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Review

Mechanisms of Salinity Tolerance in Plants[1]

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ABSTRACT

The mechanisms of salt stress response and tolerance have eluded definition despite reasonable success in defining their physiological manifestations. In this review, we consider the integrated salt metabolism of plants, essentially as a problem in meganutrient physiology. Two critical aspects of cellular and organismal metabolism are given particular attention—those involved in the control and integration of Na^+ acquisition and allocation in plants and those involved in readjustment of other aspects of metabolism, especially those involving carbon as a resource.

The responses of plants to salt and other environmental stresses have been important to students of agronomy, ecology, and physiology since the disciplines were first defined. It is, therefore, all the more frustrating that, in spite of years of research attention, the mechanisms which impart salt tolerance to some plants and sensitivity to others are still unresolved.

In this review, we will discuss these mechanisms using references selected as representative of recent work and as suitable entrance points to the relevant literature. We will restrict our consideration to Na^+ and to plants lacking salt glands or other excretory appendages (were we to emphasize Cl^- instead, the conclusions would be similar, but based on fewer data). Then, to avoid some of the problems associated with semantic differences, we will issue three simplifying proclamations. First, the mechanisms of salt tolerance cannot be known, because salt tolerance itself is a qualitative descriptor, largely reflecting correlations between size or mortality and external salinity. Second, the term salt stress is uninterpretable at the mechanistic level, because it is based on manipulations of an external environmental state which is only indirectly linked to readjustment of cellular and integrated organismal metabolism. Third, the usual classification of plants according to 'strategy' as 'includers' and 'excluders' imposes terminology sufficiently imprecise to obstruct the definition of mechanistic research problems. Even the most salt-sensitive plants accumulate salt when it is available, and the fact that degree of accumulation varies is not inherently important to the fundamental questions.

Therefore, it is more reasonable to be concerned with the metabolism of salt itself, essentially as a specialized problem in meganutrient physiology. For this review, we will designate the mechanisms of interest as (a) those involved in transport and in the control and integration of Na^+ acquisition and allocation in plants and (b) those involved in readjustment of other aspects of metabolism, especially carbon.

Transmembrane Sodium Movements. The majority of the research on Na^+ metabolism in plants has been concerned with the initial uptake across the 'root cell plasmalemma.' Beyond that, variations in the sophistication of the integrated transport network are responsible for the designations of salt includer and salt excluder, but it is unclear how many different types of transporters must actually be involved. At a minimum, it is likely that the plasmalemma and tonoplast have different systems, and though the salt relations of organelles in the cytosolic milieu have not been studied extensively, the apparent control of chloroplast Na^+ contents suggests an additional system there (13).

To date, however, only a tonoplast transport system has been addressed by *in vitro* studies. Blumwald *et al.* (2), for example, have claimed the existence of Na^+/H^+ antiport in tonoplast vesicles of sugar beet based upon the response to Na^+ of pH-dependent acridine orange fluorescence quenching. The Na^+ effect is increased by Na^+ pretreatment, sensitive to amiloride (an inhibitor of an analogous transporter in various animal systems) and to a number of promising amiloride analogs (2). Unfortunately, there have not so far been reports which have included direct measurement of Na^+ fluxes or confirmation of these results with different probes. Nevertheless, the results to date show promise for the difficult task of isolating and identifying a membrane ion transporter.

At the plasmalemma, if Na^+ entry is not always down a substantial electrochemical gradient, rather little addition of Na^+ to the external medium is required to make it so. In halophytes as well as mesophytes, the cell potential is determined by external K^+ and active H^+ efflux, and steady state potentials are largely insensitive to external Na^+. Under conditions of even moderate salinity (*e.g.* 50–100 mol m^{-3}), plants grow without extreme root Na^+ accumulation under conditions in which the cellular equilibrium Na^+ would be multimolar (3).

At the cellular level, the steady state must be maintained either by the very effective exclusion of Na^+ initially or by the extrusion or turnover of internal pools. No plant is a perfect excluder; even the most easily killed species have significant Na^+ levels in their roots. Rapid turnover rates, on the other hand, are probably common in both mesophytes and halophytes (3, 9). Influx appears to be passive down an electrochemical gradient and independent of either H^+ or K^+ movements. Both influx and efflux of Na^+ to roots are unresponsive to modifiers of plasmalemma H^+ pumping, energization level, or transport activity such as fusicoccin, *N,N'*-dicyclohexylcarbodiimide (DCCD) and *p*-fluoromethoxycarbonyl cyanide phenylhydrazone (FCCP) (3). Though the evidence is far from complete, it should also not be discounted that Na^+ movement involves mechanisms other than those mediated by transmembrane transporters; for example, it has recently been resuggested, based on very high estimates of unidirectional Na^+ movements, that the fluxes may involve vesiculation and turnover of a sub-cytoplasmic compartment (9).

Finally, there are numerous complexities in the study of Na^+ uptake and organismal response which cloud the interpretation of even apparently straightforward studies. These include the interactions of Na^+, Ca^{2+}, K^+, membrane surface properties, root cell development, and growth (5). Beyond this, the nature of the transport systems involved in the distribution and compartmentation of Na^+ at the organismal level is largely unknown. Though the potential, integrated system complexity is great, in-

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cluding, at least, sequestration within specific cells and tissues of the root, stem base, and leaves and retransport from shoots or sequestered pools to the roots for excretion, it is possible to model (schematically) the acquisition and allocation of Na^+ without additional basic cellular-level transporting systems.

Cellular Sodium Compartmentation. Beyond the initial uptake, the aspect of Na^+ metabolism which has received the most attention is cellular level compartmentation, and it is widely accepted that Na^+ must be excluded from the bulk cytoplasm. This hypothesis is, in part, based on the sensitivity of enzyme activities to very high NaCl levels *in vitro* (the fact that enzymes in general are equally tolerant [or intolerant] of high levels of K^+ [11] does not seem to concern many physiologists). It is also, in part, an outgrowth of the study of 'compatible osmotica.' Although the processes of physiological folklore have elevated this to a belief in the almost paranoic avoidance of cytoplasmic Na^+, it is not at all clear what levels of Na^+ are actually biochemically unacceptable.

If we consider plant responses to be 'of interest' only so long as there is continued growth without severe necrosis, cellular level Na^+ compartmentation may actually have limited physiological significance. For example, Seemann and Critchley (14), using x-ray microanalysis found little difference in cytoplasm plus chloroplast *versus* vacuolar Na^+ and Cl^- levels in bean leaves at 150 mM external NaCl, though by that point, growth was reduced 70%. Similarly, Robinson *et al.* (13) reported chloroplast Na^+ and Cl^- concentrations in spinach of about 100 mol m^{-3}. Chloroplast levels varied little with much larger variations in total leaf concentrations. More recently, Binzel *et al.* (1), using both x-ray microanalysis and compartmental efflux methods, reported cytoplasmic Na^+ concentrations of 100 mol m^{-3} in salt-adapted tobacco culture cells. In the root cortical cells of corn, Hajibagheri *et al.* (6) reported cytoplasmic Na^+ concentrations of 40 and 70 mol m^{-3} under 'salt-free' conditions. At a salinity of 100 mol m^{-3} externally, those levels rose as high as 140 mol m^{-3}. Vacuolar only exceeded cytoplasmic concentrations under conditions more saline than the species might normally be expected to survive. Thus, so long as the total Na^+ concentration in a tissue is below the level acceptable for the cytoplasm, postulates of more sophisticated compartmentation may not be required.

Organismal Integration. It should be obvious that without control of the quantity of salt that reaches the leaves, intracellular compartmentation would, in any case, be a very limited solution. Some higher level integrating mechanism must exist, regardless of its effectiveness at producing 'tolerance.' One major difficulty in discussing such a mechanism is that few studies have addressed it. It is quite common, for example, for transport studies to be restricted to root systems, even though a substantial portion of the total accumulated salt will be in the shoot. Similarly, Na^+ compartmentation studies in plants under realistic growth conditions generally emphasize the shoot.

The study of organismal growth and resource partitioning has drawn heavily on mathematical methods of analysis and modeling. Recently, emphasis has turned once again to consideration of relative shoot and root growth and to the internal distribution of carbon and other resources, particularly nitrogen. The apparent stability of physiological partitioning parameters once led to the concept of the functional equilibrium, now the regulated homeostasis, and recently the mathematical description of above and below ground 'perceived stress' (7). Though there are no mechanistic extensions of these concepts, *i.e.* no indications of the systems of communication and control involved, their existence is not unacceptable and the concepts are useful to physiological studies.

The acquisition and allocation of Na^+ at meganutrient but nontoxic levels are also demonstrably under control. The most simple manifestation of this is exemplified by those cases in which compartmentation of Na^+ to particular root cell types has been associated with exclusion from shoots. In rice, equally sophisticated control has been indicated by the exclusion of Na^+ from developing and young leaves and its preferential sequestering in older leaves (16). Thus, the sequential leaf development pattern may have associated systems producing discontinuous salt distributions, such that mature leaves gradually accumulate toxic levels of salt and protect younger leaves. Such control of distribution is well accepted for other nutrients, such as nitrogen, and is not unreasonably postulated for Na^+ in plants other than rice, dicots as well as monocots.

In the small coastal halophyte *Spergularia marina*, consideration of the relationship between Na^+ accumulation and growth under steady or variable environmental conditions has made it clear that the apparently simple 'inclusion' strategy of salt management is no less complex and sophisticated; the delivery of Na^+ to a shoot or leaf from which it cannot be removed must be highly integrated to growth in order that the Na^+ concentration remain within acceptable limits. In this species, Na^+ does not accumulate to high levels either within the shoot or within any age class of leaves throughout the preflowering period. Its co-regulation with, but independence from, the processes of K^+ accumulation and allocation has also been experimentally indicated. The control of Na^+ release to the shoot has been related to growth rate, to root:shoot ratio, and to plant size. When growth of *S. marina* was altered by changes in light intensity, for example, Na^+ transport was similarly altered such that Na^+ levels in the shoot remained constant (4).

Thus, we can conclude that control systems exist. We can describe their physiological manifestations, though perhaps not yet their underlying mechanisms. Next, we will consider the implications of the connection between Na^+ metabolism and growth and the control and integration of carbon acquisition and allocation.

Carbon Acquisition and Allocation. Both in halophytes and mesophytes, the effects of salinity clearly depend upon external factors which affect the rate of carbon acquisition. Effects of salinity on photosynthesis *per se*, as would be expected, include both stomatal and nonstomatal responses. For example, Seemann and Critchley (14) considered the effects of salt stress on the gas exchange characteristics of bean (*Phaseolus vulgaris*). Under conditions of severely reduced growth and leaf accumulation of Cl^-, in particular, stomatal limitations were manifested by a decrease in intercellular CO_2 and $\delta^{13}C$. Nonstomatal reductions reflected effects on both the photochemical processes (a decrease in quantum efficiency for CO_2 uptake) and on apparent, *in vivo* (but not *in vitro*) ribulose bisphosphate carboxylase activity. Both the total leaf N and carboxylase concentrations were little changed.

Robinson *et al.* (13) showed a similar result in their study of spinach leaves and chloroplasts, comparing plants grown with 0 and 200 mol m^{-3} NaCl. Overall growth was reduced approximately 65% as was stomatal conductance, which in this case became the more significant limiting factor in total carbon fixation. Leaf photosynthetic capacity, *i.e.* the maximal, unlimited rate per unit area or unit Chl, was altered only about 10%, however, and variable fluorescence was unchanged. In isolated, intact chloroplasts, CO_2-dependent O_2 evolution was reduced only 20 to 50%, and electron transport was unchanged by salinity.

It is now frequently noted, as it was in these studies, that the reduction of growth is greater than the decrease in realized or potential photosynthesis, and the reduction of shoot growth is much greater than the reduction of root growth. Such single point comparisons should be interpreted with caution, however. Their experimental convenience may have little to do with their biological information content because, first, they are based upon plants in that highly abnormal state, 'control,' in which there are

essentially no limitations placed on growth by resource availability; and second, the adjustment of growth or metabolism in a variable environment with limited resources is the quintessence of organismal function in plants. In defining the more general systems for control and integration, therefore, more attention must be directed toward the dynamics of adjustment as environmentally imposed limitations change.

Seemann and Critchley (14) commented in closing that the overall reduction in long-term growth probably reflected the reduction in carbon allocation to new leaves and long-term potential photosynthesis. In source-sink studies, it has frequently been shown that an increase in carbon usage may increase the rate of fixation. The importance of photosynthetic responses in the organismal context should, therefore, also consider the fate of the fixed carbon. The range of responses was nicely demonstrated in a comparative study of salinity responses in three San Francisco Bay area halophytes by Pearcy and Ustin (12). They demonstrated that at moderate salinity (for these plants) salinization was accompanied by an *increase* in photosynthetic capacity (per unit leaf area) with little change or a slight *increase* in net fixation under growth lighting and CO_2 conditions. Overall growth responses were considerably different, however, ranging from stimulation to severe reduction, reflecting the degree to which carbon was reallocated from shoot to root growth. In *Salicornia*, the small growth response was associated with a 33% decrease in root-shoot ratio. In *Scirpus*, a drastic reduction in total growth (80%) was associated with a 90% increase in root-shoot ratio.

It is broadly acceptable that total carbon usage can be partitioned to growth (production of cell walls and integral cellular machinery), maintenance (turnover and repair), transport (generally not separable from maintenance in practice), and storage. Analysis of that partitioning is by no means trivial, and there are numerous large gaps in our understanding of it. The best resolved and reviewed effects of salinity are those on maintenance costs of shoots. As would be expected, the maintenance respiration of rapidly growing plants is generally much higher than that of more slowly growing, enviromentally less responsive species. Salinity-induced changes are also greater. This probably reflects, in part, the additional costs of transport associated with 'exclusion.' Increased maintenance costs, however, cannot explain all loss of growth.

Particularly important to the understanding of salinity responses is allocation or diversion of carbon to storage, and increases in carbohydrate accumulation with salinity have been known for at least 40 years. Though this use does not result in loss of carbon from the plant (*i.e.* it is not measured as respiration), it may well remove it from the pool available for immediate metabolism or growth. For example, storage would include accumulation of nonstructural carbon in association with osmotic adjustment and turgor maintenance. It might be expected, then, that the degree of that accumulation would be related to the extent of salt exclusion from the shoot; includers have the alternative of salt accumulation for adjustment.

It is clear from numerous similar studies of water and salt relations, however, that turgor maintenance alone does not assure continued leaf expansion (10, 15). It may be that photosynthetic capacity is insufficient to provide the carbon both for wall synthesis and for turgor-driven cell expansion. Or, it may be that some higher level controls operate to limit expansion in spite of the available turgor potential. Munns and Termaat (10), for example, argued that shoot growth was not limited by the lack of substrate. Instead, the existing carbohydrates were metabolically unavailable for wall synthesis, and they supported the hypothesis that the controlling message originated in the roots.

Conclusion. Thus, for carbon as for Na^+, the importance of organismal integration of response to salinity conditions is clear. How, if at all, can we achieve a better understanding of the processes or mechanisms involved in that integration? We can start by dismissing the explanation that the increase in cellular salt content creates an environment unsuitable for cellular biochemistry. Though this could explain lethal responses of single cells, it fails otherwise. Organisms are complex, their control systems are sophisticated, and that must be allowed and appreciated in seeking mechanisms.

It follows both logically and empirically (based on years of plant breeding efforts), that there will be no single gene or gene product which determines 'salt tolerance.' On the other hand, it is not much comfort to realize that after years of study, we have not identified a single gene or gene product directly involved in Na^+ (or Cl^-) metabolism.

The next obvious approach is to compare the mRNA or protein synthesis patterns in plants under different growth conditions. The limitations of such an approach, however, are well illustrated by an excellent study of Hurkman and Tanaka (8). Though they found two major proteins that increased in barley roots under salinity stress, there were no less than 46 visible differences in control and salinized plants and 32 in the microsomal fraction alone. Assignment of these to primary and secondary, positive and negative, or related or unrelated responses is a daunting project. Clearly, comparison of species or even cultivars is no more helpful.

There is no simple way around this barrier, but one tool which should be explored in more depth is the use of single-gene mutants defective in some aspect of salt metabolism. This approach has proved useful, for example in the study of primary carbon metabolism; though no one gene may confer tolerance, absence of any number of single genes may confer intolerance. Still, the technique is not certifiably problem free, and we should not forget that 30 years after the wilty mutants of tomato were generated, the single altered genes are still unidentified. It takes, of course, little imagination to develop a long list of potentially insurmountable problems in this case as well, but with some imagination, we can at least find hope. For example, we can speculate about the possibilities of generating mutants by transformation, in which case the original genes might be recoverable.

Finally, we can hypothesize mechanistic understanding of a type not immediately within the purview of molecular genetics. Such understanding requires, instead, the powers of physiologists as synthesizers of the obvious and purveyors of verisimilitude. Because it may be difficult to accept that such truths are possible, we will consider a simple analogy. The in-depth understanding of the mechanisms by which washing machines operate will not suffice to explain how a pile of dirty laundry on a child's bedroom floor becomes neatly folded in the bureau drawer. More component systems are required, and each must be integrated in an overall scheme of laundry management. Further, barring the need for repair, the overall system can be quite satisfactorily understood and manipulated with no understanding of the machines themselves. In plants, the analogous systems underlie organismal integration and resource management. Despite current pejorative connotations, these are the systems for which 'descriptive understanding' is still badly needed. And these are the systems which, in any integrated, organismal analysis, are the mechanisms of salinity tolerance in plants.

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