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Genetic analysis and physiology of a trait for enhanced K^+/Na^+ discrimination in wheat

BY J. GORHAM[1], J. BRIDGES[1], J. DUBCOVSKY[2], J. DVORAK[2], P. A. HOLLINGTON[1], M.-C. LUO[2] AND J. A. KHAN[1]

[1] *Centre for Arid Zone Studies and School of Biological Sciences, University of Wales, Bangor, Deiniol Road, Bangor, Gwynedd LL57 2UW, Wales*
[2] *Department of Agronomy and Range Science, University of California, Davis, CA 95616, USA*

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SUMMARY

Variation for K^+ and Na^+ accumulation at low salinities in hydroponic (water) culture were observed in shoots of different wheat species. Greater discrimination (in favour of K^+ and against Na^+ accumulation) was shown by hexaploid bread wheat (*Triticum aestivum* L.) than by tetraploid durum wheat (*T. turgidum* L.). Since *Aegilops tauschii* Cosson (*A. squarrosa* L.), the source of the D genome in bread wheat, also exhibited high discrimination between K^+ and Na^+, it was concluded that the character resided in the D genome. Studies of aneuploid bread wheat lines and disomic substitution lines of D genome chromosomes for their A and B genome homoeologues in durum wheat cv. Langdon revealed that the trait was controlled by the long arm of chromosome 4D. Since the aneuploid and disomic substitution lines showed better relative salt tolerance than durum wheat, but had lower yield potentials, we recombined chromosome 4D with chromosome 4B in a tetraploid wheat background using a homoeologous pairing mutant. This produced families of 4D/4B recombinant lines, some of which exhibited the enhanced K^+/Na^+ discrimination trait. RFLP analysis confirmed that the trait was controlled by a single gene (*Kna1*) which was completely linked to five markers on the distal third of the long arm of 4D. A second cycle of homoeologous recombination was employed to remove the distal 4D genetic material from the recombined *Kna1* 4B/4D chromosome and to map *Kna1* in greater detail. By this strategy, *Kna1* was mapped within a short 2 cM region. Genetic analysis of K^+ : Na^+ ratios showed very high LOD scores in this region for plants grown in solution culture, but lower values for plants grown in the field.

In general, recombinant lines which exhibited the enhanced K^+/Na^+ trait were slightly more tolerant of salinity in the field and in sand culture than recombinants lacking the trait. There was, however, considerable variation between individual lines. Ion discrimination and relative tolerance were also higher in a *Kna1* recombinant (line no. R3) than in a *kna1* recombinant (line no. R165) in sodic conditions. In these two lines the enhanced K^+/Na^+ discrimination trait did not alter responses to low potassium or calcium supply.

Key words: *Triticum aestivum* (bread wheat), *Triticum turgidum* (durum wheat), salt tolerance, ion accumulation, QTL.

INTRODUCTION

In experiments on the ancestors of modern wheat species, differences in K^+ and Na^+ accumulation at low (< 100 mol m^{-3} NaCl) salinities in hydroponic (water) culture were observed in shoots of the different wheat and *Aegilops* species (Wyn Jones, Gorham & McDonnell, 1984; Shah *et al.*, 1987). Greater discrimination (in favour of K^+ and against Na^+ accumulation) was shown by hexaploid bread wheat (*Triticum aestivum* L.) than by tetraploid durum wheat (*T. turgidum* L.). Since *Aegilops tauschii* Cosson., the source of the D genome in bread wheat, also exhibited high discrimination between K^+ and Na^+, it was concluded that the character resided in the D genome. The S-genome *Aegilops* species, which are related to the genome of wheat, did not show enhanced K^+/Na^+ discrimination (Gorham, 1990*b*). Further investigations (Gorham *et al.*, 1991; Gorham, 1993) suggested that

* To whom correspondence should be addressed.
E-mail: j.gorham@bangor.ac.uk

although the trait was exhibited by diploid wheats (*T. monococcum* L. and *T. urartu* Thum.), and survived in tetraploid *T. timopheevi* Zhuk. (genome SSAA), it had been lost during the evolution of *T. turgidum* (genome BBAA). Within the tribe Triticeae the enhanced K^+/Na^+ discrimination trait was also observed in U-, M- and T-genome *Aegilops* species (Gorham, 1990*b*), rye and triticales (Gorham, 1990*a*) and synthetic hexaploid wheats (Gorham 1990*c*) derived from tetraploid wheat and *Aegilops tauschii*. It was not exhibited by cultivated barley (*Hordeum vulgare* L.) or its wild relative *H. spontaneum* (Gorham *et al.*, 1990), or by perennial wheatgrasses (Gorham, 1994), both of which are more salt-tolerant than bread wheat (see King *et al.*, 1997). For other wild barley species the picture is not clear (Gorham, 1992, 1993).

Physiological investigations showed that the trait affects transport of K^+ and Na^+ to the shoots, with little effect on root ion concentrations or on anion accumulation in leaves, and that the main site of action is probably at xylem loading in the roots (Gorham, Wyn Jones & Bristol, 1990). Although the trait can be demonstrated at all salt concentrations, it is most apparent at low salinities (< 100 mol m^{-3}). At higher salinities other mechanisms which control ion accumulation appear to be more important.

Experiments with disomic substitution lines in which D-genome chromosomes replaced their A and B genome equivalent in durum wheat cv. Langdon revealed that the trait was controlled by the long arm of chromosome 4D (Gorham *et al.*, 1987). Further investigations with aneuploid bread wheat lines based on cv. Chinese Spring located the trait on the long arm of chromosome 4D. Gene dosage had little effect on expression of the trait since lines tetrasomic for chromosomes 4D had similar leaf K^+ and Na^+ concentrations to disomic 4D lines.

Since some aneuploid lines showed better relative salt tolerance than durum wheat, but (as aneuploids) had low yield potentials, we recombined 'Chinese Spring' chromosome 4D from the 'Langdon' disomic 4D(4B) substitution line with chromosome 4B in a tetraploid wheat background using the homoeologous pairing mutant *ph1c* in cv. Senator Cappelli (Dvorak & Gorham, 1992). Initially this produced 39 families of 4D/4B recombinant lines, and nine out of 27 lines tested in hydroponics exhibited the enhanced K^+/Na^+ discrimination trait. Na^+ concentrations in young leaves fell into two non-overlapping classes, and subsequent RFLP analysis of 129 families (Dubcovsky *et al.*, 1996*b*) confirmed that the trait was controlled by a single gene (*Kna1*) which was completely linked to five markers (*XWg199*, *Xabc305*, *Xbcd402*, *Xpsr567* and *Xpsr375*) on the distal third of the long arm of 4D. To remove the distal 4D genetic material from the *Kna1* recombined chromosomes, and to further map the *Kna1* locus, two lines were subjected to another round of homoeologous recombination in the absence of the *ph1* locus (Luo *et al.*, 1996). By this procedure, *Kna1* was mapped in a 1·1 cM region on the 4B/4D map. This region spans markers *Xmwg2112* and *Xpsr375*. This region was partially mapped in *T. monococcum* where the distance from *Xwmg2112*, a marker immediately preceding *Xpsr375*, is 2·8 cM (Dubcovsky *et al.*, 1996*a*). This estimate of the length of the region including *Kna1* is similar to its estimate from the 4B/4D map, 2·2 cM, obtained by doubling the length of the region on the homoeologous 4B/4D map, since the homoeologous and homologous maps are in a 1:2 proportion at the end of the chromosome.

QTL analysis of K^+/Na^+ ratios in young leaves of glasshouse (hydroponic)- and saline-field-grown plants (Dvorak *et al.*, 1994) showed very high LOD scores in this region for plants grown in hydroponics, but lower values for plants grown in the field. In general, recombinant lines which exhibited the enhanced K^+/Na^+ trait were slightly more tolerant of salinity in the field than recombinants lacking the trait (Dvorak *et al.*, 1994). There was however, considerable variation between individual lines, which is understandable considering that they originated from a hybrid involving into very different genetic backgrounds. We now report on the effect of *Kna1* on the performance of tetraploid wheat in a variety of conditions.

MATERIALS AND METHODS

Triple line sprinkler, Spain

Field studies were performed in 1990 and 1991 using a triple line source (TLS) sprinkler system developed and evaluated by Aragüés, Royo & Faci (1992). This consisted of three parallel sprinkler irrigation lines, with the lateral spacing equal to the wetted radius of the central sprinkler. The outer lines applied fresh water, while the central line applied saline water made up of a 1:1 (w/w) ratio of NaCl and $CaCl_2$. This arrangement gave two continuous salinity gradients on each side of the central line, while maintaining uniform application of water.

Plots of hexaploid wheat cv. Siete Cerros, tetraploid wheat cv. Langdon, the Langdon 4D(4A) disomic substitution line (Joppa & Williams, 1988) and an amphiploid (Forster, Gorham & Miller 1987) between hexaploid wheat cv. Chinese Spring and *Thinopyrum bessarabicum* (Savul. and Rayss) Löve were established on a loamy soil (mixed, mesic Typic Torrifluvent) on the experimental farm of the Servicio de Investigation Agraria, Diputación General de Aragón at Zaragoza, Spain. Sowing dates were 20 January 1989 and 19 November 1990. Ten individual plots of each cv. were sown in each of two replicates, parallel to the sprinkler lines, at a seed rate of 62·5 seeds m^{-1}. Plots were of two rows, 200

mm apart and 1·2 m long. There were 80 plots in total in each year. Sprinkler irrigation was applied on about 30 occasions, with gradually increasing salinity up to 20 dS m^{-1} at the fifth irrigation.

Leaf samples were taken at the booting stage for ion analysis (see below). At harvest, the total number of plants and spikes per plot were recorded. The ten tallest primary tillers from each plot were analysed for grain yield and yield components.

Sand culture

Sand and sodic soil culture experiments were performed in glasshouses at Pen-y-Ffridd Field Station, University of Wales, Bangor. The minimum temperatures were 18/16 °C day/night with a photoperiod of 16 h d^{-1} (natural daylight supplemented with 400 W Son-T® high pressure sodium lamps; Osram, UK). Pots were filled with washed sand and three seeds were sown per pot. The pots were placed over metal mesh benches for free drainage. Eight recombinant genotypes (Dvorak & Gorham 1992) were used, five *Kna1* (R3, R63, R112, R146, R173) and three *kna1* (R21, R23, R165). Of the latter, one was a short-arm recombinant genotype (R21), two were long-arm recombinant genotypes. Two seeds were sown per 2 dm^3 pot on 24 July 1993 and were thinned to one uniform plant 1 wk after germination. Two salinity treatments, 0 (control) and 150 mol m^{-3} NaCl, were studied, each with 10 replicates. Salt was applied on alternate days in Phostrogen®-based nutrient solution at 0·5 g l^{-1} with 0·5 ml l^{-1} micronutrient solution (Hoagland & Arnon, 1950) when the plants were at the two-leaf stage. Calcium chloride was added to the solution to maintain a $Na^+:Ca^{2+}$ ratio of 20:1. Six plants were harvested 5 wk after germination, three for chemical analysis and three for fresh and dry weights. The other four plants were used for grain yield parameters.

Sodic soil

To establish exchangeable sodium percentages (ESP) of 12·5, 25·0 and 50, compost (John Innes Number 1) was passed through a 3·2-mm mesh screen and then treated with sodium bicarbonate solution. The initial cation exchange capacity (CEC) and ESP of the compost were 17·5 and 3·2 meq per 100 g respectively. Compost (91 kg) was spread 2·54 cm thick on a polyethylene sheet and sprayed with 7·5 l of 0·535 mol m^{-3} sodium bicarbonate solution by means of a knapsack sprayer. Two other lots of compost were treated similarly with double and triple the above concentration of sodium bicarbonate. The separate lots of treated compost were covered for 3 d with another polyethylene sheet to reduce evaporation and to allow time for the compost to establish equilibrium with the sodium bicarbonate solution. The cover was then removed and each lot was raked, mixed and allowed to dry for 10 d with four rakings and mixings each d. Each lot was then analysed for CEC and ESP.

To determine the CEC, 5 g of air-dried, sieved (< 2 mm) compost was transferred into a 100 ml centrifuge tube, 40 cm^3 of 1 kmol m^{-3} ammonium acetate was added, the tube stoppered and shaken thoroughly to disperse the soil. The tubes were left for a few minutes and then placed in a hot water bath for 5 min to promote flocculation. They were then centrifuged for 5 min at 2000 rpm. The supernatant was decanted avoiding any loss of fine material. The process was repeated three times, bulking the decanted supernatant from each soil and making it up to 200 cm^3 with 1 kmol m^{-3} ammonium acetate in graduated flasks. Each flask contained all the exchangeable cations from the corresponding solid samples. These solutions were used for exchangeable sodium determination. The remaining soil was washed three times with 40-cm^3 portions of 95% ethanol, rejecting the washings but avoiding any loss of soil material. The ethanol-washed soil was leached on the filter paper with successive 30-cm^3 portions of 1 kmol m^{-3} KCl solution, allowing the funnel to drain between each addition and collecting the extracts in 100-cm^3 flasks and making up to 100 cm^3 with KCl. The flasks containing the displaced NH_4^+ were used to estimate the CEC of 5 g of the soil. A 40 cm^3 aliquot was transferred to a large boiling tube and the NH_4^+ was determined by distillation with NaOH, trapping in 50 cm^3 of boric acid with added indicator and titrating with 0·025 kmol m^{-3} H_2SO_4.

Two seeds per pot were sown on 6 July 1993 in 1-l pots which were placed in saucers. Seedlings were thinned to one uniform plant 1 wk after germination. The soil in the pots was saturated with the Phostrogen-based nutrient solution at 0·4 g l^{-1} with micronutrient solution at 0·4 ml l^{-1} on every third day, whilst the rest of the nutrient solution was applied in the saucers. On each subsequent application, salt in the saucers was dissolved with nutrient solution and applied to the pots. Four ESP levels, 3·2 (control), 12·5, 25 and 50, each with 10 replicates, were studied for their effects on the two recombinant genotypes R3 and R165. Five wk after germination, five plants were harvested for chemical analysis, and the rest left to mature.

Hydroponic culture with varying K^+ and Ca^+ nutrition

The hydroponic experiments were conducted in a similar glasshouse but in a temperature range of 25/15 °C day/night. Aerated hydroponic culture is considered better than sand culture in terms of control over salinity levels. Seeds were washed and sown in P84 plug trays (Plantpak Ltd, Maldon, Essex) filled with compost (John Innes Number 1) with one seed per cell. The trays were placed over

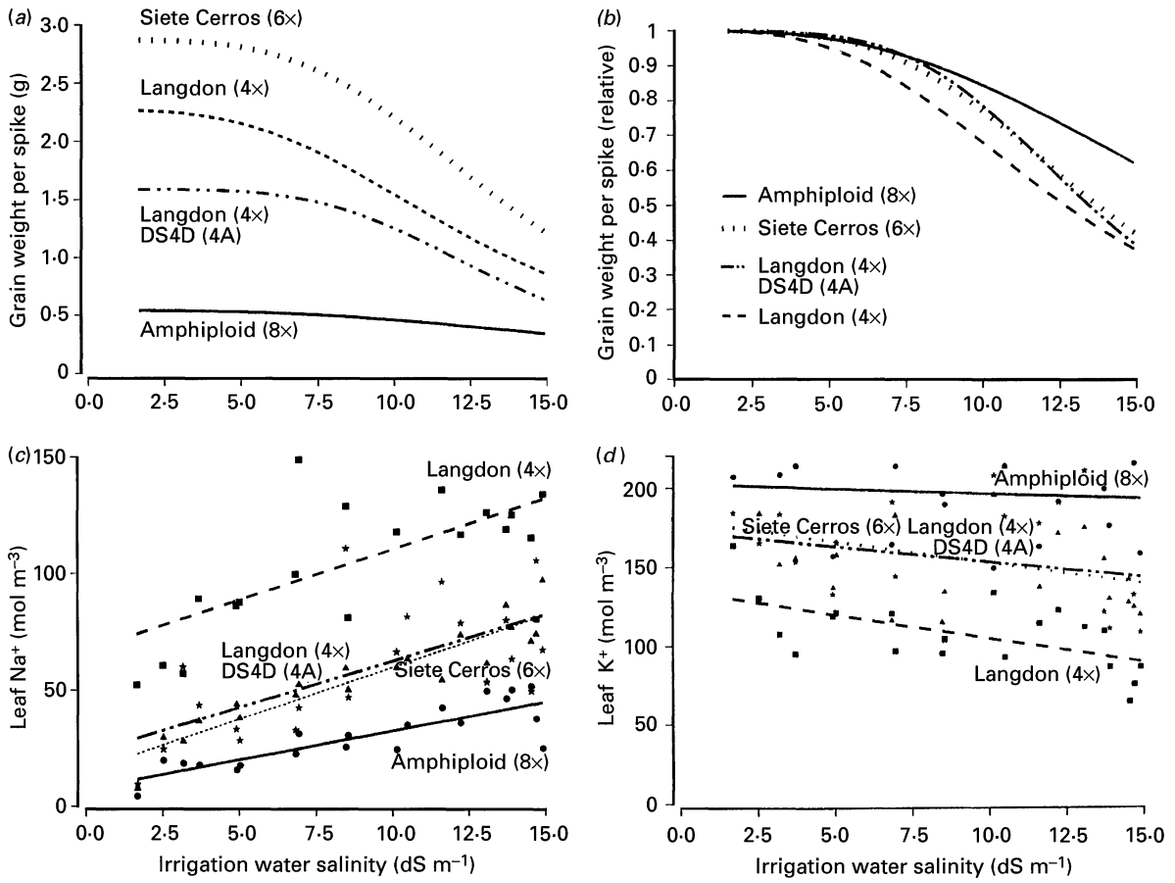

Figure 1. Performance of wheat species in a triple line sprinkler system, Zaragoza, Spain. Data are fitted according to the van Genuchten (1983) model for (*a*) grain weight per spike, (*b*) relative grain weight per spike compared with 0 salinity, (*c*) Na^+ and (*d*) K^+ concentrations in the third oldest leaf. The amphiploid is the amphidiploid hybrid between *Triticum aestivum* cv. Chinese Spring and *Thinopyrum bessarabicum*.

moistened vermiculite and were watered regularly. After *c.* 6 d, when the seedlings had two emerging leaves (one expanded and the other expanding) and well developed roots, young seedlings were transplanted into hydroponic culture. The plugtrays were suspended over 25-dm^3 tubs (Mailbox International Ltd, Stalybridge, Cheshire) containing a full-strength Hoagland nutrient solution with micronutrients. The solution in each tub was aerated constantly by an air compressor. The cells without plants were covered with black plastic sheets to give a uniform environment and minimize the risk of salt absorption through leaves.

Salt treatment commenced 2–3 d after transplanting. To avoid a salt shock, plants were exposed stepwise to salinity i.e. be adding salt to the nutrient solution at 30 mol m^{-3} d^{-1} until the desired final concentration was reached. A bulk solution for each treatment was then prepared and applied in the tubs. Daily evaporation losses were replaced by adding water. Solutions were changed at weekly intervals. Additional supports for the plants were provided during later stages of growth.

Two levels of salinity (0 and 6 mol m^{-3} NaCl), two levels of Ca^{2+} (0 and 4 mol m^{-3}) and three levels of K^+ (6, 1 and 0·1 mol m^{-3}) were studied for the two recombinant genotypes R3 and R165, giving 12 treatments with four replicates each. Seeds of the two genotypes were sown on 30 June 1994 in the trays as described above. The seedlings were transplanted to the tubs 2 wk after germination. Salinity treatments were started on 17 July 1994. Three plants of each genotype from each tub were harvested on 6 August 1994 for chemical analysis. Ion accumulation was determined for leaves (8 and 7) and roots. However, only data for leaf 7 (the leaf showing clear differences for the two genotypes) are given here. The experiment was terminated before maturity.

Sap extraction and ion analysis

Replicated samples of leaves, stems and roots of each genotype were cut, blotted dry with tissue paper and individually stored in 1·5 cm^3 microcentrifuge tubes and sealed. The roots were washed for 2 min in sorbitol solutions of the same osmolality as the corresponding treatment with 1 mol m^{-3} calcium acetate (to maintain osmotic pressure and avoid losses of ions) and dried with tissue paper. The tubes were frozen in a commercial freezer (at −18 °C) for a minimum of 24 h. The tubes were taken out of the

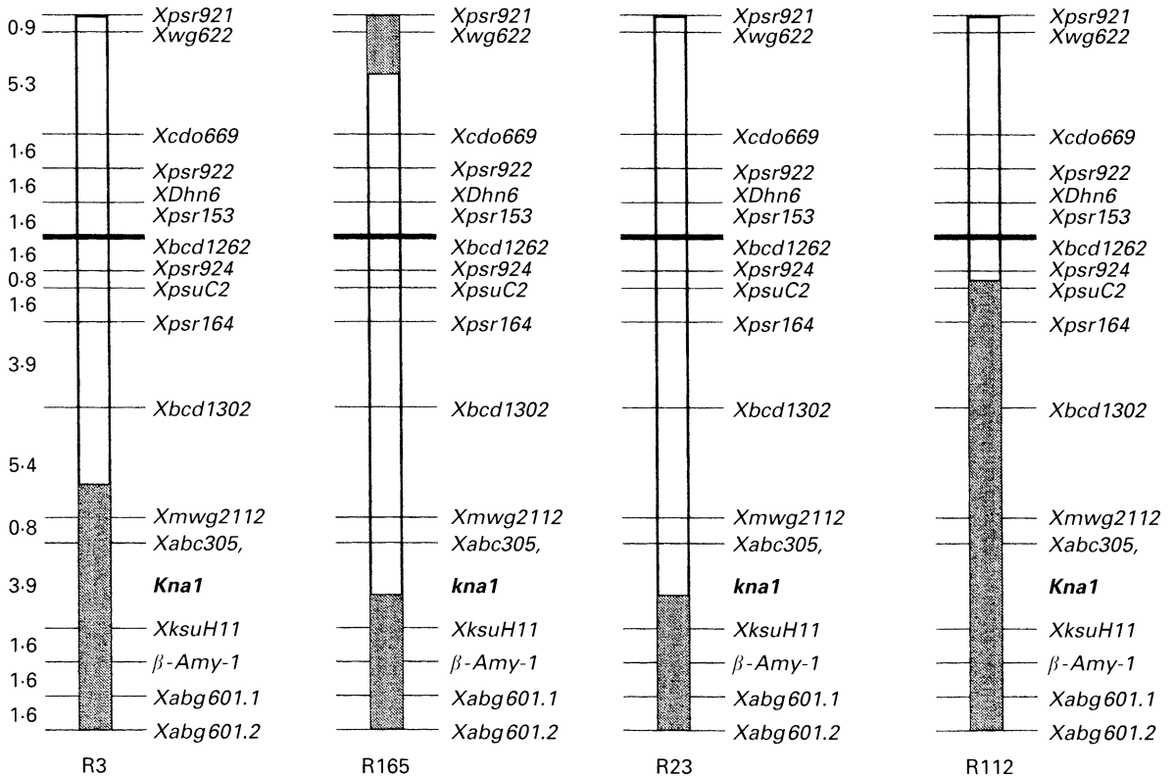

Figure 2. Maps of recombined chromosome 4B/4D showing the extent and position of sections derived from chromosome 4D (▨) in various recombinant tetraploid lines (data from Dubcovsky *et al.*, 1996*b*).

freezer and after thawing for some time, the tissues were crushed with a steel rod with a tapered end. Two holes were bored using a pin, one at the base and another at the top in the cap of the tube. Each tube was placed in another empty microcentrifuge tube and centrifuged at 8000 ***g*** for 10 min. The sap was collected in the lower tube and tissue residue was retained in the upper tube. The sap in the tubes was analysed or stored frozen for subsequent analysis.

For cation analysis (Na^+, K^+, Ca^{2+} and Mg^{2+}) 20 mm^3 of sap was diluted in a 2 cm^3 autoinjector vial with 1·4 cm^3 of cation eluant (20 mol m^{-3} methanesulphonic acid in deionized water, > 18 $M\Omega\ cm^{-1}$) and analysed on a Dionex 2000i ion chromatograph (Dionex (UK) Ltd, Camberley, Surrey) fitted with a CS12 cation exchange column and a self-regenerating cation suppressor operated in auto-regeneration mode. The column was operated at 50 °C. The system was automated by coupling to a Spark-Holland 'Marathon' autosampler fitted with a 5 mm^3 PEEK sample loop, and a Shimadzu CR5A plotting integrator linked to an Atari 1040 computer.

Statistical analysis

Results were analysed using the Minitab (DESCRIBE, ANOVA and GLM) software package to assess significant differences among the genotypes, treatments, blocks and their interactions. The sand and hydroponic culture experiments were completely randomized designs. The variances of the untransformed data were assumed to be normal and homogeneous. The data are presented with SE of the means and were tested for significant differences between means at $P > 0{\cdot}05$.

RESULTS

Spain

Figure 1 presents data from the triple line sprinkler system in Zaragoza. It can be seen that the *T. aestivum* × *Th. bessarabicum* amphiploid had the lowest yield potential (yield at low salinity), followed by the 'Langdon' disomic 4D(4A) substitution line (Fig. 1*a*). The highest yield was exhibited by the hexaploid wheat cv. Siete Cerros, whilst the yield of 'Langdon' was somewhat lower. Expressing the grain yield for each genotype as a percentage of that obtained at low salinity (according to the model of van Genuchten (1983)), it is evident that 'Langdon' was the least tolerant in relative terms, whereas the amphiploid had the highest relative yields at high salinities (Fig. 1*b*). 'Siete Cerros' and the disomic substitution line were intermediate between these two.

Analysis of Na^+ and K^+ concentrations in the third youngest leaf (Fig. 1*c*, *d*) showed opposite trends for the two ions. Na^+ was highest in 'Langdon' and lowest in the amphiploid, whereas K^+ was highest in the amphiploid and lowest in 'Langdon'. The

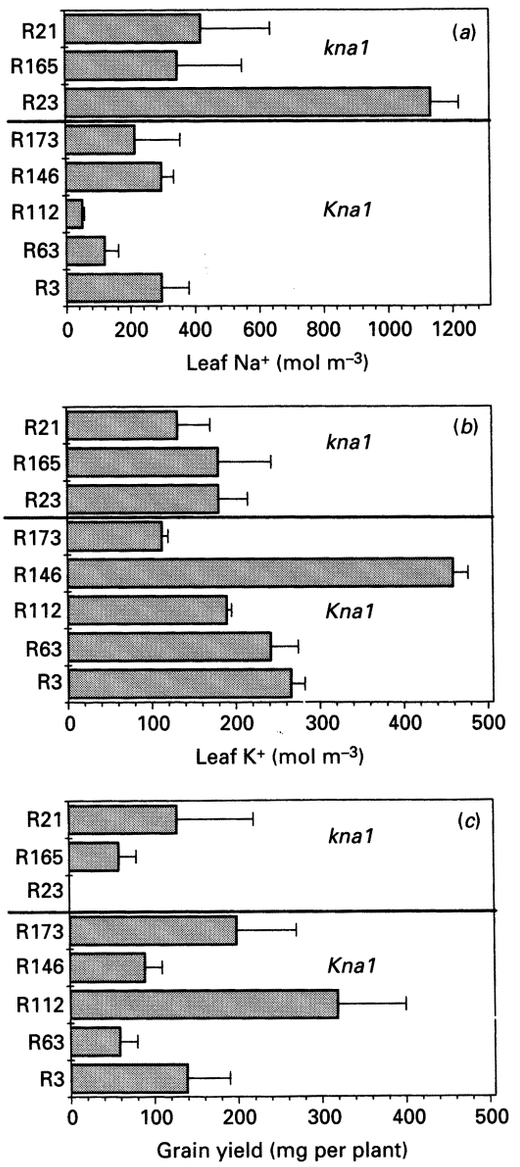

Figure 3. Leaf 8 (numbered from the base) Na^+ and K^+ concentrations and grain yields from recombinant tetraploid wheat families differing in enhanced Na^+/K^+ discrimination (*Kna1*, *kna1*). Plants were grown in sand culture with 150 mol m^{-3} NaCl and 7·5 mol m^{-3} $CaCl_2$.

disomic substitution line and the hexaploid wheat had similar values intermediate between those of the tetraploid wheat ('Langdon') and the amphiploid.

Sand culture

Maps showing the regions of 4D chromosome transferred into tetraploid wheat in selected recombinant lines used in this investigation are shown in Figure 2. R3 and R112 both included the *Kna1* allele of 4D, but differed in the position of the exchange point proximal to *Kna1*. R165 and R23 (*kna1*) differed by a small portion of the distal part of the 4D short arm which was present in R165 but not R23.

Na^+ concentrations were much higher in R23 than in the other lines (Fig. 3*a*), and generally higher in

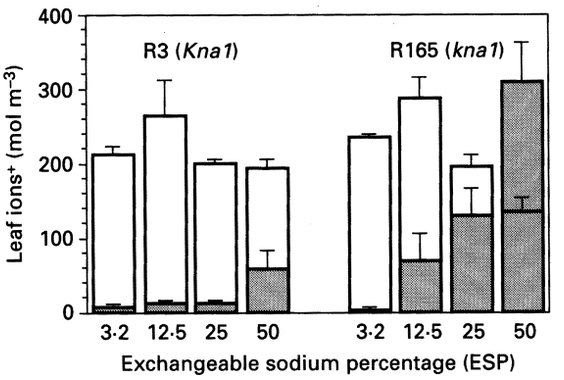

Figure 4. Leaf 8 (numbered from the base) Na^+ (▨) and K^+ (□) concentrations and grain yields from two recombinant tetraploid wheat families differing in enhanced Na^+/K^+ discrimination (*Kna1*, *kna1*). Plants were grown in sodic soil with different exchangeable sodium percentages.

the *kna1* than the *Kna1* lines. In general, the reverse was true for potassium (Fig. 3*b*), except that among the *Kna1* lines R173 had low K^+ concentrations and R146 had exceptionally high K^+ concentrations. R112 had the lowest Na^+ concentrations.

Grain yield (Fig. 3*c*) was highest in R112 and non-existent in R23, giving for these two lines a very good inverse correlation between Na^+ accumulation and yield under salinity. Looking at the other lines, there were considerable differences within the two groups (*Kna1* and *kna1*), but overall the *Kna1* group had the higher mean yield.

Sodic soil

A consistent increase was observed in Na^+ accumulation with higher ESP levels in R165 (Fig. 4), whereas in R3 there were non-significant differences between the Na^+ concentrations at 3·2 and 12·5 ESP. A gradient in the Na^+ concentration for leaves of different ages for both genotypes was also observed and higher Na^+ concentrations were observed in older leaves than in younger ones (data not shown).

Both genotypes had highest K^+ concentrations at 12·5 ESP, whilst a reduction was found at 50 ESP only for R165. A gradient was also observed in K^+ concentrations of different leaves of both genotypes and higher concentrations were measured in younger leaves than in older leaves (data not shown).

R3 produced taller plants at all ESP levels, but grain yield was not significantly affected by sodicity except at 50 ESP where it was reduced (data not shown). Absolute grain yields were similar in both lines, but relative grain yield (not shown) decreased more in the *kna1* line, R165.

Hydroponic culture

Only data for 6 mol m^{-3} NaCl and 4 mol m^{-3} Ca^{2+} are shown in Figure 5. In the absence of Ca^{2+}, accumulation of Na^+ was higher, whilst in the

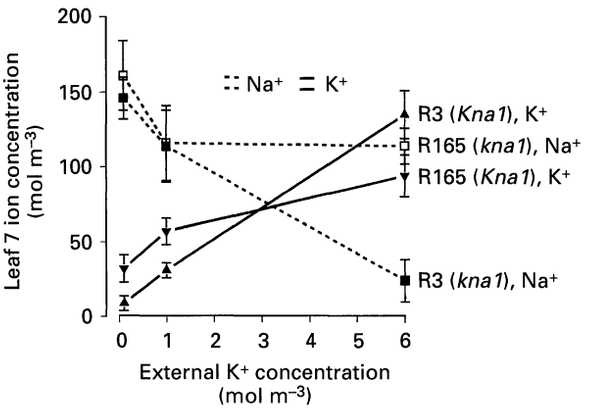

Figure 5. Leaf 7 (numbered from the base) Na^+ (––) and K^+ (—) concentrations in plants of two recombinant tetraploid wheat families differing in enhanced Na^+/K^+ discrimination (*Kna1*, *kna1*) grown in hydroponic culture with 6 mol m^{-3} NaCl, 4 mol m^{-3} Ca^{2+} and 0, 1 and 6 mol m^{-3} K^+.

absence of Na^+ in the culture solution it was almost undetectable in the leaves. No particular differences between the *Kna1* and *kna1* lines were observed under these conditions.

An increase in Na^+ concentration was observed with a decrease in K^+ level for the two lines, but the differences between R3 and R165 were only significant at the higher K^+ level. There was a change between 1 and 6 mol m^{-3} K^+ in the response of the two lines for K^+ accumulation in the leaves. At low K^+ nutrition R165 had higher leaf K^+ than R3, but the reverse was true at 6 mol m^{-3} K^+.

DISCUSSION

The data quoted by Dubcovsky *et al.* (1996*b*) point to differences in the extent of K^+/Na^+ discrimination between field and hydroponic culture. Taking R3 as representative of the *Kna1* group and R165 as representing the *kna1* lines (Dvorak & Gorham, 1992), it is clear that differences in ion accumulation between the two lines in hydroponics (Fig. 5) are not as obvious in sand culture (Fig. 3) or perhaps in the field, although data from Figure 1 suggest that such differences might still be detectable at least between 'Langdon' and the 4D(4A) disomic substitution line. From the sand culture experiments (Fig. 3), experiments in soil in a flood bench (Gorham & Bridges, unpublished), and from the field data of Dvorak *et al.* (1994), it is also apparent that the *Kna1* gene is not the only factor controlling ion accumulation or yield under salinity. Indeed, the recombinant lines vary considerably in yield under non-saline conditions (Dvorak *et al.*, 1994).

There are two main sources of this complicating variation. The first is the genetic material collaterally transferred into tetraploid wheat on other parts of the 4D chromosome (Fig. 2), since the amount of material transferred in all of the lines is still large in relation to the whole chromosome. The second problem comes from the hybrid background nature of the tetraploids themselves, with recombination between 'Langdon' and 'Senator Cappelli' homologues, and the added possibility of homoeologous recombination between A and B chromosomes in the *ph1* plants. The variation in the background can be eliminated by recurrent backcrossing and selection to one of the parents, or by developing a *ph* mutant in 'Langdon' and starting the process again. The amount of D chromosome genetic material transferred has been further reduced by a second round of homoeologous pairing (Luo *et al.*, 1996). Whatever the source of the variation, and the consequences for demonstrating the relevance of *Kna1* to salt tolerance, the material should be viewed as a valuable source of variation for durum wheat breeding.

We have attempted to answer here two further questions about the *Kna1* trait. Does it have any implications for sodicity tolerance and is it useful in conditions of low K^+ nutrition? For the first question, the data in Figure 4 suggest that the trait might be beneficial for sodic as well as saline conditions, although again, one must be careful when comparing glasshouse experiments, especially in well-drained artificial soils, with field situations. Figure 5 would indicate that there is no advantage under conditions of K^+ deficiency, and that the cation discrimination is clearer at high K^+ concentrations in the medium. This might suggest that passive, rather than high-affinity, uptake is involved.

Allen, Wyn Jones & Leigh (1995) could not detect any differences in two Na^+ transport processes in membrane vesicles from tetraploid and hexaploid wheat, but this would not be expected if the trait acts specifically on xylem loading (Gorham *et al.*, 1990). Progress in the genetics and physiology of high-affinity transporters and low-affinity channels in wheat (Schachtman & Schroeder, 1994; Smart *et al.*, 1996) might help to clarify other processes involved in K^+/Na^+ discrimination.

Progress in molecular mapping of wheat chromosomes (Devos *et al.*, 1995; Dvorak *et al.*, 1995) should help to explain the loss of expression of *Kna1* in the evolution of the A genome from diploid to tetraploid (durum) wheats (Gorham *et al.*, 1991) (see also Law & Worland, 1997).

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