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Salt resistant crop plants

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Soil salinity is a major constraint to agriculture. To improve salinity tolerance of crops, various traits can be incorporated, including ion exclusion, osmotic tolerance and tissue tolerance. We review the roles of a range of genes involved in salt tolerance traits. Different tissues and cells are adapted for specific and often diverse function, so it is important to express the genes in specific cell-types and to pyramid a range of traits. Modern biotechnology (marker-assisted selection or genetic engineering) needs to be increasingly used to introduce the correct combination of genes into elite crop cultivars. Importantly, the effects of introduced genes need to be evaluated in the field to determine their effect on salinity tolerance and yield improvement.

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Introduction

Soil salinity is a major environmental constraint to crop production, affecting an estimated 45 million hectares of irrigated land, and is expected to increase due to global climate changes and as a consequence of many irrigation practices [1,2]. The deleterious effects of salt stress on agricultural yield are significant, mainly because crops exhibit slower growth rates, reduced tillering and, over months, reproductive development is affected [2]. The ultimate aim of salinity tolerance research is to increase the ability of plants to maintain growth and productivity in saline soils relative to their growth in non-saline soils — that is, to reduce effects of salinity on growth and yield. A range of biotechnologies can facilitate this by speeding gene (and allele) discovery and speeding the delivery of crops with improved salt tolerance (using both marker-assisted selection and genetic modification).

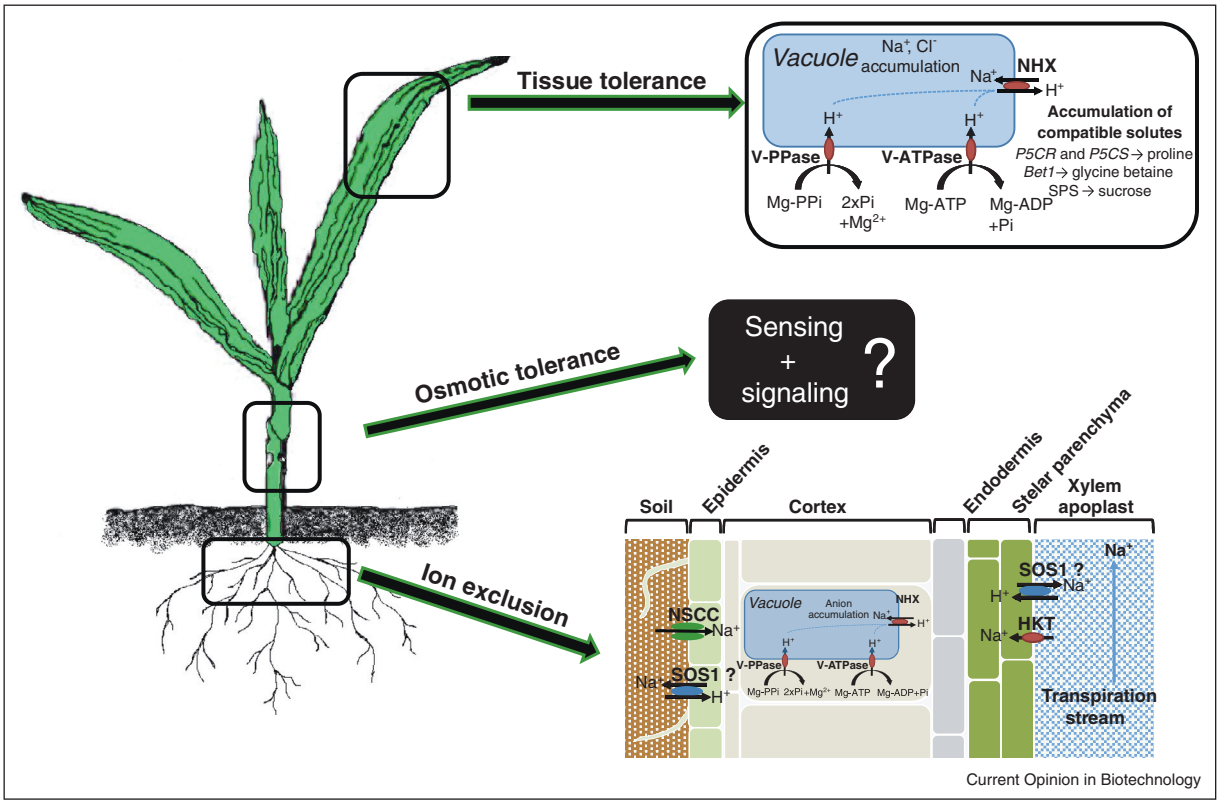
Growth is reduced by salinity via several quite distinct processes, which are related either to the accumulation of salt in the shoot, or which are independent of shoot salt accumulation. These can be experimentally distinguished by measuring effects immediately (within minutes to a few days) upon addition of salt (before there has been time for salt to accumulate in the shoot) or measured after much longer times (several days to weeks), after there has been time for salt to accumulate in the shoot and affect shoot growth. Within minutes of application of salt in an experimental system, there are several rapid responses occurring. Because of their rapid onset, these effects are clearly independent of the accumulation of salts in the shoot. The two best-documented effects are stomatal closure, with concomitant increases in leaf temperature [3]; and inhibition of shoot elongation [4,5]. Hence, the primary consequence is the overall reduction in production of new leaves and a significant reduction in shoot growth. This was termed the 'osmotic phase' by Munns and Tester [2], but there is evidence consistent with it not being due just to the effect of salt on water potential [4]. It is perhaps better described as a 'shoot salt accumulation independent effect'.

In the second phase of plant responses to salinity, there is a slower onset inhibition of growth (occurring over several days to weeks), which is due to accumulation over time of salt, especially in the older leaves, causing the premature senescence of those older leaves. This is termed the 'ionic phase' of salt toxicity. This is due to both the accumulation of salts, and the inability of the shoot to tolerate the salt that has accumulated to toxic concentrations [2].

Mechanisms of salinity tolerance — overview

Just as salinity has many different effects on a plant, so there are also many mechanisms for plants to tolerate this stress. These mechanisms can be classified into three main categories: firstly, osmotic tolerance, which is regulated by long distance signals that reduce shoot growth and is triggered before shoot Na^+ accumulation; secondly, ion exclusion, where Na^+ and Cl^- transport processes in roots reduce the accumulation of toxic concentrations of Na^+ and Cl^- within leaves; and finally,

Figure 1



The three main mechanisms of salinity tolerance in a crop plant. Tissue tolerance, where high salt concentrations are found in leaves but are compartmentalized at the cellular and intracellular level (especially in the vacuole), a process involving ion transporters, proton pumps and synthesis of compatible solutes. Osmotic tolerance, which is related to minimizing the effects on the reduction of shoot growth, and may be related to as yet unknown sensing and signaling mechanisms. Ion exclusion, where Na^+ and Cl^- transport processes, predominantly in roots, prevent the accumulation of toxic concentrations of Na^+ and Cl^- within leaves. Mechanisms may include retrieval of Na^+ from the xylem, compartmentation of ions in vacuoles of cortical cells and/or efflux of ions back to the soil.

tissue tolerance, where high salt concentrations are found in leaves but are compartmentalized at the cellular and intracellular level (especially in the vacuole) (Figure 1).

Very little, if anything, is known about tolerance to the 'osmotic phase'. This process must involve rapid, long-distance signaling, perhaps via processes such as ROS waves [6•], Ca^{2+} waves (Simon Gilroy, personal communication), or even long distance electrical signaling [7]. Differences in osmotic tolerance may be due to differences in this long-distance signaling, or they may involve differences in the initial perception of the salt or differences in the response to the signals. This is still an area of salinity research with many unknowns, and further research is required to obtain a better understanding of osmotic tolerance (Figure 1).

Much more is known about the 'ionic phase', which is due to the accumulation of Na^+ and Cl^- in the leaf blade (a trait that is relatively easy to phenotype). Plants can reduce toxicity during the ionic phase by reducing accumulation of toxic ions in the leaf blades (Na^+ and Cl^- exclusion), and/or by increasing their ability to tolerate the salts that they have failed to exclude from the shoot, such as by compartmentation into vacuoles (tissue tolerance) (Figure 1). Both of these processes involve a range of transporters and their controllers at both plasma membrane and tonoplast [8,9]. Tissue tolerance, involving the removal of Na^+ from the cytosol and compartmentalizing it in the vacuole before the ion has a detrimental effect on cellular processes, is also likely to require the synthesis of compatible solutes and higher level controls to coordinate transport and biochemical processes, thus having a role in both osmoprotection and osmotic adjustment [2,10] (Figure 1).

Suffice to say, it is clear from this brief overview that there are many mechanisms of salinity tolerance, and many of these can be present in a particular plant. To date, there is neither evidence that these mechanisms are mutually exclusive (i.e. that, e.g., ion exclusion prevents tolerance to the 'osmotic phase' of salt toxicity), nor that a particular

plant is committed to only one strategy (e.g. a plant may have ion exclusion as its primary tolerance mechanism at moderate salinity, but then has tissue tolerance as its main tolerance mechanism when the exclusion processes are 'swamped' at high salinity). It is possible that some tolerance mechanisms are more effective in particular circumstances. For example, Na^+ exclusion may be more effective in conditions of higher salinity (e.g. see [11••]), whereas 'osmotic tolerance' may be more important in moderately saline conditions. Interactions with other abiotic stresses, such as low water availability, are also likely to be important.

As such, it is clear that salinity tolerance can be complex and involve many genes, as has been pointed out for several decades — for example, programs designed to specifically introgress salinity tolerance using traditional breeding methods appear to have frequently failed (as measured by the apparent absence of commercial products), which has usually been attributed to the multigenic nature of salinity tolerance [12,13]. It is therefore necessary not to study the molecular genetic basis of salinity tolerance as a particular trait in itself, but to study the mechanisms of traits that are hypothesized to contribute to salinity tolerance. The most intensively studied of these traits is exclusion of Na^+ from leaf blades, mainly because it is relatively straightforward to phenotype. Focusing on this has led to significant increases in salinity tolerance, as measured by yield in the field, at least in durum wheat (which has poor Na^+ exclusion) when grown in highly saline sites where yield has already been greatly reduced [11••,14].

Differences in traits must then be correlated with differences in tolerance, as measured by performance in the field — yield in a saline site relative to yield in a less saline site. Traits can be measured in any system that enables the trait to be quantified, using necessary experimental manipulations, such as described earlier to access the 'osmotic tolerance' trait — key is to test the relevance of the trait being measured with salinity tolerance in field conditions.

Candidate genes likely to contribute to tolerance traits in crops

There are numerous candidate genes that might be usefully used to transform crops to improve salinity tolerance. We focus in this review on the three main traits that are proposed by Munns and Tester [2] to be the primary mechanisms for salinity tolerance — shoot ion exclusion, shoot tissue tolerance and 'osmotic' tolerance — and propose genes that might contribute to each of these traits. A particular gene (or gene family) may well contribute to more than one trait, just as one trait can be conferred by more than one gene. We discuss a particular gene family in the section where it is likely to be making a significant contribution — this does not preclude members of this gene family also contributing to other traits, and we try to acknowledge this in the text below. We have taken this approach to try to encourage a focus more on the traits that contribute to salinity tolerance, rather than on the traditional functional categorizations of gene families. This is an attempt to come more 'from the plant's perspective' of functional effects of the genes at the whole plant level, rather than categorizing genes based on the immediate activity of their encoded proteins.

- Ion exclusion
  The *high affinity potassium transporter* (*HKT*) gene family [2,15–22] and the salt overly sensitive (SOS) pathway [23–29] have both been implicated in having an important role in regulating Na^+ transport within a plant. Manipulation of the expression of these genes has been frequently reported to alter accumulation of Na^+ in the shoot (Table 1 and Supplementary Table 1). However, to date the application of this knowledge into generating successful crop plants in the field has been limited. Of the two families, the *HKT1* group of *HKTs* have perhaps the greatest potential for improving the salinity tolerance of crops, frequently appearing as the most likely candidate for quantitative trait loci when phenotyping for salt tolerance and/or Na^+ exclusion in mutant and mapping populations [30–33]. A marker assisted selection (MAS) approach was used successfully to incorporate novel *HKT* alleles from *Triticum monococcum* to improve the salinity tolerance of durum wheat [11••,14]. In contrast, transgenic approaches to improve salinity tolerance using *HKT1*s have been only moderately successful (Table 1 and Supplementary Table 1). An *HKT2* has been reported to increase salinity tolerance, although not through Na^+ exclusion [34•]. These genes appear to require cell type specific expression to be effective (Table 1 and Supplementary Table 1) [35]. If stress inducible and cell type specific expression of these genes can be realized in an effective way in crops, the potential for improving crop salinity tolerance through ion exclusion is possible.

- Shoot tissue tolerance
  To date, three main mechanisms contributing to shoot tissue tolerance have been targeted: accumulation of Na^+ in the vacuole, synthesis of compatible solutes and production of enzymes catalyzing detoxification of reactive oxygen species (Table 1 and Supplementary Table 2). Increasing the abundance of vacuolar Na^+/H^+ antiporters (NHX), vacuolar H^+ pyrophosphatases (e.g. AVP1), proteins involved in the synthesis of compatible solutes (such as proline and glycinebetaine) and enzymes responsible for the detoxification of reactive oxygen species have had differing degrees of success in improving crop salinity tolerance (Table 1 and Supplementary Table 2). Although there is still uncertainty about the primary ions being transported by NHX proteins *in planta* [36,37,38•] (and the potential role of these proteins in K^+ transport needs

Table 1

Genes that have been overexpressed to improve specific salinity tolerance traits in crops

Transgene	Gene isolated from	Promoters used	Transgenic crop	Reported transgenic plant performance during salt stress
Ion exclusion (transporters)				
Na^+/H^+ antiporter (*SOS1*)	*Arabidopsis*	Constitutive	Tobacco	Altered shoot and root accumulation of Na^+ and K^+
Na^+/H^+ antiporter (*SOD2*)	*Salicornia brachiata*	Stress inducible	Rice	Improved biomass production
Na^+ transporter (*HKT* subfamily 1)	Yeast	ABA responsive	Barley	Improved germination
Na^+/K^+ transporter (*HKT* subfamily 2)	Barley			
Na^+ ATPase (*ENA*)	*Physcomitrella patens*			
Tissue tolerance (transporters/proton pumps)				
Na^+/H^+ antiporter (*NHX*)	*Arabidopsis*	Constitutive	Buckwheat	Improved shoot and root biomass production
Na^+/H^+ antiporter (*nhaA*)	*Atriplex gmelini*		Cotton	Altered Na^+ and K^+ accumulation
Vacuolar H^+ pyrophosphatase	Rice		Tomato	Increased proline content
(*vacuolar H^+-PPase*)	Cotton		Poplar	
	Hordeum brevisubulatum		Kiwifruit	
	Pennisetum glaucum		Fescue	
	Aeluropus littoralis		Rice	
	Salicornia brachiata		Wheat	
	Salsola soda		Brassica	
	Malus domestica		Bentgrass	
	E. coli		Sugar beet	
			Alfalfa	
			Tobacco	
			Apple	
Tissue tolerance (Compatible solutes)				
Trehalose-6-phosphate synthase (*TPS*)	Yeast	Constitutive	Alfalfa	Increased compatible solute accumulation
Trehalose-6-phosphate phosphatase (*TPP*)	Rice	Stress inducible	Tomato	Improved plant survival
Mannitol-1-phosphate dehydrogenase (*mt1D*)	*E. coli*	Shoot expression	Rice	Increased growth
L-myo-Inositol-1-phosphase synthase (*MIP*)	*Porteresia coarctata*	Protein often targeted to chloroplast	Tobacco	Reduced wilting
Myo-inositol O-methyltransferase	*Mesembryanthemum crystallinum*		Wheat	Maintenance of photosynthetic efficiency
Betaine aldehyde dehydrogenase (*BADH*)	Spinach		Sweet potato	
Choline oxidase/dehydrogenase (*codA*/*betA*)	Moth bean		Wheat	
Δ1-pyrroline-5-carboxylate synthetase (*P5CS*)			Potato	
Tissue tolerance (Degradation of reactive oxygen species)				
Ascorbate peroxidase (*APX*)	*Arabidopsis*	Constitutive	Tobacco	Maintenance of photosynthetic efficiency
Glutathione S-transferase (*GST*)	Tomato	Protein often targeted to chloroplast or cytosol	Rice	Maintenance of growth
Superoxide dismutase monodehydroascorbate reductase (*MDR*)	Tobacco			Improved maintenance of photosynthesis
Catalase	Mangrove			Improved germination
	Pea			Improved growth of seedlings
	E. coli			Increased antioxidant enzyme activity
Signaling/regulatory pathways				
Calcineurin-B like interacting protein kinases (*CIPK*)	*Arabidopsis*	Constitutive	Barley	Altered Na^+, K^+ and Cl^- accumulation
Mitogen-activated protein kinase (*MAPK*)	Chickpea		Tobacco	Improved biomass production

Table 1 (*Continued*)

Transgene	Gene isolated from	Promoters used	Transgenic crop	Reported transgenic plant performance during salt stress
Sucrose nonfermenting1-1 type protein kinase	Rice		Rice	Reduced leaf senescence
	Apple Cotton Tomato		Tomato	
Transcription factors				
DREB	*Pennisetum glaucum*	Constitutive and inducible	Tobacco	Improved germination
AP2/ERF	Cotton		Wheat	Improved biomass
MYB	Soybean		Tomato	Improved chlorophyll retention
NAC	Tomato Rice Chrysanthemum Wheat		Rice	Altered Na^+ accumulation

to be kept in mind [38•]), and a new role has recently been proposed for AVP1 [39•], salinity tolerant plants appear to have been developed by the overexpression of *NHX* and vacuolar pyrophosphatase genes (Table 1 and Supplementary Table 2). While approaches to improve the tissue tolerance of crops through increasing compatible solutes (Supplementary Table 2) and enzymes involved in ROS metabolism (Table 1 and Supplementary Table 2) also appear to have been successful, there are often reports of low performance by the transgenic lines in low stress environments [40–45]. Such negative effects might be avoided by use of tightly regulated stress-inducible promoters [41,46–49].

With a large number of papers reporting success in increasing plant salinity tolerance by improving shoot tissue tolerance (Table 1 and Supplementary Table 2) in the greenhouse or growth room, particularly by improving accumulation of Na^+ in vacuoles, it might be tempting to suggest this is the best mechanism for improving crop performance. However, there is, as yet, insufficient quantitative data and field trial data available to be able to draw such a conclusion. Exclusion of ions from the shoot has frequently been shown to be an important salt tolerance mechanism. MAS for improved salinity tolerance through improved exclusion has been successful in the field [11••,14]. The limited success to date of transgenic manipulations to increase ion exclusion, for example using the *HKT* gene family, is more likely to be due to the inability to express important exclusion genes in a cell type specific manner rather than a reflection of either the candidate gene or the effectiveness of the tolerance mechanism. HKT1 proteins are reported to affect salinity tolerance by retrieving Na^+ from the xylem in roots [16,17,19–22]. However, it is often the case that genes encoding transporters involved in the transport of Na^+ across the plasma membrane cannot be constitutively overexpressed, unlike those genes encoding transporters involved in ion tissue tolerance that transport Na^+ across the vacuole. The cell type in which the transport of Na^+ across the plasma membrane occurs has a fundamental effect on the overall accumulation in the whole plant [9]. To retrieve Na^+ from the root xylem, enhanced Na^+ influx into stelar cells are required — a phenotype that is opposite to that desired in root epidermal cells, where Na^+ influx should be minimized. Therefore successful manipulation of transporter genes involved in ion exclusion processes requires a cell type-specific expression, which is not yet possible in most crops.

- Osmotic tolerance

  We are currently not aware of any specific candidate genes for osmotic tolerance, although some genes highlighted above may be involved. Differences in osmotic tolerance are likely to involve long-distance signaling, control of cell cycle and processes involving perception of signals from the roots in the shoots. Given crop plants will usually be exposed to low levels of salinity throughout the growing season, or levels of salinity that start low but which build up toward the end of the growing season, genes encoding for osmotic tolerance traits have the potential to have a significantly larger impact on the salinity tolerance of crops than those involved in ion exclusion. The introgression of *TmHKT1;5-A* from *T. monococcum* into the durum wheat, Tamaroi, resulted in a significant improvement in grain yield in salt stressed, field grown durum by increasing its ion exclusion, but only in plots with highly saline soils [11••,14]. However, the yield of Tamaroi with *TmHKT1;5-A* was similar to that observed in the Tamaroi cultivar without the introgressed gene, under low and moderate saline conditions [11••,14] suggesting that osmotic stress was having a greater effect on the end yield of these plants growing in low to moderate salinity, than ionic stress. The identification of genes for osmotic tolerance should therefore be a priority for improving salinity tolerance of crops growing in low to moderate saline soils.

- Signaling/regulatory pathways
  An attractive alternative to manipulating specific salinity stress tolerance mechanisms is to adjust the detection, signaling and regulatory pathways involved in global salinity tolerance. Potentially altering one component of these pathways can have significant secondary effects for downstream processes, such as ion exclusion, tissue tolerance and osmotic tolerance. ROS signaling has recently been shown to be important for regulating plant responses to many abiotic stresses [6•,50•], and is involved controlling shoot Na^+ accumulation by regulating vasculature Na^+ concentrations [51•]. Many aspects of plant growth and development are mediated by Ca^{2+}. Environmental cues are perceived by receptors on the cell membrane, activating a Ca^{2+} signaling cascade, resulting in the regulation of gene expression and protein activities [23,24,52–54]. Overexpression of genes encoding proteins in Ca^{2+} signaling pathways have been shown to improve the growth of crop plants, such as rice, apple, barley, tobacco and tomato during salt stress (Table 1 and Supplementary Table 3).
  Similarly, overexpression of transcription factors as a viable way of improving crop salinity tolerance has also been described numerous times in model species, such as *Arabidopsis* [55–58], and to a lesser extent in crops, such as rice, wheat, tomato and alfalfa (Table 1 and Supplementary Table 3). As key transcription factors may regulate the induction and/or repression of a range of salinity tolerance genes and mechanisms, it is tempting to suggest that manipulation of transcription factors may result in the greatest effect on crop salinity tolerance with the least amount of genetic modification. However, as for genes encoding compatible solute synthesis, yield penalties can often be apparent in low stress environments when transcription factors are constitutively expressed [59–61], presumably due to the effects of the transcription factors on a wide range of processes. Therefore, while these genes maybe have the potential to have the greatest effect on improving salinity tolerance, care should be taken in manipulating the expression levels of transcription factors. Use of stress inducible promoters may address many of these issues.

It is important to recognize that different tissues and cells in a plant are adapted for specific and often very different purposes. It is therefore not surprising that the expression levels of genes will be different from tissue to tissue and cell to cell, depending on the cell's function — as an obvious example, genes involved in photosynthesis will not normally be expressed in cells not exposed to light, for example roots. Care must therefore be taken in the choice of promoter used to drive the expression of salinity tolerance transgenes, so as not to disrupt or negatively affect the plant phenotype. There exist many examples where the constitutive expression of genes encoding transcription factors, ion transporters or proteins involved in compatible solute synthesis can lead to undesirable phenotypes, especially under non-stressed conditions [35,40–45,47,55,59–62]. However, when these genes are controlled by a promoter that is either cell type specific and/or stress inducible or have a tag on the protein that directs it to the correct cellular organelle, then the desired salt tolerant phenotype can be observed [35,40,42,48,49,55,63–68]. It is therefore imperative that promoters of genes which allow cell type and/or stress inducible expression are identified for controlling the expression of genes which can be used to obtain the desired phenotype. The gene is only half the story.

It is also important to recognize that altering expression of a gene is only one way to alter the protein function. In addition to allelic variation of the primary protein sequence, post-translational modifications that activate or suppress the protein's activity along with the location of the protein within a cell are also important factors that can be modified to alter a plant's salinity tolerance [24,69–71]. The calcium signaling pathways, which involve protein kinases to phosphorylate key proteins involved in salinity tolerance (such as SOS1) [23,24,26,27,52,72,73•], are a good example of where post-translation modifications can enable a plant to respond quickly (and reversibly) to environmental changes.

It must be made clear that the choice of salinity tolerance mechanism to manipulate in crops plants will be largely dependent on the underlying salt tolerance mechanisms within individual crop species. For example, barley has greater salinity tolerance than wheat, and a greater ability to accumulate high concentrations of Na^+ in its shoot (higher tissue tolerance). Barley may therefore benefit from the addition of, for example, osmotic tolerance mechanisms, rather than improved ion exclusion. However, transferring the tissue tolerance mechanisms of barley into crops such as wheat and rice may significantly increase salinity tolerance of these crops. Again, incorporation of osmotic tolerance and improved signaling traits may also benefit these species. Unfortunately, there is not enough data available to make critical comments on which salinity tolerance strategy would work best for a certain crop. Proper field evaluations of crops developed by MAS or transgenic approaches are now required to determine the effect of the modification on crop yield and the best strategy to improve the salinity tolerance of specific crop species. To date very few studies have been able to examine the effect of modifying salinity tolerance traits on the yield of field grown crop plants, and assess their yield penalty in non-saline soils.

Delivery to crops

Discoveries of mechanisms of salinity tolerance now need to be applied to crops to improve crop performance

in the field. One way that this can be done is by using conventional breeding, accelerated by the use of molecular markers linked to the tolerance trait being introgressed, MAS. The effectiveness of the research outputs in salinity tolerance can be observed in the newly developed durum wheat lines with *TmHKT*1;5-A (a gene conferring Na^+ exclusion) introgressed by MAS, which showed a yield increase of 25% when compared to control wheat, at least when grown in saline fields [11••,14]. Several studies evaluated natural allelic variants of HKT genes in response to salinity in *Arabidopsis* [33,74•], rice [32,75•,76,77] and barley [78]. This allelic variation in the gene of interest can provide both a novel source of genetic material for MAS, and can also be used to design an easy, cheap molecular marker, thus providing a perfect marker for salinity tolerance — or, at least, for a trait contributing to salinity tolerance. If variation in a particular gene is insufficient within the relevant germplasm or if the gene is only found in a model plant species [79•], then alteration of the trait using GM technologies can be deployed. It is essential that those experiments involving GM plants have not only greenhouse evaluation of the salinity tolerance of the plant but field evaluation as well. A plant that shows salinity tolerant in the greenhouse may not necessarily have improved grain yield in the field. Only a few studies (e.g. [80••,81]) have evaluated the performance of GM plants for any abiotic stress tolerance mechanism in the field. It also must be taken into consideration that a GM approach for edible crops may not be desirable due to public perception; therefore the use of biotechnology techniques that will not generate a GM crop may often be more appropriate.

These emerging biotechnologies include ones where plants go through a GM phase, then have the GM construct segregated back out. Designer nuclease-based approaches to alter genetic composition, such as Transcription Activator-Like Effector Nucleases (TALEN) [82–85], are reported to increase the mutagenesis efficiency of endogenous targets by specifying their binding sites with single nucleotide precision [85]. The potential promise of this strategy was tested in rice to enhance disease resistance [86]. Another emerging genome engineering tool is the CRISPR/Cas system (short for Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas)) [87,88]. Several groups are optimizing and exploring the potential of Cas9 RNA-guided endonuclease system in human cells [89,90] and zebra fish [91]. These approaches are examples of new technologies that give promise to the future of crop biotechnology, whilst avoiding the presence of problematic transgenes or antibiotic markers.

To conclude, salinity tolerance is too complex to be easily amenable to improvement through selection as a trait in itself, but traits that are hypothesized to contribute to salinity tolerance are more genetically tractable and genes underlying these can be discovered using molecular genetics tools and genomics. Alterations in crops can then be made using both marker-assisted selection and genetic modification, and the relevance of such traits on whole plant salinity tolerance can then be tested, as measured by yield maintenance in saline conditions.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.copbio.2013.12.004

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A good example of how to perform research using transgenic crops, importantly using null segregants as biological controls in addition to wild type plants. The authors not only show that the transgene was present and expressed in their transformed cotton but additionally demonstrated the location of the AVP1 protein. Transgenic cotton expressing *AVP1* produces more biomass and have higher yields under both salinity and drought stress in the greenhouse, and also yielded more in dry-land field conditions over two years of field trials.

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