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RESEARCH PAPER

A screening method to identify genetic variation in root growth response to a salinity gradient

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Abstract

Salinity as well as drought are increasing problems in agriculture. Durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) is relatively salt sensitive compared with bread wheat (*Triticum aestivum* L.), and yields poorly on saline soil. Field studies indicate that roots of durum wheat do not proliferate as extensively as bread wheat in saline soil. In order to look for genetic diversity in root growth within durum wheat, a screening method was developed to identify genetic variation in rates of root growth in a saline solution gradient similar to that found in many saline fields. Seedlings were grown in rolls of germination paper in plastic tubes 37 cm tall, with a gradient of salt concentration increasing towards the bottom of the tubes which contained from 50–200 mM NaCl with complete nutrients. Seedlings were grown in the light to the two leaf stage, and transpiration and evaporation were minimized so that the salinity gradient was maintained. An NaCl concentration of 150 mM at the bottom was found suitable to identify genetic variation. This corresponds to a level of salinity in the field that reduces shoot growth by 50% or more. The screen inhibited seminal axile root length more than branch root length in three out of four genotypes, highlighting changes in root system architecture caused by a saline gradient that is genotype dependent. This method can be extended to other species to identify variation in root elongation in response to gradients in salt, nutrients, or toxic elements.

Key words: Architecture, branch, breeding, elongation, selection.

Introduction

Salinity is a common problem in agriculture in arid and semi-arid regions, whether due to irrigation with water containing dissolved salts, to rising water tables carrying naturally-deposited salts to the surface, or to subsoil salinity not associated with groundwater rise (Rengasamy, 2010). Soil salinity and water limitations are likely to increase in agriculture this century. Urban spread is forcing agriculture into drier or more marginal lands, and global food requirements are projected to increase 70% by 2050, requiring gains in agricultural productivity with less land and water (Fischer *et al.*, 2010). There is an imperative to develop crops with root and shoot traits that enable higher yields in soils with higher salt and limited water.

Durum wheat is a high value crop, used for the production of pasta and couscous. It is relatively salt sensitive compared with bread wheat and yields poorly on saline soil (reviewed in Munns *et al.*, 2006). Field studies indicate that roots of durum wheat do not proliferate as extensively as bread wheat in saline soil (Zubaidi *et al.*, 1999).

In order to look for genetic diversity in root growth response to saline stress, a rapid screening method was developed to identify genetic variation in the rates of root elongation of durum wheat in saline solution. Methods used for *Arabidopsis* on agar plates (Sun *et al.*, 2008) are not applicable to cereals as the seed and root embryo size and the consequent rates of root expansion require greater space to avoid the impedance of root growth. High throughput mutant screens selecting for root growth have discovered new and important genes for the control of Na^+ transport, the most significant being the plasma membrane Na^+/H^+

antiporter SOS1 (Wu *et al.*, 1996) and the Na^+ transporter AtHKT1 (Rus *et al.*, 2001). These confer salinity tolerance under conditions of K^+ deficiency. Leaf-based screens have also revealed important genes that limit the rate of transport of Na^+ from roots to shoots, such as the HKT1:4 and HKT1;5 transporters in wheat (Huang *et al.*, 2006; Byrt *et al.*, 2007). However, the main limitation to root and leaf growth in wheat and other temperate cereals may be the osmotic stress, for which no single major gene is known (Munns *et al.*, 2006). Yet there is certainly genetic diversity that is worth pursuing.

Salinity is rarely uniform down the soil profile, and more commonly strong gradients occur (see Fig. 1, adapted from Dang *et al.*, 2006). When a crop is sown, salinity is mostly low at the surface and greater at depth, as sowing usually follows an irrigation or rain event. In Mediterranean climates, seeds are sown following heavy rain that can leach the salt to 50 cm below the surface (Rengasamy, 2010). Seeds therefore germinate in low salinity, and root tips reach high salinity 1–2 weeks later. The bulk of the upper part of the root system is exposed to low salinity soil, and water is preferentially taken up from the low salinity solution (Bazihizina *et al.*, 2009).

The aim of this study was to reproduce a salinity gradient for roots of a germinating seed to grow into, without exposing the seed to salt, and to document genotypic differences in the salinity response in seminal root elongation and branch root elongation. The screen was adapted from a maize mutant screen successfully used by Hochholdinger and colleagues to identify genes regulating the presence or absence of root system components (Hetz *et al.*, 1996; Woll *et al.*, 2005; Taramino *et al.*, 2007). The germplasm chosen was known to differ in salt tolerance in terms of shoot growth rate (James *et al.*, 2008; Rahnama *et al.*, 2010), but no studies had been made on root elongation rate. Salt tolerance in that work was identified from a long-term growth screen where the roots are flushed with saline solution and supported by quartz gravel, but pots were only 15.8 cm high and only 6.5 cm wide, which would have constrained root elongation and not revealed genetic differences in root growth potential.

Materials and methods

Development of salt gradient

Experiments to create a gradient in salt were first carried out without plants. The screen was conducted in rolls of germination paper (25 cm wide×38 cm long) (catalogue number OP1015, Hoffman Manufacturing Inc., Albany, OR 97321, USA) using a method modified from that used to screen maize root mutants (Hetz *et al.*, 1996) (Fig. 2A). Papers without seeds were rolled by making a 3 mm crease along the long side, and then rolling the paper tightly. The roll was then dipped into a tray of tap water to saturate the whole roll and transferred for 15 min to PVC™ tubes (37 cm long× 11 cm wide) sealed with a plumbing lid bottom with

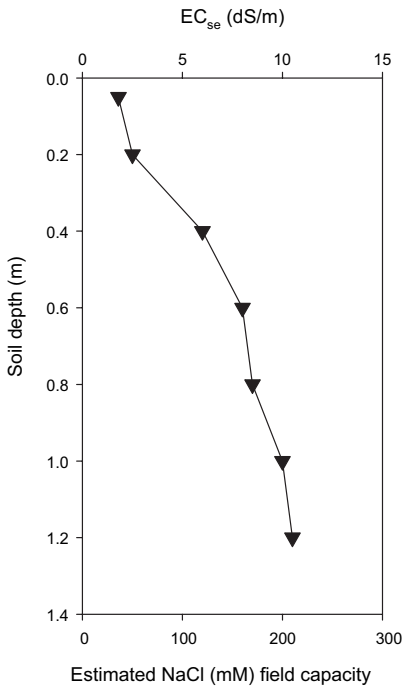

Fig. 1. Distribution of salt in a soil profile in a field with a high constraint to crop yields (adapted from Dang *et al.*, 2006, Fig. 3, by kind permission of CSIRO Publishing: web link http://www.publish.csiro.au/nid/40/paper/AR05268.htm.). EC=electrical conductivity; NaCl concentration estimated by multiplying the EC values times 20 to relate to concentrations used in this study.

Fig. 2. Growth conditions for durum wheat. (A) Plants at the 1.5 leaf stage, rolled in germination papers. (B) Unrolled germination leaf stage paper showing approximately five seminal axile roots (one indicated by a black arrow) emerged per seed. (C) PVC™ tube with paper rolls with wheat plants and sealed with a plastic bag to prevent evaporation from the top of the paper and movement of the salt gradient.

a clear base. The tubes had 1500 ml of one of five concentrations of NaCl (0, 50, 100, 150, and 200 mM NaCl) in half-strength modified Hoagland's solution (Rahnama *et al.*, 2010) which immersed the rolled papers to a depth of approximately 21 cm. After 15 min, papers were transferred to identical PVC™ tubes with 500 ml of the same solution, which immersed the rolls to 7 cm. The tops of the tubes were sealed with Parafilm™ and kept in a growth cabinet at 18 °C and a 12 h photoperiod with photosynthetic photon flux of 500 μmol m^{-2} s^{-1} at plant height. After 3, 4, and 5 d, rolls were taken out of solution, and immediately cut into five parts (7 cm each) (Fig. 3A). Each part was weighed fresh, immersed in 50 ml of distilled water, agitated several times, and after 1 h removed and the electrical conductivity (EC) of the solution measured with a digital conductivity meter (CDM 210, Radiometer Analytical SAS, Lyon, France). The section of the roll was dried at 65 °C for 24 h. The EC of the solution in the paper was calculated by the following equation:

$$EC_{(\text{paper})} = EC_{(\text{extract})} \times \text{volume}_{(\text{extract})} / \text{volume}_{(\text{paper solution})}$$

$$= EC_{(\text{extract})} \times 50\text{ml} / FW\text{–}DW_{(\text{paper})}\,(\text{g})$$

EC is expressed in units of dS m^{-1} and was converted to mM NaCl by multiplying by 10, since 1 dS m^{-1} is equivalent to approximately 10 mM NaCl.

Plant growth conditions

Cultivars of durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) were originally sourced from the Australian Winter Cereals Collection, and grown prior to the present studies in a glasshouse to obtain uniform seed from the same environment. Seeds of up to eight cultivars were selected that were uniform in size and weight, soaked in tap water for 2 h to imbibe and then surface-sterilized with Thiram solution (1.4 g l^{-1}) for 5 min. Before rolling the seeds in the germination paper, a crease was made 2 cm from the top of the shorter side (25 cm) for seed placement, 3 mm of paper was folded twice along the long side (28 cm) to provide a starting fold for rolling, and water was misted along the crease to help secure the seeds. Seeds were placed along the crease spaced a few centimetres apart with the embryo facing the bottom of the page. The paper was rolled tightly, dipped whole into a tray of water and put into a PVC™ tube with 500 ml tap water. Tubes were sealed with Parafilm™, refrigerated at 4 °C in the dark for 2 d and then the Parafilm™ was removed and the tube transferred to the growth cabinet set to the conditions described above. Coleoptiles appeared at the tip of the papers after 2 d.

Experiment 1

To determine the concentration to distinguish genotypic responses to salt, a salt-tolerant (Coulter) and a salt-sensitive (Candicans) durum cultivar was rolled and grown according to the conditions described above. These two durum wheat cultivars were found to differ in salinity tolerance in terms of shoot biomass production after 7 weeks in the soil (Rahnama *et al.*, 2010). Five days after coleoptiles emerged at the top of the rolls, roots were exposed to the salt gradient described above by first dipping the roll in a PVC tube with 1500 ml of 0, 50, 100, 150, or 200 mM NaCl in half-strength modified Hoagland's solution for 15 min, then transferring to tubes with 500 ml of the same salt solution for 5 d until harvested. Tubes were covered with a sealed plastic bag to avoid

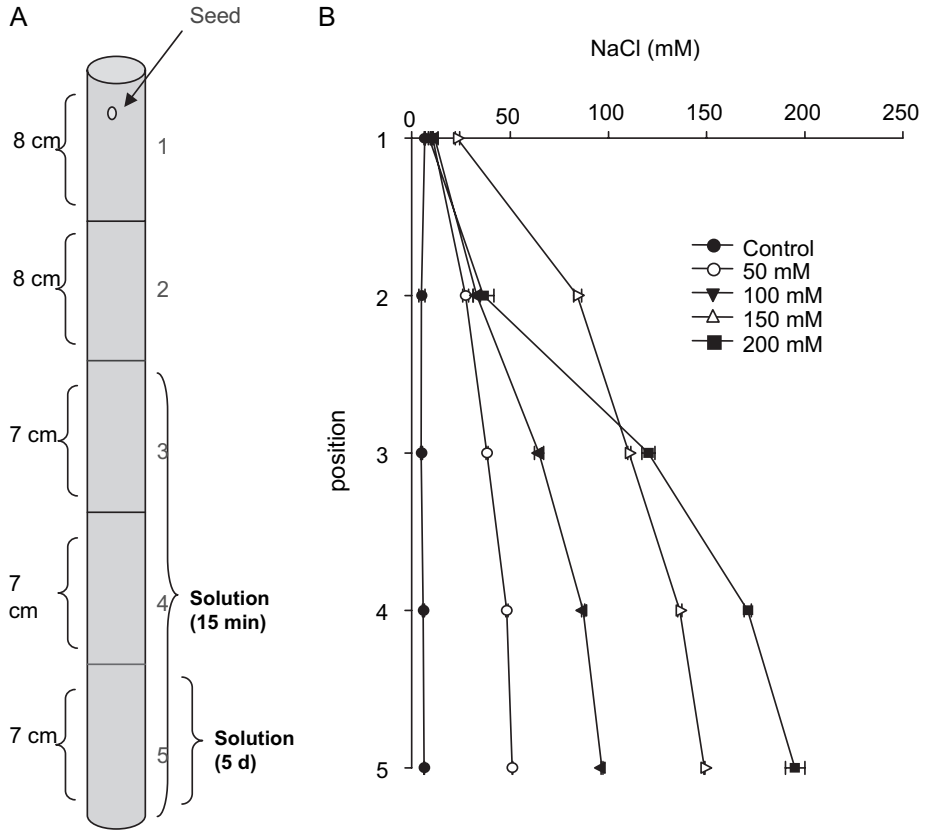

Fig. 3. Salt gradient. (A) Diagram of a paper roll indicating the height (21 cm) dipped in salt for 15 min (sections 3 to 5) and height (7 cm) of the paper sitting in solution for the remainder of the experiment (5 d for Experiments 1 and 2; 12 d for Experiment 3). (B) Salt concentration of the sections of the paper roll shown in (A) after exposure to different concentrations (Control to 200 mM) for 5 d (similar profile to that after 2 d and 4 d).

evaporation throughout the 5 d (Fig. 2C). At harvest, shoots and roots were preserved in 50% ethanol until the length of each seminal root and shoot were measured with a ruler. The electrical conductivity of 7 cm sections of the paper was measured as described above immediately after harvest.

Experiment 2

Eight durum cultivars (Coulter, Seklavi, Emblem, and Hercules, considered tolerant based on shoot traits; Brkulja, Candicans, Koelz, and Durex, considered sensitive based on shoot traits) were grown as in Experiment 1 with 0 or 150 mM NaCl in half-strength modified Hoagland's solution, to test the range of genetic variation in root growth in saline conditions. Plants were harvested after 5 d of exposure to the saline gradient as above, and preserved in 50% ethanol until seminal axile roots were measured with a ruler. The electrical conductivity in different parts of the papers was also measured.

Experiment 3

In order to determine the relative responses of axile and branch roots to the saline gradient, four cultivars (Coulter, Seklavi, Brkulja, and Candicans), were grown in the conditions described above. They were screened with 0 and 150 mM NaCl as above for 12 d, harvested, and preserved in 50% ethanol. Seminal axile root lengths were measured with a ruler, in addition to the distance between the axile root tip of the longest seminal root and emergence of the most distal branch root. Total root length and average diameter was determined with WINRhizo software and an Epson 1680 modified flatbed scanner (Regent Instrument Inc., Quebec, CA) according to Watt *et al.* (2005). The length of the branch root was the total length minus the total axile root length, previously measured with a ruler.

Statistical analysis

Analyses of variance were carried out using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Comparisons between mean values were made using MSTAT-C version 2.1 (Russell D Freed, MSTAT Director Crop and Soil Sciences Department, MSU, USA), using least significant differences (LSD) between means at a significance level of P=0.05.

Results

Generation of a standing gradient in salinity

It was considered important to generate a stable gradient of salinity, from very low at the top increasing gradually to the base of the rolls. This would mimic most field situations where seed is sown after rain or irrigation which leaches salt downwards (Fig. 1). It was also essential to reduce evaporation from the top of the pot otherwise salt would concentrate over time at the top of the rolls.

A stable gradient in salinity was generated by first wetting the paper with water, then immersing the lower 60% for a short time in the required salinity, then the lower 20% for the remainder of the experiment (Fig. 3A). This created a continuous and stable salt gradient along the paper (Fig. 3B).

When evaporation from the top of the paper was minimized by covering the papers with a plastic bag (Fig. 2C), the gradient was stable over 3 d in the light (data not shown; 3 d and 4 d after exposure, the gradient was similar to the 5 d gradient shown in Fig. 3B), and presumably for longer as long as the shoots could be enclosed in a high humidity environment. This was necessary in order to prolong the experiment sufficiently for lateral roots to be initiated and their elongation rate included in the total root assessment. Seeds therefore germinate in low salinity, and root tips reach high salinity 1–2 weeks later.

When evaporation was not minimized, salt rose to the top and reached a concentration of 600–700 mM (data not shown). Ideally, evaporation should be inhibited but transpiration allowed, however, it was found impossible to seal around the base of every shoot. This would be possible with one plant per roll, and one roll per tube but not with ten rolls.

Optimum salt concentration to distinguish genotype differences

A range of salinity concentrations at the base of the tube from 50–200 mM was chosen to represent the range of field concentrations that commonly occur, to see which concentration provided the most distinction between different genotypes. A concentration of 100 mM or above was found to be the most suitable concentration for differentiating total root length and the length of the two longest seminals of Coutler and Candicans (Fig. 4). Candicans had a greater reduction in total root length than Coulter at 100 mM NaCl

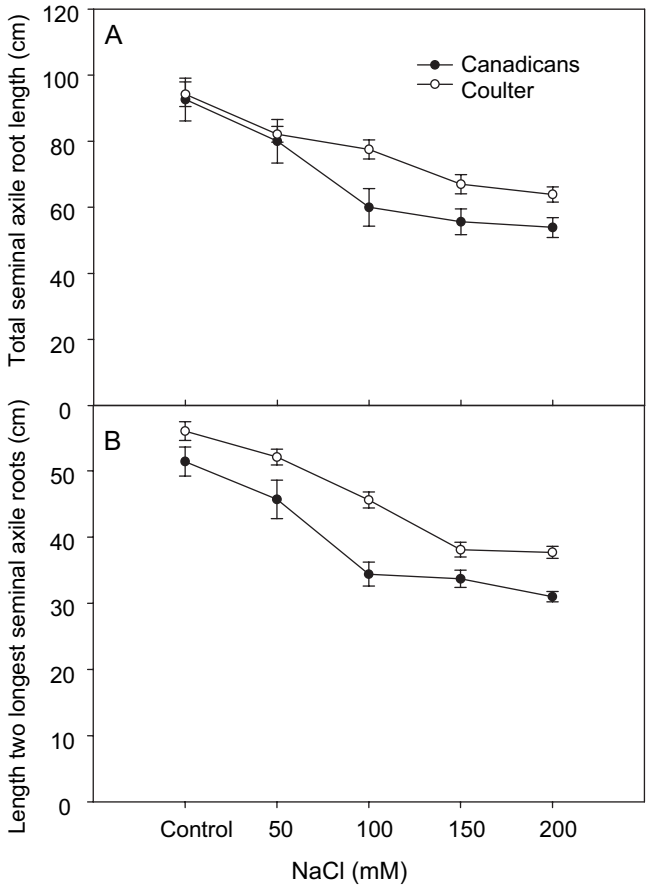

Fig. 4. Root growth of two durum cultivars after exposure to different concentrations of salt gradient for 5 d. Seminal axile roots measured with a ruler. Length in (B) is the sum of the two longest axile roots. Mean ±standard error of 20 plants per line.

or above. 150 mM was therefore chosen for further experiments. It also equated with the concentration found to discriminate the shoot growth and stomatal response of these two lines (Rahnama *et al.*, 2010).

Variation among genotypes

The eight cultivars of durum showed genetic variation in total seminal axile root length ranging from 79% to 96% of the control (Fig. 5; see Supplementary Table S1 at *JXB* online). Candicans, Koelz, and Durex were significantly reduced by the salinity gradient, while the other cultivars were not significantly reduced. These three cultivars were also in the sensitive group (James *et al.*, 2008; Rahnama *et al.*, 2010) based on their leaf growth and stomatal sensitivity.

Response of axile and branch roots to saline gradient

After 12 d exposure to the 150 mM NaCl gradient, the Coulter total root system length did not show a response to salinity (Fig. 6A), similar to that observed after 5 d exposure in Fig. 5. That of Seklavi, Brkulja and Candicans, however, declined significantly (Fig. 6A; see Supplementary Table S2 at *JXB* online). It is possible that the longer exposure in this experiment provided enough time for the sensitivity of total root length to salinity to become evident in these lines. When the seminal axile and branch root length were examined separately, it was found that the seminal axile root elongation decreased in response to the salt in the four genotypes (Fig. 6B), while the branch root length increased in three of the four genotypes (Fig. 6C), helping to compensate for the reduction in seminal axile root length. The distance between the seminal axile root tip and the most distal branch root was shortened at least 6-fold in the four genotypes (from at greater than 15 cm to about 3 cm) (Fig. 6D). The architecture of the root systems was modified by salt through inhibition of the seminal axile roots and promotion of the branch roots in all but Seklavi, showing that the proportion of the root system component is modified by the salinity gradient. This difference may have been caused by the time of exposure to salt, as the branch roots would have initially emerged and grown into the to 20 cm of the gradient, where NaCl concentration was less than 100 mM, while the seminal axile roots would have been exposed to 150 mM of salt for at least 7 d. It is also possible that the salt gradient caused a shift in tissue differentiation along the axile roots, and this is discussed below.

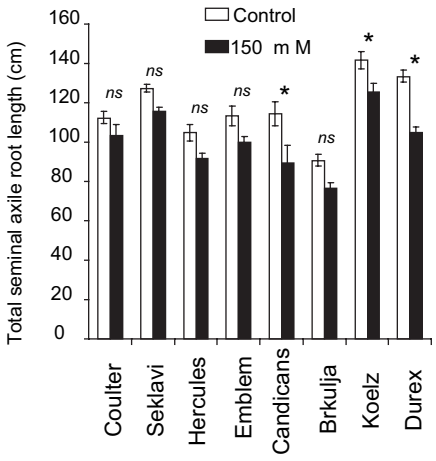

Fig. 5. Root growth responses to salt by eight durum genotypes. (A) Total seminal axile root length measured with a ruler after exposure to 150 mM salt gradient for 5 d compared with the control, half-strength nutrient solution without salt.
Mean ±standard error of 20 plants per line. Asterisks indicate a probability of significant difference of 95% between the control and 150 mM salt for a line; ns=no significant difference.

Discussion

Features of screen and other applications

This paper reports on a rapid screen to identify genotypic variation in whole root system growth responses to a salinity gradient. Many saline soil conditions have very low salt at the surface where the seed is sown and germinates, and increasing salt concentration with depth that would be encountered by the root system as it penetrated deeper (Fig. 1). Salt gradient conditions were simulated here in germination paper rolls that were quick to set up, easy to transfer between saline solutions, and easy to reveal the intact root systems for rapid ruler measurements or scanning at harvest. Importantly, the papers were readily cut and segmented for extraction to measure salt concentration with the EC meter to determine the local salt conditions after time and around a given portion of the root system. In the salt gradient, seminal axile root growth continued vertically into the regions of higher salinity, and did not show any loss of gravitropism as found by Zolla *et al.* (2010) with *Arabidopsis*, indicating a realistic response.

This screen may help to bridge the gap between controlled environment screens and the field, as hydroponics where solutions are continually washed over entire root systems can give quite different results from pot experiments in soil, in terms of growth response and transport of salt into the shoot (Tavakkoli *et al.*, 2010).

The method could be adapted to represent the chemical composition of particular saline soils, for example, by adding more Ca^{2+} or sulphate, or increasing the pH with bicarbonate. The method could also be useful to select for tolerance of toxic compounds such as boron or heavy metals, as the gradient will give more information than just for single concentrations, for example, in the conventional B screen (Chantachume *et al.*, 1995). It could be used for gradients in nutrient supply and in waterlogging studies with a subsoil perched water table. It might also be related to soil water deficit caused by drought, if validated with other osmotica such as concentrated macronutrients (Munns *et al.*, 2010).

A major limitation of the screen is that the shoot is enclosed in a plastic bag to limit evaporation from the top of the paper roll and migration of the salt towards the seed. This limits the use of the screen to young seedling stages of

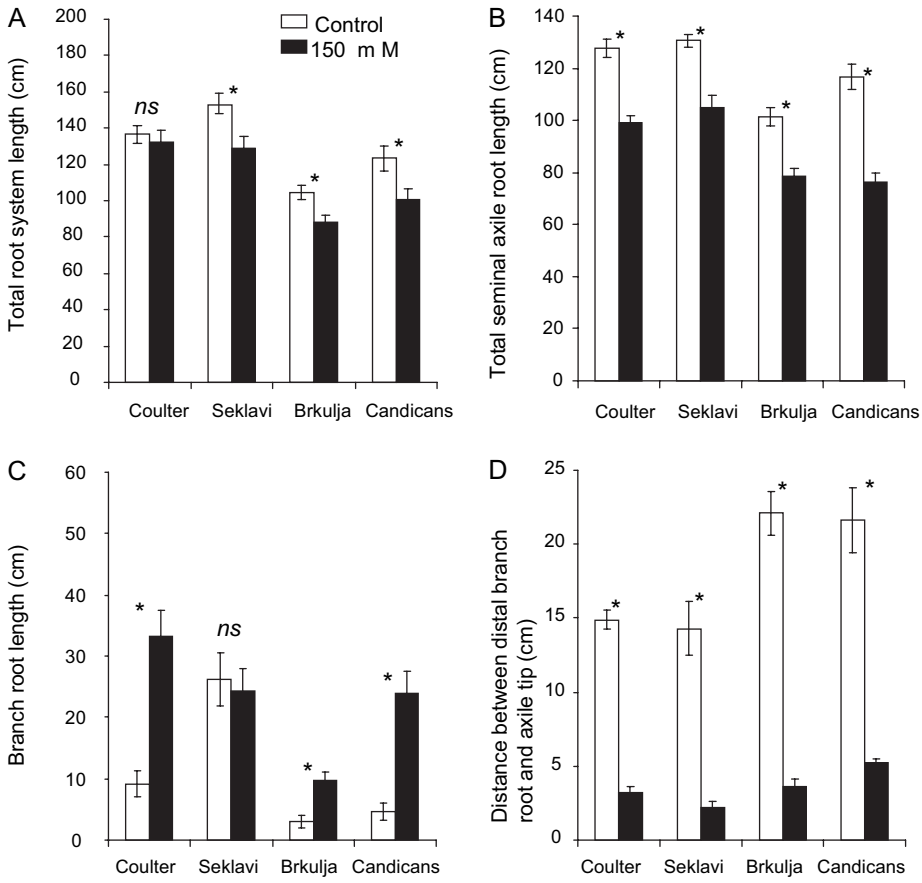

Fig. 6. Responses of root system components (axile and branch roots) to 150 mM salt gradient in four genotypes. (A) Total root system length including branch roots measured by scanning and image analysis. (B) Seminal axile root lengths measured with a ruler. (C) Branch root length obtained by subtracting axile root length from total root system length. (D) Distance along longest axile root length between tip and position of emergence of most distal branch root measured with a ruler. Mean ±standard error of 20 plants per line. Asterisks indicate a probability of significant difference of 95% between control and 150 mM salt for a line; ns=no significant difference.

wheat and other grain cereals, although *Brachypodium* could readily reach the 5-leaf-stage within the conditions because of its small shoot and root size (Watt *et al.*, 2009).

Variations in axile and branch root growth in response to salt gradient

The most striking effect of the screening conditions was the shift in contribution to total root system length from the seminal axile roots to the branch roots after 12 d exposure to the salt gradient (Fig. 6). All genotypes showed a significant. reduction in seminal axile length at that stage, including Coulter which had limited reduction at 5 d exposure (Figs 4, 5). However, in all genotypes but Seklavi, branch root growth increased greatly in the presence of the gradient (Fig. 6). The adaptive advantage of increased branch root growth when the axile tip is slowed could be the maintenance of total root length for access to water and nutrients, as was seen in Coulter here, where axile roots were shortened by the salt but the concomitant increase in branch roots compensated, maintaining root length.

This phenotype of shorter axile roots with a concomitant increase in branch roots suggests that differentiation of the pericycle cells proceeded relative to the advancement of the axile, parent root which was slowed by the salt. There may have been a loss of apical dominance in the axile root tips mediated by the external salt and osmotic conditions, while sugars and water continued to be delivered to the branch root primordia via the phloem to enable growth (Boyer *et al.*, 2010). In the salt gradient of this screen, the upper part of the root systems were not exposed to salt and would have been able to take up similar amounts of water as the controls in that region, to help maintain root growth in the lower portions, and shoot growth and photosynthesis for sugar supplies to the roots. Since branch roots are generally thinner than axile roots, more root length can be generated per unit carbon in branch roots, perhaps explaining the shift from axile elongation. Given that some of the branch root primordia were within the salty segments of the paper during the screen, it is possible that branch root tips are less sensitive to salt than axile roots. This needs to be tested in future experiments perhaps using time-lapse imaging of entire root systems with measurements of local salt concentrations around branch and axile primordia (Watt *et al.*, 2006; Fig. 3).

Earlier studies have reported that root system biomass may even be greater in dry or saline soils than in well-watered

and non-saline conditions (reviewed by Munns and Sharp, 1993). This may be due to the stimulation of lateral root initiation, as shown for plants in dry soil by Jupp and Newman (1987), or to stimulation of the rate of individual root elongation, as reported for plants in saline solution by Kurth *et al.* (1986). Recently, Zolla *et al.* (2010) found that *Arabidopsis* root systems proliferated branch roots while axile (tap) root length slowed when grown on agar with basal nutrient medium with salt. The medium probably contained sucrose for the maintenance of branch and axile root growth, suggesting that the axile tips are more sensitive to the salt conditions than the branch roots and that differentiation of stelar pericycle cells was maintained to generate branch root primordia and their growth in salt.

Previous studies of other plant species growing in salty conditions that surrounded the entire root systems have found that vascular root tissues continue to differentiate despite slowing of axile tip elongation. In detailed anatomical studies of cotton tap roots growing in vermiculite with 150 mM or 200 mM NaCl, Reinhardt and Rost (1995*a*) showed that, as axile root (tap root) elongation slowed, xylem vessel differentiation proceeded such that mature vessels were found closer to the tips of salt-stressed plants than control plants without exposure to salt. They also found that branch roots emerged about three times closer to the tip when the tap elongation was severely restricted (over ten times slower than the control) in 200 mM NaCl compared with the control (Reinhardt and Rost, 1995*b*). Other soil stresses also shorten the distance between the tip and positions of tissue differentiation. The growth zone of maize primary roots grown at low water potential in vermiculite shortened progressively as the water potential decreased and elongation decreased (Sharp *et al.*, 1988). High aluminium concentration in solution induced rapid branch root development near the tips of roots of an Al-sensitive maize line (Doncheva *et al.*, 2005). In hard soil, root hairs and branch roots emerged closer to the axile tip than in soft soil (Watt *et al.*, 2003). Working with bread wheat seminal axile roots in a soil compaction system that allowed the estimation of root elongation rate and the ages of tissues at distances from the tip, it was found that root hair and branch root differentiation accelerated relative to the tip, and hairs and branch roots emerged earlier in hard soil than in loose. All soil stresses, however, do not result in a shorter distance between tips and the developmental differentiation of cells, as cool soil temperatures do not uncouple root elongation rate and differentiation rate of root hairs and branch roots behind the tip in wheat and maize (reviewed in Watt *et al.*, 2006).

Genotypic variation and possible mechanisms of salt tolerance in roots

This study revealed genotypic differences in root elongation response in durum wheat using a salt gradient from 0–150 mM NaCl, a typical salt profile of saline soils that limit the yield of wheat (Rengasamy, 2010). The germplasm chosen was known to differ in salt tolerance in terms of shoot growth rate and in stomatal response that indicated photosynthetic limitations (Rahnama *et al.*, 2010). In that study, the genetic variation in the shoot growth response was shown to be due to the osmotic effect of the salt, and not to an ion-specific effect of the NaCl, because the response occurred immediately the roots were exposed to salt, that is before any Na^+ or Cl^- would have entered the shoot, and because KCl had the same effect on stomatal conductance as did NaCl (Rahmana *et al.*, 2010). In this present study, the four of the five genotypes without significant inhibition in seminal axile root elongation response after 5 d exposure to the saline gradient (Fig. 5) were also those previously identified with the least inhibition of stomatal conductance and shoot growth rate. After 12 d exposure however, only Coulter (with tolerant shoot traits in previous studies) had no significant inhibition to total root length. All genotypes showed a shift in root architecture (Fig. 6) in response to the salinity gradient. There may be differences in shoot and root responses to saline conditions that are genotype specific, and this needs to be explored more thoroughly in future studies with concurrent shoot and root measurements.

It is probable that the roots exposed to the higher salinity were reduced in growth mainly because of the osmotic effect of the salt, rather than a salt-specific or toxic effect. The mechanism of salt tolerance at the cellular level is compartmentation of salt in vacuoles and the maintenance of a low concentration of Na^+ in the cytoplasm. What constitutes a toxic concentration of Na^+ in the cytoplasm of cells is hard to know, but could be as little as 30 mM (Munns and Tester, 2008). As meristematic cells have only small vacuoles, the tissue concentration as a whole is indicative of the cytoplasmic concentration. Na^+ concentration in the meristematic zone of root tips (0–1 mm from the tip) of the halophyte *Atriplex amnicola* was only 20 μmol ml^{-1} tissue volume (approximately 20 mM) when the plants were growing in 200 mM NaCl (Jeschke *et al.*, 1986). On the other hand, a detailed study of Na^+ deposition rates in cotton roots growing in 150 mM NaCl and 1 mM Ca^{2+} showed that Na^+ concentrations were 100 μmol g^{-1} FW (approximately 125 mM), and were reduced to about 40 μmol g^{-1} FW when Ca^{2+} was increased to 10 mM (Zhong and Läuchli, 1994). This was accompanied by an increase in growth rate (Zhong and Läuchli, 1993). In the present study, the Ca^{2+} concentration was 2 mM, a concentration shown previously to be adequate for ion homeostasis and growth rate in roots of durum wheat (Husain *et al.*, 2004). The Na^+ concentration in roots as a whole was about 0.8 mmol g^{-1} DW (about 50 mM) at 150 mM NaCl and was not affected significantly when the Ca^{2+} concentration was increased from 2 mM to 10 mM (Husain *et al.*, 2004).

Whether root growth in wheat is limited by the toxic effect of Na^+ could be tested with other media. In *Arabidopsis*, mannitol had a similar effect to NaCl at similar osmotic strength on total root growth (Sun *et al.*, 2008; Zolla *et al.*, 2010), although analysis of axile versus branch roots found that the main effect of mannitol was on lateral root length whereas that of NaCl was on primary root length (Zolla

et al., 2010). Alternatively, another inorganic osmoticum such as KCl could be used. KCl had the same effect on stomatal response as NaCl (Rahnama *et al.*, 2010), but the roots were not measured. It is unlikely that Cl^- is the toxic ion (Munns and Tester, 2008), but this could be checked with balanced concentrated salts, but not simply by substituting Cl^- with SO_4^{2-} or NO_3^- as they are more toxic than Cl^- when given as a single salt (Termaat and Munns, 1986).

In more extended studies, shoot growth was more inhibited than root growth in saline and dry soils (see review by Munns and Sharp, 1993), however, in this study, there was little effect on shoot growth (data not shown). Thus, assimilate supply was not limiting root growth. Shoot growth was presumably little affected because the roots were not experiencing the salt until they had grown nearly 20 cm and the first leaf was half developed, unlike standard experiments in hydroponic or sand culture when all the roots are exposed to the same concentration of salt. In this study, the roots encountered the salt in a gradual incremental manner, and the bulk of the root system remained in contact with low salinity throughout the experiment, and would be taking up water from the low salinity solution. Split root studies have shown that the plants extract water from the least saline solution. This complicates the interpretation of results, however, it mimics the real conditions in the field.

Conclusions and future work

The salt gradient set up on paper rolls presents a rapid new screen for root growth responses that can be applied to other species and to other osmotica, nutrients, waterlogging, or toxic elements. The screen was useful for measuring the growth responses of the primary axile roots and the branch roots, and gave a measure of changes in the overall root system architecture in a realistic environment. The benefits for the establishment of a screening medium for cereals are that they can be used in a number of model grass systems, such as rice and *Brachypodium*, and for different abiotic stresses. Ultimately, the results with the paper assay need to be validated in real soils.

Supplementary data

Supplementary data can be found at *JXB* online.

Supplementary Table S1. Effect of salinity levels on total seminal axile root length of eight durum genotypes (5 d of salt treatment).

Supplementary Table S2. Effect of salinity levels on total root system length, total seminal axile root length, branching root length, and distance between distal branch root and axile tip of four durum genotypes (12 d of salt treatment).

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