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Screening methods for salinity tolerance: a case study with tetraploid wheat

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Abstract

Fast and effective glasshouse screening techniques that could identify genetic variation in salinity tolerance were tested. The objective was to produce screening techniques for selecting salt-tolerant progeny in breeding programs in which genes for salinity tolerance have been introduced by either conventional breeding or genetic engineering. A set of previously unexplored tetraploid wheat genotypes, from five subspecies of *Triticum turgidum*, were used in a case study for developing and validating glasshouse screening techniques for selecting for physiologically based traits that confer salinity tolerance. Salinity tolerance was defined as genotypic differences in biomass production in saline versus non-saline conditions over prolonged periods, of 3–4 weeks. Short-term experiments (1 week) measuring either biomass or leaf elongation rates revealed large decreases in growth rate due to the osmotic effect of the salt, but little genotypic differences, although there were genotypic differences in long-term experiments. Specific traits were assessed. Na^+ exclusion correlated well with salinity tolerance in the *durum* subspecies, and K^+/Na^+ discrimination correlated to a lesser degree. Both traits were environmentally robust, being independent of root temperature and factors that might influence transpiration rates such as light level. In the other four *T. turgidum* subspecies there was no correlation between salinity tolerance and Na^+ accumulation or K^+/Na^+ discrimination, so other traits were examined. The trait of tolerance of high internal Na^+ was assessed indirectly, by measuring chlorophyll retention. Five landraces were selected as maintaining green healthy leaves despite high levels of Na^+ accumulation. Factors affecting field performance of genotypes selected by trait-based techniques are discussed.

Introduction

New sources of salinity tolerance are needed for crops grown on salt-affected land. This would be particularly effective in areas with subsoil salinity, which is extensive in many landscapes dominated by sodic soils. 'Transient salinity', i.e., natural salinity not associated with groundwater or with rising saline water tables, can occur under sodic topsoils (Rengasamy, 2002). In Australia, two-thirds of the agricultural area has a potential for transient salinity. 'Dryland salinity', i.e., land affected by clearing and resultant rising water tables, is difficult to reclaim and make productive, but rising water tables can be controlled by planting deep-rooted perennial species. Trials in Australia have shown that lucerne (*Medicago alfalfa*) can lower a water table sufficiently to allow subsequent cropping (Ridley et al., 2001). Salinity tolerance is required, not only for the 'de-watering' species, but also for the annual crops to follow, as salt will be left in the soil when the water table is lowered. Increasing the salt tolerance of crops will also allow the more effective use of poor quality irrigation water. Ability to grow high-return crops such as durum wheat on salt-affected land will boost farm incomes and support changed farm management practices to address salinisation.

Improving the salt tolerance of crop and pasture species requires access to new genetic diversity (either natural or transgenic), and efficient techniques for identifying salt-tolerance. There is probably a

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wide range of genetic diversity in salinity tolerance in international collections that is undiscovered or underutilised.

Screening methods based on growth or yield

Screening large numbers of genotypes for salinity tolerance in the field is difficult, due to spatial heterogeneity of soil chemical and physical properties, and to seasonal fluctations in rainfall. A field study in Syria using ICARDA's advanced durum breeding lines indicated that significant genetic variation for salt tolerance might exist, but the confounding presence of drought stress made it difficult to identify genotypes with salt tolerance (Srivastava and Jana, 1984). They conclude that 'the lack of reliable large-scale field screening techniques still seems to be the biggest problem in genetic improvement of salt and drought tolerance of crop plants'. Screening techniques that can be carried out under controlled environments have therefore often been used (Table 1).

Large numbers of bread and durum wheat genotypes have been screened for salt tolerance in glasshouses, the criteria being biomass production at high salinity (up to 250 m*M* NaCl) relative to biomass in non-saline conditions (e.g., Kingsbury and Epstein, 1984; Martin et al., 1994). A screen by Sayed (1985) of 5000 wheat lines, based on survival of high salinity, showed considerable genetic diversity amongst hexaploid and tetraploid lines. However, little has come from such work, presumably due to a lack of correlation between glasshouse selection (survival of high salinity) and performance in the field.

Possibly, application of the glasshouse based screening methods would be greater if genetic differences at moderate salinity (50–100 m*M* NaCl) could be found. However, this has proved difficult to do. Table 1 lists techniques that have been used to screen large numbers of genotypes for salinity tolerance. Many studies have used measurements of leaf or root elongation rate to identify genetic differences in response to moderate salinity, but the results so far have not been usilised in the field. Frequently, short-term growth experiments have revealed little difference between genotypes that differ in long-term biomass production or yield. For example, in a comparison between 20 cultivars of wheat, barley and triticale there were no significant differences between the leaf elongation rate in the first 10 days of salinisation of any cultivar (Munns et al., 1995), including that a of durum wheat that proved to be the most sensitive and a barley that proved the most tolerant (Rawson et al., 1988). Many short-term growth experiments measuring whole shoot biomass revealed little difference between wheat genotypes in their response to salinity, even between those known to differ in long-term biomass production or yield (Nicolas et al., 1993; Rivelli et al., 2002; Shah et al., 1987; Weimberg, 1987). Longer-term experiments have been necessary to detect genotypic differences in the effects of salinity on growth: it has been necessary to expose plants to salinity for at least two weeks, and sometimes several months (Fortmeier and Schubert, 1995; Francois et al., 1986; Kingsbury and Epstein, 1984; Munns et al., 1995). Even with rice, a fast growing and salt sensitive species, it has been necessary to grow plants for several weeks to be confident of obtaining reproducible differences in salinity tolerance between genotypes (Aslam et al., 1993; Zhu et al., 2001).

However, long-term experiments are demanding of labour and resources, even when limited to a relatively few number of genotypes, so other techniques have been utilised.

Screening methods based on damage, or tolerance to very high salinity levels

Techniques that can handle large numbers of genotypes include germination or plant survival in high salinity, leaf injury as measured by membrane damage (leakage of ions from leaf discs), premature loss of chlorophyll (using a hand-held meter), or damage to the photosynthetic apparatus (using chlorophyll fluorescence). These are listed in Table 1, and are discussed in some detail at the end of this article. These methods can identify genotypes able to germinate in, or survive, very high salinities (over 200 m*M* NaCl), but do not discriminate between genotypes in ability to tolerate the low or moderate salinities typical of many saline fields (50–100 m*M* NaCl). This could be why genotypic variation in germination or survival has rarely been replicated in the field or in long-term growth experiments.

A major limitation to the use of injury or survival to identify salt-tolerant germplasm arises when the cause of injury is not known. The injury could be due to water stress, the Na^+ or Cl^- accumulating within the leaf, or to K^+ or Ca^{2+} deficiency (Greenway and Munns, 1980). Alternatively, leaf death could be due to accelerated senescence due to osmotic effect of the salt. This becomes particularly relevant when considering the reproducibility of the responses in the field.

Table 1. Techniques used to screen large numbers of genotypes for salinity tolerance in glasshouses or controlled environments. Comments indicate whether a control (non-saline) treatment is necessary, particular advantages of the technique that relate to its experimental feasibility, whether the responses measured are due to the osmotic or the salt-specific effect of the salinity treatment, and how long the treatment needs to be imposed. Avoiding the need to grow controls plants is a major advantage.

Technique[A]	Controls needed	Advantages	Osmotic or salt-specific effect	Length of treatment (weeks)
Screening techniques for tolerance to moderate salinity (50–150 m*M* NaCl)				
Measurements of growth:				
Root elongation	Yes	Can be used with very young seedlings	Osmotic	1
Leaf elongation	Yes	Not destructive	Osmotic	2
Biomass	Yes	More likely to relate to field	Both	4
Yield	Yes	Most likely to relate to field	Both	16
Measurements of injury:				
Leakage from leaf discs	No*	Not destructive	Either or both	3–4
Chlorophyll content	No*	Not destructive and quick (using hand-held meter)	Either or both	3–4
Chlorophyll fluorescence	No*	Not destructive	Either or both	3–4
Specific traits:				
Na^+ exclusion	No	Not descrutive, and a single easy analysis	Salt-specific	1–2
K^+/Na^+ discrimination	No	Not destructive	Salt-specific	1–2
Cl^- exclusion	No	Not destructive	Salt-specific	1–2
Screening techniques for tolerance to high salinity (200–300 m*M* NaCl)				
Germination	Yes	Very large numbers easily handled	Osmotic	1
Survival	No*	Limited experimental period, if high salinity used. Highly tolerant genotypes stand out.	Either or both	2–8, depending on salinity

*Assumes all genotypes under control conditions have no leakage, the same leaf longevity, fluorescence parameters typical of healthy plants, and 100% survival.

[A]Not listed are photosynthesis, osmotic adjustment, enzyme activity, gene expression, compatible solutes, ABA or ethylene, as these are not feasible screening techniques. These measurements can be made on only small numbers of genotypes at the one time. Not listed, also, are stomatal conductance and transpiration efficiency. These are feasible screening techniques, and can be used for large numbers of genotypes, but are specific traits for drought tolerance.

The effect of the field, when salinity might be increasing at the same time the soil water content falls, means additional factors come in such as increased osmotic stress, and the inability of roots to access nitrogen and other nutrients. For instance, N deficiency will cause accelerated leaf senescence due to demand for N to be remobilised from old leaves.

Screening methods based on physiological mechanisms

Because of the complex nature of salinity tolerance, as well as the difficulties in maintaining long-term growth experiments, trait-based selection criteria are recommended for screening techniques (Noble and Rogers, 1992; Yeo and Flowers, 1986; Yeo et al., 1990). Traits used for screening germplasm for salinity tolerance have included Na^+ exclusion (Garcia et al., 1995), K^+/Na^+ discrimination (Asch et al., 2000) and Cl^- exclusion (Rogers and Noble, 1992).

We have focussed on improving the salinity tolerance of durum (tetraploid) wheat. Current durum wheat cultivars are more sensitive to soil salinity than bread wheat (hexaploid) cultivars (Francois et al., 1986; Maas and Grieve, 1990; Shah et al., 1987; Rawson et al., 1988). The first mechanism that we used as a basis for a screening technique was Na^+ exclusion, as genetic differences in Na^+ exclusion are highly correlated with differences in salinity tolerance between tetraploid and hexaploid wheat (Francois et al., 1986; Gorham et al., 1987). We looked for genetic diversity in the trait of Na^+ exclusion from leaves in a set of tetraploid relatives of durum wheat grown at

150 m*M* NaCl (Munns et al., 2000). Three landraces with the trait of Na^+ exclusion were identified, but their salinity tolerance was not verified (Munns et al., 2000). The first set of experiments described here assessed the relationship between the trait of Na^+ exclusion and salinity tolerance. Anticipating we would be backcrossing the trait from the landrace into durum cultivars, we examined environmental variables that might influence the uptake of Na^+ and so interfere with assessing the trait in glasshouse or field. The two variables we considered most important were root temperature and light. The relationship between salinity tolerance and K^+/Na^+ discrimination was also considered, because K^+/Na^+ rather than Na^+ alone has been used as an index of salinity tolerance for cultivar comparisons in wheat (Chhipa and Lal, 1995; Dvořák et al., 1994) and rice (Asch et al., 2000; Zhu et al., 2001).

The second mechanism of salinity tolerance we considered was tissue tolerance of high internal Na^+ concentrations. Tissue tolerance cannot be measured directly, and is difficult to quantify. Yet it is clearly important; overexpression of the vacuolar Na^+/H^+ antiporter that sequesters Na^+ in vacuoles (NHX1) improved the salinity tolerance of *Arabidopsis*, tomato and brassica (Aharon et al., 2003). Variation in salinity tolerance not associated with Na^+ exclusion was observed for a small number of accessions of the diploid wheat ancestor *Triticum tauschii* (syn. *Aegilops squarrosa*) accessions (Schachtman and Munns, 1992; Schachtman et al., 1991), so it is likely that variation exists within the tetraploid wheat ancestors. To assess genetic variation in the tetraploid lines, we looked for leaf longevity, lack of necrosis, and prolonged growth despite very high accumulation of Na^+.

Materials and methods

Germplasm

A collection of 54 *Triticum turgidum* selections comprising the sub-species *durum, turgidum, polonicum, turanicum*, and *carthlicum* were provided by Dr Ray Hare (NSW Agriculture, Tamworth) as representing a wide range in genetic diversity (Table 2). This set of genotypes had previously been screened for rates of Na^+ uptake and K^+/Na^+ discrimination (Munns et al., 2000).

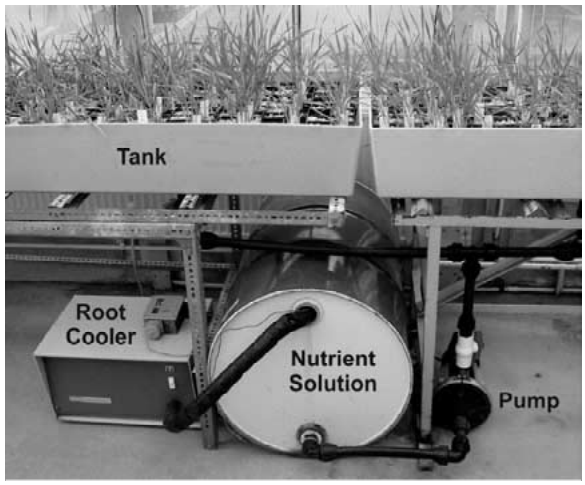

Figure 1. Supported hydroponic method for screening plants for salinity tolerance. Pots are filled with quartz gravel, 144 pots per tank, and the tanks are subirrigated every 30 min with nutrient or saline solution from a 44 gallon drum, by a pump activated by a timer. Solution drains back into the drum when the pump turns off. A cooling coil is inserted into the drum to maintain the nutrient solution close to 20 °C.

Growth conditions and the gravel-based hydroponic method

Seeds were selected by weight, surface sterilised with 1% hypochlorite for 15 min, and germinated in Petrie dishes for 3 days. Germinated seeds were planted into 6.5 × 15.8-cm pots containing quartz gravel, one plant per alternate pot, in 90-L plastic moulded trays containing 144 pots. Trays were subirrigated with either saline or non-saline nutrient solution, as described in Munns et al. (1995), and illustrated in Figure 1. This gravel culture was preferred to other forms of hydroponic culture, as roots are supported, each plant is a separate replicate, the frequent subirrigation and drainage avoids hypoxia, and there is no breakage of lateral roots as occurs in unsupported hydroponics when the solution is changed (Miller, 1987). The nutrient solution at full strength was Hoagland and Arnon solution No 2, containing 4 m*M* Ca^{2+} and 1 m*M* P.

Seedlings were watered initially with tap water, then half strength nutrient solution was introduced 2 days after emergence (DAE), and increased to full strength at 3 DAE. Commencing at 4–10 DAE, 25 m*M* NaCl was added to the irrigation solution twice daily over 3 days to a final concentration of 150 m*M*. Supplemental Ca^{2+} was added (as $CaCl_2$) to bring the total concentration of Ca^{2+} to 10 m*M*, and the molar ratio of Na^+:Ca^{2+} to 15:1. Control treatments al-

Table 2. Cultivars and numbers of tetraploid selections used in the individual experiments

Genotypes	Experiments					
	Exp 1	Exp 2	Exp 3	Exps 4, 5	Exp 6	Exp 7
Tetraploid accessions:						
T. turgidum L. ssp. *durum* (Desf.)		3	8	7	3	17
T. turgidum L. ssp. *polonicum* (L.) Thell.	1	3	5	5	1	11
T. turgidum L. ssp. *turgidum*	1		4	4		7
T. turgidum L. ssp. *carthlicum* (Nevski)		2	2	2		6
T. turgidum L. ssp. *turanicum* (Jakubz.)	1	3	1	4		6
Durum wheat cultivars:	Wollaroi Altar		Wollaroi	Wollaroi Tamaroi	Wollaroi	Wollaroi Tamaroi
Bread wheat cultivars:	Kharchia Genaro 81 Quarrion Halberd Condor Currawong Spear			Janz Machete	Janz	Janz Machete Kharchia Westonia
Barley cultivar:						Skiff
Total number of genotypes tested:	12	11	21	26	6	54

ways had 1 m*M* NaCl added to the nutrient solution. The pH was measured twice weekly and adjusted as needed to pH 6.0 with HCl. Root temperature was controlled using condensers in the solution reservoirs and monitored every 5 min using thermocouples. All experiments were conducted in a glasshouse with natural light and controlled air temperature (conditions during individual experiments are given below). Salinity tolerance was calculated as shoot dry weight as a percentage of control shoot dry weight.

Experimental series

Variation for salinity tolerance in hexaploid and tetraploid wheat (Experiment 1)

A collection of seven hexaploid wheat (*Triticum aestivum* L.) genotypes and five tetraploid wheat (*Triticum turgidum* L.) genotypes (Table 2) were grown in control (1 m*M* NaCl) and salt treatments of 150 m*M* NaCl with 10 m*M* $CaCl_2$. Nine replicate seedlings per genotype were used for each treatment, randomly spaced within each treatment. Shoots were harvested 45 DAE (corresponding to 35 days in treatment), dried (70 °C for 4 days) and weighed. Average daily PAR was 12.8 mol m^{-2} day^{-1}. Daily glasshouse air temperature ranged from between 23 °C (day) and 18 °C (night).

Leaf elongation rate evaluation (Experiment 2)

A selection of 11 tetraploid lines representing four *T. turgidum* subspecies (Table 2) were grown in control and salt treatments as described above ($n = 5$). Leaf elongation rate (LER) of the mainstem growing leaf (leaf 3) was measured with a ruler between 4 and 6 days after the commencement of the treatments. Shoots were harvested 30 DAE (corresponding to 25 days in treatment). Average daily PAR was 33.1 mol m^{-2} day^{-1}. Daily glasshouse air temperature ranged from between 25 °C (day) and 16 °C (night). Root temperatures were maintained between 22 °C (day) and 16 °C (night).

Trait evaluation (Experiment 3)

A collection of 21 tetraploid lines representing five subspecies were randomly selected from all tetraploid subspecies (Table 2) and grown in control and salt treatments described above ($n = 10$). Leaf 3 from salt-treated seedlings ($n = 5$) was harvested 10 days

after its appearance (coinciding with approximately 10 days after the salt treatment started for all genotypes). Remaining shoots ($n = 10$) were harvested at 28 DAE (24 days in treatments). Average daily PAR was 43.6 mol m^{-2} day^{-1}. Daily glasshouse air temperature ranged from between 32 °C (day) and 20 °C (night).

Effect of root temperature and light on Na^+ and K^+ accumulation and K^+/Na^+ (Experiments 4 and 5)

A collection of 22 tetraploid lines with two durum and two bread wheat cultivars (Table 2) were grown in the salt treatment described above in two experiments that differed in light and root temperature ($n = 4$). In the first experiment, average daily PAR was 13.5 mol m^{-2} day^{-1}, whereas average daily PAR in the second experiment was about double at 29.9 mol m^{-2} day^{-1}. Average daily root temperature treatments were 15.4 °C, 19.6 °C and 24.3 °C, respectively, in the first experiment and 15.6 °C, 20.0 °C and 24.0 °C respectively in the second experiment. Average daily air temperature was 20.3 °C in both experiments. Seedlings were randomly spaced in four replicate blocks in each root temperature treatment. Leaf 3 was harvested at 10 days after appearance (and also 10 days after the final salt concentration was reached). Remaining shoots were harvested 24 DAE (18 days in salt), and dried and weighed.

Effect of salinity-induced phosphorus accumulation on leaf injury (Experiment 6)

A selection of four tetraploid lines, with a bread wheat and durum cultivar (Table 2), were grown in control and salt treatments as described above, which contained 1 m*M* P. Ten replicate seedlings per line were used for each treatment and replicates were randomly spaced within each treatment. Seedlings were harvested 22–24 DAE (corresponding to 15–17 days in treatment) and visually assessed for leaf injury. Blades of leaf 1, 2 and 3 (most recently fully expanded leaf) were ground. Average daily PAR was 8.0 mol m^{-2} day^{-1}. Average daily glasshouse air temperature was 20.2 °C, and average daily root temperature was 20.9 °C.

Screen for tissue tolerance to Na^+ in a collection of tetraploid landraces (Experiment 7)

A selection of 47 tetraploid lines, two durum cultivars, four bread wheat cultivars and a barley cultivar were screened for symptoms of salinity-induced leaf injury in salt treatment as described above except that P was reduced to 50 μM. Lines were randomly double spaced within a block design (n = 4). Entire shoots were harvested at 28 DAE, which corresponded to 3 weeks in 150 m*M* NaCl. Leaf blades were separated into green and dead leaf portions. The percentage dead leaf was calculated as the weight of dead leaf as a percentage of total leaf weight. Immediately prior to harvest, chlorophyll content was estimated using a leaf chlorophyll meter (SPAD-502 meter, Minolta, Osaka, Japan). Mean leaf chlorophyll content for each genotype was derived from three readings taken at the base, middle and tip of leaf 1, 2 and 3. Average daily PAR was 15.6 mol m^{-2} day^{-1}. Average daily glasshouse air temperature was 19.1 °C, and average daily root temperature was 19.9 °C.

Chemical analyses

Harvested leaf blades were rinsed with deionised water, dried at 70 °C for 3 days, weighed and extracted in 500 m*M* HCl at 80 °C for 1 h and analysed for Na^+ and K^+ by an atomic absorption spectrophotometer (Varian Spectra AA-300, Melbourne Australia). Phosphorus was analysed on dried (70 °C for 3 days), finely ground and pelleted leaf material using an X-ray fluorescence spectrometer (Phillips PW 1404, Eindhoven, The Netherlands) according to the method described by Norrish and Hutton (1977).

Data analysis

Data were analysed by analysis of variance, and LSD ($P = 0.05$) was used to compare genotype means. Data from Experiments 4 and 5 were analysed using an analysis of variance–split plot design, where light was assigned as whole plots, root temperature as subplots and replicates as blocks. LSD ($P = 0.05$) was used to compare treatment means.

Results

Growth experiments: how to measure salinity tolerance

Biomass (Experiment 1)

The first step in this study was to see if there was significant genetic variation in salinity tolerance in tetraploid wheat genotypes, and to compare the tolerance with durum cultivars and bread wheat cultivars. Salinity tolerance was expressed as the percent biomass in saline versus control treatments. A level of

150 m*M* NaCl was chosen as a preliminary experiment at 100 m*M* NaCl failed to produce statistically significant differences between any genotypes over a period of 6 weeks, including those that were expected to differ in salt tolerance (data not shown).

The first experiment used a small set of hexaploid and tetraploid wheats (Table 2), and showed that after 35 days in 150 m*M* NaCl there were significant differences between hexaploids and tetraploids, and that there was genetic variation within each species (Table 3). Most importantly, it showed that some tetraploid landraces had a salinity tolerance significantly greater than that of the two durum cultivars. The most salt tolerant genotypes were the bread wheats Genaro 81and Quarrion, which were significantly more tolerant than the Indian landrace Kharchia that has long been used in the subcontinent for its tolerance to sodic/saline soils (Joshi et al., 1979). However, three tetraploid genotypes showed tolerance greater than the durum cultivars and equal to some of the bread wheats, so it was considered worthwhile to screen a larger number of tetraploid wheats.

To maintain a larger number of genotypes for 5 weeks, the length of time required to find genotypic differences in salinity tolerance was not feasible, because of the space required to maintain the controls. This was particularly important when comparing landraces with cultivars, as the taller landraces shaded the cultivars and the shorter landraces, and made comparisons of biomass production under control conditions impossible to make in the limited space of a glasshouse. Another problem arose in longer-term experiments when floral initiation occurred earlier in the cultivars than the landraces, even when the latter were vernalised, and the resulting shift from leaf area production to stem elongation in the cultivars meant that the growth rates of the two types could no longer be compared.

Alternative means to screen for salinity tolerance were sought, that avoided long term experiments, or avoided the need to grow plants in control conditions. Leaf elongation rates are a quick and convenient measure of short-term growth responses.

Leaf elongation (Experiment 2)

Leaf elongation rates were measured on a random selection of landraces from four subspecies, after growing for just 4 days of salt treatment, and compared with shoot biomass after 25 days of salt treatment. Elongation rate in the control treatment differed between genotypes, from 32.8 ± 0.6 to 52.1 ± 1.4 mm day^{-1}, so the effect of salinity was expressed as a percentage of controls. Elongation rate was most affected by salt in the three *turanicum* lines, their elongation rate being 60, 61 and 65% of controls, and was least affected in the three *durum* lines, which were 79, 82 and 85% of controls (Figure 2). However, there was only a small correlation with shoot biomass production after 25 days ($r^2 = 0.23$).

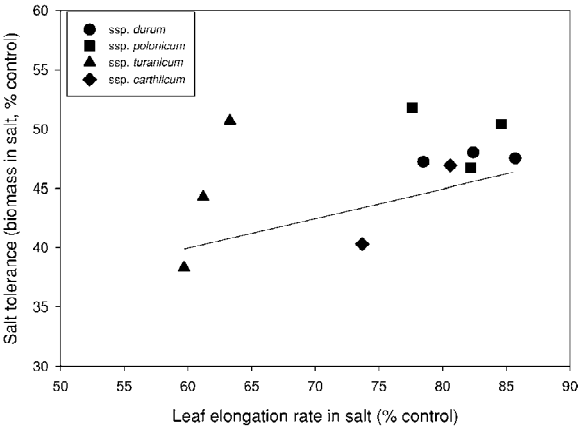

Figure 2. Relationship between salinity tolerance and leaf elongation rate in 11 tetraploid selections (Experiment 2). Biomass was measured after 25 days in treatments (control and 150 m*M* NaCl) and LER was measured on leaf 3 between 4 and 6 days of treatment. Values are means ($n = 5$). Fitted linear regression is $y = 0.21x + 30.8$ ($r^2 = 0.23$).

The low correlation between short-term leaf elongation rate and long-term biomass production could be because the effect of salinity on leaf width could vary between genotypes (i.e., the increase in leaf length is not proportional to the increase in leaf area), or because measuring the elongation rate of the leaf on the main stem is an insensitive index of total leaf area increase as the effect on the number of tillers is more important. However, it is most likely that it was because genotypic differences in the effect of salinity on rate of leaf growth take time to appear.

Having established that long-term experiments were not feasible with large numbers of genotypes, trait-based screening methods were tested. The aim of the following experiments was to identify and validate traits that could enable early detection of genetic differences in salinity tolerance.

Table 3. Salinity tolerance of hexaploid and tetraploid cultivars and selections grown in control or salt treatment (150 m*M* NaCl) after 35 d (Experiment 1). Values are means ($n = 9$)

Category	Genotype name or line number	Shoot dry weight (g)		Salt tolerance (%) control
		Control	Salt treatment	
Hexaploids:				
Cultivars	Genaro 81	2.06	1.38	67
	Quarrion	2.53	1.32	52
	Halberd	2.80	1.33	48
	Condor	2.56	0.99	39
	Currawong	3.76	1.42	38
	Spear	2.98	0.92	31
	Kharchia	3.76	1.36	36
Tetraploids:				
Cultivars	Wollaroi	2.50	0.63	25
	Altar	1.95	0.69	35
Selections	Line 543	2.29	0.99	43
	Line 248	2.87	1.21	42
	Line 354	2.49	1.06	43
	$LSD_{(0.05)}$	0.36	0.14	6

The trait of sodium exclusion – rates of ion accumulation in leaves

Trait evaluation (Experiment 3)

To assess the relationship between Na^+ exclusion and salinity tolerance in a range of tetraploid germplasm, the leaf Na^+ level at 10 days of treatment was compared with biomass production at 24 days, by which time genetic difference in tolerance had appeared (Figure 3). Leaf 3 was chosen to measure as it was the first leaf to have fully developed after the salt treatment. The time period of 10 days of treatment, coinciding with 10 days after the emergence of leaf 3 from the sheath of leaf 2, was chosen as previous experiments had indicated that genotypic differences were greatest then. However, any subsequent leaf at any stage of development would probably have shown the same genotypic differences. Subsequent leaves have lower NA^+ concentrations than early leaves, but the genetic differences remain the same (Rivelli et al., 2002). Na^+ correlated well with salt tolerance across all tetraploids ($r^2 = 0.65$), but this was dominated by the durum selections with $r^2 = 0.74$ (Figure 3A). Na^+ did not correlate with salt tolerance in the other tetraploid subspecies (Table 4). K^+/Na^+ correlated well with salt tolerance across all subspecies, but the regression coefficent was not as high as with Na^+ (Table 4). Again, the correlation of K^+/Na^+ with salt tolerance was driven by the durum selections (Table 4). The poorer correlation of K^+/Na^+ than of Na^+ with salt tolerance was a result of a poorer correlation between K^+ and salt tolerance (Table 4).

Na^+ exclusion or K^+/Na^+ discrimination did not correlate with salinity tolerance in the non-durum tetraploid genotypes (Table 4), yet some had high salinity tolerance (Figure 3B). *Polonicums* as a subspecies performed well, and the *carthlicums* and *turgidums* poorly. This suggests that some of these tetraploids have a greater capacity to handle the salt accumulated in the leaf, the trait of tissue tolerance.

Effect of root temperature and light on Na^+ and K^+ accumulation and K^+/Na^+ in leaves (Experiments 4 and 5)

Environmental influences that might affect Na^+ accumulation in leaves, namely root temperature (affecting ion uptake and transport to shoots) and light levels (affecting transpiration and growth rates), were examined

Table 4. Linear regression coefficients (r^2) between ion concentration and salinity tolerance in 21 *T. turgidum* selections from 5 ssp. (see Table 2), and in the *durum* and non-*durum* ssp. (Experiment 3). The numbers of genotypes are shown in brackets. Na^+ and K^+ concentrations were measured on leaf 3 after 10 days in 150 m*M* NaCl, and biomass was measured after 24 days in control and salt treatments. The same Na^+ data is shown in Figure 3

	Linear regression coefficients (r^2) between ion concentration and shoot biomass		
	All tetraploid ssp. (21)	*Durum* ssp. (9)	Other tetraploid ssp. (12)
Na^+	0.65	0.74	0.08
K^+/Na^+	0.29	0.54	0.08
K^+	0.13	0.40	0.03

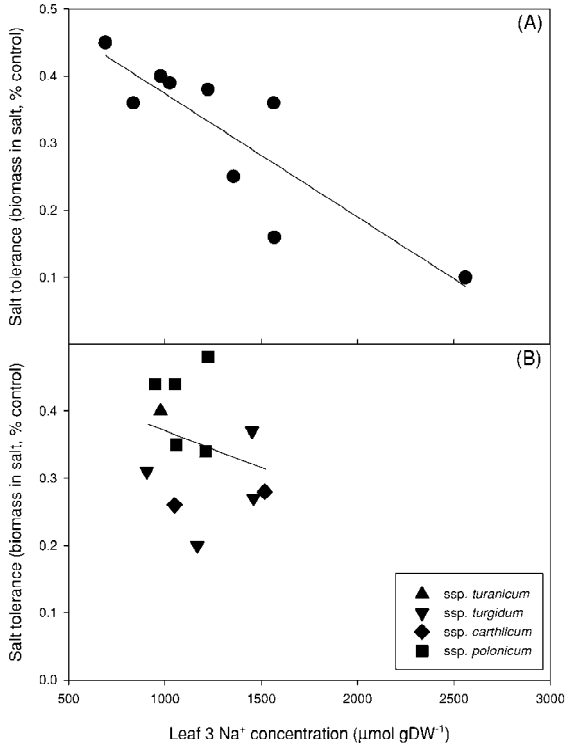

Figure 3. Relationship between salinity tolerance and leaf Na^+ concentration in (A) subspecies *durum* selections (●) and (B) other tetraploid subspecies selections (Experiment 3). Na^+ concentrations were measured on leaf 3 after 10 days in 150 m*M* NaCl and biomass after 24 days in control and salt treatments. All values are means ($n = 5$). Fitted linear regressions are (A) $y = -1.84E{-}04x + 0.74$ ($r^2 = 0.74$) and (B) $y = -1.1E{-}04\text{x} + 0.47$ ($r^2 = 0.08$).

to assess the degree of genotype by environment interaction.

In the low light experiment, there was no effect of root temperature on leaf Na^+ accumulation. However, in the high light experiment, Na^+ accumulation decreased with the higher root temperatures; the mean leaf Na^+ concentrations at 20 and 24 °C were significantly lower than at 15 °C. Figure 4A shows the relationship of Na^+ uptake for the 24 tetraploid lines and two hexaploid cultivars for the two temperature extremes (15 and 24 °C) at the higher light conditions, i.e., for the conditions giving the biggest changes in leaf Na^+ concentrations. The effect of root temperature was most pronounced in the four genotypes with the lowest Na^+, where it dropped to almost half (Figure 4A). Although root temperature affected leaf Na^+ concentrations in some lines more than others, it had little effect on the overall ranking of these lines.

The effect of higher light was to increase the mean leaf Na^+ concentration by about 10% at all temperatures (Figure 4B). To determine whether light altered the ranking of genotypes, Na^+ accumulation was compared at low and high light for a given root temperature (15 °C). While the Na^+ increased in most lines with higher light, there was a high correlation ($r^2 = 0.89$) between the accumulation of Na^+ in the leaves of individual lines at low and high light conditions (Figure 4B). High light significantly increased shoot biomass across all root temperatures, by almost 2-fold (Table 5), yet this had relatively little effect on Na^+ accumulation, indicating independent controls of Na^+ transport and shoot growth rates. That is, there was no effect of shoot vigour on Na^+ accumulation.

High light generally increased K^+ concentrations in leaves. There was also an interaction of light with root temperature, where K^+ increased significantly with increasing root temperatures in high light, but not in low light. There was no effect of light on K^+/Na^+, and while there was a small effect of root temperature at the high light, the K^+/Na^+ of the 22 tetraploid selections with high Na^+ levels remained relatively constant across temperature and light regimes (Table 5). There was, however, a large effect of root temperature on the K^+/Na^+ of the two low-Na^+ tetraploid selections, where Na^+ decreased and K^+ increased with root temperature, giving a substantial increase in K^+/Na^+ (data not shown). There appeared to be no clear relationship between K^+ and Na^+ concentrations in the leaves and thus no apparent linkage between the control of K^+ and Na^+ transport with changing environment conditions.

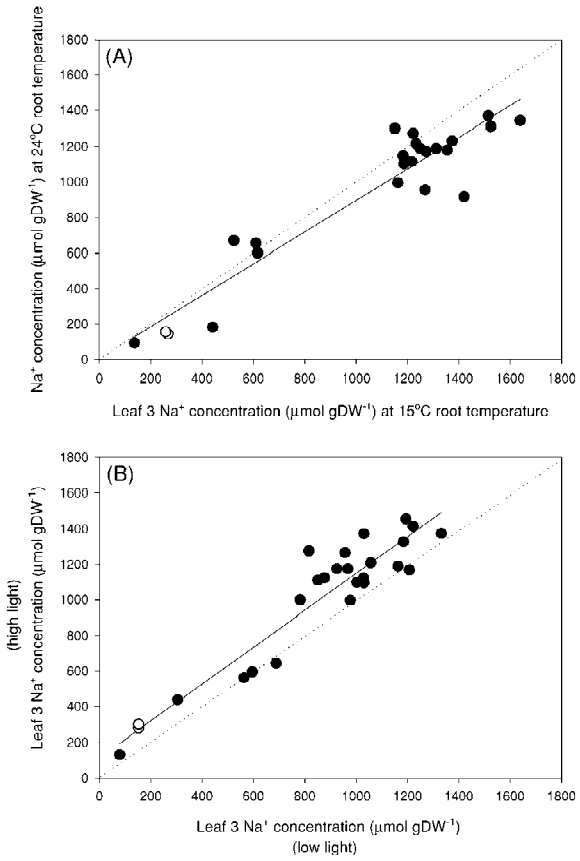

Figure 4. Relationship of leaf Na^+ concentration of 24 tetraploid selections (●) and two hexaploid cultivars (○) in (A) root temperature of 24 versus 15 °C in high light, and in (B) high light versus low light at a root temperature of 15 °C (Experiments 4 and 5). Na^+ concentration was measured on leaf 3 after 10 days in 150 m*M* NaCl. Values are means ($n = 4$). Fitted linear regressions are (A) $y = 0.89(x) + 7.93$ ($r^2 = 0.90$), and (B) $y = 1.04(x) + 112.9$ ($r^2 = 0.89$). Dotted line indicates 1:1 relationship between different temperatures and light levels.

The trait of tissue tolerance – high leaf Na^+ without injury

An earlier experiment showed that several genotypes of the non-durum tetraploid subspecies had a high degree of salinity tolerance, despite having very high leaf Na^+ levels (Figure 3B). These genotypes may have a special ability to tolerate high internal levels of Na^+. This trait of tissue tolerance to Na^+ cannot be measured directly, and is difficult to quantify. It has been assessed from the association of leaf death with rising leaf Na^+ concentrations, the genotype with the highest level of Na^+ at the onset of leaf death being likely to have a mechanism for tissue tolerance (Yeo and Flowers, 1983). This mechanism could be due to compartmentation of Na^+ in vacuoles, or retention of Na^+ in certain cell types such as epidermal cells. However, leaf injury or death could be caused by a number of things. Plants grown at high root temperatures and high light had extensive leaf injury, even though there was little increase in Na^+ over those grown at lower temperature and light (Table 5), which indicated that something other than Na^+ was causing leaf death. Elemental analysis of leaves showed that all elements except P fell within recommended levels (data not shown). Levels of P exceeded 1% in all lines, whereas 0.6% P is considered as the maximum level (Reuter and Robertson, 1986). These data indicated that P toxicity may contribute to leaf death in the salt-treated plants.

Salinity-induced P toxicity (Experiment 6)

To ascertain whether the leaf death was due to P toxicity rather than Na^+ toxicity, six genotypes with contrasting degrees of Na^+ accumulation were compared. Plants were grown with and without NaCl in full strength nutrient solution containing the standard P concentration of 1 m*M*. Figure 5 shows the relationship between leaf death and P concentration of leaves from seedlings grown in control or salt treatment. There was genotypic variation in P uptake, with a 2–3-fold range in both control and salt conditions, that lead to greater leaf death in those lines with higher P uptake and was unrelated to Na^+ toxicity. Na^+ concentrations in control leaves were less than 100 μmol g^{-1} DW and in the salt-treated leaves ranged from 110 to 1150 μmol g^{-1} DW, but there was little correlation between the Na^+ concentration and leaf death ($r^2 = 0.14$). Leaf death and P concentrations above 1.8% were highly correlated ($r^2 = 0.92$ for the control and 0.98 for the salt treatment), but the slope was much greater for the salt than the control treatment. Salinity increased the sensitivity of these lines to P, causing leaf death at lower P levels than for those in the control conditions. For example, approximately 3% P resulted in 50% leaf death in salt conditions compared to about 4% P causing the same degree of leaf death in control conditions (Figure 5).

Further experiments indicated that 50 μM P lead to levels of 0.6% P in both dead leaf and green leaf material which is considered an optimal level for wheat leaves (Reuter and Robertson, 1986). This level had been recommended by Epstein and colleagues for use in nutrient solutions in experiments on salinity tolerance in bread wheat (e.g., Colmer et al., 1995) and was therefore used in the following experiment.

Table 5. The effect of root temperature and light levels on the accumulation of Na^+, K^+, and K^+/Na^+ in leaf 3 and shoot biomass of 22 tetraploid selections after 20 days in 150 m*M* NaCl (Experiments 4 and 5). The photosynthetically active radiation (PAR) of the low light experiment was 13.5 mol m^{-2} day^{-1} and the PAR of the high light experiment was 29.9 mol m^{-2} day^{-1}. Values are means of 22 genotypes ($n = 4$)

Light	Average root temperature (°C)	$[Na^+]$ (μmol g DW^{-1})	$[K^+]$ (μmol g DW^{-1})	K^+/Na^+	Shoot DW (g)
Low light	15	1049	649	0.68	0.241
	20	982	643	0.72	0.278
	24	1061	635	0.66	0.240
High light	15	1213	624	0.60	0.488
	20	1118	679	0.69	0.462
	24	1123	713	0.73	0.444
LSD ($P = 0.05$)					
Light		42	17	ns	0.012
Root temperature		49	12	0.04	0.019
Light × root temperature		ns	18	0.05	0.023

ns – not significant.

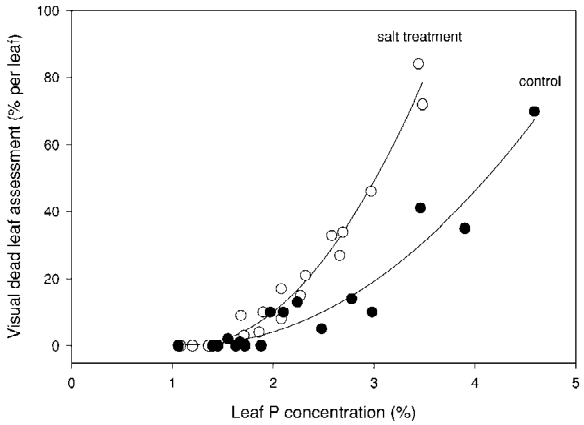

Figure 5. Relationship between leaf injury (assessed visually) and leaf P concentration (% DW) in main stem leaves 1, 2 and 3 from control (●) and salt-treated (○) tetraploid seedlings grown in control or 150 m*M* NaCl for 17 days (Experiment 6). Values are means ($n = 10$).

Screen for tissue tolerance of Na^+ (Experiment 7)

Fifty-four genotypes were harvested after 21 days of salt treatment, to identify genotypes with the least leaf injury associated with highest leaf Na^+concentration. Barley was included as a benchmark, because of its established reputation for salinity tolerance coupled with high rates of salt accumulation, and our previous observations that it was slow to develop leaf injury. Significant variation in percent dead leaf (weight of dead leaf as percent of total leaf dry weight) was found between individual tetraploid lines, the percent dead leaf ranging from 2 to 29% (Figure 6A and Table 6). The barley cultivar Skiff had a low degree of leaf injury as expected (only 3% of leaves were dead). Bread wheat cultivars and the durum cultivar Tamaroi had a relatively low percent dead leaf (6–8%) but the durum cultivar Wollaroi was higher (15%). There was a 2–3-fold range in percent dead leaf in four of the tetraploid subspecies, and a 13-fold range in ssp. *carthlicum.* The *carthlicum* selection Line 414 had the lowest degree of leaf injury, even less than barley (Table 6).

Chlorophyll concentration of the three oldest leaves (estimated with a SPAD meter) was measured to determine if there was a relationship between this simple non-destructive measure of leaf injury and the total plant injury measured with a destructive harvest. If chlorophyll concentrations correlated with percent dead leaf (in lines established as having high Na^+ accumulation) then this could provide a useful screening tool when assessing tissue tolerance in breeding populations. Selections having the lowest total percent dead leaf also had the highest chlorophyll estimates for leaves 1–3, with the exception of three genotypes with high degree of death (Figure 7). Further, selections from four of the five tetraploid subspecies with the lowest percent dead leaf, also had the highest mean chlorophyll content (Table 6). The satisfactory correl-

Table 6. Mean (n=4) and range of percent dead leaf (%DL), mean chlorophyll estimate (leaves 1, 2 and 3 on main stem), total leaf Na^+ content per percentage dead leaf , and Na^+ concentration in dead leaf, in a number of genotypes from different subspecies of *T. turgidum* (the number of genotypes shown in brackets), and cultivars of durum wheat, bread wheat and barley. Plants were grown in 150 m*M* NaCl for 21 days (Experiment 7)

Genotypes	Assessment	%DL (%)	Mean chlorophyll estimate of leaves 1, 2 and 3 (SPAD units)	Total leaf Na^+ per %DL (μmol)	Na^+ concentration in dead leaf (mmol g DW^{-1})
Ssp. *durum*	Min	5.1	12.2	15	1.46
selections (17)	Max	15.2	35.2	58	4.68
	Mean	10.1	22.1	38	3.72
	Line 139	5.1	30.8	42	3.94
Ssp. *polonicum*	Min	4.2	17.3	47	3.81
selections (11)	Max	10.3	31.5	93	5.11
	Mean	7.8	20.9	62	4.29
	Line 255	4.2	31.5	93	3.81
Ssp. *turgidum*	Min	5.6	16.0	28	3.60
selections (7)	Max	12.5	29.9	88	4.32
	Mean	9.0	23.2	58	3.94
	Line 362	5.6	29.9	88	3.69
Ssp. *carthlicum*	Min	2.2	10.7	17	2.97
selections (6)	Max	28.4	32.7	108	4.23
	Mean	18.1	20.1	43	3.54
	Line 414	2.2	32.7	108	3.87
Ssp. *turanicum*	Min	6.0	19.7	27	2.52
selections (6)	Max	13.2	36.6	66	3.69
	Mean	10.1	24.5	38	3.31
	Line 528	6.0	36.6	66	2.52
Durum wheat	Wollaroi	14.5	13.9	14	2.86
cultivars	Tamaroi	8.4	28.7	37	4.30
Bread wheat	Janz	8.0	19.9	23	2.97
cultivars	Machete	7.6	20.7	21	3.83
	Westonia	6.6	23.6	22	3.90
	Kharchia	5.8	23.6	32	3.57
Barley cultivar	Skiff	3.0	31.2	107	4.08
	$LSD_{(0.05)}$	5.5	9.1	29	1.06

ation between mean chlorophyll content and percent dead leaf ($r^2 = 0.51$) suggests that a simple non-destructive measure of chlorophyll content (measured using a SPAD meter) would be an adequate surrogate for measuring extremes in percent dead leaf when screening breeding populations.

The total leaf Na^+ content of individual genotypes did not correlate with the percent dead leaf (Figure 8),

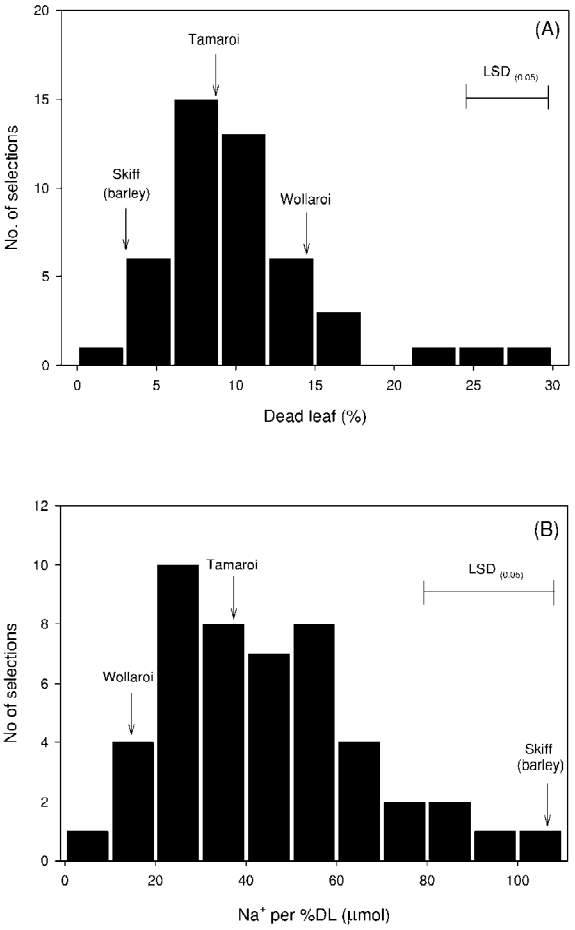

Figure 6. Frequency distribution of (A) percentage dead leaf and (B) Na^+ content per percentage dead leaf of 47 tetraploid wheat selections, grown in 150 m*M* NaCl for 21 days (Experiment 7, $n = 4$). Bars represent LSDs at $P = 0.05$ for among selection comparisons.

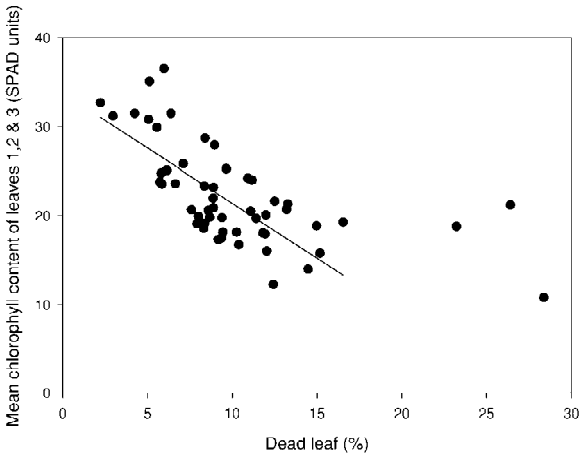

Figure 7. Relationship between mean chlorophyll content and percent dead leaf of main stem leaves 1, 2 and 3 of 47 tetraploid wheat selections, grown in 150 m*M* NaCl for 21 days (Experiment 7, $n = 4$). A linear regression line is fitted for all genotypes with less than 20% dead leaf, $y = -1.24(x) + 33.8$ ($r^2 = 0.51$).

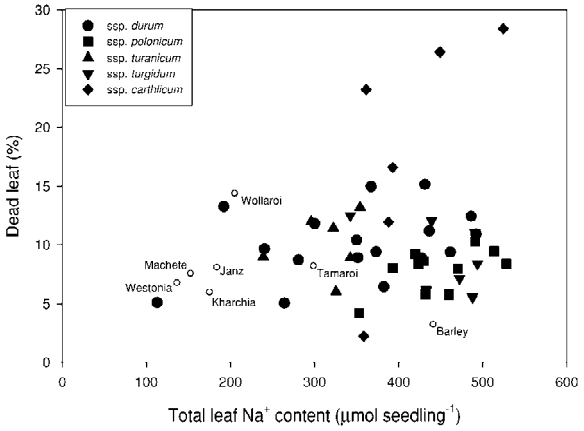

Figure 8. Relationship between percent dead leaf and total leaf Na^+ content of 47 tetraploid wheat selections, four hexaploid wheat genotypes, two durum cultivars and a barley cultivar, grown in 150 m*M* NaCl for 21 days (Experiment 7). Values are means ($n = 4$).

suggesting there might be genotypic variation in the ability to tolerate the Na^+ at the tissue or cellular level. The ratio of Na^+ content to percent dead leaf (whole shoot basis) was calculated as an index of tolerance to Na^+ in the leaves. A higher Na^+ content per percent dead leaf might indicate a higher degree of tissue tolerance to Na^+. This ratio ranged from 15–108 μmol Na^+ per percent dead leaf, with Skiff at the high end of that range with a value of 107 (Figure 6B). Analysis of variance showed that there were significant differences ($P = 0.05$) between a number of tetraploid selections and both Wollaroi and Tamaroi. The selections with the lowest percent dead leaf in four of the five subspecies also had the highest Na^+ content per percent dead leaf and were similar to that of Skiff (Table 6). The bread wheat cultivars, however, while excluding 2–3 times the amount of Na^+ from the leaves, displayed similar levels of leaf injury to a number of tetraploid selections, indicating greater sensitivity to tissue Na^+ levels (Figure 8, Table 6).

The Na^+ concentration in the dead leaf material was measured on the presumption that tissue tolerant selections might tolerate unusually high Na^+ concentrations prior to leaf death. This could provide a simple and non-destructive measure of tissue tolerance of Na^+. However, there was little genotypic variation in Na^+ concentration in the dead leaf material (Table 6), and the anticipated negative correlation with percent

dead leaf was not found ($r^2 = 0.04$). As some leaves may have died a week or more before harvest, it is possible that the lack of variation in Na^+ concentration was due to them acting as a wick and taking up Na^+ passively.

In summary, this experiment revealed five tetraploid genotypes with an exceptional combination of high Na^+ accumulation and low leaf injury, indicating they may have an exceptional ability to tolerate high Na^+ levels in tissues.

Discussion

Leaf elongation rates of the mainstem leaves were measured in each experiment for which biomass production in control and saline treatments was measured. There were differences between genotypes, but there was no correlation between the effect of salinity on leaf elongation rate and subsequent shoot mass. This may be because we did not take into account effects of salinity on leaf width, which determines area, or leaf thickness, which determines specific leaf area. Shoot growth rates depend not just on leaf area production, but also on specific leaf area and leaf weight ratio. Alternatively, it may be because genotypic differences in subsequent shoot mass is determined by the rate of leaf death rather than the rate of new leaf production.

The failure of many short-term experiments to distinguish genotypic differences in salinity tolerance is because the early response to salinity is to the osmotic effects of the salt, the salt outside the roots (Munns, 1993). It takes more time for the salt-specific effects to show up, i.e., the effects of the salt inside the plant. The osmotic stress of the salt outside the roots reduces the rate of formation of new leaves and the rate of tiller production (Munns, 2002). This response is probably under the control of hormonal signals from roots (Munns, 2002). Leaf growth generally responds in linear proportion to the osmotic strength of the soil solution (e.g., Rawson et al., 1988), with some species being more sensitive than others (e.g., Cramer, 2003, this issue). The salt-specific effect takes time to develop. In genotypes in which salt is not effectively excluded from the transpiration stream, salt will build up to toxic levels in the leaves that have been transpiring the longest (Munns, 1993). The rate at which they die in relation to the rate at which new leaves are produced is crucial. If old leaves die faster than new leaves are produced, then the assimilation rate of the plant falls, and reduced supply of assimilate to the growing regions further reduces their growth.

We found that screening large numbers of genotypes for salinity tolerance itself was not feasible. Plants needed to be grown for many weeks in saline and control conditions, and the amount of space needed to maintain control plants at their optimal growth rate became prohibitively high. We therefore focussed on trait-based screening methods.

Screening for the trait of Na^+ exclusion

For the durum subspecies, low Na^+ in the leaf blade correlated well with salinity tolerance ($r^2 = 0.74$), whereas K^+ or K^+/Na^+ had a lower regression coefficient. Leaf blade Na^+ accumulation and K^+/Na^+ discrimination were found to be independent of root temperature and factors that might influence transpiration rates such as light level. Most importantly, there was little influence of rates of shoot growth on leaf blade Na^+ accumulation or K^+/Na^+ discrimination, indicating separate controls of ion transport and shoot growth rate. There was no effect of shoot vigour on ion leaf blade accumulation, in contrast to rice (Yeo et al., 1990).

Recent glasshouse experiments have shown that landraces with low Na^+ accumulation yield better than high Na^+ genotypes at moderate salinity (Husain, 2002), so we consider that Na^+ exclusion is a robust trait that should help to confer salinity tolerance in the field. We have found no advantage in measuring K^+/Na^+. The trait of Na^+ exclusion has a high heritability, and we have recently mapped at QTL and identified a molecular marker for this trait (Munns et al., 2002), which is being used in a breeding program.

Screening for the trait of tissue tolerance

Tolerance of high internal Na^+ levels is evidenced by an absence of leaf injury despite high leaf concentrations of Na^+. Concentrations of Na^+ above 100 m*M* will start to inhibit most enzymes (reviewed by Munns et al., 1983), so when tissue concentrations are over 100 m*M*, which corresponds to about 0.5 mmol g^{-1} DW (assuming a leaf water content of 5 g H_2O g^{-1} DW), there must be effective compartmentation of Na^+ in vacuoles. This mechanism is exemplified in halophytes, which can tolerate much higher Na^+ concentrations than 100 m*M*, yet have no metabolic adaptation to high salt (Flowers et al., 1986; Greenway and Osmond, 1972). Greenway and Os-

mond (1972) showed that *in vitro* activities of enzymes extracted from the halophytes *Atriplex spongiosa* and *Suaeda maritima* were just as sensitive to NaCl in the assay media as were enzymes extracted from the common bean or green pea. Glycophytes have a degree of compartmentation ability, as levels of Na^+ up to 1 mmol g^{-1} DW are quite common in photosynthetically active leaves of many species. For example, in a study of two tetraploid wheat genotypes, we concluded that Na^+ became potentially toxic only when concentrations exceeded 1.25 mmol g^{-1} DW (equivalent to about 250 m*M* Na^+ in the leaf tissue water), as this level correlated with the onset of non-stomatal reductions in photosynthesis in durum wheat (James et al., 2002).

The ability to cope with high internal Na^+ levels was identified in a number of tetraploid landraces. Five landraces from various *T. turgidum* subspecies maintained a high percentage of green healthy leaves despite having high levels of Na^+, indicating that they may have the ability to tolerate high internal Na^+ at the tissue or cellular level. These data suggest that we have found genetic material with the potential to improve the internal Na^+ tolerance of durum cultivars and even of bread wheat.

Leaf injury, however, could arise from a number of reasons. First there would be the osmotic effects of salt in the soil solutions, causing accelerated senescence due to leaf water deficit or hormonal effects arising from root signals. Second, there could be nutrient imbalances resulting in deficiencies or excesses of other ions. Third, there could be toxic effects of salts in the leaves, due to excessive salt build up in cytoplasm or cell wall. It is only the last effect that is relevant to compartmentation of Na^+ and hence tissue tolerance, but it is difficult to separate from the other effects. It is essential to know what the cause of the injury is, if the germplasm is to be used in a breeding program. Further experiments will be done to verify that these genotypes are tolerant to high levels of Na^+, and not to other factors.

Going to the field

Although the glasshouse, or other controlled environments is necessary to provide reproducible treatments, there are factors in the field that may make glasshouse selections invalid. In the field, low Ca^{2+} availability, as in sodic soils, may influence cation uptake or transport. Screening techniques based on leaf injury are particularly prone to interference by other factors, such as other mineral toxicities or deficiencies, or by the high pH typical of many sodic/saline soils. Another major factor influencing leaf senescence is N availability. In the field, a saline soil will almost certainly at some stage be a drying soil, so as N becomes less available, remoblisation from old leaves will induce premature senesence, something that does not occur in hydroponics. Indeed, it is common for salt treatment in hydroponic solution to prolong leaf longevity (Rawson et al. 1988b, Husain, 2002).

In the field, additional traits become important, such as those conferring water use efficiency (Munns and Richards, 1998). Genotypes with high transpiration efficiency and deep roots, for instance, may do better on saline soils than those for which selections have been based solely on the ability to handle salt.

In the field, salinity varies throughout the growing season. The soil is usually least saline at the time of planting, as sowing follows soon after an irrigation or rainfall event. In a Mediterranean environment, waterlogging may occur for several weeks during the early growth of a crop (Setter and Waters, 2003). With an annual crop, salinity usually increases with time over the season, and reaches its maximum at grain maturity, so the period of grain filling needs to be most salt tolerant. At this time, leaf longevity is much more important than new leaf production. In summary, factors promoting leaf longevity, root extraction of deep water, and water use efficiency, are of critical importance to crop yield in a saline environment.

Are other methods suitable for screening large numbers of genotypes?

Germination is a convenient test for large numbers of genotypes, but little or no correlation has been found between genotypic differences in germination and later growth in salinity for species as diverse as bread wheat (Ashraf and McNeilly, 1998; Francois et al., 1986; Kingsbury and Epstein, 1984; Srivastava and Jana, 1984; Torres et al., 1974), durum wheat (Almansouri et al., 2001), barley (Norlyn and Epstein, 1982), tomato (Kurth et al., 1986) and lucerne (Rogers et al., 1995). This is presumbly because the processes that drive cell expansion during germination and during subsequent growth are entirely different. The water uptake and swelling that allows imbibition and radicle emergence is a physico-chemical process, in contrast to the biochemical and molecular processes that drive subsequent cell division and expansion. Many species, such as wheat and barley, have the ability to germin-

ate at very high salinity (over 300 m*M* NaCl), but the emerged radicle cannot grow further at this level of salinity. The physico-chemical nature of the swelling process during germination may explain why there is relatively little difference between species in salt tolerance to germination. For example, halophytes are no more salt tolerant than glycophytes at germination, although they quickly show their superior tolerance at the start of hypocotyl elongation (Malcolm et al., 2003).

There seems little value in screening for salinity tolerance of germination *per se*, at very high salinity, as it would be rare for a field to have a soil solution of EC more than 20 dS m^{-1} (equivalent to 200 m*M* NaCl) at the time of planting. Fields are usually at their least saline at the time of planting. Seedling emergence from the soil is most likely to be more important than germination, particularly if the soil surface is sodic and hard, when vigorous growth of the coleoptile and roots is critical (Hollington, 1998).

Survival at high salinities is also a convenient test, and has been used in a number of studies (Kingsbury and Epstein, 1984; Sayad, 1985) but this is more relevant to perennials than annuals, because perennials may have an opportunity to recover from a period of high salinity. Survival has been used successfully to select for the most tolerant genotypes within the perennial halophyte, tall wheatgrass (Shannon, 1978). In any case, screening species for survival of high salinity, rather than growth, carries the risk of selecting against productivity. As salinity is usually variable within a field, and most of the yield comes from the least saline areas, selecting for performance in a high-salinity environment alone may not be productive (Richards, 1983).

Leaf injury can be measured by various methods, ranging from leaf disc leakage to chlorophyll fluorescence. We found that the dark-adapted fluorescence parameters Fv/Fm were no more sensitive an index of salt injury than chlorophyll content itself; both Fv/Fm and chlorophyll started to decrease at the same time (James et al., 2002). However, the light-adapted photochemical quenching parameter NPQ was a more sensitive index of leaf injury; this started to increase at the same time we observed non-stomatal effects on assimilation. This response coincided with a buildup of Na^+ and Cl^- above 250 m*M*, raising the possibilty of a toxic ion effect on photosynthesis (James et al., 2002).

Tissue culture has been tried as method to generate and select for genetic variation. However, plants regenerated from selected cells or calluses have usually shown no increase in tolerance over established cultivars (Dracup, 1993). This is probably because salt tolerance is not due to the action of a single cell alone, but depends on the structural and physiological integrity of the whole plant.

Stomatal conductance could provide a positive screen for salinity tolerance, and be better than a negative screen such as leaf injury. In a study with two tetraploid genotypes, stomatal conductance was reduced early in the life of the leaf, and was the initial cause of the reduced assimilation (James et al., 2002). It is likely that the reduced stomatal conductance was due to osmotic stress, generated via root signals, as it occurred before NaCl started to build up to high levels in the leaf. Even so, if this is the major limitation to photosynthesis and growth, screening for stomatal conductance may be the most effective way of selecting genotypes that will continue to grow fast in saline soil. Screening for conductance could be done with a viscous flow porometer, which is much faster than a diffusion porometer (Rebetzke et al., 2000). Measuring gas exchange itself or even chlorophyll fluoresence is not feasible as a screen, but can be valuable to investigative a small number of germplasm selections that have been obtained by quicker means.

Future need for screening methods

Rapid and specific screening methods will be needed for screening large germplasm collections, for developing molecular markers, for gene discovery, and for pyramiding traits or genes.

International collections undoubtedly hold many treasures, including variation in salinity tolerance. But to screen these is still a formidable task. Feasible screening methods should avoid the need to grow plants under control conditions, and avoid pleiotropic or interactive effects of other variables such as genetic differences in growth rate, morphology or phenology.

Molecular markers can reduce the work involved in phenotypic screens. Once a locus (QTL) or gene associated with a specific trait is identified, a PCR-based molecular marker can be developed. Markers can be tested on seeds or seedlings, and provide a cost-effective way of screening large numbers of individuals in a segregating population. Molecular markers then represent the ultimate in a selection technique – their use is non-destructive, and does not require controls or salt treatments. However, to identify a marker in the first place, a specific phenotype is needed, and

this is best developed from a physiologically based selection technique.

For gene discovery using microarray approaches, specific screens will be more useful than just 'salinity tolerance', i.e., growth in saline versus control conditions. Specific screens will show whether the function of the candidate gene is for osmotic or salt specific adaptation to salinity. Even in genetic engineering, a specific phenotype will be useful to validate the function of the transgene in different genetic backgrounds, and to allow pyramiding of different genes or traits.

Future breakthroughs in salinity tolerance will come through an understanding of processes controlling the transport of Na^+ and Cl^- within the plant. These require specific cell types in specific locations within the plant catalysing transport in a coordinated manner. For example, genes controlling the efflux of Na^+ across the plasmamembrane may function usefully only in epidermal cells of roots; efflux of Na^+ into the apoplast would be useless to cells in leaves at the end of the transpiration stream, and presumably also in the internal cells in roots while the plant was transpiring. A deep understanding of whole plant tolerance requires more knowledge of cell-specific transport processes and the consequences of manipulation of transporters and signalling elements in specific cell types (Tester and Davenport, 2003). Understanding of the molecular genetics and physiology of the traits conferring salinity tolerance will form the basis for further improvements in the salinity tolerance of agricultural species

Acknowledgements

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