Tansley Insight

Salinity tolerance of crops – what is the cost?

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Contents

Summary

Soil salinity reduces crop yield. The extent and severity of salt-affected agricultural land is predicted to worsen due to inadequate drainage of irrigated land, rising water tables, and global warming. The growth and yield of most plant species is adversely affected by soil salinity, but varied adaptations can allow some crop cultivars to continue to grow and produce a harvestable yield under moderate soil salinity. Significant costs are associated with saline soils: the economic costs to the farming community, and the energy costs of plant adaptations. We briefly consider mechanisms of adaptation and highlight recent research examples through a lens of their applicability to improving the energy efficiency of crops under saline field conditions.

Keywords: salinity, salt, tolerance, yield, cost, energy, mechanism, adaptation

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I. Soil salinity and its economic costs

Soil salinity can reduce crop, horticulture and forage production in arid and semi-arid regions. Salt may arise naturally in the subsoil, or be introduced by brackish irrigation waters. Salinity is becoming more extensive due to land clearing and unsustainable irrigation practices, and through pressures for bringing marginal land into production. Agronomic and engineering solutions are being exhausted, so to minimise the impact of saline land on global food production the way forward is to breed greater salt tolerance into present crops, and to introduce new species for cultivation.

In this article we focus on the costs of soil salinity. One cost, relevant to farmers, is the economic cost of reduced yield (Box 1). The second cost – and the cause of the reduced yield that underpins the economic loss to the farmer – is the energy cost incurred by the plant when exposed to soil salinity. We briefly consider these two costs and highlight recent research with potential for improving crop salinity tolerance.

II. Mechanisms of plant adaptation to saline soil and potential energy costs

The majority of energy acquired by photosynthesis and fixed into carbon (C) compounds is used by plants in general maintenance (Amthor, 2000; Jacoby *et al.*, 2011). Only a small proportion (10-40%) is used directly for biomass accumulation even under optimal conditions (Figure 1). Stress can be defined in terms of energy costs; we consider stress to be occurring when the amount of energy acquired by plants is reduced (due to a reduction in photosynthetis rate or leaf area) and/or when energy is redistributed from growth into stress defence (Figure 1). By improving the energy efficiency of plant metabolism and physiology, especially during floral development and grain fill, more fixed C could be allocated to grain, improving yield. When crops are exposed to stress, the less energy plants need to use in tolerating salt the more will be available for grain yield (Figure 1).

Plants deploy a variety of traits to combat salt in soil solution. The most essential trait is osmotic adjustment – all cells must accumulate sufficient solutes to balance extra osmotic pressure in the soil solution to maintain turgor. To achieve this, plants use two strategies to varying degrees: (A) excluding Na^+ and Cl^-, particularly from leaves, and relying on organic solutes for osmotic adjustment ("ion exclusion"); or, (B) ~~allowing~~ accumulating ~~uptake of~~ sufficient Na^+ and Cl^- to balance that in the soil solution but having strict ionic regulation in various cell compartments ("tissue tolerance").

In general, salt tolerant species have high Na^+ and Cl^- concentrations in leaves – higher than the external solution. This is particularly true for halophytes and the more salt tolerant non-halophytes like barley, where the trait of tissue tolerance is clearly evident. Such plants must compartmentalise most of the leaf Na^+ and Cl^- in vacuoles to keep the cytosolic and organellar concentrations below toxic levels, and use organic osmolytes (and K^+) to balance the osmotic pressure in these cytoplasmic compartments (Shabala, 2013). The concentration where Na^+ (or Cl^-) becomes toxic in the cytoplasm is unclear, and is a priority area for research; cytosolic estimates are ~30 mM Na^+ (Munns & Tester, 2008; Conn and Gilliham, 2010), whereas chloroplasts and mitochondria appear to tolerate 100–200 mM Na^+ and Cl^- (Flowers *et al.*, 2015). Estimates of osmotic adjustment costs using organic molecules versus Na^+ and Cl^- (Greenway & Munns, 1983; Raven, 1985; Yeo, 1983) indicate that the energy demands are significant and could restrict growth rates at high salinity, either in the diversion of C or N compounds from growth to storage pools, or in costs of controlling Na^+ and Cl^- transport across membranes. Table 1 indicates that 200 mM NaCl is the limit of growth for species that have low Na^+ and Cl^- concentrations in leaves and rely on organic solutes for osmotic adjustment.

The more sensitive species tend to have low Na^+ concentrations in leaves, lower than in the external solution, i.e. they rely on "ion exclusion" as the major adaptive trait. ~~Interestingly, there is a paradox that seemingly contradicts the notion that "tissue tolerance" is the most cost-efficient strategy.~~ Within any species, where significant genotypic variation in Na^+ accumulation in leaves exists, there is a correlation between salt tolerance and Na^+ exclusion. This is true for sensitive species like rice and durum wheat (reviewed in Munns, 2005), but it may also be true for the more salt tolerant species like barley (e.g. Chen *et al.*, 2005). This presents ~~Interestingly, there is~~ a paradox that seemingly contradicts the notion that "tissue tolerance" is the most cost-efficient strategy; it~~.~~ ~~This~~ indicates that there are significant costs of compartmentation of Na^+ and or Cl^- in leaf cells, and that reducing the salt load on a leaf confers a benefit. This presumably becomes important over time when the initial osmotic adjustment has occurred, and salt toxicity threatens as ions continue to be transported to leaves.

Future work is needed to quantify the costs of the different traits for salt tolerance to test the limits of each strategy, and to provide new ideas for research approaches to increase the salt tolerance of crops.

III. New insights into salinity tolerance mechanisms

Salinity research is predominantly performed on model systems. Very few fundamental research findings relevant to salinity tolerance have been applied to crop plants. An exception is the application of AtNHX1 (Na^+/H^+ antiporter 1 proteins) to improve salt compartmentation in the vacuoles of tomato vegetative tissue, which improved yield without increasing salt in the tomato fruit (cited in Bassil & Blumwald, 2014). More recently, *AtCIPK16*, a SNF1-related kinase/CBL-interacting protein kinase underlying a QTL for Na^+ exclusion in the *Arabidopsis thaliana* Bay-0 x Shahadara mapping population, was expressed in barley and found to improve Na^+ exclusion and biomass in a saline field (Roy *et al.*, 2013). Here, we discuss recent research that has the potential for improving salt tolerance in the field, which is also summarised at both the cellular and organ level in Figure 2.

Cellular mechanisms of salt tolerance

Reactive oxygen species (ROS) act as a signal during salt stress but can also damage plant root and shoot tissue during salinity stress by perturbing enzyme, cell wall and membrane function. Several genes involved in ROS detoxification have been cloned from SR3 wheat which is a hybrid with a high level of salinity tolerance (e.g. Dong *et al.*, 2013). Overexpression of genes involved in ROS scavenging have resulted in lower cellular damage, the maintenance of photosynthetic energy capture, and an improvement in shoot and root growth under saline conditions (Roy *et al.*, 2014). Many of these transgenics have reduced growth under non-saline conditions so the energetics of ROS detoxification is important to quantify, as are the implications of ROS detoxification on final grain yield.

Ion transport can account for the majority of respiratory costs in plants (Van der Werf *et al.*, 1988). Ion transporters (e.g. Osakabe *et al.*, 2014) and their localisation in key cell-types underpin plant salinity tolerance. Root xylem parenchyma cells represent 'gatekeeper' cell types for shoot NaCl exclusion as they have a physical location and unique protein circuitry primed for this role (Henderson & Gilliham, 2015). TaHKT1;5-D is responsible for maintaining high cytosolic K^+/Na^+ ratios in bread wheat shoots; it underpins the *Kna1* locus, resides on the (PM) membrane of root xylem parenchyma cells and reduces Na^+ load in the xylem prior to entering the shoot (Byrt *et al.*, 2014). Orthologous proteins in sequence and function are found in Arabidopsis, durum wheat and rice (Henderson & Gilliham, 2015).

Introgression of the *Triticum monococcum* (*HKT1;5-A*) into durum wheat improved shoot Na^+ exclusion and improved grain yield in the field by 25% (Munns *et al.*, 2012). Other salt tolerance factors expressed in the root stele include the *salt overly sensitive* (*SOS*) pathway genes and *AtCIPK16* (Roy *et al.*, 2013). Root stelar cells also confer control shoot Cl^- accumulation, which can induce salinity toxicity in crops, however, we know little about the proteins involved (Henderson *et al.*, 2014).

Aquaporin proteins, a large multigenic family that regulate a large proportion of water transport across membranes, are rapidly influenced both transcriptionally and post-translationally by salt (Chaumont & Tyerman, 2014). Overexpression of a PM intrinsic protein in soybean increased shoot Na^+ exclusion and increased seed yield from a saline field (Zhou *et al.*, 2014). Wheat TIP2;2 is regulated by methylation following salt treatment (Xu *et al.*, 2013), as is HKT1 in Arabidopsis (Sani *et al.*, 2013). The role of methylation and aquaporins in salt tolerance is worth further exploration.

The emerging role of the endomembranes and endosomes

In salt-acclimated tobacco BY2 cells (Garcia de la Garma *et al.*, 2015) reported extensive vesicle trafficking of Na^+ between the PM and the Na^+-rich vacuolar compartment. This novel mechanism of salt deposition presumably avoids raising cytosolic Na^+, so its application is worth further exploration. The role of RAB6 GTPase ARA6 and VAMP727-mediated endocytotic machinery in salt tolerance was also shown in Arabidopsis roots; when *ARA6* was overexpressed it improved salt tolerance whereas *ara6/vamp727* knockout plants were salt hypersensitive (Ebine *et al.*, 2011).

The CPA1 family of Na^+/H^+ antiporters, NHX1 (tonoplast-localised) and NHX7/SOS1 (PM-localised) are often reported to confer Na^+ compartmentation or exclusion under high salt loads but their role is less clear under moderate salinities. Double knockouts of *nhx1/nhx2* are not ~~salt~~ sensitive to moderate external Na^+ concentrations, whereas they are sensitive to moderate external K^+ concentrations (reviewed in Bassil & Blumwald, 2014). In contrast, the trans-golgi network-localised NHX double knockouts, *nhx5/nhx6*, are hypersensitive to moderate salinity and disrupt vesicle trafficking to the vacuole (Bassil & Blumwald, 2014). Another CPA family member, a cation/H^+ exchanger (CHX), GmSALT3, improves shoot Na^+ exclusion and salt tolerance in soybean (Guan *et al.*, 2014). CHX proteins, including GmSALT3, have frequently been localised to the ER using fluorescent protein fusions. If these membrane localisations are to be trusted this is further evidence to suggest that

endosomal-localised transport proteins have crucial roles in salt tolerance – possibly in endosomal pH or cation homeostasis, or vesicle trafficking, but their exact roles are still to be determined.

Can we better exploit beneficial soil micro-organisms to improve salinity tolerance?

Rhizospheric fungi and plant growth promoting rhizobacteria (PGPR) can increase plant yield under stressed and non-stressed conditions (De-la-Pena & Loyola-Vargas, 2014; Nadeem *et al.*, 2014). Salt tolerant PGPR populations can reduce Na^+ content of shoots, increase the expression of stress responsive transcription factors, induce greater proline synthesis, enhance ROS scavenging, and improve plant biomass under salinity stress (De-la-Pena & Loyola-Vargas, 2014; Nadeem *et al.*, 2014). Arbuscular mycorrhizal fungal colonisation of roots can improve plant salt tolerance by increasing water acquisition and shoot K^+ whilst decreasing shoot Na^+ concentration ~~content~~ (Auge *et al.*, 2014). Therefore, treatment with rhizospheric organisms is an attractive option to improve crop yields under saline conditions, so the quantification of their costs and expansion of trials to the field should be encouraged.

How does root system architecture ~~(RSA)~~ influence salinity tolerance of cereals?

Root systems are key to improving crop salt tolerance through their potential for improving access to water and nutrients and limiting salt acquisition (Jung & McCouch, 2013). Salt, reportedly through its osmotic effects, decreases root epidermal cell division and elongation rates reducing primary root growth but initiating lateral root development in Arabidopsis and wheat (Rahnama *et al.*, 2011; Jung & McCouch, 2013). This would assist plants to mine non-saline areas for water and minerals until exploitation of saline areas is necessary. In the field, soil salinity is always heterogeneous and usually increases with depth. A complex set of intersecting hormone-mediated pathways control ~~RSA~~ root system architecture in Arabidopsis (Jung & McCouch, 2013), with the mechanisms little explored in crops (Rogers & Benfey, 2015). Arabidopsis roots exposed to a band of high NaCl in sterile culture exhibit negative halotropism, i.e. they grow away from salt. This asymmetric root growth response is initiated by a rise in cytosolic Na^+ mediated by clathrin-mediated endocytosis of the PIN-FORMED 2 (PIN2) auxin efflux carrier, and an active redistribution of the auxin gradient to the side of the root facing the salt (Galvan-Ampudia *et al.*, 2013). Whether halotropism exists in crops, rather than root growth being inhibited purely by decrease water potential of the soil, is yet to be reported and the costs of changing ~~RSA~~ root architecture are unexplored.

IV. Better yield under non-saline conditions equals better salt tolerance?

Elite cultivars that perform particularly well under optimal conditions are also often best yielding under water-limited conditions (Richards *et al.*, 2014), and this principle may apply to saline conditions as long as the enhanced yields are due to energy-efficient processes. Overexpression of the Arabidopsis vacuolar proton pumping pyrophosphatase (H^+-PPase) improves the salinity tolerance of various crop species under controlled conditions and was shown by Schilling *et al.* (2014) to increase growth and yield of transgenic barley under saline conditions in both greenhouse and field. Notably, the *AtAVP1* overexpressing barley also produced greater shoot biomass and grain yield *under non-saline conditions*. The mechanism for these improvements were unclear and is the source of further research (Schilling *et al.*, 2014). The transgenic manipulation of a crop to improve yield under both control and saline conditions is an exciting development and warrants further exploration.

In semi-arid regions, phenology is a primary factor determining grain yield. Worryingly, climate change models predict increased temperature and decreased rainfall in certain semi-arid regions (Anwar *et al.*, 2015). This may leave crops susceptible to terminal droughts, and very high salt concentrations in the soil during grain filling, which reduces grain size. Planting and flowering time is therefore crucial to maximise opportunities for photosynthetic capture and translocation of photosynthate to grain. Salinity affects flowering time, and can delay or advance it according to species and level of salinity (Munns & Rawson, 1999; Kim et al., 2013). Research that has recently highlighted novel genes that have an impact on this salinity-flowering time interaction are summarised in Supporting Text S1. Further understanding of the molecular controls of flowering time and their interaction with soil salinity is needed to explain and exploit the difference in the salt-induced phenology responses between genotypes and species.

Whilst the salinity tolerance of many cereals remains poor, breeders are still producing yearly incremental improvements in grain yield. It has been suggested that the narrowing crop genetic diversity following domestication and intensive breeding has reduced the potential for large gains in stress tolerance (Munns *et al.*, 2012). Useful natural variation clearly exists in 'exotic' cereals, for instance many Tibetan wild barley lines show higher than normal levels of salt tolerance in terms of biomass accumulation (Wu *et al.*, 2011). Exploiting such germplasm has great potential for improving crop salt tolerance.

V. What does the future hold for stress tolerance research?

This insight has highlighted mechanisms with potential for improving crop stress tolerance. It has also highlighted that we lack basic information on the energy costs of salinity tolerance. There is a rationale for revisiting questions posed over thirty years ago and quantify the costs of salt to plants. The challenge is to gain quantitative data for the role of specific salt tolerance mechanisms at the genetic level through to single cells and whole plants so we can develop models that predict which pathways lead to energy gains. The desired outcome will be the informed selection of crops with lower energy costs and greater yields. A rigorous understanding of the plant economy when faced with salt, and the natural variation that exists in this economy will provide a foundation for a targeted approach to crop breeding for stressful environments that has not yet been possible.

A role of pre-breeding is to provide germplasm to breeders that produces significant increases in yield in stressful environments (e.g. Schroeder *et al.*, 2013). Fundamental research on Arabidopsis has led to interesting insights into salt tolerance mechanisms, but how much of this can be applied to crop plants in the field? (Figure 2). Affordable next generation sequencing and novel transformation techniques now allow fundamental research to be performed on crops. The greater available natural variation within crops, and their more complex genomes, will likely lead to greater yield improvements than has been possible with model plants, providing tangible research impacts towards food security targets.

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Table 1. Demand for organic solutes (in hexose units) versus Na^+ and Cl^- for osmotic adjustment (OA). The contribution of osmotica to the dry weight is calculated from the concentration needed to balance the osmotic pressure of the external solution and the water content of a typical cereal leaf. (Note that 1 mole of NaCl is equivalent in osmolarity to 2 moles of hexose, and the molecular weight of hexose and NaCl is 180 and 60 respectively.) Most plants accumulate sucrose as the preferred organic osmotica, which has twice the mass as does hexose, thus setting 200 mM NaCl has an upper limit for growth in saline soil as it would comprise 70% of the dry weight.

$NaCl_{ext}$	Leaf H_2O/DW	**Hexose for OA**		**NaCl for OA**	
mM	g/g	g/L H_2O	g/g DW	g/L H_2O	g/g DW
50	6	18	0.102	3	0.018
100	5.5	36	0.198	6	0.033
200	5	72	0.360	12	0.060
300	4.5	108	0.486	18	0.072

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Box 1. The economic cost of salinity

The major economic cost of salinity is the reduced income to farmers caused by the reduced yield. Areas where salinity occurs are always arid or semi-arid, so crops are always limited by water, but they can also be limited by the salt concentration in the soil especially when rainfall is below average.

The economic costs differ from one country to another, and are influenced largely by the cost of farmer inputs versus the profit they can make in the seasons with average rainfall. In broad-acre dryland farming, the inputs to the crop (including off-farm subsidies) may cost as much as \$300 ha^{-1}. The water-limited yield may be 3 tonnes ha^{-1}, and at a good market price (say \$200 per tonne) the crop will return a gross income of \$600 ha^{-1} with a net return of 300 ha^{-1}. If the yield is reduced by salinity to 2 tonnes ha^{-1}, the gross income drops to \$400 $ha^{-1}$and the net return is only \$100 ha^{-1}. Consistent losses to salinity due to climate change or rising water tables may mean that cropping is impossible, and the land usage reverts to pasture production, using salt-tolerant grasses or other species including halophytes. This usually brings a much lower return to farmers, but the inputs are fewer, so farming may still be viable. Farmers have many other expenses on the farm and are always living close to the margin of profit or loss, and a small decrease in yield or an enforced change in land use may have devastating economic consequences.

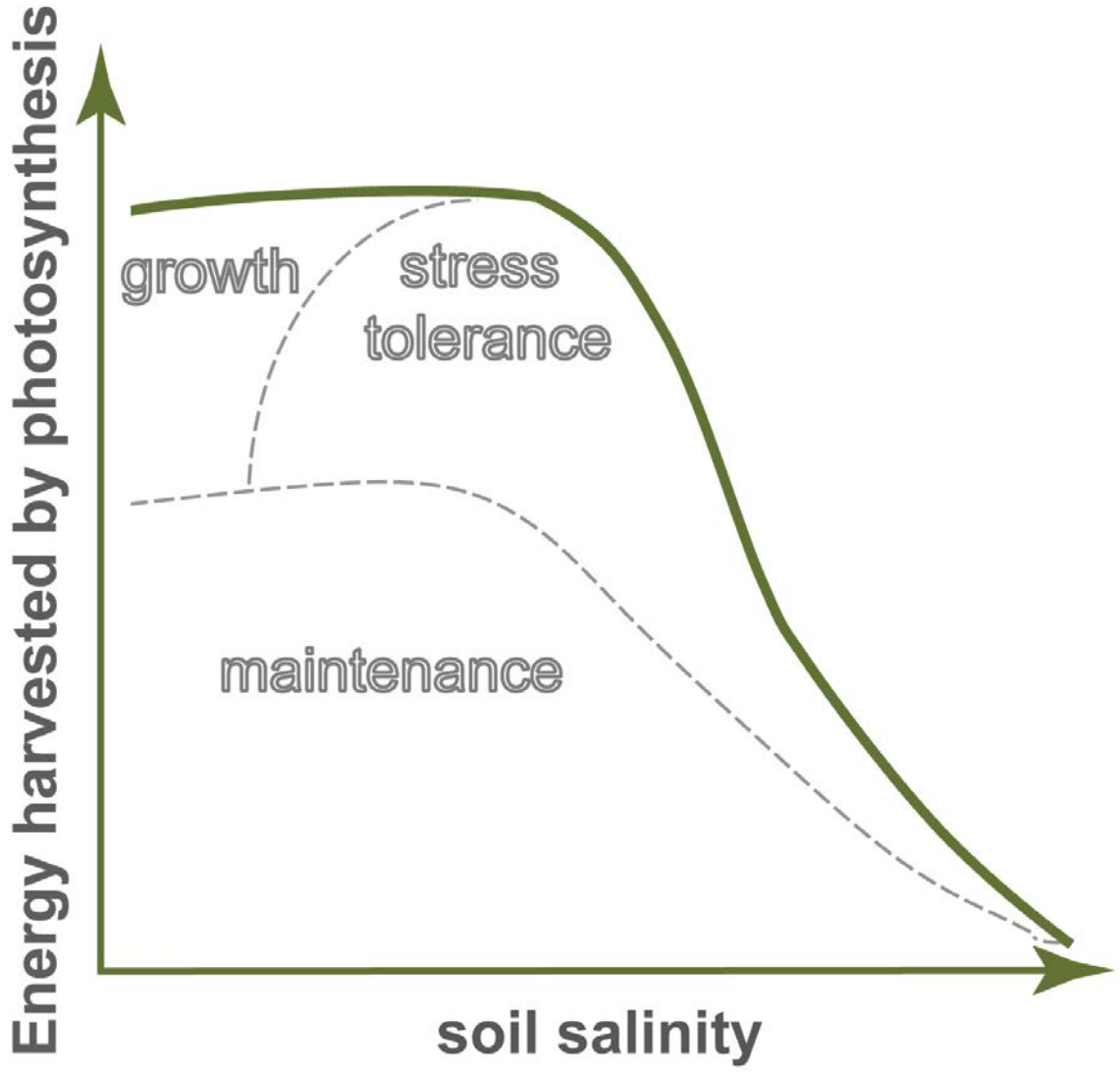

Figure 1. Schematic of energy gain and use of a crop plant under salinity stress. At any given time there is a finite amount of energy that can be harvested by the plant through photosynthesis. Plants use the majority of this energy in processes necessary for maintenance of biomass including protein turnover, synthesis of lipids and carbohydrates, maintaining ion gradients, gaining nutrients and source to sink transfer. Growth also requires the investment of energy in these processes, whether this is biomass accumulation or grain yield depends on the developmental stage of the plant. The proportion of energy used in maintenance, growth and stress defence is portrayed under the dotted lines. The relative proportions will change depending on the developmental stage of the plant – maintenance costs will be greater when plants are larger. Total energy gain will decrease with greater salinity by decreasing photosynthetic rate following induced closure of stomata and damage to cellular and photosynthetic machinery. Stress tolerance mechanisms represent additional costs to the plant

required to deal with the salt load in the soil (for example but not limited to greater costs in ion exclusion or compartmentation, and ROS detoxification). At high salinity there will be zero growth as the total costs to the plant equal energy gain, when costs exceed energy gained then tissue will senesce. Adapted from concept by A.H. Millar and H. Lambers, based on data and reasoning of Amthor, 2000 and van der Werf *et al.*, 1988.

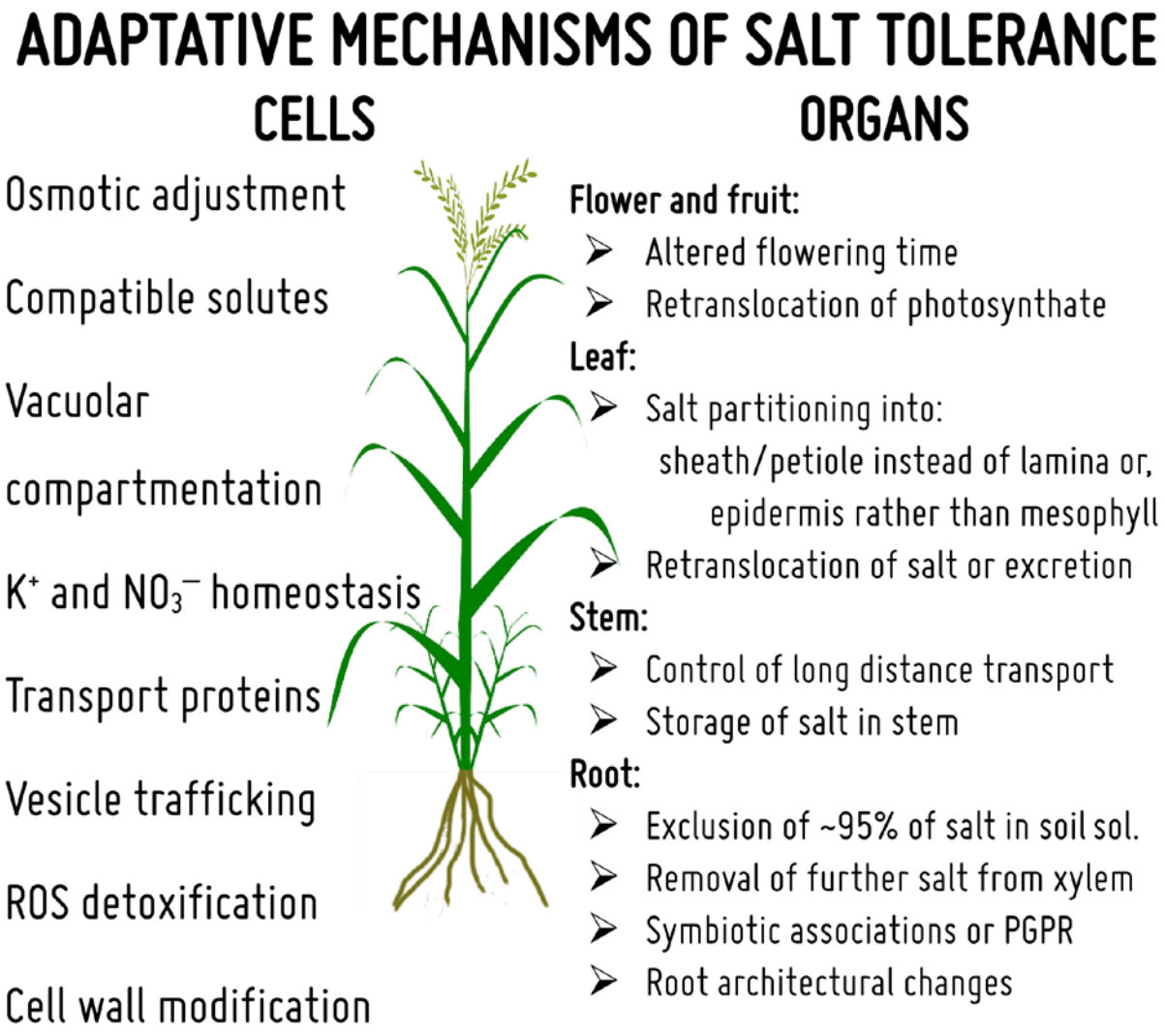

Figure 2. Adaptive mechanisms of salt tolerance. On the left are listed the cellular functions that would apply to all cells within the plant. On the right are the functions of specific tissues or organs. Exclusion of at least 95% (19/20) of salt in the soil solution is needed as plants transpire 20 times more water than they retain (Munns, 2005). Most of these functions are explained in the text. Omitted for space, and lack of recent advances, is limitation that Cl^- can impose on growth through its antagonistic accumulation against the nitrogen form NO_3^- (NO_3^- homeostasis) (Henderson *et al.*, 2014) and the differential capacity and sensitivity of different cell types and tissues to accumulate Na^+ and Cl^- e.g. NaCl accumulation within photosynthetic cells incurs a larger cost than accumulation in root cortical cells (Conn and Gilliham, 2010).

Supporting Text S1.

The impact of flowering time on salinity tolerance

In Arabidopsis, salinity has been shown recently to delay flowering through at least two mechanisms. Salt-induced protein degradation of GIGANTEA (GI) resulting in delayed activity of the *Flowering Locus* (*FT*) (Kim *et al.*, 2013) and activated expression of *Brother of Flowering Time Locus* (*BFT*) with the resulting protein competing with FT for binding to the bZIP transcription factor (FD), which is required for floral initiation (Ryu *et al.*, 2014). Interestingly, Kim *et al.* (2013) also found that GI interacts with SOS2 (salt overly sensitive)/CIPK24 and upon salt degradation of GI, SOS2 is released to activate the plasma membrane Na^+/H^+ antiporter SOS1, which results in greater cytoplasmic Na^+ exclusion. This provides a link between flowering and a key mechanism of salt tolerance first found in Arabidopsis, which appears to also be active in wheat. TdSOS1 in wheat (Feki *et al.*, 2014) was upregulated by salt – as is *AtSOS1* – and can complement the mutant phenotype of the *Atsos1* knockout.

Feki K, Quintero FJ, Khoudi H, Leidi EO, Masmoudi K, Pardo JM, Brini F. 2014. A constitutively active form of a durum wheat Na^+/H^+ antiporter SOS1 confers high salt tolerance to transgenic Arabidopsis. *Plant Cell Reports* **33**: 277-288.

Kim W-Y, Ali Z, Park HJ, Park SJ, Cha J-Y, Perez-Hormaeche J, Javier Quintero F, Shin G, Kim MR, Qiang Z, Ning L, Park HC, Lee SY, Bressan RA, Pardo JM, Bohnert HJ, Yun D-J. 2013. Release of SOS2 kinase from sequestration with GIGANTEA determines salt tolerance in Arabidopsis. *Nature Communications* **4:**1820.

Ryu JY, Lee H-J, Seo PJ, Jung J-H, Ahn JH, Park C-M. 2014. The Arabidopsis floral repressor BFT delays flowering by competing with FT for FD binding under high salinity. *Molecular Plant* **7**: 377-387.