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ORIGINAL PAPER

Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress

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Abstract Worldwide, dryland salinity is a major limitation to crop production. Breeding for salinity tolerance could be an effective way of improving yield and yield stability on saline-sodic soils of dryland agriculture. However, this requires a good understanding of inheritance of this quantitative trait. In the present study, a doubled-haploid bread wheat population (Berkut/Krichauff) was grown in supported hydroponics to identify quantitative trait loci (QTL) associated with salinity tolerance traits commonly reported in the literature (leaf symptoms, tiller number, seedling biomass, chlorophyll content, and shoot Na^+ and K^+ concentrations), understand the relationships amongst these traits, and determine their genetic value for marker-assisted selection. There was considerable segregation within the population for all traits measured. With a genetic map of 527 SSR-, DArT- and gene-based markers, a total of 40 QTL were detected for all seven traits. For the first time in a cereal species, a QTL interval for Na^+ exclusion (*wPt-3114-wmc170*) was associated with an increase (10%) in seedling biomass. Of the five QTL identified for Na^+ exclusion, two were co-located with seedling biomass (2A and 6A). The 2A QTL appears to coincide with the previously reported Na^+ exclusion locus in durum wheat that hosts one active *HKT1*;*4* (*Nax1*) and one inactive *HKT1*;*4* gene. Using these sequences as template for primer design enabled mapping of at least three *HKT1*;*4* genes onto chromosome 2AL in bread wheat, suggesting that bread wheat carries more *HKT1*;*4* gene family members than durum wheat. However, the combined effects of all Na^+ exclusion loci only accounted for 18% of the variation in seedling biomass under salinity stress indicating that there were other mechanisms of salinity tolerance operative at the seedling stage in this population. Na^+ and K^+ accumulation appear under separate genetic control. The molecular markers *wmc170* (2A) and *cfd080* (6A) are expected to facilitate breeding for salinity tolerance in bread wheat, the latter being associated with seedling vigour.

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Introduction

Worldwide, dryland salinity is a major limitation to agriculture (Rengasamy 2006). Dryland salinity can be classified into two types: seepage salinity and transient salinity

(Rengasamy 2002). Seepage salinity is caused by rising water tables, often due to clearing of vegetation that alters the hydrological balance of the landscape. This mobilises salts in the soil and brings them into the root zone. Transient salinity refers to naturally-occurring high salt loads of sodic soils (Rengasamy 2002). Worldwide, there is approximately 5.8×10^6 km^2 of sodic soils that have the potential to develop transient salinity (Rengasamy 2006). In Australia, it is estimated that 67% of the cropping land is at the risk of transient salinity, while 16% is likely to be affected by seepage salinity (Rengasamy 2002). Breeding for improved salinity tolerance could be an effective way of improving yield and yield stability on transiently saline soils of dryland cropping systems. Salinity tolerance reflects the ability of a genotype to grow and yield well in a saline environment. It is generally measured as the relative biomass production or relative yield under saline and non-saline conditions (Munns et al. 2002).

Similar to other agronomical traits, breeding for salinity tolerance requires (a) economic justification, (b) genotypic variation, (c) a rapid and reliable selection method and (d) understanding of genetic control. The first two criteria are already satisfied, but the third and fourth criteria require further work. The current situation is that salinity tolerance is difficult to assess in the field due to spatial and temporal variation. Although alternative screening methods have been developed (Munns and James 2003), they are generally time-consuming, expensive (Lindsay et al. 2004) and require validation in the field. Salinity tolerance also remains complex both physiologically and genetically (Koyama et al. 2001; Colmer et al. 2005; Munns and Tester 2008; Table 1). To circumvent these problems, it has been suggested that breeding for salinity tolerance should focus on trait-based selection (Shannon and Noble 1990; Yeo et al. 1990; Flowers and Yeo 1995; Rajendran et al. 2009) rather than phenotypic selection for yield or yield components. Pyramiding of salinity tolerance traits into breeders lines using quantitative trait loci (QTL) mapping and subsequent marker-assisted selection (MAS) have a great potential to accelerate the breeding process using the trait-based approach.

A number of QTL associated with salinity tolerance traits have already been identified in rice, barley and wheat (Table 1). It is interesting to note that most of these QTL studies were short term (1–2 weeks) and investigated either morphological (seedling growth) or physiological traits (chlorophyll, Na^+ and/or K^+ concentrations in leaves), but only very few studies investigated both morphological and physiological traits together (Ma et al. 2007; Xue et al. 2009; Rajendran et al. 2009). In addition, only two of these studies (wheat, Ma et al. 2007; barley, Xue et al. 2009) were conducted at different levels of salinity stress (control or salt). Screening at two levels of stress distinguishes between constitutive and non-constitutive expression of traits, and thereby identifies those traits that are specifically related to improved growth under salinity stress. The barley study reported that most of the QTL varied with treatments, while the wheat study presented the ratios of trait values under saline and non-saline conditions rather than actual trait values; hence, it is not clear if QTL differed with the levels of salinity stress or were expressed constitutively.

Of all the traits assessed, Na^+ concentration in leaves is one of the most studied. The basis for this has been the correlations reported between low Na^+ concentrations in leaves and salinity tolerance (Schachtman et al. 1992; Munns and James 2003; Husain et al. 2003) although other studies did not find such correlations (Ashraf and McNeilly 1988; Bagci et al. 2007; Genc et al. 2007) or only weak associations (Hollington 2000; Huang et al. 2006a). The summary in Table 1 shows that there are already a number of QTL identified for Na^+ exclusion in wheat, barley and rice, but their effects on yield or biomass at either phenotypic or genetic levels have not yet been determined. Potassium (K^+) concentration in leaves is also frequently used as a salinity tolerance parameter since higher K^+ concentrations have been occasionally associated with higher salinity tolerance in barley and wheat (Chen et al. 2007; Bagci et al. 2007; Cuin et al. 2008). Despite its importance in salinity tolerance, to our knowledge, only three studies in rice have reported QTL for K^+ concentration (Koyama et al. 2001; Bonilla et al. 2002; Lin et al. 2004). Similar to Na^+ concentration, the effects of K^+ concentration QTL on yield or biomass have not yet been demonstrated. Further, there has been very little data generated on the genetic control of salinity tolerance beyond 2–3 weeks of seedling growth. In light of recent findings that low Na^+ concentration and/or high K^+ concentration in leaves does not always correlate with salinity tolerance (based on biomass) in bread wheat (Genc et al. 2007), further studies are required to identify QTL associated with salinity tolerance traits. Moreover, it is important to verify whether QTL for physiological traits such as low Na^+ and/or high K^+ concentrations are also associated with higher biomass and/or yield at the phenotypic and the genetic level.

In the present study, a doubled-haploid wheat mapping population, derived from broadly adapted South Australian (Krichauff) and CIMMYT (Berkut) parents, was grown under non-saline and saline supported hydroponic conditions to (1) identify QTL associated with salinity tolerance traits, (2) understand the relationships amongst these traits and their contribution to salinity tolerance and (3) determine their genetic value for marker-assisted selection.

Table 1 A list of commonly reported QTL associated with salinity tolerance in wheat, barley and rice

Trait	Species	Ch.	Wheat homeologous group[a]	Method	Comments	Reference
Germination	Rice	6, 7	7, 2	Filter paper	Seedling (14 days at 8.6 dS m^{-1})[b]	Prasad et al. (2000)
	Barley	4H, 5H, 6H, 7H	4, 5, 6, 7	Filter paper	Imbibiton (24 h at 25–30 dS m^{-1})[b]	Mano and Takeda (1997)
	Bread Wheat	3, 4, 5, 7	3, 4, 5, 7	Filter paper	Seedling (7 days at 25 dS m^{-1})[b]	Ma et al. (2007)
Visual scores/seedling vigour	Rice	1, 3	3, 4	Soil and solution culture	Seedling (14 days at 8.6 and 12 dS m^{-1})[b]	Lee et al. (2007)
	Rice	7	2	Saline pool	Maturity (at 8.6 dS m^{-1})[b]	Zhang et al. (1995)
	Rice	6	7	Filter paper	Seedling (14 days at 8.6 dS m^{-1})[b]	Prasad et al. (2000)
Seedling survival	Rice	1, 6, 7	3, 7, 2	Solution culture	Seedling (10 days at 14 dS m^{-1})[b]	Lin et al. (2004)
Tiller number	Barley	7H	7	Solution culture	Seedling (14 days at 15 dS m-1)[b]	Ellis et al. (2002)
	Barley	4H	4	Field irrigated with saline water	Stem elongation (20 dS m^{-1})[b]	Xue et al. (2009)
Dry matter	Rice	5, 6, 10	1, 7, 1	Filter paper	Seedling (14 days at 8.6 dS m^{-1})[b]	Prasad et al. (2000)
	Rice	6	7	Solution culture	Seedling (12 days at 5 dS m^{-1}, then 8 days at 10 dS m^{-1})[b]	Koyama et al. (2001)
	Barley	2H, 4H, 5H	2, 4, 5	Solution culture	Seedling (14 days at 15 dS m^{-1})[b]	Ellis et al. (2002)
	Barley	7H	7	Solution culture	Seedling (8 days at 17.5 dS m^{-1})[b]	Ellis et al. (1997)
	Barley	1H, 2H, 5H 6H	1, 2, 5, 6	Solution culture	Seedling (28 days at 50 dS m^{-1})[b]	Mano and Takeda (1997)
	Barley	2H	2	Field irrigated with saline water	Maturity (20 dS m^{-1})[b]	Xue et al. (2009)
	Bread Wheat	1, 3	1, 3	Solution culture	Seedling (whole shoots, 25 days at 25 dS m^{-1})[b]	Ma et al. (2007)
Chlorophyll content	Bread Wheat	3, 7	3, 7	Solution culture	Seedling (25 days at 25 dS m^{-1})[b]	Ma et al. (2007)
Shoot Na^+ conc.	Rice	4, 6	2, 7	Solution culture	Seedling (12 days at 5 dS m^{-1}, then 8 days at 10 dS m^{-1})[b]	Koyama et al. (2001)
	Rice	7	2	Solution culture	Seedling (10 days at 14 dS m^{-1})[b]	Lin et al. (2004)
	Rice	1	3	Solution culture	Seedling (16 days at 12 dS m^{-1})	Bonilla et al. (2002)
	Barley	1H	1	Soil culture and field	Seedling (28 days at 1.9 dS m^{-1} measured in 1 soil:5 water extracts); penultimate leaves in field trials with varying salinity levels measured in paste extracts (ECe 1.9–3.6 and 7.2–8.5 dS m^{-1} in topsoil and subsoil, respectively)	Rivandi (2009); also see Rivandi (2009) for studies which focused on abiotic stresses other than salinity but identified the same locus
	Barley	2H	2	Field irrigated with saline water	Maturity (20 dS m^{-1})[b]	Xue et al. (2009)
	Barley	7H	7	Solution culture	Seedling (leaf 3 blades, 10 days at 15 dS m^{-1})[b]	Shavrukov et al. (2010)
	Bread Wheat	7	7	Solution culture	Seedling (leaf 3 blades, 10 days at 10 dS m^{-1})[b]	Edwards et al. (2008)
	Bread Wheat	2, 3, 4, 6, 7	2, 3, 4, 6, 7	Solution culture	Seedling (leaf 3 blades, 10 days at 15 dS m^{-1})[b]	Ogbonnaya et al. (2008)
	Durum Wheat	2	2	Gravel culture with automatic sub-irrigation	Seedling (leaf 3 blades, 10 days at 15 dS m^{-1})[b]	Lindsay et al. (2004)

Table 1 continued

Trait	Species	Ch.	Wheat homeologous group[a]	Method	Comments	Reference
Shoot K^+ conc.	Rice	1, 4	3, 2	Solution culture	Seedling (12 days at 5 dS m^{-1} then 8 days at 10 dS m^{-1})[b]	Koyama et al. (2001)
	Rice	1	3	Solution culture	Seedling (16 days at 12 dS m^{-1})	Bonilla et al. (2002)
	Rice	1	3	Solution culture	Seedling (10 days at 14 dS m^{-1})[b]	Lin et al. (2004)
Na^+:K^+ ratio	Rice	1, 4	3, 2	Solution culture	Seedling (12 days at 5 dS m^{-1}, then 8 days at 10 dS m^{-1})[b]	Koyama et al. (2001)
	Rice	1	3	Solution culture	Seedling (16 days at 12 dS m^{-1})	Bonilla et al. (2002)
	Barley	6H	6	Field irrigated with saline water	Maturity (20 dS m^{-1})[b]	Xue et al. (2009)
K^+:Na^+ ratio	Bread Wheat	4	4	Solution culture	Seedling (YEBs, 14 days at 5.0 dS m^{-1})[b]	Dubcovsky et al. (1996)
Grain yield	Barley	6H	6	Field irrigated with saline water	Maturity (20 dS m^{-1})[b]	Xue et al. (2009)
	Bread Wheat	1, 2, 3, 4, 5, 6, 7	1, 2, 3, 4, 5, 6, 7	Field irrigated with saline water	Maturity (8.2 and 16.4 dS m^{-1})	Quarrie et al. (2005)

YEBs youngest fully emerged leaf blades

[a] Designation of barley chromosomes homeologous to wheat chromosome groups has been adapted according to the convention at the 7th International Barley Genetics Symposium held at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada, on August 5, 1996 (http://wheat.pw.usda.gov/ggpages/bgn/27/reslt xt.html); designation of rice chromosomes homeologous to wheat chromosome groups is based on macrosynteny as depicted in Sorrells et al. (2003)

[b] Values were converted from mM NaCl to dS m^{-1} to (10 mM NaCl = 1 dS m^{-1})

Materials and methods

Plant material

A doubled-haploid population of 152 lines derived from a cross between bread wheat (*Triticum aestivum* L.) cultivars Berkut (Irene/Babax//Pastor) and Krichauff (Wariquam//Kloka/Pitic-62/3/Warimek/Halberd/4/3-Ag-3/Aroona) was used in the study. Previous screening of the parental genotypes over a range of NaCl concentrations in supported hydroponics showed significant differences in salinity tolerance (i.e. relative biomass) and shoot Na^+ concentrations between the two genotypes (Genc et al. 2007). Two check varieties of known salinity tolerance (the salt tolerant Kharchia 65, Naqvi and Tandon 1991; the salt sensitive Baart 46, Richards et al. 1987) were also included in the bioassay. The seeds of 152 DH lines, the parental genotypes and check varieties were increased in University of California soil mix (Barker et al. 1998) under glasshouse conditions to obtain healthy seed for the bioassay. To ensure purity of the seed, all heads were covered with paper bags during anthesis.

Phenotyping

A supported hydroponic method was used in this study as described elsewhere (Genc et al. 2007). Briefly plants were grown in cylindrical PVC tubes (4 cm diameter × 28 cm deep) filled with cylindrical black polycarbonate pellets (approximately 2–4 mm long and 1–2 mm diameter) in a series of 52-L tubs each of which contained 42 PVC tubes. Each tub filled and drained with 25 L of nutrient solution every 30 min. The nutrient solution was a modified Hoagland solution (mM); NH_4NO_3 (0.2); KNO_3 (5); $Ca(NO_3)_2{\cdot}4H_2O$ (2); $MgSO_4{\cdot}7H_2O$ (2); KH_2PO_4 (0.1); Na_2SiO_3 (0.5); NaFe(III)–HEDTA (0.05); H_3BO_3 (0.01); $MnCl_2{\cdot}4H_2O$ (0.002); $ZnSO_4{\cdot}7H_2O$ (0.003); $CuSO_4{\cdot}5H_2O$ (0.0005) and Na_2MoO_3 (0.0001). Solutions were changed every 7 days, at which time the pH was adjusted to 6.0. The pH of the solution was monitored using a pH meter (Model PHM 92, Radiometer, Copenhagen, Denmark) several times a week and maintained within a range of 6.0–7.0.

Uniform-sized seeds of each genotype were surface-sterilised in 70% ethanol for 1 min followed by soaking in 3% sodium hypochlorite for 5 min and three lots of rinsing with deionised water. Seeds were germinated on filter paper in petri dishes at room temperature for 4 days. The seedlings were then transplanted into PVC tubes (one seedling per genotype; two replications per treatment in each of the two runs) filled with cylindrical black polycarbonate pellets (approximately 2–4 mm long and 1–2 mm diameter). At 8 days after transplanting (DAT) when the

third leaf was just appearing in a majority of the genotypes, the salt treatment began and sodium chloride (NaCl) salt was added to the nutrient solution in increments of 25 mM NaCl day^{-1} together with supplemental calcium as $CaCl_2 \cdot 2H_2O$ until the final concentration of 100 mM NaCl was achieved. 100 mM NaCl was selected based on a previous study, which gave the best discrimination of salinity tolerance (relative shoot DW) amongst several wheat genotypes. Supplemental calcium was added to the salt treatment giving a Na^+:Ca^{2+} ratio of 15. This ratio was identified in a previous experiment as being the optimum for growth under saline conditions (Genc et al. 2010). The electrical conductivities of the nutrient solutions, measured by a portable conductivity meter (Model TPS-LC81, TPS Ltd, Brisbane, QLD, Australia), were 1.9 and 12.1 dS m^{-1} for control and salt treatments, respectively.

Plants were grown in a growth cabinet set at 20/15°C day/night temperature, and 14/10 h day/night day length regime. The intensity of photosynthetically active radiation was measured using a quantum sensor (Model LI-1000, LI-COR, United States of America) and varied from 220 to 340 μmol m^{-2} s^{-1} at plant height. After 2 weeks of 100-mM NaCl stress, the presence and severity of Na-induced leaf chlorosis on the first leaf was recorded for all entries (entire population, parental and check cultivars). A scale of 1–5 was used in the assessment of Na-induced leaf chlorosis (1 healthy green leaves, 2 chlorosis starting on the tips of leaves, 3 chlorosis extending, 4 leaves turning pale yellow and 5 dead leaves). At 40 DAT (28 days in 100 mM NaCl), plants were harvested. At harvest, tiller number, maturity (based on Zadoks' growth scale; Zadoks et al. 1974) and chlorophyll content of youngest fully expanded leaf blades (measured with Minolta SPAD 502 Chlorophyll Meter) were recorded for all entries in both runs. As Berkut/Krichauff population segregates for maturity, this trait was also measured to see if it influenced salinity tolerance traits as screening studies often involve germplasm with varying maturity. Harvested shoots were rinsed in double-deionised water for about 5 s, and oven-dried at 65°C for 48 h for dry weight measurement. Na^+ and K^+ concentrations in dry shoot samples of salt treatment (all four replications) were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Zarcinas et al. 1987).

DNA extraction and genotyping

DNA extraction

DNA extraction of the two parental and 152 doubled-haploid lines of the Berkut/Krichauff mapping population was carried out as described in Williams et al. (2006).

Genotyping

Out of a total of 1,150 SSR markers that were screened on DNA of the population parents Berkut and Krichauff, 233 markers were polymorphic. 17 out of 233 markers were unlinked and could not be assigned to linkage groups. SSRs used were previously described: *barc* (Song et al. 2002; 2005), *cfa* (Sourdille et al. 2003), *cfd* (Guyomarc'h et al. 2002), *gdm* (Pestsova et al. 2000), *gwm* (Röder et al. 1998) and *wmc* (Gupta et al. 2002). Genotyping using SSRs was carried out using standard PCR conditions and subsequent gel electrophoretic separation on 8% polyacrylamide gels. Multiplexed PCR amplification and product separation were performed on an ABI3730 capillary sequencer (Applied Biosystems, Warrington, UK) as described previously by Hayden et al. (2008). Subgenome-specific primers of *Vrn1* genes were used according to Fu et al. (2005).

In addition to the 216 SSRs and *Vrn* genes, 311 DArT markers were mapped by Triticarte Pty Ltd. (http://www.tritcarte.com.au). Genomic DNA of the mapping population parents and doubled-haploid lines was hybridised to the wheat DArT array Version 2.0 containing 5,137 clones, and polymorphisms detected and scored as previously described by Akbari et al. (2006).

Sequence analysis of HKT1;4 family members in bread wheat

HKT1;*4* was previously known as *HKT7* (Platten et al. 2006). The fragments amplified using six PCR primers (Table S1; Figure S1) and genomic DNA from Berkut and Krichauff were subcloned into plasmid pCR2.1 (Invitrogen, Carlsbad, CA, USA), sequenced with M13 vector primers on an ABI 3730 DNA Sequencer (PE Applied Biosystems, USA) and compared with the two *Triticum monococcum* *TmHKT7*-A1 (EF062820) and *TmHKT7*-A2 (EF062819) sequences (Huang et al. 2006b) using Vector NTI software (Invitrogen, USA). Sequences were also used to query the NCBI databases at http://www.ncbi.nlm.nih.gov using BLAST algorithms (Altschul et al. 1997). For genetic mapping of *HKT1*;*4*-like genes in bread wheat, a nulli-tetrasomic and ditelosomic aneuploid Chinese Spring set was used (Sears 1954) as template for PCR analysis using primers spanning 74% of the predicted first intron region in the *TmHKT7*-A1 sequence (Table S1; Figure S1).

Map construction

The linkage map was constructed using Map Manager QTX version QTXb20 (Manly et al. 2001) using the Kosambi mapping function with a threshold value of $P = 0.01$. Genotypic data from the doubled-haploid population was initially arranged into groups via the "Make Linkage Groups"

function. New markers were integrated into these chromosomes using the "Links report" function, then in conjunction with the "ripple" function and published maps, an order of markers was established, with the aim of minimising double recombinants and chromosome length. Marker order was verified using RECORD with ripples = 0, EQV threshold = 0 (Van Os et al. 2005). Segregation ratios of two genotype classes (Berkut allele and Krichauff allele) at each locus were tested using a chi-square test $P < 0.05$). The segregation ratio at a locus deviating from the expected ratio of 1:1 indicated distorted segregation (Table 2). These markers were excluded from QTL analysis.

Table 2 Segregation distortion of 29 loci in Berkut*Krichauff DH mapping population

Locus	Ch.	Allelic frequencies (observed)		*n*	χ^2**
		A*	B*		
wPt-9857	1A	104	36	140	33.04
wmc036b	1B	110	42	152	30.43
wPt-4647	1D	79	56	135	3.93
wPt-9951	2A	83	58	141	4.44
cfa2278	2B	53	84	137	7.02
wPt-3632	2B	55	80	135	4.64
cfd044	2D	92	57	149	8.23
gwm296	2D	91	59	150	6.83
wmc11	2D	89	63	152	4.45
gdm035	2D	88	63	151	4.15
GBM1209	2D	24	43	67	5.40
wmc343	3A	92	59	151	7.22
cfa2262	3A	89	63	152	4.45
gwm299	3B	65	31	96	12.05
wPt-7627	3B	86	59	145	5.03
wPt-7266	3B	87	60	147	4.97
wPt-4597	3B	87	63	150	3.85
wPt-9065	3D	87	59	146	5.38
wPt-4237	3D	79	55	134	4.31
gwm304	5A	86	58	144	5.45
wPt-3376	6B	89	60	149	5.65
wPt-7662	6B	83	56	139	5.25
wPt-4218	6B	84	60	144	4.01
wPt-2305	7B	81	55	136	4.98
gwm344a	7B	44	88	132	14.67
gwm132b	7B	55	87	142	7.22
wmc014b	7B	61	88	149	4.90
wPt-2677	7B	63	88	151	4.15
wPt-5674	7D	81	57	138	4.18

* A and B represent Berkut and Krichauff genotype, respectively

** χ^2 value greater than 3.84 ($df = 1$) indicates distortion at $P = 0.05$

Experimental design and statistical analysis

The experiment consisted of two runs in a growth cabinet. In each run, there were four trolleys on each side of the growth cabinet (blocks), each controlling two tubs. Each tub consisted of 9 rows and 5 columns with 3 positions being allocated to input and output of the supported hydroponics and hence not having a line assigned. In each replication, the 152 DH lines were randomly allocated across two trolleys (and hence four tubs) with the two parents (Berkut and Krichauff) and two other standard lines (Baart 46 and Kharchia 65) being also randomly allocated to each tub; thus there were four replicates of each of the DH lines. The assignment was undertaken using the DiGGer software (Coombes 2002), which allows for possible spatial effects and provides a row-column design within the constraints of the experiment. The two treatments, a control and salt solution, were randomly allocated to pairs of adjacent trolleys within each side of the growth cabinet (for each run). The purpose of having both treatments was to determine if QTL varied with the levels of salinity stress or were constitutively expressed. If QTL were constitutive, this would simplify the breeding procedure. Some traits (leaf symptoms, tiller number, seedling biomass, chlorophyll content and maturity) were measured on both the control and salt treatment, whereas others (Na^+ and K^+ concentrations) were measured only under the salt treatment. Na^+ and K^+ concentrations were divided by 1,000, while seedling biomass was log-transformed (due to variance heterogeneity) and multiplied by a factor of 10 to improve the scale for both estimation and reporting.

The whole genome average interval mapping (WGAIM) approach of Verbyla et al. (2007) forms the basis of the QTL analysis. This approach requires a baseline model to be developed, in which a polygenic effect and non-genetic sources of variation are included. The non-genetic variation may be the result of an experimental design (as in our analysis) together with model-based terms that allow for obvious additional aspects of the experiment. For example, correlation between experimental units may be required. For the growth cabinet experiment and for traits measured under both treatments, the initial baseline model was (in symbolic form):

Trait = Run.Treat.Type + **Run.Block + Run.Block.Trolley + Run.Block.Trolley.Tub + Run.Block.Trolley.Tub.Row + Run.Block.Trolley.Tub.Column + Run.Treat.Variety + Run.Block.Trolley.Tub.Pot**

The factor Type has 5 levels (DH for doubled haploid and the 4 non-DH lines), and including this factor ensures that genetic variance relates to the doubled-haploid lines only. The terms in bold are random effects, and terms like Run.Treat.Type are interactions. Not all the terms with

Row or Column were required. The Variety effects were modelled across Run and Treatment combinations using a factor analytic model along the lines of Smith et al. (2001). The baseline model for those traits measured only under the salt treatment was the same with the factor Treat omitted.

All QTL analyses required an extension of the WGAIM approach as outlined in Verbyla and Cullis (2010). This multivariate extension allows for heterogeneity across runs of the experiment, and hence, results are produced for both runs in a single analysis and allow for correlation between runs. For those traits observed under both treatments (Control and Salt), the QTL results are across both runs and treatments, allowing QTL by treatment and QTL by environment (runs) and their interaction to be included.

While numerous QTL intervals were found in the present study, only those consistent and significant (in most cases $P \leq 0.001$–0.0001) between two runs and at least under one set of growth conditions (saline or non-saline) were presented (Tables 5, 6).

Results

Linkage map of Berkut/Krichauff DH population and marker segregation

A total of 547 SSR-, DArT- and gene-based markers were mapped on 21 linkage groups (Fig. 1). There was an average of 25 markers per chromosome with an average spacing of 6 cM. However, individual chromosomes consist of several linkage groups with some of the chromosomal assignment being based on the genetic maps of other doubled-haploid wheat populations (http://www.genica.net.au). The segregation ratios of two genotype classes in most loci fitted the expected Mendelian ratio of 1:1. Segregation distortion (deviation from Mendelian ratio) was observed in 29 out of 527 (5.5%) mapped loci ($P < 0.05$) (Table 2). The frequency of Berkut alleles was high at loci on chromosomes 1A, 1D, 2A, 2D, 3A, 3B, 3D, 5A, 6B, 7B and 7D (23 loci, 4.4%), while the frequency of Krichauff alleles was high at loci on chromosomes 2B, 2D and 7B (6 loci, 1.1%).

Responses to salinity stress, distributions and relationships between traits

Considerable variation was observed in all traits measured (Table 3). For example, later maturing Berkut had higher seedling biomass than earlier maturing Krichauff under saline (15% greater) and non-saline conditions (18% greater). Berkut also had slightly higher Na^+ concentration in shoots than Krichauff while K^+ concentration was similar in Berkut and Krichauff. Krichauff had higher chlorophyll content and more severe leaf symptoms than Berkut. While the differences between the parents were not as great as those observed in previous experiments in which a lower concentration of supplementary Ca^{2+} was used (Genc et al. 2007), responses of the parents were consistent with earlier work, with Berkut having higher Na^+ concentration than Krichauff, but slightly higher salinity tolerance based on seedling biomass. Under salinity stress, Na^+ concentrations were very high (toxic to plant growth), while other nutrients (Mg, Ca, P, S, Fe, Mn, B, Cu and Zn) were within an adequate range for normal growth (data not shown; Reuter and Robinson 1997). No other toxicities or deficiencies which could have confounded the present results are therefore thought to have been involved.

There were significant genetic correlations amongst a number of the traits (Table 4). A negative and weak correlation was found between the severity of leaf symptoms and seedling biomass ($r_g = -0.37$). As expected, severity of leaf symptoms was correlated with increased Na^+ concentration ($r_g = 0.40$). However, this correlation was not very strong indicating that some lines had higher Na^+ concentration, but showed few leaf symptoms, which suggests a higher level of tissue tolerance. The significant but not strong correlation ($r_g = -0.43$) between Na^+ concentration and seedling biomass suggested that better Na^+ excluders were not necessarily higher yielding. There was no relationship between Na^+ concentration and either K^+ concentration or chlorophyll content. In addition, Na^+ concentration was not influenced by maturity or tillering ability, while K^+ concentration was significantly and positively correlated with tillering ($r_g = 0.85$). Finally, there was a positive and strong correlation between maturity and chlorophyll content ($r_g = 0.77$), while the correlation between K^+ concentration and either chlorophyll content ($r_g = -0.78$) or maturity ($r_g = -0.94$) was negative but strong.

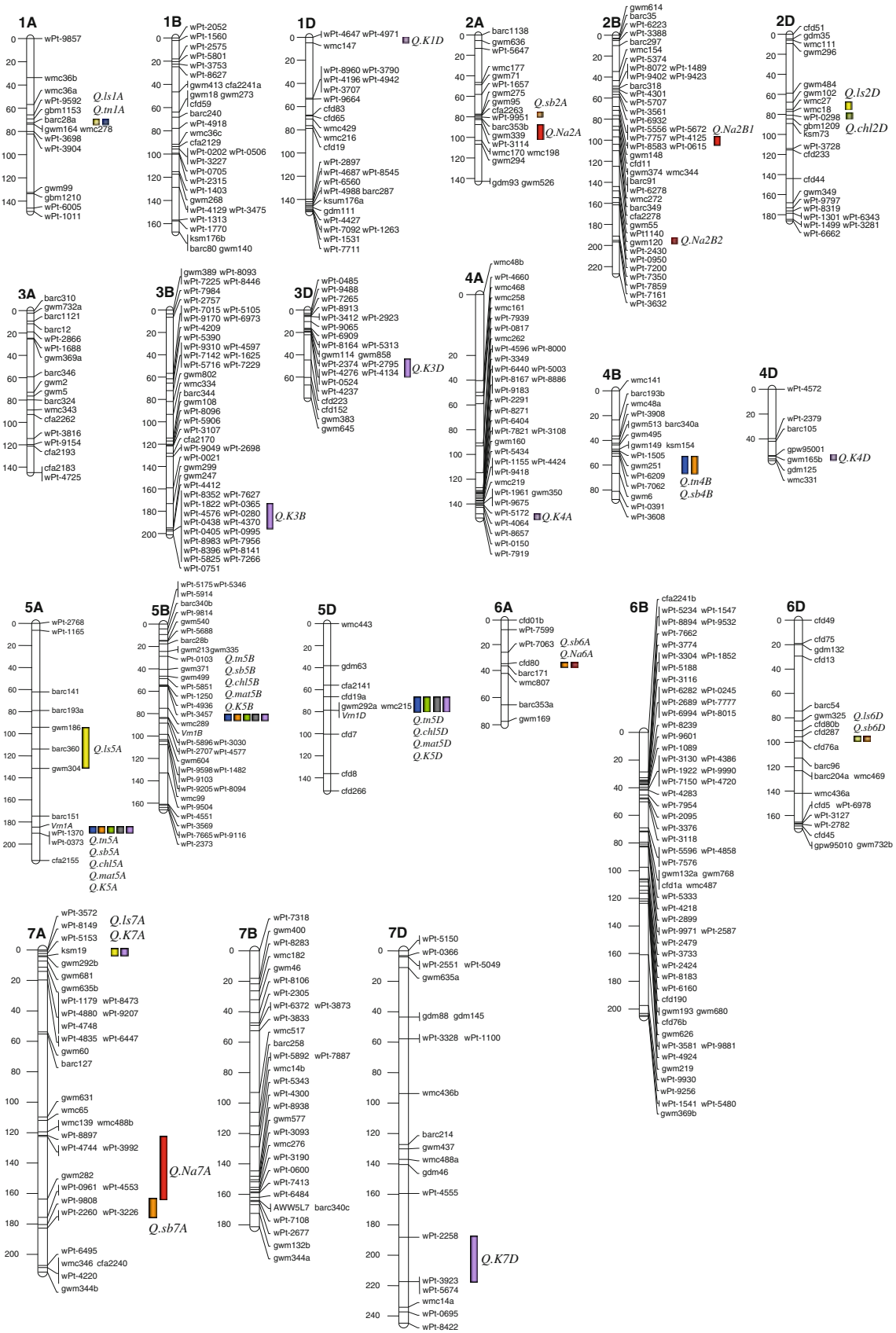
QTL analysis

Leaf symptoms

Six QTL intervals (*Q.ls1A*, *Q.ls2D*, *Q.ls5A*, *Q.ls6D*, *Q.ls7A1* and *Q.ls7A2*) were found for leaf symptoms (Table 5), all with low LOD scores (<3). The Berkut allele increased severity of leaf symptoms on chromosomes 1A and 7A, while the Krichauff allele contributed to severity of leaf symptoms on chromosomes 2D, 5A, 6D and 7A.

Tiller number

Five QTL were detected for tiller number on chromosomes 1A, 4B, 5A, 5B and 5D (*Q.tn1A*, *Q.tn4B*, *Q.tn5A*, *Q.tn5B* and *Q.tn5D*) and in all cases the same QTL were identified in both the control and the salt treatments (Table 5).


1A
wPt-9857
wmc36b
wmc36a
wPt-9592
gbm1153
barc28a
gwm164 wmc278
wPt-3698
wPt-3904
gwm99
gbm1210
wPt-6005
wPt-1011
Q.ls1A
Q.tn1A
1B
wPt-2052
wPt-1560
wPt-2575
wPt-5801
wPt-3753
wPt-8627
gwm413 cfa2241a
gwm18 gwm273
cfd59
barc240
wPt-4918
wmc36c
cfa2129
wPt-0202 wPt-0506
wPt-3227
wPt-0705
wPt-2315
wPt-1403
gwm268
wPt-4129 wPt-3475
wPt-1313
wPt-1770
ksm176b
barc80 gwm140
1D
wPt-4647 wPt-4971
wmc147
wPt-8960 wPt-3790
wPt-4196 wPt-4942
wPt-3707
wPt-9664
cfd83
cfd65
wmc429
wmc216
cfd19
wPt-2897
wPt-4687 wPt-8545
wPt-6560
wPt-4988 barc287
ksum176a
gdm111
wPt-4427
wPt-7092 wPt-1263
wPt-1531
wPt-7711
Q.K1D
2A
barc1138
gwm636
wPt-5647
wmc177
gwm71
wPt-1657
gwm275
gwm95
cfa2263
wPt-9951
barc353b
gwm339
wPt-3114
wmc170 wmc198
gwm294
gdm93 gwm526
Q.sb2A
Q.Na2A
2B
gwm614
barc35
wPt-6223
wPt-3388
barc297
wmc154
wPt-5374
wPt-8072 wPt-1489
wPt-9402 wPt-9423
barc318
wPt-4301
wPt-5707
wPt-3561
wPt-6932
wPt-5556 wPt-5672
wPt-7757 wPt-4125
wPt-8583 wPt-0615
gwm148
cfd11
gwm374 wmc344
barc91
wPt-6278
wmc272
barc349
cfa2278
gwm55
wPt1140
gwm120
wPt-2430
wPt-0950
wPt-7200
wPt-7350
wPt-7859
wPt-7161
wPt-3632
Q.Na2B1
Q.Na2B2
2D
cfd51
gdm35
wmc111
gwm296
gwm484
gwm102
wmc27
wmc18
wPt-0298
gbm1209
ksm73
wPt-3728
cfd233
cfd44
gwm349
wPt-9797
wPt-8319
wPt-1301 wPt-6343
wPt-1499 wPt-3281
wPt-6662
Q.ls2D
Q.chl2D
3A
barc310
gwm732a
barc1121
barc12
wPt-2866
wPt-1688
gwm369a
barc346
gwm2
gwm5
barc324
wmc343
cfa2262
wPt-3816
wPt-9154
cfa2193
cfa2183
wPt-4725
3B
gwm389 wPt-8093
wPt-7225 wPt-8446
wPt-7984
wPt-2757
wPt-7015 wPt-5105
wPt-9170 wPt-6973
wPt-4209
wPt-5390
wPt-9310 wPt-4597
wPt-7142 wPt-1625
wPt-5716 wPt-7229
gwm802
wmc334
barc344
gwm108
wPt-8096
wPt-5906
wPt-3107
cfa2170
wPt-9049 wPt-2698
wPt-0021
gwm299
gwm247
wPt-4412
wPt-8352 wPt-7627
wPt-1822 wPt-0365
wPt-4576 wPt-0280
wPt-0438 wPt-4370
wPt-0405 wPt-0995
wPt-8983 wPt-7956
wPt-8396 wPt-8141
wPt-5825 wPt-7266
wPt-0751
Q.K3B
3D
wPt-0485
wPt-9488
wPt-7265
wPt-8913
wPt-3412 wPt-2923
wPt-9065
wPt-6909
wPt-8164 wPt-5313
gwm114 gwm858
wPt-2374 wPt-2795
wPt-4276 wPt-4134
wPt-0524
wPt-4237
cfd223
cfd152
gwm383
gwm645
Q.K3D
4A
wmc48b
wPt-4660
wmc468
wmc258
wmc161
wPt-7939
wPt-0817
wmc262
wPt-4596 wPt-8000
wPt-3349
wPt-6440 wPt-5003
wPt-8167 wPt-8886
wPt-9183
wPt-2291
wPt-8271
wPt-6404
wPt-7821 wPt-3108
gwm160
wPt-5434
wPt-1155 wPt-4424
wPt-9418
wmc219
wPt-1961 gwm350
wPt-9675
wPt-5172
wPt-4064
wPt-8657
wPt-0150
wPt-7919
Q.K4A
4B
wmc141
barc193b
wmc48a
wPt-3908
gwm513 barc340a
gwm495
gwm149 ksm154
wPt-1505
gwm251
wPt-6209
wPt-7062
gwm6
wPt-0391
wPt-3608
Q.tn4B
Q.sb4B
4D
wPt-4572
wPt-2379
barc105
gpw95001
gwm165b
gdm125
wmc331
Q.K4D
5A
wPt-2768
wPt-1165
barc141
barc193a
gwm186
barc360
gwm304
barc151
Vrn1A
wPt-1370
wPt-0373
cfa2155
Q.ls5A
Q.tn5A
Q.sb5A
Q.chl5A
Q.mat5A
Q.K5A
5B
wPt-5175 wPt-5346
wPt-5914
barc340b
wPt-9814
gwm540
wPt-5688
barc28b
gwm213 gwm335
wPt-0103
gwm371
gwm499
wPt-5851
wPt-1250
wPt-4936
wPt-3457
wmc289
Vrn1B
wPt-5896 wPt-3030
wPt-2707 wPt-4577
gwm604
wPt-9598 wPt-1482
wPt-9103
wPt-9205 wPt-8094
wmc99
wPt-9504
wPt-4551
wPt-3569
wPt-7665 wPt-9116
wPt-2373
Q.tn5B
Q.sb5B
Q.chl5B
Q.mat5B
Q.K5B
5D
wmc443
gdm63
cfa2141
cfd19a
gwm292a wmc215
Vrn1D
cfd7
cfd8
cfd266
Q.tn5D
Q.chl5D
Q.mat5D
Q.K5D
6A
cfd01b
wPt-7599
wPt-7063
cfd80
barc171
wmc807
barc353a
gwm169
Q.sb6A
Q.Na6A
6B
cfa2241b
wPt-5234 wPt-1547
wPt-8894 wPt-9532
wPt-7662
wPt-3774
wPt-3304 wPt-1852
wPt-5188
wPt-3116
wPt-6282 wPt-0245
wPt-2689 wPt-7777
wPt-6994 wPt-8015
wPt-8239
wPt-9601
wPt-1089
wPt-3130 wPt-4386
wPt-1922 wPt-9990
wPt-7150 wPt-4720
wPt-4283
wPt-7954
wPt-2095
wPt-3376
wPt-3118
wPt-5596 wPt-4858
wPt-7576
gwm132a gwm768
cfd1a wmc487
wPt-5333
wPt-4218
wPt-2899
wPt-9971 wPt-2587
wPt-2479
wPt-3733
wPt-2424
wPt-8183
wPt-6160
cfd190
gwm193 gwm680
cfd76b
gwm626
wPt-3581 wPt-9881
wPt-4924
gwm219
wPt-9930
wPt-9256
wPt-1541 wPt-5480
gwm369b
6D
cfd49
cfd75
gdm132
cfd13
barc54
gwm325
cfd80b
cfd287
cfd76a
barc96
barc204a wmc469
wmc436a
cfd5 wPt-6978
wPt-3127
wPt-2782
cfd45
gpw95010 gwm732b
Q.ls6D
Q.sb6D
7A
wPt-3572
wPt-8149
wPt-5153
ksm19
gwm292b
gwm681
gwm635b
wPt-1179 wPt-8473
wPt-4880 wPt-9207
wPt-4748
wPt-4835 wPt-6447
gwm60
barc127
gwm631
wmc65
wmc139 wmc488b
wPt-8897
wPt-4744 wPt-3992
gwm282
wPt-0961 wPt-4553
wPt-9808
wPt-2260 wPt-3226
wPt-6495
wmc346 cfa2240
wPt-4220
gwm344b
Q.ls7A
Q.K7A
Q.Na7A
Q.sb7A
7B
wPt-7318
gwm400
wPt-8283
wmc182
gwm46
wPt-8106
wPt-2305
wPt-6372 wPt-3873
wPt-3833
wmc517
barc258
wPt-5892 wPt-7887
wmc14b
wPt-5343
wPt-4300
wPt-8938
gwm577
wPt-3093
wmc276
wPt-3190
wPt-0600
wPt-7413
wPt-6484
AWW5L7 barc340c
wPt-7108
wPt-2677
gwm132b
gwm344a
7D
wPt-5150
wPt-0366
wPt-2551 wPt-5049
gwm635a
gdm88 gdm145
wPt-3328 wPt-1100
wmc436b
barc214
gwm437
wmc488a
gdm46
wPt-4555
wPt-2258
wPt-3923
wPt-5674
wmc14a
wPt-0695
wPt-8422
Q.K7D

◀ **Fig. 1** Linkage map of Berkut*Krichauff DH population. QTL and their positions are indicated: for leaf symptoms (*Q.ls1A*, *Q.ls2D*, *Q.ls5A*, *Q.ls6D*, *Q.ls7A1* and *Q.ls7A2*), tiller number (*Q.tn1A*, *Q.tn4B*, *Q.tn5A*, *Q.tn5B* and *Q.tn5D*), seedling biomass (*Q.sb2A*, *Q.sb4B*, *Q.sb5A*, *Q.sb5B*, *Q.sb6A*, *Q.sb6D* and *Q.sb7A*), chlorophyll content (*Q.chl2D*, *Q.chl5A*, *Q.chl5B* and *Q.chl5D*), maturity (*Q.mat5A*, *Q.mat5B* and *Q.mat5D*), Na^+ shoot concentration (*Q.Na2A*, *QNa2B1*, *QNa2B2*, *Q.Na6A* and *Q.Na7A*) and K^+ shoot concentration (*Q.K1D*, *Q.K3B*, *Q.K3D*, *Q.K4A*, *Q.K4D*, *Q.K5A*, *Q.K5B*, *Q.K5D*, *Q.K7A* and *Q.K7D*). Where more than one QTL is indicated, colours from *left* to *right* correspond to QTL names from *top* to *bottom*

QTL on group 5 chromosomes had large effects on tiller number, and those on 5A and 5B were associated with the vernalisation genes *Vrn1A* and *Vrn1B*. The effects of QTL differed with salinity treatment. For example, at the 5A locus the effects were greater under saline than non-saline conditions, while at the 1A locus the reverse was observed. Tiller number was increased by the Berkut allele on 5A, and by the Krichauff allele on 5B and 5D. The interval on 5A had the largest effect on tiller number (control 0.6; salt 0.9), and this was followed by intervals on 5B (control 0.6; salt 0.6) and 5D (control 0.6; salt 0.5).

Seedling biomass

Seven intervals were identified as putative QTL for seedling biomass on 2A, 4B, 5A, 5B, 6A, 6D and 7A (*Q.sb2A*, *Q.sb4B*, *Q.sb5A*, *Q.sb5B*, *Q.sb6A*, *Q.sb6D* and *Q.sb7A*) in both saline and non-saline conditions (Table 5). With the exception of the QTL on 6D, which was expressed under saline conditions only, all the other QTL were present under both sets of conditions. In general, the effects of intervals were greater under non-saline than saline conditions. The QTL on 4B and 5A were the same as those identified for tiller number. The Berkut allele on chromosomes 2A, 6A, 6D and 7A increased seedling biomass, while the Krichauff allele on 4B, 5A and 5B contributed to seedling biomass. The intervals on 5A and 5B were both associated with vernalisation gene homeologs, *Vrn1A* and *Vrn1B*.

Chlorophyll content

Four QTL intervals were found for chlorophyll content on chromosomes 2D, 5A, 5B and 5D (*Q.chl2D*, *Q.chl5A*, *Q.chl5B* and *Q.chl5D*; Table 5). In general, the QTL effects were again greater under non-saline than saline

Table 3 Mean (±SD) and ranges for traits measured in the present study

Trait	Treatment	Parental means		DH lines	
		Berkut	Krichauff	Mean	Range
Leaf symptoms (*1* healthy leaves; *5* dead leaves)	Salt	2.5 ± 0.5	3.5 ± 0.9	2.4 ± 0.8	1.0–4.5
Tiller number	Control	5.8 ± 0.7	5.2 ± 0.4	5.6 ± 1.4	3.0–10.5
	Salt	3.2 ± 0.2	3.3 ± 0.2	3.8 ± 1.5	1.8–9.0
Seedling biomass (g $plant^{-1}$)	Control	1.918 ± 0.222	1.670 ± 0.093	1.882 ± 0.382	0.827–2.955
	Salt	1.556 ± 0.054	1.318 ± 0.077	1.547 ± 0.235	0.935–2.217
Chlorophyll content (SPAD unit)	Control	33.5 ± 0.8	40.3 ± 1.1	36.7 ± 3.1	30.6–46.5
	Salt	40.7 ± 0.5	47.6 ± 1.2	44.3 ± 2.7	35.7–51.7
Maturity (Zadok's growth scale)	Control	28.5 ± 0.6	36.6 ± 0.8	33.5 ± 5.0	23.5–42.0
	Salt	32.9 ± 1.7	42.0 ± 0.7	38.2 ± 6.4	25.0–50.5
Na^+ concentration (mg kg^{-1} DW)	Salt	6,308 ± 296	5,942 ± 442	5,691 ± 1,135	2,850–9,733
K^+ concentration (mg kg^{-1} DW)	Salt	50,417 ± 438	49,667 ± 892	49,026 ± 6,109	37,667–62,667

Table 4 Genetic correlation coefficients (*r*) between each pair of traits under salinity stress

Trait	Leaf symptoms	Tiller number	Seedling biomass	Chlorophyll content	Maturity	Na^+ conc.
Tiller number	−0.41**					
Seedling biomass	−0.37**	−0.10				
Chlorophyll content	0.31**	−0.69**	0.16*			
Maturity	0.36**	−0.84**	0.20*	0.77**		
Na^+ conc.	0.40**	0.01	−0.43**	−0.07	−0.10	
K^+ conc.	−0.40**	0.85**	−0.17*	−0.78**	−0.94**	0.07

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively

Table 5 QTL associated with leaf symptoms, tiller number, seedling biomass, chlorophyll content and maturity

Trait	QTL	Ch.	Interval	Run	Additive effect[a]	Allele[b]	*P*
Leaf symptoms	*Q.ls1A*	1A	*gbm1153/barc028a*	Salt 1	0.002	B	0.0164
				Salt 2	0.002	B	0.0004
	Q.ls2D	2D	*gwm102/wmc027*	Salt 1	−0.002	K	0.0016
				Salt 2	−0.002	K	0.0131
	Q.ls5A	5A	*gwm304/gwm186*	Salt 1	−0.002	K	0.0131
				Salt 2	−0.002	K	0.0164
	Q.ls6D	6D	*cfd287/cfd076a*	Salt 1	−0.002	K	0.0053
				Salt 2	−0.002	K	0.0278
	Q.ls7A	7A	*wPt-8897/wPt-4744*	Salt 1	−0.002	K	0.0006
				Salt 2	−0.002	K	0.0030
	Q.ls7A	7A	*wPt-5153/ksm019*	Salt 1	0.002	B	0.0069
				Salt 2	0.002	B	0.0102
Tiller number	*Q.tn1A*	1A	*barc028a/gwm164*	Control 1	−0.320	K	0.0000
				Salt 1	−0.238	K	0.0015
				Control 2	−0.318	K	0.0001
				Salt 2	−0.288	K	0.0010
	Q.tn4B	4B	*wPt-7062/gwm6*	Control 1	−0.318	K	0.0001
				Salt 1	−0.139	K	0.0949
				Control 2	−0.335	K	0.0001
				Salt 2	−0.337	K	0.0005
	Q.tn5A	5A	*wPt-1370/Vrn1A*	Control 1	0.702	B	0.0000
				Salt 1	0.943	B	0.0000
				Control 2	0.592	B	0.0000
				Salt 2	0.711	B	0.0000
	Q.tn5B	5B	*Vrn1B/wPt-5896*	Control 1	−0.570	K	0.0000
				Salt 1	−0.622	K	0.0000
				Control 2	−0.548	K	0.0000
				Salt 2	−0.484	K	0.0000
	Q.tn5D	5D	*cfd19a/Vrn1D*	Control 1	−0.595	K	0.0000
				Salt 1	−0.579	K	0.0000
				Control 2	−0.557	K	0.0000
				Salt 2	−0.448	K	0.0000
Seedling biomass	*Q.sb2A*	2A	*gwm095/cfa2263*	Control 1	0.810	B	0.0000
				Salt 1	0.588	B	0.0000
				Control 2	0.808	B	0.0000
				Salt 2	0.606	B	0.0000
	Q.sb4B	4B	*wPt-7062/gwm6*	Control 1	−0.527	K	0.0006
				Salt 1	−0.007	K	0.9522
				Control 2	−0.641	K	0.0000
				Salt 2	−0.203	K	0.0969
	Q.sb5A	5A	*wPt-1370/Vrn1A*	Control 1	−0.894	K	0.0000
				Salt 1	−0.372	K	0.0008
				Control 2	−0.926	K	0.0000
				Salt 2	−0.223	K	0.0394
	Q.sb5B	5B	*gwm213/wPt-0103*	Control 1	−0.275	K	0.0404
				Salt 1	−0.445	K	0.0001
				Control 2	−0.406	K	0.0006
				Salt 2	−0.254	K	0.0203

Table 5 continued

Trait	QTL	Ch.	Interval	Run	Additive effect[a]	Allele[b]	*P*
	Q.sb6A	6A	*cfd080/barc171*	Control 1	0.739	B	0.0000
				Salt 1	0.577	B	0.0000
				Control 2	0.735	B	0.0000
				Salt 2	0.463	B	0.0000
	Q.sb6D	6D	*cfd287/cfd076a*	Control 1	0.226	B	0.0949
				Salt 1	0.306	B	0.0053
				Control 2	0.240	B	0.8103
				Salt 2	0.471	B	0.0000
	Q.sb7A	7A	*gwm282/wPt-0961*	Control 1	0.355	B	0.0114
				Salt 1	0.251	B	0.0332
				Control 2	0.321	B	0.0091
				Salt 2	0.302	B	0.0085
Chlorophyll content	*Q.chl2D*	2D	*gbm1209/wPt-0298*	Control 1	−0.961	K	0.0000
				Salt 1	−0.620	K	0.0008
				Control 2	−1.116	K	0.0000
				Salt 2	−0.903	K	0.0000
	Q.chl5A	5A	*wPt-1370/Vrn1A*	Control 1	−2.072	K	0.0000
				Salt 1	−1.672	K	0.0000
				Control 2	−1.946	K	0.0000
				Salt 2	−1.511	K	0.0000
	Q.chl5B	5B	*Vrn1B/wPt-5896*	Control 1	0.693	B	0.0002
				Salt 1	1.027	B	0.0000
				Control 2	0.678	B	0.0002
				Salt 2	0.975	B	0.0000
	Q.chl5D	5D	*cfd19a/Vrn1D*	Control 1	0.807	B	0.0001
				Salt 1	0.776	B	0.0001
				Control 2	0.959	B	0.0000
				Salt 2	0.889	B	0.0000
Maturity	*Q.mat5A*	5A	*wPt-1370/Vrn1A*	Control 1	−4.179	K	0.0000
				Salt 1	−4.232	K	0.0000
				Control 2	−3.636	K	0.0000
				Salt 2	−4.213	K	0.0000
	Q.mat5B	5B	*wmc289/Vrn1B*	Control 1	1.605	B	0.0000
				Salt 1	2.596	B	0.0000
				Control 2	1.224	B	0.0000
				Salt 2	2.831	B	0.0000
	Q.mat5D	5D	*cfd19a/Vrn1D*	Control 1	1.872	B	0.0000
				Salt 1	2.473	B	0.0000
				Control 2	1.849	B	0.0000
				Salt 2	2.891	B	0.0000

[a] Positive and negative values indicate that Berkut and Krichauff alleles increased the phenotypic values, respectively

[b] B and K indicate Berkut and Krichauff, respectively

conditions with the exception of the QTL on 5B where the effect was reversed. In addition, the QTL on 5A was associated with tiller number and biomass as was the QTL on 5B (biomass) and both coincide with the *Vrn1* gene locus. The Krichauff allele on 2D and 5A was linked to higher chlorophyll content while it is the Berkut allele that

Table 6 QTL associated with Na^+ and K^+ concentrations in whole shoots under salinity stress

Trait	QTL	Ch.	Interval	Run	Additive effect[a]	Allele[b]	*P*
Na^+ concentration	*Q.Na2A*	2A	*wPt-3114/wmc170*	Salt 1	−380	K	0.0000
				Salt 2	−463	K	0.0000
	Q.Na2B1	2B	*wmc272/barc349*	Salt 1	−296	K	0.0000
				Salt 2	−316	K	0.0003
	Q.Na2B2	2B	*wPt-7859/wPt-7161*	Salt 1	212	B	0.0013
				Salt 2	236	B	0.0048
	Q.Na6A	6A	*cfd080/barc171*	Salt 1	−145	K	0.0251
				Salt 2	−238	K	0.0048
	Q.Na7A	7A	*wPt-4744/gwm282*	Salt 1	−397	K	0.0000
				Salt 2	−504	K	0.0000
K^+ concentration	*Q.K1D*	1D	*wPt-4647/wmc147*	Salt 1	618	B	0.0088
				Salt 2	949	B	0.0010
	Q.K3B	3B	*gwm299/gwm247*	Salt 1	822	B	0.0037
				Salt 2	816	B	0.0188
	Q.K3D	3D	*cfd223/cfd152*	Salt 1	204	B	0.0000
				Salt 2	863	B	0.0048
	Q.K4A	4A	*wPt-7919/wPt-0150*	Salt 1	727	B	0.0016
				Salt 2	747	B	0.0080
	Q.K4D	4D	*gpw95001/gwm165b*	Salt 1	−616	K	0.0078
				Salt 2	−745	K	0.0085
	Q.K5A	5A	*wPt-1370/Vrn1A*	Salt 1	5,443	B	0.0000
				Salt 2	4,811	B	0.0000
	Q.K5B	5B	*Vrn1B/wPt-5896*	Salt 1	−2,494	K	0.0000
				Salt 2	−2,500	K	0.0000
	Q.K5D	5D	*cfd19a/gwm292a*	Salt 1	−4,518	K	0.0000
				Salt 2	−4,283	K	0.0000
	Q.K7A	7A	*wPt-5153/ksm019*	Salt 1	−770	K	0.0008
				Salt 2	−926	K	0.0011
	Q.K7D	7D	*wPt-2258/wPt-3923*	Salt 1	1,236	B	0.0000
				Salt 2	1,201	B	0.0006

[a] Positive and negative values indicate Berkut and Krichauff alleles increased the phenotypic values, respectively

[b] B and K indicate Berkut and Krichauff, respectively

increased chlorophyll content on 5B and 5D. Of these four intervals, the interval on 5A had the largest effect on chlorophyll content, and was again associated with vernalisation gene, *Vrn1A*.

Maturity

This population segregated for maturity, which resulted in identification of three putative intervals on 5A, 5B and 5D (*Q.mat5A*, *Q.mat5B* and *Q.mat5D*; Table 5). The effects were usually greater under saline than non-saline conditions. The Krichauff allele on 5A accelerated maturity while the Berkut allele on 5B and 5D hastened maturity. All three intervals were associated with the vernalisation gene homeologs on chromosome group 5, *Vrn1A*, *Vrn1B* and *Vrn1D* (Fu et al. 2005). *Vrn1A* had the largest effect on maturity (by up to 4 days).

Shoot Na^+ concentration

Five QTL intervals were found for shoot Na^+ concentration on chromosomes 2A, 2B, 6A and 7A (Table 6). The Krichauff alleles on 2A, 2B, 6A and 7A increased shoot Na^+ concentration by between 300 and 500 mg kg^{-1} DW while the contribution by the Berkut allele on 2B was approximately 200 mg kg^{-1} DW. The QTL on 6A coincided with a QTL for seedling biomass, with the Krichauff allele associated with higher Na^+ concentration and lower biomass.

Shoot K^+ concentration

A total of ten QTL intervals were found for shoot K^+ concentration on chromosomes 1D, 3B, 3D, 4A, 4D, 5A, 5B, 5D, 7A and 7D (Table 6). The intervals on chromosomes 5A, 5B and 5D were the same as those found for maturity, but their effects were reversed. K^+ concentration was increased by the Berkut allele on 5A, while the increase in K^+ concentration was associated with the Krichauff alleles on 5B and 5D. The QTL on 5A was the only interval that was also associated with seedling biomass. The intervals on 1D, 3B, 3D, 4A, 4D, 7A and 7D were independent of maturity, and K^+ concentration was increased by either the Krichauff allele (7A) or the Berkut allele (1D, 3B, 3D, 4A, 4D and 7D).

Chromosome location of HKT1;4 gene family members in bread wheat

To verify that *HKT1*;*4* gene family members similar to the *T. monococcum* genes are present in bread wheat, three primer pairs (ExInt1-F/-R, Intr1-1F/-1R and Intr1-2F/-2R; Table S1) were designed spanning 74% of the published first intron region in *TmHKT7*-A1 (EF062820; Huang et al. 2006b), which is predicted to be co-located to the *Nax*1 gene (EF062819, *TmHKT7*-A2) on chromosome 2AL. Results of the sequence comparisons of genomic fragments from Berkut and Krichauff with *TmHKT7*-A1 are summarised in Table S2. No sequence polymorphisms between the bread wheat parents were detected. Two bread wheat sequences closely (about 97% each) matched the corresponding sequence in *T. monococcum* when amplified with primer pair ExInt1-F/-R. Primer pairs Intr1-1F/-1R and Intr1-2F/-2R both amplified a single bread wheat sequence of about 85 and 91% identity to the *T. monococcum* sequence, respectively (Table S2).

The lack of polymorphisms in these sequences between Berkut and Krichauff did not allow for mapping the three sequences in our mapping population. Nevertheless, chromosome mapping using the nulli-tetrasomic and ditelosomic aneuploid Chinese Spring set (Sears 1954) positioned all three fragments onto chromosome 2AL (Figure S2, A–C). Accordingly, a fragment in bread wheat using the *TmHKT7*-A2 (*Nax*1)-specific primers A2F/A2R (Huang et al. 2006b) was mapped onto the same chromosome 2AL in the aneuploid wheat lines (Figure S2, D).

Discussion

Segregation distortion

Segregation distortion is frequently reported in various crops including wheat (Quarrie et al. 2005; Peleg et al. 2008; Francki et al. 2009). It can be observed in all types of mapping populations, but it is most frequent in recombinant inbred line (RIL) populations due to 5–6 generations of selection forces (Singh et al. 2007). Peleg et al. (2008) reported segregation distortion in 29% of the skeleton loci in an F_6 RIL population, while it was only 5.5% in the DH population tested in the present study. The female parent Berkut had a higher percentage of segregation distortion than did the male parent Krichauff. Segregation distortion in favour of the female parent in the present study agrees with most studies on this topic although limited studies have also shown that it can favour the male parent (Singh et al. 2007). There are various causes of segregation distortion (Peleg et al. 2008), but whatever the reason, it may lead to a biased estimate of marker–trait association; therefore, markers with distorted segregation should not be used in the analysis of marker–trait association (Gupta 2002). In the present study, only one distorted locus (*wPt-9951*; Table 2) showed association with seedling biomass, and it was discarded from the analysis.

A putative marker (wmc170) for Na^+ exclusion

Three QTL were associated with biomass production and Na^+ exclusion under salt stress on chromosomes 2A, 6A and 7A (Tables 5, 6), but the identification of the QTL on 2A is the most significant finding. Preliminary analysis of field trials conducted in South Australia also identified the Na^+ exclusion locus on 2A (*Q.Na2A*) (Genc et al., unpublished). The QTL on chromosome 6A was also associated with early crop vigour and kernel weight in the field trials conducted in South Australia over 3 years (Genc et al., unpublished) which was very similar to the findings by Spielmeyer et al. (2007). Therefore, it is likely that the association between Na^+ concentration and biomass for *Qsb6A* is a result of the greater vigour of lines with a large seed size, which results in a dilution of Na^+ concentration in the plant. While coincident QTL for Na^+ exclusion and seedling biomass were identified on chromosome 7A, further bivariate analysis using the same type of model as outlined in "Materials and methods" indicated that there were two separate intervals on 7A. Therefore, the QTL on 2A is potentially of greatest importance for selection for Na^+ exclusion to improve biomass production.

The Na^+ exclusion QTL (*wPt-3114*/*wmc170*) on chromosome 2AL was associated with a 10% increase in seedling biomass in the screening experiments (Fig. 2). To our knowledge, this type of co-location has not been reported previously in a cereal species. The QTL also overlaps a QTL interval flanked by the *gwm312*/*wmc170* previously shown to have close association with the gene for Na^+ exclusion from the leaf blade in durum wheat, *Nax1* (Lindsay et al. 2004). However, the durum study did not report seedling

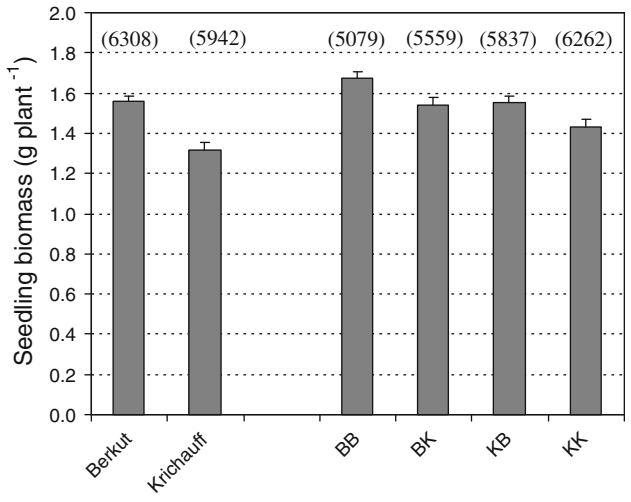


Fig. 2 Mean seedling biomass under salinity stress for Berkut, Krichauff and four genotypic classes of Berkut/Krichauff doubled-haploid lines: BB (Berkut alleles at *wmc170-2A* and *cfd080-6A*), BK (Berkut at *wmc170-2A* and Krichauff at *cfd080-6A*), KB (Krichauff at *wmc170-2A* and Berkut at *cfd080-6A*) and KK (Krichauff alleles at *wmc170-2A* and *cfd080-6A*). *Error bars* are the standard error of the means. Shoot Na^+ concentrations (mg kg^{-1} DW) are given in *parentheses* on *top of the bars*

biomass or related QTL, so it is not known if the Na^+ exclusion QTL also affected seedling biomass. Bivariate analysis of Na^+ exclusion and seedling biomass was used to test whether there may be one QTL controlling both Na^+ exclusion and plant biomass on 2A, and this indicated that there was one interval controlling these two traits on 2A. Given that marker positions are estimates and can vary depending on the population used, it is reasonable to assume that the QTL interval in our study may also carry a gene with a function similar to that of the Na^+ exclusion gene *Nax1* in durum wheat. However, at the phenotypic level the effect of the Na^+ exclusion gene *Nax1* in durum wheat appears to be considerably greater (38%) (Lindsay et al. 2004) than that of the Na^+ exclusion QTL on chromosome 2A in the present study (10%) (Fig. 2). The reasons for this difference could be due to (a) allelic variation of this gene between durum wheat and bread wheat, (b) the presence or absence of other minor exclusion genes at other loci, (c) a possibly different molecular basis for the measured low Na^+ concentration and/or (d) different experimental conditions (10 days in 150 mM NaCl in the durum study; 28 days in 100 mM NaCl in the present study) leading to different expression levels. Whatever the reasons, there is adequate evidence (Table 1 and the present study) that 2A in bread wheat also carries Na^+ exclusion gene(s) similar to *Nax1* gene.

Nax1 locus in bread wheat

Several lines of evidence support the hypothesis that the Na^+ exclusion locus *Nax1*, which was identified in durum wheat, also exists also in bread wheat. There is the apparent overlap between the Na^+ exclusion QTL on 2AL in bread wheat in this study (*wPt-3114*/*wmc170*) and the QTL interval (*gwm312*/*wmc170*) in durum wheat (Lindsay et al. 2004) mentioned earlier. Although it was not possible to map *Nax1* in the Berkut/Krichauff mapping population due to the lack of polymorphisms, we were able to map the two *HKT1*;*4* genes located at the *Nax1* locus (Huang et al. 2006b) on chromosome 2AL using the nulli-tetrasomic and ditelosomic Chinese Spring lines, confirming their chromosome position proposed by Huang et al. (2008) using Southern blot hybridisations on the same Chinese Spring aneuploid set. While Huang et al. (2008) proposed that there are two *HKT1*;*4* genes (A1 and A2), our sequence comparisons of *HKT1*;*4* gene fragments suggest that there are at least two genes in bread wheat very similar (97.4, 96.8%) to *TmHKT7*-A1 alone in addition to a gene matching the *TmHKT7*-A2 sequence. Our PCR-based mapping and sequence analysis are still in agreement with a Southern blot hybridisation proposing two genes because Southern blot hybridisation can only provide a conservative estimate on the number of gene copies, especially if gene family members are very similar and thus share the same restriction enzyme pattern. Our sequence comparisons suggest that locus Q.*Na2A* hosts a third *HKT1*;*4* gene family member that could be responsible for the observed contribution to Na^+ exclusion in current bread wheat population.

In the present study, there was no evidence of segregation for the only other known Na^+ exclusion gene *Nax2* (5AL) identified in durum wheat (James et al. 2006) since no Na^+ exclusion QTL were detected on any of the group 5 chromosomes or chromosome arms 4AL carrying a 5AL translocation (Liu et al. 1992). *Nax2* confers a reduced rate of transport of Na^+ from root to shoot and has a higher rate of K^+ transport, thus resulting in enhanced K^+ versus Na^+ discrimination (known as K^+/Na^+ ratio) in the leaf (James et al. 2006). *Kna1* locus was initially mapped to chromosome 4D in bread wheat (Dubcovsky et al. 1996) and later shown to be homeologous to the location for *Nax2* on chromosome 5AL in durum wheat (Byrt et al. 2007). The greater Na^+ exclusion in bread wheat than in durum wheat has been attributed to *Kna1*. However, our previous work with bread wheat germplasm (Genc et al. 2007) and the present study indicated that K^+/Na^+ trait may not be a reliable measure of salinity tolerance in bread wheat, and it does not necessarily result from higher K^+ concentration and lower Na^+ concentration. In addition, there are potential problems with mapping K^+/Na^+ because it is a derived ratio, not an independent variable, and does not tell us whether it is driven by Na^+ or K^+. However, when mapped in Berkut/Krichauff to illustrate this point, K^+/Na^+ QTL co-located with the K^+ QTL as well as Na^+ exclusion QTL (data not shown). We propose that future mapping studies

should consider presenting the more informative Na^+ and K^+ QTL separately as seen in Koyama et al. (2001) not K^+/Na^+ or Na^+/K^+ QTL.

Na^+ exclusion and salinity tolerance

Historically, low Na^+ concentration has been a surrogate for salinity tolerance (Schachtman et al. 1992; Ashraf and O'Leary 1996; Rashid et al. 1999; Munns and James 2003; Poustini and Siosemardeh 2004), but often there is little or no correlation with biomass under salinity stress (Ashraf and McNeilly 1988; Hollington 2000; Huang et al. 2006b Bagci et al. 2007; Genc et al. 2007). While the present study found a negative genetic correlation between Na^+ concentration and seedling biomass under salinity stress (Table 6) Na^+ concentration only accounted for approximately 18% of the genetic variation in seedling biomass. This implies that there is still about 80% of the genetic variation is associated with traits other than Na^+ exclusion. This is not surprising as salinity tolerance is governed by several traits other than Na^+ exclusion (tissue tolerance and osmotic adjustment) any of which can be controlled by several genes (Flowers 2004; Munns and Tester 2008). Further research is required to verify the effects of Na^+ exclusion loci on growth and grain yield and to quantify the contribution of different salinity tolerance traits to overall salinity tolerance. Eventually this will lead to the development of appropriate selection criteria, so they can be employed in breeding programs.

K^+ concentration, salinity tolerance and QTL

The maintenance of cellular K^+ concentration above a certain threshold in the presence of excess external Na^+ is critical for growth and salinity tolerance (Zhu et al. 1998), but the role of K^+ homeostasis in salinity tolerance amongst species is not clear. There have been few reports of a positive relationship between K^+ and salinity tolerance (Chen et al. 2007; Bagci et al. 2007; Cuin et al. 2008), while others have found no relationship (Genc et al. 2007). The lack of relationship between Na^+ and K^+ concentrations at the genetic level (Table 4) combined with the fact that QTL for Na^+ and K^+ concentrations mapped to separate chromosomes would support for wheat the earlier suggestion in rice that pathways for Na^+ and K^+ accumulation are separate (Garcia et al. 1997). There have been no K^+ QTL reported previously in barley or wheat, but three rice studies have reported QTL on rice homologous group 3 (Koyama et al. 2001; Bonilla et al. 2002; Lin et al. 2004; Table 1). This group was one of the homologous groups that carried K^+ QTL in the present study (*Q.K3B*, *Q.K3D*). However, the most significant QTL were co-located with the vernalisation genes on 5A, 5B and 5D. The question arises whether vernalisation genes also govern K^+ accumulation or whether vernalisation and K^+ accumulation gene(s) are tightly linked and cannot be separated due to a lack of markers within those regions of 5A and 5D.

Constitutive expression of traits under saline and non-saline conditions

A majority of the QTL associated with shoot growth and development were present under both control and saline conditions (Table 5), indicating that genes responsible for those traits were constitutively expressed and not specific to salinity stress. The significant positive correlations between biomass or grain yield under saline and non-saline conditions in bread wheat (Bagci et al. 2007; Genc et al. 2007), barley (Huang et al. 2006b) and rice (Shannon et al. 1998) is consistent with this observation. Although Na^+ concentrations were measured only in the salt treatment, previous studies under saline and non-saline conditions have suggested constitutive expression of genes involved in Na^+ uptake (Taeb et al. 1992). These findings suggest that selection for biomass or yield at one level of stress (which could even be non-saline conditions) may capture some of the variation required to improve yield in saline soils which would simplify the selection for improved productivity under salinity stress.

Parameters for breeding for salinity tolerance

Many traits have been examined for screening and in QTL studies for salt tolerance in wheat, barley and rice (Table 1). In rice, leaf symptoms correlate well with grain yield (Gregorio 1997) and have been used to select for salt tolerance. However, in the present study, the genetic correlation between leaf symptoms and seedling biomass was low ($r_g = -0.37$), which suggests that it may be of little value as a single selection criterion. This finding is further supported by the observation of various minor QTL with no apparent practical value (Table 1). Tissue Na^+ concentration has been studied extensively in all three species (Table 1). However, to date, selection for Na^+ exclusion has not been shown to increase seedling biomass or grain yield in wheat. In the present study, there was a negative genetic correlation between Na^+ concentration and seedling biomass and two coincident QTL for Na^+ concentration and seedling biomass were identified (2A and 6A). These results indicate that selection for Na^+ exclusion may provide some improvement in salinity tolerance. As for seedling biomass under salt stress, given its genetic complexity, it is not surprising that seedling biomass QTL were identified on all 7 homeologous groups (Table 1). However, loci on chromosome groups 1, 2 and 7 appear more frequently, and hence, should be targeted in future studies. Given the QTL on 6D

was the only locus linked to seedling biomass under salt stress (approx. 6% increase in seedling biomass), this region could also be considered in future studies. In the present study, no relationship was observed between tillering and seedling biomass, and there does not therefore appear to be much scope for using tiller number as a selection parameter. Similarly, chlorophyll content does not appear to be a useful selection trait in this population. In addition, as we discussed earlier, the ratios of trait values under saline and non-saline conditions are problematic as far as statistical analyses and their interpretation are concerned, and hence, the use of ratios are not recommended in QTL studies. Across all loci observed to segregate in this population, the most promising for further investigation are the Na^+ exclusion QTL on 2A (*wPt-3114*/*wmc170*) and 6A (*cfd080*/*barc171*) both of which increased seedling biomass by 10%. *wPt-3114*/*wmc170* seems to be associated with Na^+ exclusion, and may have a direct effect on maintaining growth under salt stress. However, *cfd080*/*barc171* appears to be associated with seedling vigour rather than Na^+ exclusion; nevertheless, it was expressed under salt stress, and so may provide an agronomic benefit tto crops grown under salt stress. Also since soil salinity is spatially variable, with most of the yield coming from the less saline parts of a field (Richards 1983; Richards et al. 1987), improving vigour under different levels of salinity may be of benefit to yield more generally.

In conclusion, the Na^+ exclusion gene (*Nax1*) identified in a durum landrace (Lindsay et al. 2004) may also be present in some form in bread wheat. However, as earlier studies suggested that the *Nax1* gene in durum wheat may be absent in bread wheat (James et al. 2006; Munns et al. 2008), it is possible that the allele in bread wheat might be quite different from that in durum wheat. Nevertheless, when all potential Na^+ exclusion loci were considered together, at the genetic level they explained only 18% of the variation in seedling biomass under salinity stress pointing to mechanisms other than Na^+ exclusion contributing to seedling biomass in this bread wheat population. In addition, two of the QTL were the same for Na^+ concentration and seedling biomass (2A and 6A), and to our knowledge, this type of co-location has not been reported previously. Na^+ and K^+ accumulation appear under separate genetic control mechanisms, and K^+ accumulation was not associated with differences in biomass production under salt stress in this population. It is proposed that the molecular markers *wmc170* (2A) and *cfd080* or *barc171* (6A) would facilitate breeding for salinity tolerance in bread wheat. However, other traits (tissue tolerance and osmotic adjustment) contributing to salinity tolerance should be investigated in order to achieve significant improvements in salinity tolerance.

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