Use of The Plant Accelerator® to map quantitative trait loci for osmotic tolerance in wheat

Mark Crowe, Bettina Berger and Mark Tester
The Plant Accelerator, University of Adelaide Waite Campus, Hartley Grove, Urrbrae SA 5064, Australia

March 2012

The project

Osmotic tolerance is a component of salinity tolerance, a feature of major interest and value in cereal breeding. However, because it is a rapid response and overlaps in its physiological effects with other aspects of salinity tolerance, it has been difficult to investigate using conventional approaches. This project sought to address that challenge by using the high-throughput and non-destructive phenotyping capabilities of The Plant Accelerator® in a study of the response of a wheat mapping population to moderate levels of soil salinity.

Using near-daily total leaf area measurements on a mapping population of more than five hundred plants over a three week period, two putative quantitative trait loci (QTL) for osmotic tolerance were discovered; we believe that it is the first time such QTL have been identified in wheat. In addition, lines displaying low and high osmotic tolerance could be readily distinguished by their growth responses to salt treatment.

The experiment was distributed across two of The Plant Accelerator's Smarthouses, and by comparison of the growth of the population in these two 140m^2 rooms, it was shown that there is good reproducibility of results between these two rooms. The project also found that relative growth rates of the plants were reduced by as much as 30 to 50% by the levels of salinity being tested.

This project was carried out by Nawar Shamaya, a PhD student at the Australian Centre for Plant Functional Genomics.

Background

Salinity tolerance is an issue of major importance to the Australian cereal industry, as well as in many other regions around the world. With up to 80% of Australian cereal growing regions in Australia at risk from salinity problems (1), and estimates of the salinity-caused production losses in the hundreds of millions of dollars annually (2-4) any improvements to the salinity tolerance of cereal crops has potentially huge benefits.

Salinity, most commonly caused by excessive levels of sodium chloride, affects plants in two major ways:

1. Through osmotic stress; the increased osmotic pressure of the soil water caused by the dissolved salts can result in reduced water availability to the plant and bring about responses analogous to drought stress.

2. Once the salt enters the plant, it can inhibit the activity of a large number of enzymes, as well as require the diversion of significant resources into the operation of ion transport systems in an attempt to maintain an appropriate cellular environment (5).

At least three separate mechanisms are involved in determining the responses of plants to these challenges, namely:

1. the exclusion of salts from root uptake,
2. tissue tolerance to high levels of Na^+ (and sometimes Cl^-) through compartmentalization of excess ions, and
3. osmotic tolerance through water conservation responses or up-regulation of active uptake pathways.

The relative importance of these three mechanisms varies between species (6, 7) and also can depend on the particular conditions of the salt stress; and there is, of course, interaction between them as well. This complicates the identification of genes involved in aspects of salinity tolerance, and so hampers the introduction of such genes into breeding programs for the development of salt-tolerant varieties. Therefore, there are considerable potential benefits in the use of phenomics approaches to separate these three tolerance components.

Osmotic tolerance is well suited to analysis using The Plant Accelerator, since it is a rapid response (within a few days of treatment) characterised by a decrease in growth rate, both in terms of the emergence of new leaves and the expansion of those leaves. The capability of the Accelerator to accurately measure the growth of a large number of plants every one to two days is ideal for identifying and characterising variation in osmotic tolerance across a population.

Experimental design

The mapping population consists of a library of Gladius x Drysdale recombinant inbred lines (RILs) (8). To generate these RILs, the population was grown without selection until F4, and then 250 lines were selected and grown by single seed descent until F7. Two hundred of these single seed descent lines were used in this experiment, along with the two parental lines and two other commercial wheat varieties (Excalibur and Kukri).

Six replicates of each parental line and five replicates of each of the other commercial varieties were grown for each experimental treatment, along with two replicates of 40 lines of the mapping population and a single replicate of the remaining 160 lines (i.e. a 20% replication rate)

Seeds were planted in 2.5 L drained pots and grown in a conventional greenhouse for seventeen days. The plants were then transferred to the Smarthouses and set out using a randomised design layout, but with control and salt-treated plants of any given line being next to each other. Each pot was placed inside a 2 L square tray on the conveyor belt carts (Figure 1). These trays prevented water escape from the pots, and were later used for

addition of the salt solution. Approximately 65% of the plants were grown in Smarthouse 1, with the remainder grown in Smarthouse 2.

Figure 1. *Pot setup in the Smarthouse – a drained 2.5L pot stands inside a 2L square tray on a Smarthouse cart. The blue wire frame is designed to prevent leaves from drooping below the lip of the pot.*

The plants were imaged four times over the four days after transfer to the Smarthouses. On the fifth day, saline solution (25 mL of 2 M NaCl plus 65 mM compensatory $CaCl_2$, an amount equivalent to 75 mM NaCl/2.5 mM $CaCl_2$ final concentration on a soil water basis when fully diffused through the pot) was added to the tray of the 75 mM salt pots and an equal volume of water added to the tray of the control (0 mM) pots. Plants were then imaged a further twelve times over the subsequent two weeks.

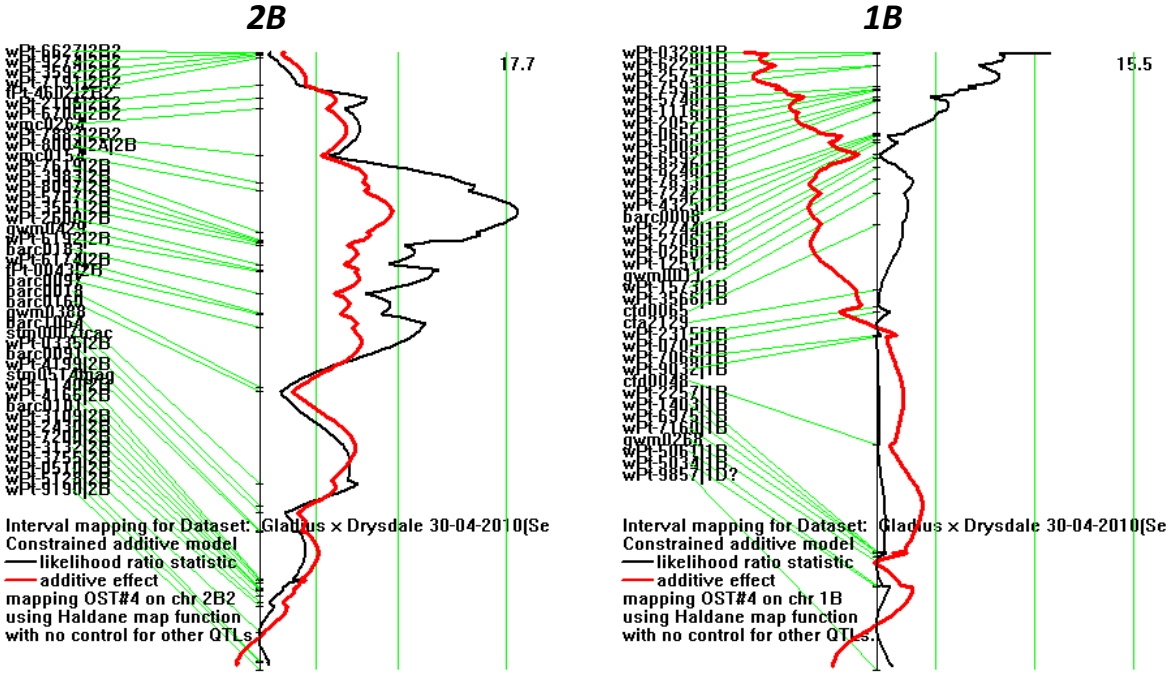
At each imaging cycle during the period in the Smarthouses, the pots were watered to field capacity from on top of the soil, using the watering to weight capabilities of The Plant Accelerator.

Outcomes

QTL identification

To date, the researchers have identified two putative QTL for osmotic tolerance in this mapping population, on chromosomes 1B and 2B (Figure 2). They believe that this is the first time any QTL for osmotic tolerance have been identified in wheat, as well as being one of relatively few instances where osmotic tolerance QTL have been found using soil grown plants rather than from hydroponics systems. They are continuing their analysis to refine and confirm these QTL and, perhaps, to find others.

Figure 2. *QTL traces for osmotic tolerance in a Gladius/Drysdale wheat mapping population. Chromosome 2B (left) has a significant QTL peak centered between markers wmc015 and wPt-7619, while Chromosome 1B contains a suggestive QTL over wPt-0328.*



Experimental design

In any forward genetic study, it is important to account for possible positional effects on plant growth, particularly when, as was the case in this project, the population is distributed across more than one room. Figure 3 shows average growth curves for control and salt-treated plants for each of the two Smarthouses used for this project, and shows good overall consistency between the observations between the two rooms. For example, the extent of inhibition of growth by salinity is similar between the two Smarthouses.

However, there appears to be slightly faster growth in Smarthouse 1 at around day 45. The reasons for this are not clear, but it does emphasise the need for good experimental design to account for such potential effects. In this particular case, a randomised layout was used overall, but each pair of treated and control plants was positioned adjacent to each other in the Smarthouses. Because osmotic stress tolerance index was used for the analysis in the project, and this index is a ratio between the growth rate of treated and untreated plants rather than the absolute rate, this design should compensate for most inter-room and intra-room variation.

Genetic variability in osmotic tolerance

The power of a mapping project lies in the analysis of the population as a whole, and with only one or two replicates of each line, it is inappropriate to put much weight on individual growth curves. However, on occasion, individual examples can be interesting and educational. Figure 4 shows the growth curve of two lines with very different degrees of osmotic tolerance: one sensitive line which shows a classic osmotic stress response of reduced growth rate within only two days of salt treatment, and one tolerant line with no apparent effect on growth for over a week after treatment.

Figure 3. *Growth curves of salt-treated and control populations in the two Smarthouses. Dotted vertical line indicates time of salt treatment. Values are the average projected leaf area for all plants with that treatment in that Smarthouse. A consistent outcome is seen between the two Smarthouses, with only very slightly faster growth in Smarthouse 1 at around day 45.*
Error bars are not shown since standard errors (n=175 and 87 for SH1 and SH2 respectively) are between 1.0 and 1.5% of actual values, and are too small to be plotted at this scale.

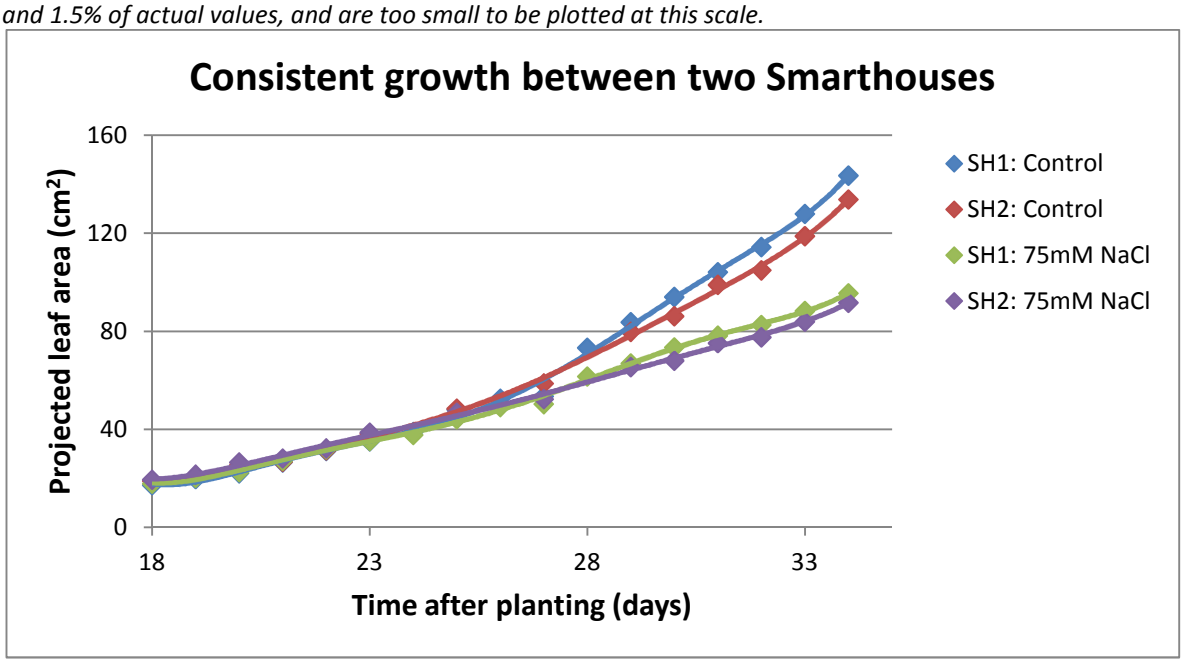
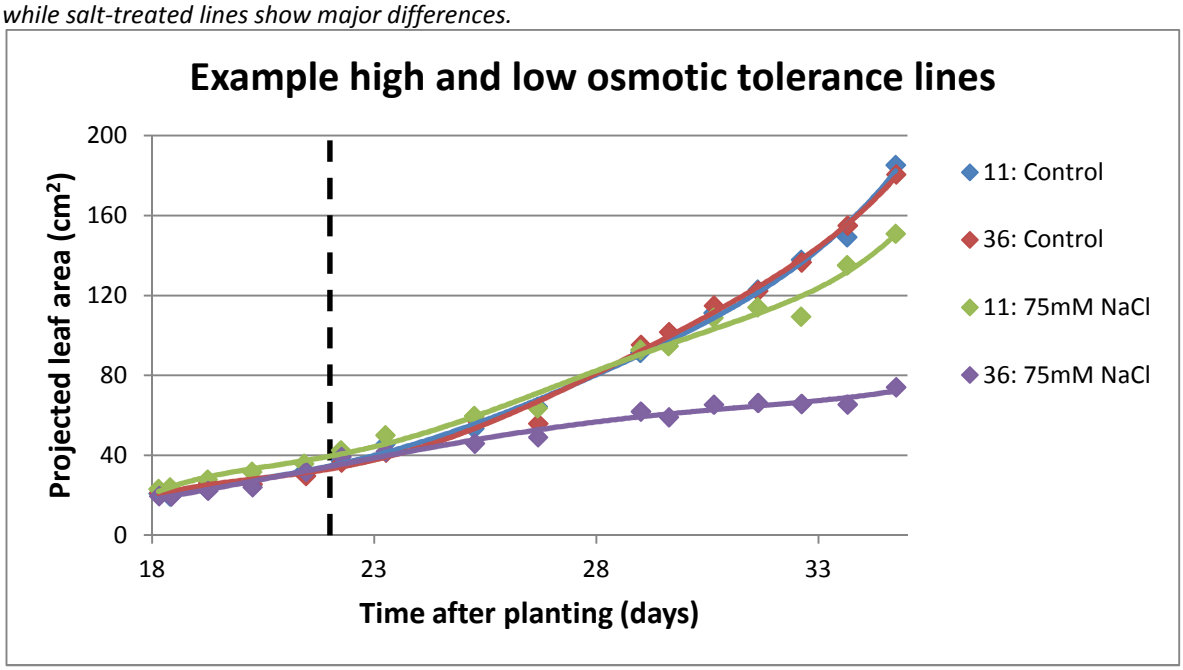


Figure 4. *Growth curves of osmotically-tolerant (line 11) and osmotically-sensitive (line 36) mapping lines. Dotted vertical line indicates time of salt treatment. Growth rates under control conditions are very similar, while salt-treated lines show major differences.*

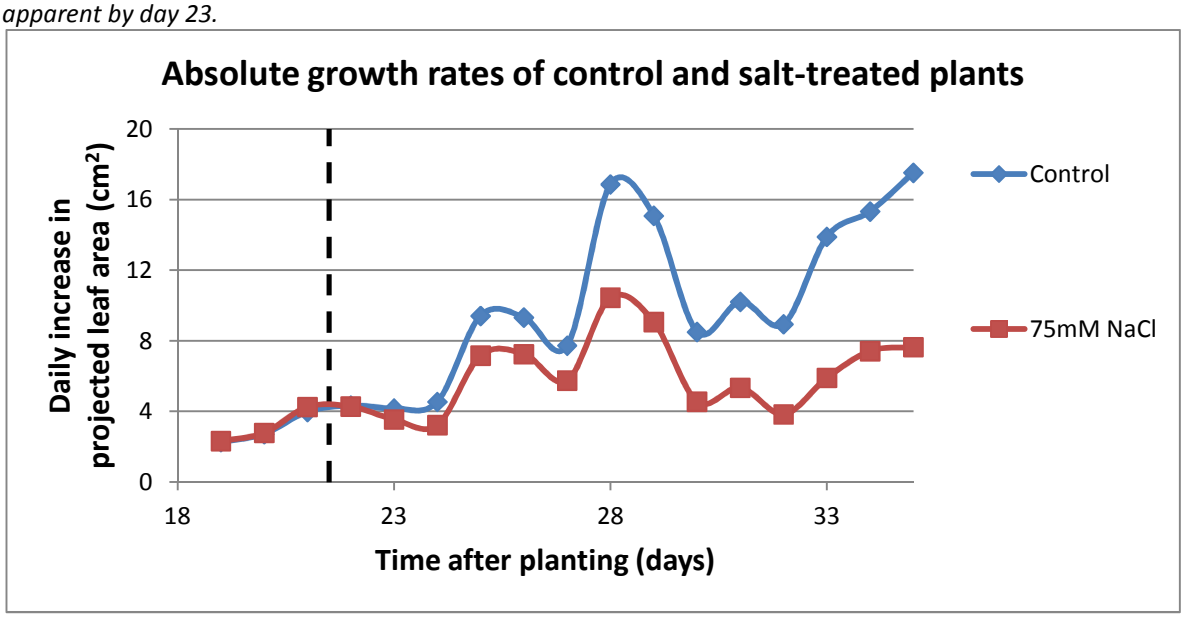


In fact, the timeframe of the response of the osmotically tolerant line 36 is more consistent with sensitivity to the ion-specific effects of growth in saline conditions rather than the osmotic stresses; this line is therefore perhaps highly osmotically tolerant but with only low or moderate tissue tolerance.

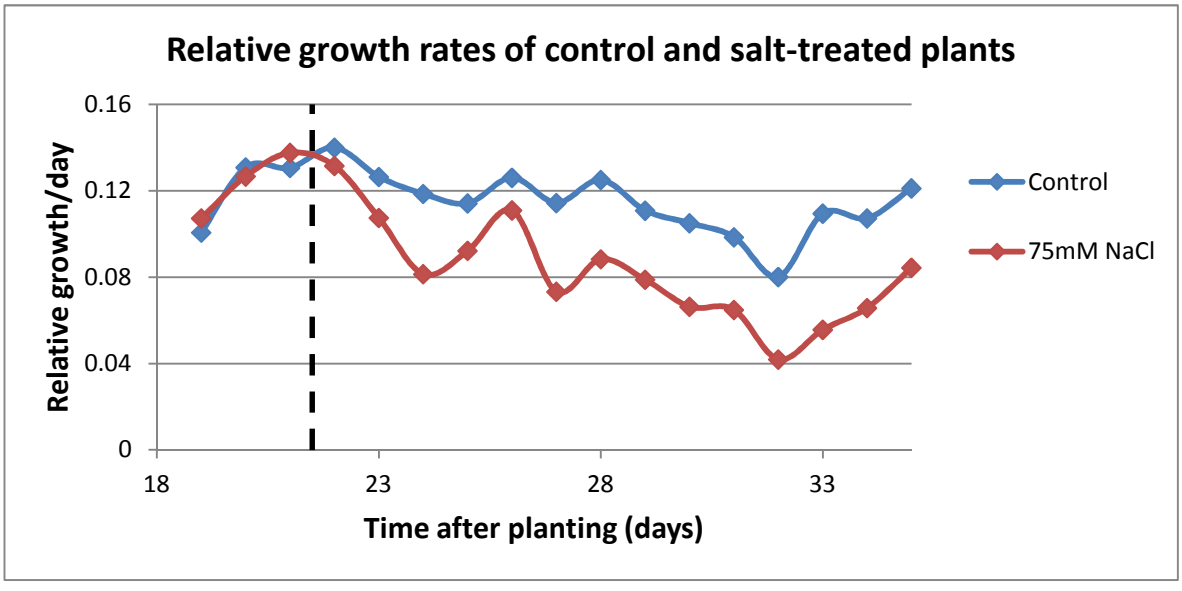
Daily growth rates

Because plants are phenotyped every one to two days at The Plant Accelerator, incremental changes in plant size provide a sensitive way of detecting rapid responses to osmotic stress. Both the absolute and relative daily growth traces indicate this is the case for this project (Figure 5); while the total plant size data from Figure 3 does not show an obvious deviation between the growth curves of the two treatments until about three days after salt application, both relative and absolute daily results show divergence within one to two days.

Figure 5. Absolute and relative daily growth for control and salt-treated populations. *Dotted vertical line indicates time of salt treatment.*
5a) Absolute growth rate – the increase in the size of a plant between two subsequent measurements, divided by the number of days between measurements. The reduced growth of salt-treated plants is apparent by day 23.



5b) Relative growth rate – the proportional increase in size of a plant from one measurement to the next, divided by the number of days between measurements. Reduced growth of salt-treated plants is apparent by day 22 or 23, and reaches a maximum by day 24, from which point it remains at an approximately constant level below the controls.



Furthermore, the relative growth curve (which corrects for the growth advantage provided by the greater photosynthetic capacity of larger plants) suggests that while there is an almost immediate effect, it takes about two days for the maximum reduction of growth rate to be reached. This reduction in growth rate, by about 30% compared to unstressed plants, is then maintained for at least the subsequent two weeks of the project.

Finally, it is apparent that daily growth rates can be quite variable; this is perhaps largely due to variable levels of sunlight during the period of the project (the Smarthouses rely on natural lighting for illumination). However, the Accelerator records the intensity of illumination every 15 minutes, so variation in light intensity can be incorporated into a project analysis if required.

Benefits of The Plant Accelerator

For accurate mapping of QTL, as well as accurate estimation of their effect, it is essential to use a large mapping population – ideally of more than 200 lines (9). Larger populations also assist in the identification of QTL of smaller effect. With appropriate replication and appropriate controls and treatments, an experiment of over 500 plants is required, as was the case for this project.

Since osmotic tolerance is defined by changes in growth rate in response to osmotic stress, and is a short-term response, it can only be effectively monitored by a detailed time course of plant size. This requires frequent and accurate measurements - for a population of this size, this would simply not be achievable without the throughput and accuracy of an automated phenotyping facility such as The Plant Accelerator.

Furthermore, because the responses to osmotic stress overlap with those caused by drought stress, it is essential for an effective study of osmotic tolerance that all plants are accurately watered to equal soil water content. It is not possible to do this by watering to capacity and allowing pots to drain, since this will flush out salt from the soil, so controlled watering to weight is required. This can be done automatically at the Accelerator another feature which was critical for this project, since manual watering to weight of more than five hundred plants would have been almost as unfeasible as measurements of plant size

Other points to note

- Although this project only recorded growth responses for a relatively short period after salt treatment (two weeks), it would have been possible to have continued phenotyping for a number of weeks longer and studied longer-term ion-specific salinity tolerance responses. Senescence can be measured at the Accelerator using either chlorophyll fluorescence or colorimetric imaging, while tissue sampling of the plants during or at the end of the experiment (using conventional manual harvesting and destructive testing) would have allowed measurements of the salt content of the shoots. The latter would provide an indication of tolerance by salt exclusion, which could then be combined with the former to measure tissue tolerance (the degree of senescence caused by a known tissue salt concentration)

- Sirault et al (10) demonstrated that infrared thermography also has the potential for use as a measure of osmotic tolerance, by monitoring differences in transpirational cooling caused by changes in stomatal conductance in response to osmotic stress. The Plant Accelerator is equipped with thermal infrared imaging chambers, and is developing techniques in infrared thermography for such applications. The Canberra node of the Australian Plant Phenomics Facility, where the Sirault et al work was conducted, has good facilities for using infrared thermography to measure leaf temperature.

Acknowledgements
This work was done as part of a PhD undertaken by Nawar Shamaya, who is supported by a scholarship from the government of Iraq. We thank Huwaida Rabie and Chris Brien at the University of South Australia for statistical analyses and The Plant Accelerator team for excellent support. The project was supervised by Bettina Berger, Mark Tester and Peter Langridge.

References

1) Rengasamy P. 2006. World salinization with emphasis on Australia. Journal of Experimental Botany 57:1017-1023.
2) Hajkowicz S, Young M. 2005. Costing yield loss from acidity, sodicity and dryland salinity to Australian agriculture. Land Degradation & Development 16: 417-433.
3) NLWRA. 2001. Australian Dryland Salinity Assessment 2000: Extent, impacts, process, monitoring and management options. Turner, ACT: National Heritage Trust - Commonwealth of Australia, 1-2.
4) CRC. 1999. The costs of soil acidity, sodicity and salinity for Australia: preliminary estimates. Glen Osmond, Adelaide: Cooperative Research Centre (CRC) for Soil and Land Management.
5) Munns R , Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59:651–81.
6) Munns R, James RA. 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant and Soil 253(1):201-218.
7) Genc Y, McDonald GK, Tester M. 2007. Reassessment of tissue Na^+ concentration as a criterion for salinity tolerance in bread wheat. Plant, Cell & Environment 30:1486-1498.
8) Fleury D, Jefferies S, Kuchel H, Langridge P. 2010. Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany 61:3211-3222.
9) Ferreira A, da Silva MF, da Costa e Silva L, Cruz CD. 2006. Estimating the effects of population size and type on the accuracy of genetic maps. Genetics and Molecular Biology 29(1):187-192
10) Sirault XRR, James RA, Furbank RT. 2009. A new screening method for osmotic component of salinity tolerance in cereals using infrared thermography. Functional Plant Biology 36:970-977