Abiotic stress tolerance is complex, but as phenotyping technologies improve, components that contribute to abiotic stress tolerance can be quantified with increasing ease. In parallel with these phenomics advances, genetic approaches with more complex genomes are becoming increasingly tractable as genomic information in non-model crops increases and even whole crop genomes can be re-sequenced. Thus, genetic approaches to elucidating the molecular basis to abiotic stress tolerance in crops are becoming more easily achievable.

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**Introduction**

Since the foundations of agriculture approximately 10,000 years ago, humans have been selectively breeding crop plants to increase yield and feed the expanding world population. With the population of the planet continuing to grow rapidly, estimated to reach 9bn by 2050 (http://www.fao.org/wsfs/world-summit/en/), it has been calculated that in order to meet the demand there must be an increase in global food production of 44 Mt each year, which is considerably more than the current annual average increase of 32 Mt [1**]. This task is challenging, as not only must we increase crop yields by a level not seen before, but we must do so in a changing climate and while considerable amounts of grain are being diverted to biofuels.

Drought, salinity, temperature extremes, nutrient deficiencies and mineral toxicities are all abiotic stresses which reduce plant growth and therefore have a major impact on crop yield [2,3**,4**,5**,6**,7**,8**]. Of great concern is that these stresses will be increasingly important due to climate change, land degradation and declining water quality [1**,4**].

**Abiotic stresses are often complex**

Unfortunately, the mechanisms by which crops maintain yield under abiotic stress are poorly understood. This is particularly problematic for drought stress, as:

i. drought can occur at different stages of the plant’s development, with different effects on plant function, and thus requires distinct mechanisms for tolerance;

ii. a variety of additional abiotic stresses commonly occur during drought, such as high temperatures, high concentrations of salt and other toxic solutes and low availabilities of nutrients [5**,9**,10**], and these vary by location and time; and

iii. there is a diversity of mechanisms and combinations of mechanisms which can be used by plants to tolerate each of these stresses [5**,9**,11**].

In addition, the sensitivity of many crops to a particular abiotic stress varies depending on their developmental stage. For example, rice is sensitive to salt stress at the young seedling stage, but much less so at the reproductive stage [12,13**]. The stress tolerance mechanisms a plant needs to employ will be controlled by a variety of genes, which are expressed at different times during the life of the plant [3**,4**,9**]. Plant adaptations to most abiotic stresses involve a range of traits which combine to contribute to overall whole plant tolerance. While individual genes have been reported to improve the stress tolerance of crops, such as the transcription factor ZmNF-YB2 which has been shown to improve drought tolerance in maize [14**], the number of environments in which these genes increase yield remains to be shown. For most cases, it is not going to be a simple matter of identifying the one gene that will provide resistance to one abiotic stress, let alone all such stresses.

**Use of forward genetics to elucidate mechanisms of abiotic stress tolerance**

The complexity of plant stress adaptation makes breeding for abiotic stress tolerance, particularly for drought, complicated. Taking a reverse genetics approach based on candidate genes to increase the tolerance of crop plants is also fraught with danger. Therefore, a more powerful approach is to identify naturally occurring variation of abiotic stress tolerance in varieties, landraces and wild relatives of crops, and to study the traits that contribute to tolerance. The genetic loci determining these traits can then be discovered by correlating trait values with the genetic variation in a large mapping population derived from the parents in which the trait was originally identified to differ. Once the molecular bases of traits contributing to tolerance have been identified using this...
forward genetic approach, marker-assisted breeding and genetic modification (GM) technologies can be used to introduce these traits (genes) into current, high yielding elite cultivars.

To undertake the above-mentioned process, it is necessary to have well-defined genetic materials and reliable and accurate quantitative phenotyping techniques [5**,15]. While some components of abiotic stress tolerance can be relatively easily measured, such as the accumulation of Na+ in leaf blades [16–18], other traits are more difficult to measure, such as growth immediately after application of salt to obtain a measure of the ‘osmotic’ component of salinity stress [3**,5**,11]. For traits where it is more difficult to control the onset of the stress, such as drought tolerance, multiple measurements over time are also required both of the plant and of the environment in which it is growing.

**Phenomics technologies assist the genetic analysis of abiotic stress tolerance**

Conventional methods for phenotyping plants are frequently laborious and destructive, and often involve the removal of plant biomass for analysis. Recent developments in high-throughput, non-destructive imaging technologies allow a researcher to obtain multiple images of the same plant at different time points and at different wavelengths, thereby offering new non-destructive methods for acquiring quantitative data on plant growth, health and water use under abiotic stress [8*,15,19].

Some of these technologies have already been used to quantify traits related to drought, salt and heat tolerance in a number of crop plants [15,20,21]. The majority of experiments, though, have focused on shoot traits. Despite the likely importance of tolerance mechanisms in roots, such as optimizing architecture to improve access to soil water, there have been few genetic studies of roots due to difficulties in phenotyping roots [9**,22*,23]. These studies have identified important root traits for abiotic stress tolerance, such as Al and B tolerance [24,25], nitrogen deficiency [26] and phosphate deficiency [27] – see also the review of Zhu et al. [23]. It is imperative, therefore, to develop accurate non-destructive root phenotyping assays to further elucidate mechanisms of abiotic stress tolerance.

In addition to the relevant phenotyping techniques, it is important to have defined and monitored growth conditions so that environmental influences on plant responses can be considered [15]. While it is ultimately desirable to study abiotic stress tolerance in environments similar to those in which crops are grown, the additional complexity of environmental variations superimposed onto the applied stress often makes it more difficult to score traits in the field. Screening for specific tolerance traits in a controlled greenhouse environment or growth chamber is often necessary to reduce the complexity of interactions between genetic and environmental effects on phenotype (so-called ‘G × E’). In controlled environments, the onset of some abiotic stresses can be clearly defined, such as the addition of salt to hydroponics, withholding water or dropping the temperature of a growth chamber. Control of the environment comes at the cost of reduced reality, in particular with plants being grown in pots rather than in the field [28], and the trade-off between control and reality needs to be carefully considered when a particular hypothesis is being tested. One way to assess the impact of this loss of reality is to relate growth room and greenhouse results in a particular population with yield in a range of field sites.

A significant challenge for genetic studies of abiotic stress tolerance is the speed at which phenotyping assays can be made. With high-throughput assays now available for gene identification and molecular marker generation, the process of phenotyping thousands of plants is becoming the limiting component, the ‘phenotyping bottleneck’. In recent years, a number of high-throughput phenotyping methods have been coming on line to help increase the speed at which plants can be phenotyped, such as rapid elemental analysis of plant tissue [29] and growth facilities equipped with conveyor belts that deliver plants to automatic imaging, watering and weighing stations, for example The Plant Accelerator® in Australia (http://www.plantaccelerator.org.au), CropDesign in Belgium (http://www.cropdesign.com) and the Leibniz Institute of Plant Genetics and Crop Plant Research in Germany (http://www.ipk-gatersleben.de/Internet). Use of these facilities, in conjunction with techniques for rapid marker generation and gene identification, will help accelerate the identification of traits and genome loci that contribute to stress tolerance in plants.

Despite the advantages of using controlled environments to reduce variability in experimentation, such experiments are likely to underestimate the plasticity in plant responses to abiotic stresses in field conditions, and will fail to adequately account for interactions with other environmental effects, both abiotic and biotic. It is therefore also necessary to undertake field trials of populations of plants over several years at several sites to test the relevance in the field of traits measured in controlled conditions and to identify components of abiotic stress tolerance which may have been overlooked or deemed unimportant in glasshouse experiments. The recent advances in phenotyping technologies in the field will also be important, such as digital RGB images and infrared thermography for shoot measurements [15,19,30], and the use of minirhizotrons, ground penetrating radar and electrical resistivity imaging for non-invasive imaging of roots [23,31]. When combined with techniques for precise characterization of growth environments, such
as high-resolution EM38 mapping linked to a global positioning system [31,32], genetic studies in field-grown conditions become possible.

Studies of abiotic stress tolerance in crops
Several crops have already been screened for their tolerance to abiotic stress, both in controlled and in field environments, and some patterns have already emerged. For example, it appears that tetraploid (durum) and hexaploid (bread) wheats are more broadly adapted to a range of environmental conditions than diploid wheats, and this phenomenon has been attributed to their large genomes [33,34]. Bread wheat is more tolerant to a range of stresses than durum wheat, which may be due, at least partly, to the addition of the third genome.

While there is still much useful variation for abiotic stress tolerance within our crops, there is not as much potential as there could be, due to the narrowing of genetic diversity within elite germplasm during the course of plant breeding [1,33–35]. It has been estimated that approximately 15% and 40% of the available genetic variation has been captured in modern wheat and barley varieties, respectively [2]. However, there is still wide genetic diversity in plants which are landraces or near wild relatives of crops. This can be seen from the comparison of molecular marker diversity studies between species such as *Hordeum vulgare* and *H. vulgare* spp. *spontaneum* [35,36,37]. These plants are likely to be sources for abiotic stress tolerance traits, and with their close genetic similarity to current cultivars, these traits could be introduced into commercial breeding lines [1,35–38]. Traits for biotic stress tolerance, such as disease and pest resistance, have already been introgressed from near wild relatives of wheat, such as *T. urartu*, *T. monococcum* *T. tauschii* and *Aegilops speltoides* [33,38,39] and from *O. nivara* for rice [40], demonstrating the potential of such an approach for abiotic stress tolerance.

Phenotyping of a large range of landraces and near wild relatives has demonstrated that there is indeed large phenotypic variation for abiotic stress tolerance traits, with some near wild relatives showing greater abiotic stress tolerance than current elite cultivars [4,17,34,36,38,40,41,42–44]. Already many traits for abiotic stress tolerance have started to be introduced into current crops. Rice with genes introgressed from *Oryza rufipogon* has been shown to have better Al tolerance when grown on toxic soils [45], while the introduction of genes from *O. logistaminata* has increased the drought tolerance of cultivated rice [38]. More recently, two Na⁺ exclusion genes, *Nax1* and *Nax2* [46,47], have been introgressed into the durum wheat variety Tamaroi, with those lines containing the *Nax2* gene having increased yield in saline soils.

Figure 1

Diagrammatic representation of the traits for salinity tolerance for which QTL have been identified and, if known, the genes responsible for the tolerance mechanism in cereals. A majority of the traits identified are in the maintenance of plant growth and yield under salt stress, while a few traits have been identified in specific tolerance mechanisms, such as the manipulation of ion transporters. For a comprehensive list of QTL, suspected tolerance mechanisms, species and references see Table 1.
Table 1

A selection of QTL and genes, where known, identified from a variety of mapping populations as being important in salinity tolerance in a variety of cereal crops. The chromosomes where these QTL and genes are found are given, along with the likely tolerance mechanism

<table>
<thead>
<tr>
<th>Process involved</th>
<th>Candidate genes or loci</th>
<th>Trait measured</th>
<th>Salt tolerance mechanism</th>
<th>Species</th>
<th>Chromosome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination</td>
<td></td>
<td>Germination rate</td>
<td></td>
<td>Rice</td>
<td>6, 7</td>
<td>[71]</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Barley</td>
<td>4H, 5H, 7H</td>
<td>[72]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bread wheat</td>
<td>3A, 4D, 6H, 7H</td>
<td>[73]</td>
</tr>
<tr>
<td>Shoot growth</td>
<td></td>
<td>Seedling vigor</td>
<td>Increased cell expansion; delayed senescence</td>
<td>Rice</td>
<td>1, 3</td>
<td>[74]</td>
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<td></td>
<td></td>
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<td>Rice</td>
<td>7</td>
<td>[75]</td>
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<td></td>
<td></td>
<td></td>
<td>Rice</td>
<td>6</td>
<td>[71]</td>
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<tr>
<td>Shoot growth</td>
<td>HKT (saltol)</td>
<td>Seedling survival</td>
<td>Increased cell expansion; delayed senescence</td>
<td>Rice</td>
<td>1, 6, 7</td>
<td>[76]</td>
</tr>
<tr>
<td>Shoot growth</td>
<td></td>
<td>Tiller number</td>
<td>Increased cell expansion; delayed senescence</td>
<td>Rice</td>
<td>6</td>
<td>[77]</td>
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<td>Barley</td>
<td>7H</td>
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<td></td>
<td>Barley</td>
<td>4H</td>
<td>[79]</td>
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<td></td>
<td></td>
<td></td>
<td>Bread wheat</td>
<td>5A</td>
<td>[18]</td>
</tr>
<tr>
<td>Shoot growth</td>
<td>Dry matter production</td>
<td>Increased cell expansion; delayed senescence</td>
<td>Rice</td>
<td>5, 6, 10</td>
<td>[71]</td>
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<td></td>
<td></td>
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<td></td>
<td>Rice</td>
<td>6</td>
<td>[80]</td>
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<td></td>
<td></td>
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<td></td>
<td>Barley</td>
<td>2H, 4H, 5H</td>
<td>[78]</td>
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<td></td>
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<td></td>
<td>Barley</td>
<td>7H</td>
<td>[81]</td>
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<td></td>
<td></td>
<td>Barley</td>
<td>1H, 2H, 5H</td>
<td>[72]</td>
</tr>
<tr>
<td>Photosynthesis</td>
<td>ERA1, PP2C, AAPK, PKS3</td>
<td>Chlorophyll content</td>
<td>Avoidance/delay in ion toxicity in chloroplasts; decreased stomatal closure</td>
<td>Rice</td>
<td>2, 3, 4</td>
<td>[82]</td>
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<td></td>
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<td>Bread wheat</td>
<td>3D, 7A</td>
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<td></td>
<td>Bread wheat</td>
<td>5B</td>
<td>[18]</td>
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<tr>
<td>Shoot Na⁺</td>
<td>HKT (Nax1, saltol, SKC1, SOS1, SOS3)</td>
<td>Shoot Na⁺ concentration</td>
<td>Increased osmotic adjustment; reduced Na⁺ transport; reduced Na⁺ exclusion</td>
<td>Rice</td>
<td>4, 6</td>
<td>[80]</td>
</tr>
<tr>
<td>Shoot K⁺</td>
<td>HKT (Nax2, SKC1)</td>
<td>Shoot K⁺ concentration</td>
<td>Selective uptake of K⁺; differential transport of K⁺</td>
<td>Rice</td>
<td>1, 4</td>
<td>[76]</td>
</tr>
<tr>
<td>Ion transport</td>
<td>HKT (Nax2, saltol, SKC1, Kna1)</td>
<td>Na⁺:K⁺ ratio</td>
<td>Unloading of Na⁺ from the xylem to reduce the accumulation of Na⁺ ions in the shoots</td>
<td>Rice</td>
<td>1, 4</td>
<td>[85]</td>
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<td>Rice</td>
<td>1</td>
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<td>Rice</td>
<td>1, 9</td>
<td>[83]</td>
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<td></td>
<td>Rice</td>
<td>1, 4, 12</td>
<td>[83]</td>
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<td>Barley</td>
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<td>Bread wheat</td>
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<td></td>
<td>Bread wheat</td>
<td>4D</td>
<td>[47, 86]</td>
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<td>Grain filling</td>
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<td>Grain yield</td>
<td>Delayed senescence</td>
<td>Rice</td>
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<td>Barley</td>
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<td></td>
<td></td>
<td></td>
<td>Bread wheat</td>
<td>1B, 2B, 3D, 4A, 4B</td>
<td>[87]</td>
</tr>
</tbody>
</table>
and importantly, no yield penalty under non-stressed conditions (Munns and Richards, unpublished results). If the genetic basis encoding the desired abiotic stress tolerance trait can be identified through the use of forward genetic approaches, such as mapping for quantitative trait loci (QTL) and positional cloning, this would make the task of breeding or engineering tolerant crops much easier.

Tolerances to abiotic stress are typically complex quantitative traits that are influenced by a number of genetic and environmental interactions. Determining the genetic basis of tolerance involves correlating the incidence of molecular markers with phenotypic scores to predict a genomic region of DNA that harbors a factor influencing the plant’s response. Factors that affect the resolution of such a mapping approach include the population structure, the phenotypic methods used to dissect the physiological components of the plant’s response to the stress and the density of molecular markers used to cover the genome. Genetic mapping strategies typically use QTL mapping with biparental mapping populations or linkage disequilibrium mapping with association mapping populations.

Biparental mapping populations are derived from a cross between two parents, for example backcrosses, doubled haploid lines, F2 progeny, recombinant inbred lines and near isogenic lines, with parents chosen which differ in physiological responses for the trait of interest. Association mapping populations are diverse collections of germplasm chosen to represent the whole spectrum of phenotypes observed for the trait of interest. Both types of populations have been extensively used in mapping abiotic QTL in crop species. QTL have been identified in numerous wheat, barley and rice populations for drought [48–54], cold [55–58], heat [59–61], mineral toxicity [24,25,62–66], salinity (Figure 1 and Table 1) and nutrient deficiencies [26,67–69]. Some approaches have utilized adaptive genetic diversity present in landraces or wild varieties to identify tolerance genes [34,36,62,63,68,69]. The reviews by Langridge et al. [2], Collins et al. [70] Genè et al. [18] and Fleury et al. [9**] provide a more comprehensive coverage of QTL linked to abiotic stress tolerance in cereals and other plant species.

Conclusions
Abiotic stress tolerance is complex, but as phenotyping technologies improve, components that contribute to abiotic stress tolerance can be quantified with increasing ease. In parallel with these phenomics advances, genetic approaches with more complex genomes are becoming increasingly tractable as genomic information in non-model crops increases and even whole crop genomes can be re-sequenced. Thus, genetic approaches to elucidating the molecular basis to abiotic stress tolerance in crops are becoming more easily achievable. This knowledge can be delivered to breeders through marker-assisted selection or genetic modification technologies.

Acknowledgements
Research in the Tester laboratory is supported by grants from the Australian Research Council and the Grains Research and Development Corporation.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest
★ of outstanding interest

1. Tester M, Langridge P: Breeding technologies to increase crop production in a changing world. Science 2010, 327:818-822. The article describes the challenges and possible solutions for increasing the global cereal production from 2350 million metric tons in 2007 to over 4000 million tons by 2050, focusing on the role of new breeding technologies, such as marker-assisted selection (MAS), and the potential contribution of genetically modified (GM) technologies, among other approaches.


3. Munns R, Tester M: Mechanisms of salinity tolerance. Annual Review of Plant Biology 2006, 58:651-681. A comprehensive review on the mechanisms of salinity tolerance detailing not only the extent of saline soils but also the two distinct phases of salinity stress on plant growth and the tolerance mechanisms employed by plants to increase their growth in saline conditions.


Table 1 in this review lists the concentration ranges at which the 14 essential plant nutrients are desired in leaf tissue, as well as the concentrations at which they, and other minerals, such as Na, Al, Pb etc., become toxic.


The paper describes the production of transgenic drought tolerant maize which is able to maintain higher rates of photosynthesis, increased chlorophyll content and reduced leaf temperature compared to non-transgenic controls. This increase in drought tolerance was linked to an improvement in yield under drought stress.


A recent review on breeding for improved water use in cereals. Table 1 has a summary of important traits and the selection method required for improving yield of cereals in water-limited environments.


The proteins encoded for by the Nax1 and Nax2 genes is shown to increase retrieval of Na⁺ from the xylem in roots, thereby reducing shoot Na⁺ accumulation. The Nax1 protein (TaHKT1;4A) is also shown to withdraw Na⁺ from the xylem in the shoot, allowing Na⁺ to be accumulated in the leaf sheath away from the leaf blade.


