaine increases more in absolute terms than that of proline, which may, however, show a higher percentage increase. Although direct evidence is difficult to obtain, simple calculations based on the relative volumes of the cytosol and the vacuoles show that to have a significant osmotic effect proline must be concentrated in the cytosol. Glycinebetaine is present in bulk samples at such high concentrations that cytosolic concentrations of several hundred mol m^{-3} would still leave most of the total glycinebetaine in the larger vacuolar compartment. Both solutes accumulate in response to salt and water stresses, but proline accumulation in salt stress is primarily a result of the osmotic component of the salt stress. Proline concentrations decline as a result of changes in synthesis or metabolism rates when the stress is removed, or after osmotic adjustment is achieved. Glycinebetaine is a metabolic dead end. Once accumulated it largely remains at the site of accumulation since there is little further metabolism and limited retranslocation in cotton. Further details and implications of this model are beyond the scope of this paper but have been reviewed by Wyn Jones and Gorham (2002).

Conclusions

Bulk analysis of the solute composition of cotton shoots (and roots) has limited value in relation to understanding the processes that occur during exposure to abiotic stress. There are considerable differences in the solute contents of organs, tissues, individual cells and subcellular compartments. Identifying these differences and integrating them with models of cotton growth and development are essential to an understanding of responses to abiotic stresses.

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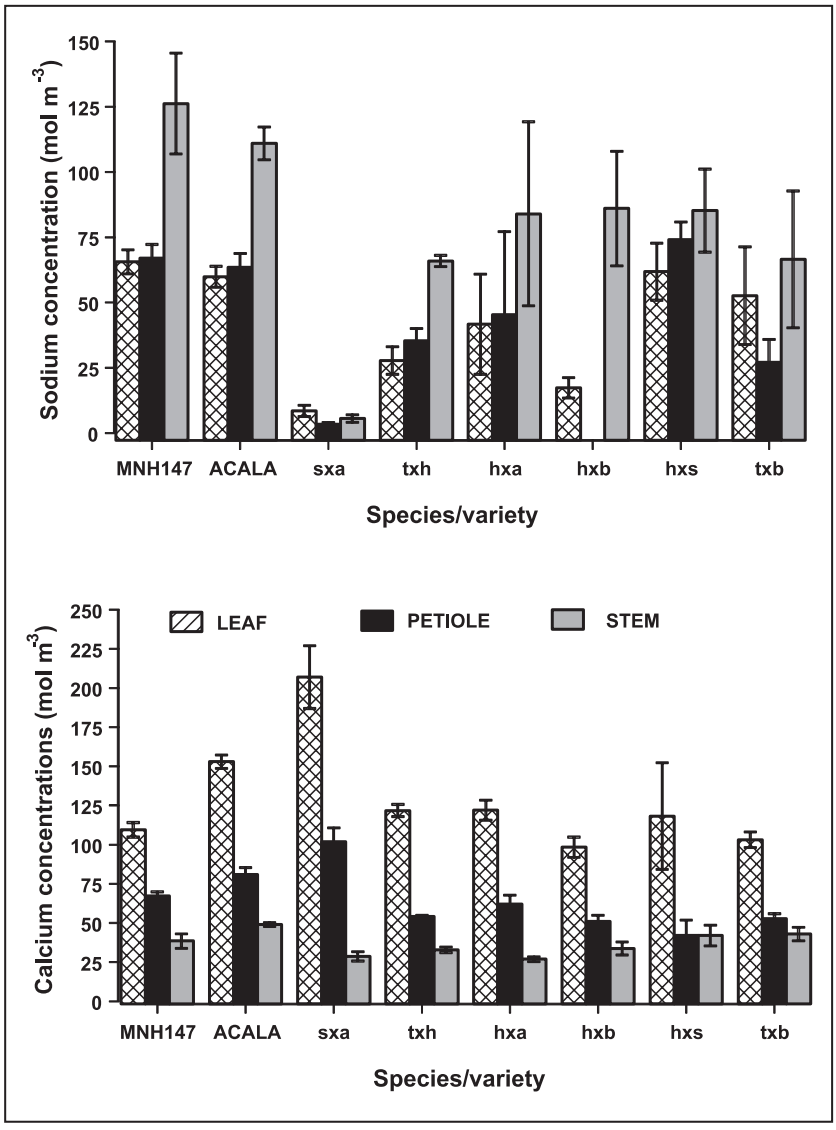

Figure 1.
Cation concentrations in expressed sap from leaves, petioles and stems of Gossypium *genotypes grown for four weeks at 250 mol m^{-3} NaCl + 12.5 mol m^{-3} $CaCl_2$ (Experiment 1).*

The genotypes are:

MNH147	G. hirsutum *cv. MNH-147*
Acala	G. hirsutum *cv. Acala SJ2*
Sxa	G. stocksii x G. hirsutum
Txh	G. trilobum x G. hirsutum
Hxa	G. hirsutum x G. australe
Hxb	G. hirsutum x G. bickii
Hxs	G. hirsutum x G. sturtianum
Txb	G. turneri x G. barbadense

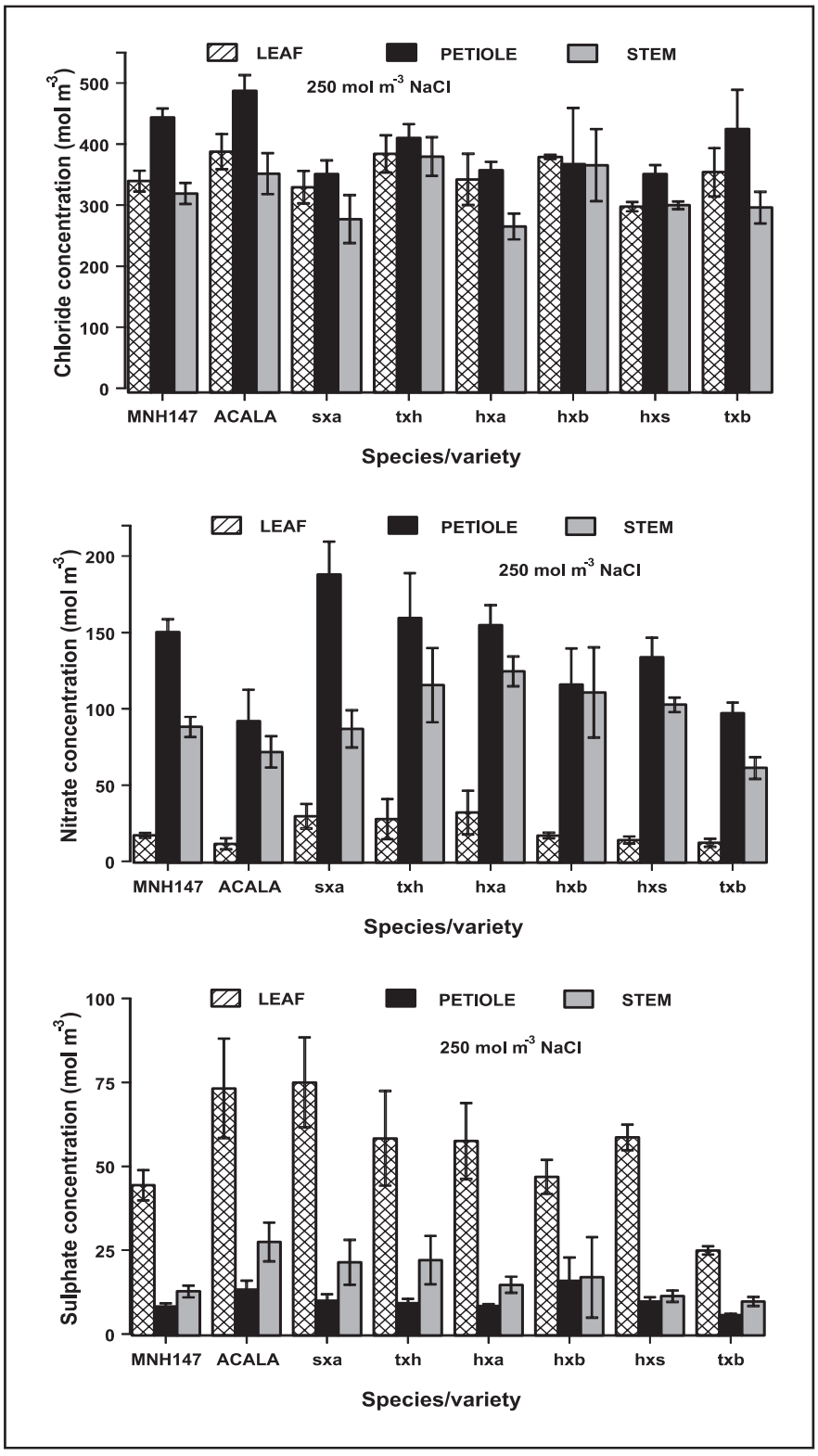

Figure 2.
Anion concentrations in expressed sap from leaves, petioles and stems of Gossypium genotypes grown for four weeks at 250 mol m^{-3} NaCl + 12.5 mol m^{-3} $CaCl_2$ (Experiment 1). The genotypes are as in Figure 1.

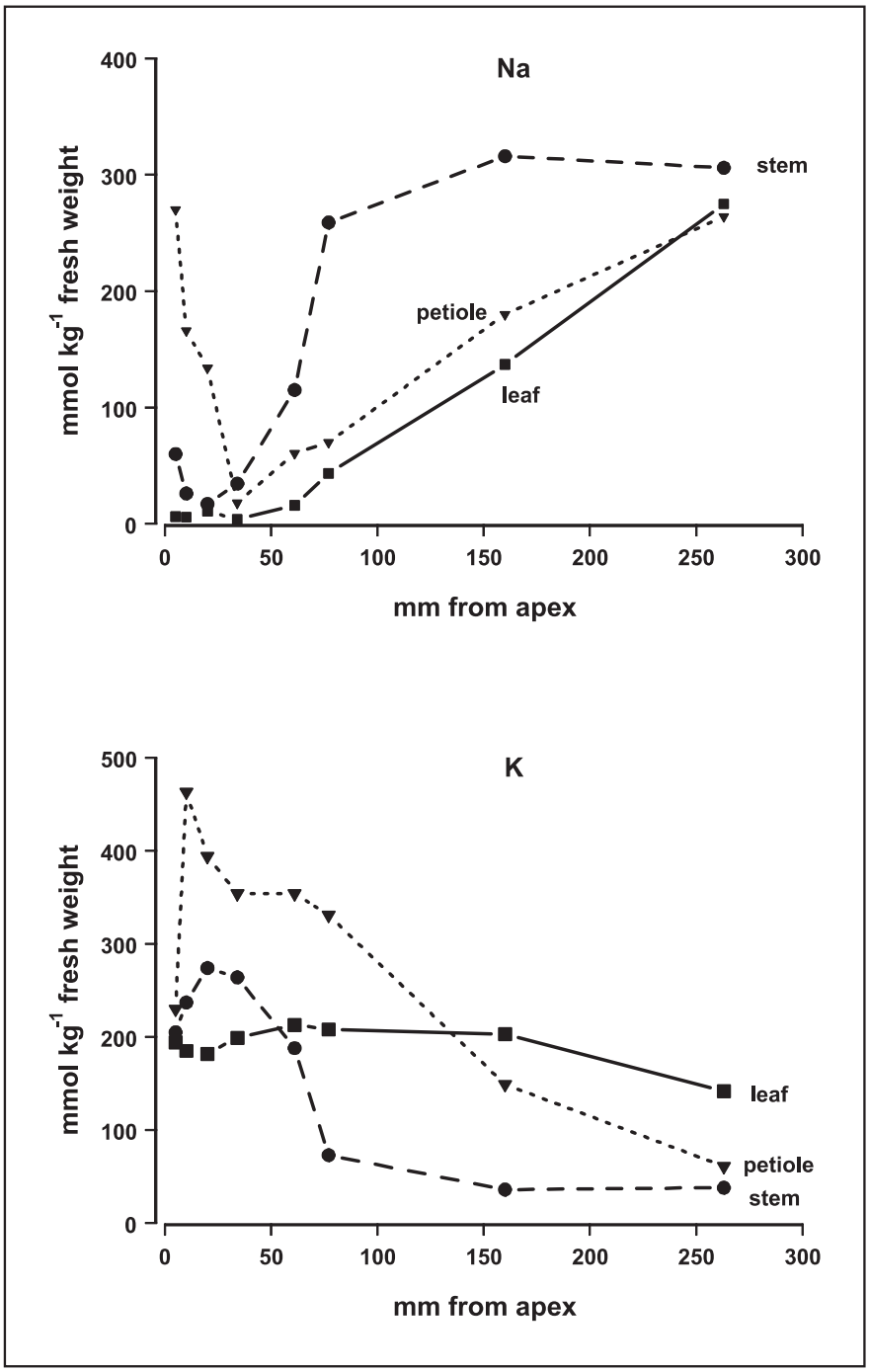

Figure 3.
Distribution of Na^+ and K^+ concentrations (mmol kg^{-1} fresh weight) in leaves, petioles and stems of a shoot of G. longicalyx *grown for 12 weeks at 200 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$ (Experiment 2).*

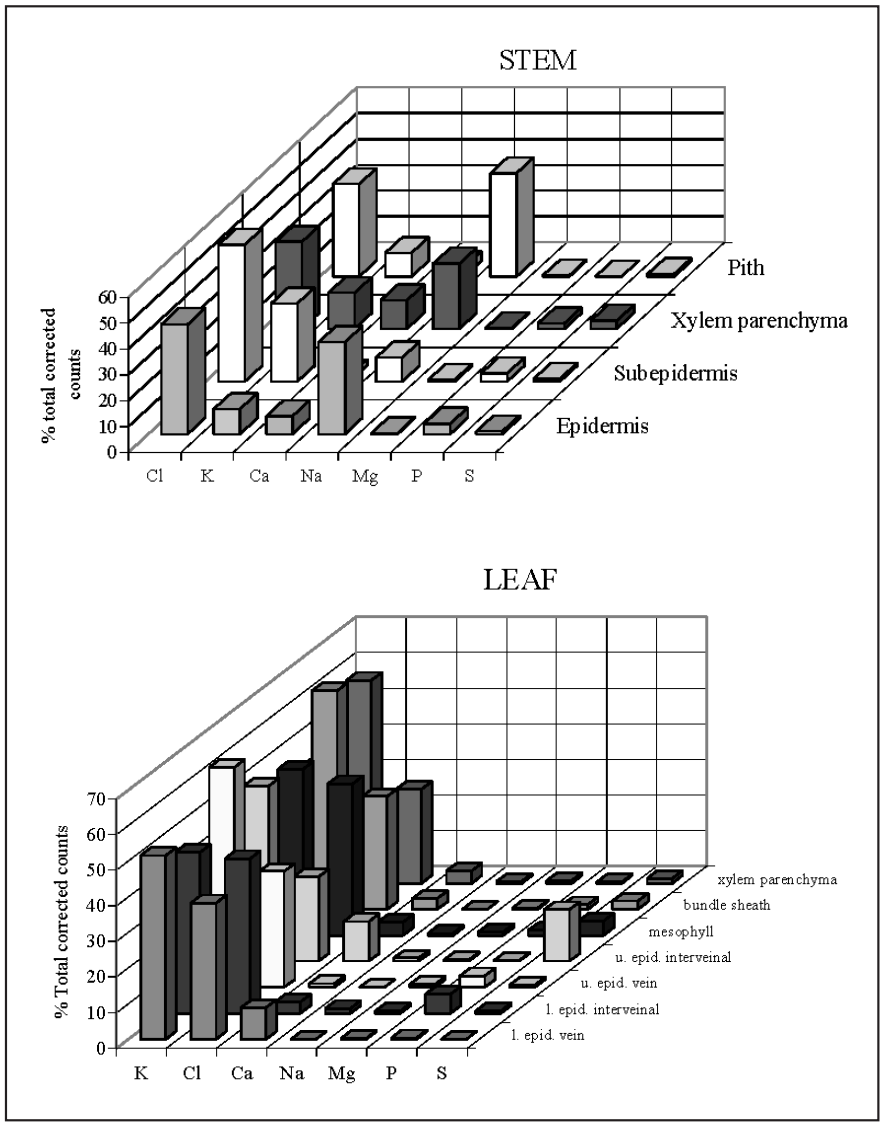

Figure 4.
X-ray microanalysis stem and leaf cells 100 mm behind the shoot apex of G. longicalyx. *Mean corrected values for individual elements are expressed as a percentage of the total corrected counts from each spectrum.*

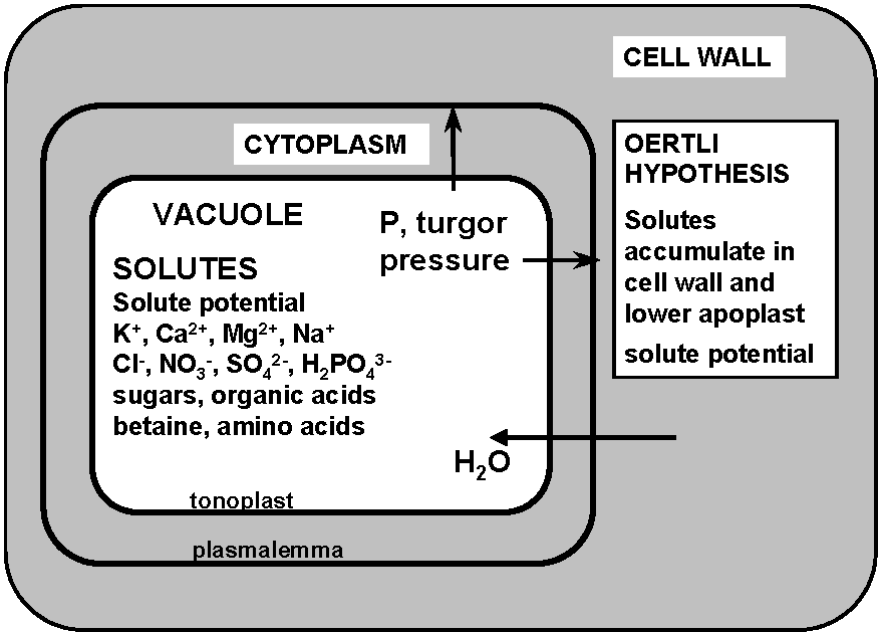

Figure 5.
A simplified model of intra-cellular solute compartmentation. Accumulation of solutes within the symplast (cytoplasm plus vacuoles) attracts water into the cell by osmosis. As water enters the cell the volume of the symplast tends to expand, but this is resisted by the cell wall, leading to a build up of hydrostatic pressure (turgor). Some solutes (mainly inorganic ions) cannot be accommodated in the cytoplasm for a variety of physiological reasons. In order to maintain an osmotic balance across the tonoplast (and maintain cytoplasmic volume and metabolite concentrations), alternative, compatible solutes such as glycinebetaine and proline are accumulated in the cytoplasm. If the supply of solutes (ions) to the apoplast around the cell (including the cell wall) exceeds the ability of the cell to take up the solutes, solutes will accumulate in the cell wall and lower the apoplast solute potential, and thus reducing the gradient in the water potential across the plasmalemma that drives osmosis.