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Genetic analysis of salt tolerance in a recombinant inbred population of wheat (*Triticum aestivum* L.)

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Abstract A population of 114 recombinant inbred lines (RILs), derived from the cross Opata85 × W7984, was used to genetically analyze the response of wheat to salt stress. This analysis resulted in the identification of 47 QTL mapping to all wheat chromosomes except 1B, 1D, 4B, 5D and 7D. Of these QTL, 10 were effective during the germination stage, and 37 at the seedling stage. Many of the traits related to salt tolerance mapped to common chromosome intervals, such as *Xglk683–Xcdo460* on chromosome 3A, *Xfbb168–Xbcd147* on chromosome 3B, *Xcdo1081–Xfbb226* on chromosome 4DL and *Xpsr106–Xfbb283* on chromosome 6DL. QTL located in the interval *Xcdo1081–Xfbb226* (chromosome 4DL) were effective during the germination stage, whereas those in the interval *Xfbb231.1–Xmwg916* (chromosome 6DL) were relevant to the seedling stage. The QTL in the intervals *Xglk683–Xcdo460* (chromosome 3AS) and *Xfbb168–Xbcd147* (chromosome 3BL) were effective at both the germination and seedling stages.

Keywords QTL · Salt tolerance · Common wheat · RIL

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Introduction

It has been estimated that approximately 20% of agricultural land and 50% of cropping land in the world suffers from soil salinity (Flowers and Yeo 1995). This represents a major constraint to food production (Yokoi et al. 2002), because it limits crop yields and restricts the use of previously uncultivated land. Wheat is one of the most important world food crops, and its productivity directly affects human survival and quality of life. Improving the salt tolerance of wheat and increasing its productivity are the major objectives of our breeding program. At the genetic level, salt tolerance has to be treated as a quantitative trait, and is significantly modulated by environment (Foolad and Jones 1993; Winicov 1998). In the last decade, the development of molecular markers had made possible the genetic analysis of a number of complex traits, even to the extent of allowing the tagging of individual

QTL (Yano and Sasaki 1997). Thus salt related QTL have been mapped in tomato (Foolad 2001; Foolad and Jones 1993), barley (Yoshiro and Kazuyoshi 1997), soybean (Lee et al. 2004), Arabidopsis (Quesada et al. 2002) and rice (Gong et al. 1998; Gu et al. 2000; Lin et al. 1998, 2004; Zhang et al. 1995). In wheat, there is a history of physiological research surrounding response to salinity stress, but genetic analysis is limited. In comparisons between hexaploid, tetraploid and diploid types, it was suggested that the D genome of wheat carries gene *Knal* that controls the relative concentrations of K and Na in the shoots of plants grown in saline hydroponic culture (Wyn Jones et al. 1984; Shan et al. 1987; Gorham et al. 1987, 1990), and this gene could be located in chromosome 4DL (Dvorak et al. 1994). Recent fine mapping confirmed that *Kna1* is a single gene (Dubcovsky et al. 1996). A number of attempts were made to map the QTL controlling Na exclusion, but only one QTL was successfully located to chromosome 2AL (Munns et al. 2002; Lindsay et al. 2004). In a study of yield under salt stress, Quarrie et al. (2005) identified two candidate QTL, mapping to probable homoeologous regions in the proximal parts of chromosomes 5B and 5D.

The identification of salt tolerance at both the germination and seedling stages is particularly important (Yoshiro and Kazuyoshi 1997), but QTL effective at these developmental stages have not been reported to date in wheat. In the present paper we report the identification and mapping of a large number of QTL associated with salinity tolerance, including salt tolerance index, salt injury index, biomass, shoot length/root length, chlorophyll content (CHLO) and proline content (PRO). Markers closely linked to some of the major QTL identified may find use in salinity breeding programs, and could open the way for map-based cloning in wheat.

Material and methods

The 114 Opata85 × W7984 F10 RILs came from an international mapping population. Control cultivars were Chadianhong (a local salt tolerant cultivar) and Chinese Spring (salt sensitive). The parental lines and RILs were evaluated for salt tolerance at two salt treatments (0 and 250 mM NaCl) in two replicates. A sample of 25 seeds of each line per replicate was sown in 9 cm petri dishes on two filter papers soaked in 9 ml distilled water or 9 ml of 250 ml NaCl, and incubated at 20–25°C. After 7 days, the germination rate was recorded, by scoring as germinated those individuals with an emerged plumule longer than 50% of the length of the seed and a radicle at least 100% of the length of the seed. A salt tolerance index at the germination stage was defined as the ratio Is/Iu, where the Is represents the germination rate under salt stress, and Iu the germination rate under the control non-saline treatment. Ten-day-old plumules and radicles emerged under 250 mM NaCl were weighed and oven-dried at 70°C for 2 days, following which dry weights were recorded. For seedling stage responses, seed was germinated at room temperature for 4 days, the most uniformly germinated individuals were sown in cells made in sheets of thin styrofoam, which were floated over a solution of half-strength Hoagland solution, aerated for 14 h per day. The salt treatment commenced as soon as one new leaf had emerged. To avoid salt shock, NaCl was added in equal daily increments over 3 days, to a final concentration of 250 mM. Each treatment (0 and 250 mM NaCl) was represented by two replicates (six seedlings per replicate). The experiment was conducted in a growth chamber with a 14 h light/10 h dark photoperiod, 25°C day/18°C night mean temperature, and a photon flux density of 300–800 unol/m^2s. The solution was changed once a week, and the pH was maintained at 5.5–6.5 and adjusted every day. Shoot PRO was determined according to the method of Bates (1973) after 4 days of salt stress. After 10 days of stress, the chlorophyll content of the second leaf was measured using a SPAD-502 chlorophyll meter (Minolta, Japan). Each RIL was measured 30 times, and the mean value used for analysis. After the plants had been kept at 250 mM NaCl for 25 days, a salt injury index (Liu et al. 2001; 0 for green leaves and 5 for leaf death) was recorded. Shoots and roots were then separately harvested, and rinsed with distilled water. Shoot and root length, and fresh weight, were

recorded, and the materials were then oven-dried to obtain dry weights. Except for the salt injury index and the chlorophyll and the proline contents, data are presented as calculated ratios (As/Au × 100%), where As corresponds to the trait measured under salt stress and Au was the control. Variance and covariance analyzes were conducted at both the germination and seedling stages with EXCEL software. We used MAPMAKER/QTL1.0 software for genetic mapping, applying thresholds of $P = 0.005$ and LOD ≥ 2.5 to declare a putative QTL.

Results

Phenotypic values of the RILs and parents

Opata85 was superior to W7984 for all traits at the germination stage (Table 1), and the mean across all RILs suggested transgressive segregation. At the seedling stage, except for salt injury index and RSR (ratio of root length to shoot length), W7984 outperformed Opata85, and once again the RIL mean values indicated the presence of transgressive segregation. Correlation coefficients between the salt tolerance index and radicle dry weight (RDWG), fresh weight and plumule dry and fresh weights during the germination stage are presented in Table 2. The salt tolerance index was positively and significantly correlated with these four traits. During the seedling stage, chlorophyll content and biomass index (but not root fresh weight (RFWS), or root dry weight (RDWS)), were negatively and significantly correlated with the salt injury index (Table 3). This result indicated that high tolerance was associated with a high biomass index and high chlorophyll content, and thus that biomass and chlorophyll content were important indicators of salt tolerance. The ratio of root length to shoot length (RSR) was positively correlated with the salt injury index and therefore may be useful as a salt sensitivity index. Significant correlations were found between PFWS, SDWS, SFWS, RDWS, RFWS and PDWS. PRO was positively, but non-significantly, correlated with SIISS. There were no significant correlations

Table 1 Traits related to the salt tolerance of Opata85 and W7984 and RILs derived from their F1 hybrid

Trait description	Trait abbreviation	Parents		RIL	
		Opata85	W7984	Mean	Range
Salt tolerance index in the germination stage	STGSS	0.87	0.23	0.53 ± 0.03	0.00–0.98
Fresh weight of radicle (g) in the germination stage under salt stress	RFWG	0.96	0.45	0.83 ± 0.37	0.25–2.02
Dry weight of radicle (g) in the germination stage under salt stress	RDWG	0.20	0.11	0.19 ± 0.07	0.07–0.40
Fresh weight of plantule (g) in the germination stage under salt stress	PFWG	0.47	0.28	0.37 ± 0.19	0.14–1.65
Dry weight of plantule (g) in the germination stage under salt stress	PDWG	0.13	0.10	0.12 ± 0.04	0.05–0.31
Salt injury index in seedling stage	SIISS	3.62	1.73	2.64 ± 0.08	1.17–4.80
Root fresh weight index in seedling stage	RFWS	18.54	53.61	32.14 ± 0.87	15.69–85.72
Shoot fresh weight index in seedling stage	SFWS	5.37	17.44	12.45 ± 0.34	2.83–24.06
Plant fresh weight index in seedling stage	PFWS	7.74	19.24	15.62 ± 0.40	4.98–35.54
Root dry weight index in seedling stage	RDWS	20.88	49.50	43.65 ± 0.81	20.00–77.08
Shoot dry weight index in seedling stage	SDWS	7.73	25.38	20.75 ± 0.45	5.00–61.26
Plant dry weight index in seedling stage	PDWS	9.17	28.52	23.37 ± 0.42	7.53–50.80
Ratio of root length and shoot length in seedling stage	RSR	1.63	0.98	0.81 ± 0.01	0.41–1.74
Proline content (mg/gFW) under salt stress	PRO	2.03	3.36	2.52 + 0.07	0.72–5.16
Chlorophyll content (SPAD value) under salt stress	CHLO	15.4	37.3	32.30 ± 0.074	11.8–48.0

Table 2 Correlation coefficients between STGSS and four germination stage traits, and among five salt tolerance indices at the germination stage

	STGSS	RFWG	RDWG	PFWG	PDWG
STGSS	1.0000				
RFWG	0.5075**	1.0000			
RDWG	0.4248**	0.8685**	1.0000		
PFWG	0.5484**	0.6970**	0.5935**	1.0000	
PDWG	0.4147**	0.6018**	0.5978**	0.8597**	1.0000

Significant at *P = 0.05, and **P = 0.01

between PRO and any of the other indices. No significant correlations were found between salt tolerance traits at the germination and the seedling stages (data not shown).

QTL mapping

QTL for salt tolerance at the germination stage were as follows (Table 4): QTL for salt tolerance index at the germination stage were detected on chromosomes 3A, 4D and 5A, explaining 26.6% of phenotypic variance. Gene *Qstgss-4D.2* had the largest individual additive effect, and was mapped to the interval *Xcdo1081–Xfbb226*, with a positive allele in Opata85. One QTL for plumule dry weight was mapped to the interval *Xfbb226–Xfba177* on chromosomes 4D, having 19.8% of the variance and carrying a positive allele in Opata85. Two QTL for plumule fresh weight were mapped to chromosomes 3A and 3B, explaining 9.84% of the overall variance. The *Qpfwg-3B.2* allele in W7984 had a positive effect, and *Qpfwg-3A.1* was a negative allele. Three RDWG QTL were detected on chromosomes 3A, 3B and 7A, explaining 29.8% of phenotypic variance, with *Qrdw-7A.3* in the interval *Xfba72–Xfba127* making the largest contribution to variance (14.1%) and carrying a positive allele in Opata85. One QTL affecting radicle fresh weight (RFWG) was identified on chromosomes 4D, with the positive allele in Opata85. We identified one shared location for RFWG, PDWG and STGSS QTL, in the interval *Xcdo1081–Xfbb226–Xfbd177* on chromosome 4DL, explaining, respectively, 15.7%, 19.8% and 15.9% of the total variance.

Table 3 Correlation coefficients between SIISS and nine seedling stage traits, and among ten salt tolerance indices at the seedling stage

	SIISS	SDWS	RDWS	PDWS	RFWS	SFWS	PFWS	RSR	PRO	CHLO
SIISS	1.0000									
SDWS	–0.3954**	1.0000								
RDWS	–0.1665	0.6836**	1.0000							
PDWS	–0.4394**	0.9685**	0.7638**	1.0000						
RFWS	–0.0891	0.3806**	0.4500**	0.4178**	1.0000					
SFWS	–0.2816**	0.6216**	0.4445**	0.5695**	0.6465**	1.0000				
PFWS	–0.2074*	0.6110**	0.4474**	0.5526**	0.7886**	0.9612**	1.0000			
RSR	0.2148*	–0.0621	–0.09475	0.1223	0.0555	0.0772	–0.05033	1.0000		
PRO	0.1839	–0.1341	0.0545	–0.1299	–0.0165	0.0167	–0.0488	0.1373	1.0000	
CHLO	–0.4657**	0.2847**	0.0591	0.2756**	0.0168	0.2312*	0.2181*	–0.0259	–0.1587	1.0000

Significant at *P = 0.05, and **P = 0.01

Table 4 Putative QTL for salt tolerance during the germination stage

Trait	QTL	Chr.	Interval	Site (cM)	LOD	A	Contribution
STGSS	*Qstgss-3A.1*	3A	*Xglk683–Xtam61*	0.01	2.43	–0.0828	4.68
	Qstgss-4D.2	4D	*Xcdo1081–Xfbb226*	0.055	3.6	–0.1527	15.9
	Qstgss-5A.3	5A	*Xbcd1871–Xcdo749*	0.04	2.9	–0.0939	6
PDWG	*Qpdwg-4D.1*	4D	*Xfbb226–Xfba177*	0.015	3.7	–0.0226	19.78
PFWG	*Qpfwg-3A.1*	3A	*Xmwg30–Xbcd372*	0.02	4.6	–0.0828	5.61
	Qpfwg-3B.2	3B	*Xfbb168–Xbcd147*	0.005	4.2	0.124	4.23
RDWG	*Qrdwg-3A.1*	3A	*Xglk683–Xtam61*	0.065	3.3	–0.3225	5.91
	Qrdwg-3B.2	3B	*Xfbb168–Xbcd147*	0.005	2.6	0.4182	9.81
	Qrdwg-7A.3	7A	*Xfba72–Xfba127*	0.095	2.6	–0.5017	14.12
RFWG	*Qrfwg-4D.1*	4D	*Xcdo1081–Xfbb226*	0.095	2.8	–0.2337	15.68

QTL for salt tolerance at the seedling stage were as follows (Table 5): five QTL for salt injury index were present on chromosomes 3A, 5B, 6B (two loci), and 6D, contributing between 5.6% and 8.4% of the variance. Opata85 carried positive alleles at all but one (*Qsii-6D.5* on 6D) of these QTL. There were five QTL for plant fresh weight (PFWS) mapping to chromosomes 3B (two loci), 3D, 4A and 7B. W7984 carried positive alleles at the *Qpfws-3B.1* (3B), *Qpfws-3B.2* (3B) loci and *Qpfws-7B.5* loci (7B). Opata85 carried positive alleles at the other QTL. Five QTL for plant dry weight (PDWS) mapped to chromosomes 2A (two loci) and 3B (three loci). Loci *Qpdws-3B.3* and *Qpdws-3B.5* mapped to the same intervals as *Qpfws-3B.1* and *Qpfws-3B.2*, respectively. *Qpdws-2A.1* and *Qpdws-2A.2* mapped to chromosomes 2AS and 2AL, respectively, with Opata85 carrying positive alleles at both. Six shoot fresh weight (SFWS) QTL mapped to chromosomes 2A, 2B, 3B (two loci), 3D and 4A, together explaining 41.2% of the phenotypic variance. Two of these QTL (*Qsfws-3B.3* and *Qsfws-3B.4*) mapped to different intervals of chromosome 3B, with W7984 carrying positive alleles at both. Two shoot dry weight (SDWS) QTL mapped to chromosomes 1A and 3B. *Qsdws-3B.2* was present in the same region as *Qsfws-3B.3*. Two RFWS and two RDWS QTL

Table 5 Putative QTL for salt tolerance during the seedling stage

Trait	QTL	Chr.	Interval	Site (M)	LOD	A	Contribution
SIISS	*Qsii-3A.1*	3A	*Xtam47–Xcdo460*	0.045	2.6	–0.2754	5.55
	Qsii-5B.2	5B	*Xfbb12.2–Xfba127*	0	2.7	–0.3116	7.15
	Qsii-6B.3	6B	*Xfba345–Xglk479*	0.015	2.5	–0.3142	7.24
	Qsii-6B.4	6B	*Xbcd2014–Xfbb364*	0.035	2.5	–0.2981	6.52
	Qsii-6D.5	6D	*Xfbb283–Xmwg916*	0.06	2.7	0.338	8.39
PFWS	*Qpfws-3B.1*	3B	*Xfbb156–Xfba220*	0	3.4	2.6464	8.59
	Qpfws-3B.2	3B	*Xfbb168–Xbcd147*	0	3.8	2.8689	10.1
	Qpfws-3D.3	3D	*XksuH15–Xbcd361*	0.02	3.6	–1.9023	4.44
	Qpfws-4A.4	4A	*Xbcd588–Xbcd129*	0.005	2.7	–1.5134	2.81
	Qpfws-7B.5	7B	*Xfba311–Xbcd178*	0.005	2.8	2.0328	5.07
PDWS	*Qpdws-2A.1*	2A	*Xfba70.1–Xcdo447*	0.005	3.1	–3.2798	7.73
	Qpdws-2A.2	2A	*Xcdo1281–Xfba106*	0.005	2.7	–3.0902	6.86
	Qpdws-3B.3	3B	*Xfbb117–Xfbb156*	0.045	3.2	3.1957	7.34
	Qpdws-3B.4	3B	*Xbcd1418–Xcdo583*	0.03	2.5	2.0603	3.05
	Qpdws-3B.5	3B	*Xfbb168–Xbcd147*	0.005	2.9	3.1978	7.35
SFWS	*Qsfws-2A.1*	2A	*Xcdo1281–Xfba106*	0.005	3.3	–1.8949	8.02
	Qsfws-2B.2	2B	*Xmwg2025–Xfbb284*	0	2.6	1.9254	8.28
	Qsfws-3B.3	3B	*Xfbb168–Xbcd147*	0	3.2	2.0652	9.53
	Qsfws-3B.4	3B	*Xfbb293–Xmwg11*	0.205	2.5	1.9433	8.43
	Qsfws-3D.5	3D	*Xbcd515–XksuD19*	0	3.2	–1.361	4.14
	Qsfws-4A.6	4A	*Xbcd588–Xbcd129*	0.005	2.7	–1.2064	3.25
SDWS	*Qsdws-1A.1*	1A	*XksuD1.2–Xcdo426*	0.105	3.5	0.798	6.21
	Qsdws-3B.2	3B	*Xfbb168–Xbcd147*	0.005	3	1.0397	10.54
RFWS	*Qrfws-4A.1*	4A	*Xbcd588–Xbcd129*	0	2.7	–3.3501	5.92
	Qrfws-6D.2	6D	*Xfbb231.1–Xpsr106*	0.015	3.5	6.1052	19.67
RDWS	*Qrdws-3D.1*	3D	*XksuH15–Xbcd361*	0.015	3.2	–4.6275	6.48
	Qrdws-6D.2	6D	*Xfbb231.1–Xpsr106*	0.005	2.5	6.4933	12.75
RSR	*Qrsr-1A.1*	1A	*Xgli1–XksuD1-2*	0.015	3.5	0.0971	5
	Qrsr-2A.2	2A	*Xcdo1410–XksuF41*	0	4.6	0.1064	9.22
	Qrsr-2D.3	2D	*Xbcd1970–Xbcd718*	0.015	2.9	–0.0874	4.02
	Qrsr-3A.4	3A	*Xfbb29-2–Xmwg12*	0.005	2.7	–0.1097	6.43
	Qrsr-3D.5	3D	*XksuH15–Xbcd361*	0	3.6	–0.0923	4.5
	Qrsr-6A.6	6A	*Xfba345–Xglk479*	0.025	4.4	–0.1181	7.4
	Qrsr-6A.7	6A	*Xfbb82–Xfbb70*	0.055	3.5	0.1079	6.2
	Qrsr-6D.8	6D	*Xfb231.1–Xpsr106*	0.015	5.8	0.1691	15.18
CHLO	*Qchlo-3D.1*	3D	*Xcdo1406–Xbcd288*	0.08	3.1	–2.9478	5.39
	Qchlo-7A.2	7A	*Xwg380–XksuD2*	0.035	3.4	3.0859	5.91

were located on chromosomes 4A, 6D (two loci) and 3D. Two QTL of *Qrfws-6D.2* and *Qrdws-6D.2* co-localized to the same interval *Xfbb231.1–Xpsr106* on chromosome 6D, explaining, respectively, 19.7% and 12.8% of the phenotypic variance. W7984 carried positive alleles at both loci. Eight RSR QTL were detected on chromosomes 1A, 2A, 2D, 3A, 3D, 6A (two loci) and 6D (Table 5), together explaining 58.1% of phenotypic variance. Of these, the largest individual effect (15.2%) was contributed by *Qrsr-6D.8.* Two PRO QTL were mapped to chromosomes 5D and 6D, each explaining about 13% of the phenotypic variance, with positive alleles in W7984, but the LOD value for two PRO QTL were only 2.1 and 2.2, respectively (data not shown). Two QTL for chlorophyll content mapped to chromosomes 3D and 7A, explaining 11.3% of the phenotypic variance. W7984 had a negative allele at *Qchlo-3D.1* and a positive allele at *Qchlo-7A.2*.

Discussion

Identifying traits related to salt tolerance

Appropriate phenotypic characterisation was necessary for mapping QTL. Several other attempts to map genes related to salt resistance in wheat have been reported (Wyn Jones et al. 1984; Shan et al. 1987; Gorham et al. 1987; Dvorak et al. 1994; Liu et al. 2001; Munns et al. 2002), using different salt tolerance indices and mapping populations. This hinders a direct comparison between our results and those in the literature. We identified not only phenotypic value (salt tolerance/injury index), but also biomass, PRO and chlorophyll content during both the germination and seedling stages. Assessment of response to salt stress at both stages was important, as noted by Yoshiro and Kazuyoshi (1997) and needs to be further extended to the mature stage of plant growth. Apart from phenotypic indices, salt tolerance index, and salt injury index, we also made measurements of radicle length, plumule and seedling biomass, seedling shoot length, and seedling root length, allowing a more in-depth view of salinity response.

The percentage of biomass treatment/control ratio is an important salt tolerance index (Levitt 1980; Munns et al. 2002). In our experiments, SFWG, SDWG, PFWG and PDWG were negatively correlated with salt injury index in seedling stage, with high coefficients. It showed that the percentage of biomass treatment/control ratio can be used as a salt tolerance index in wheat. Under the imposed osmotic stress, shoot growth was inhibited more severely than root growth, so the RSR increased under abiotic stress (Zerihun et al. 2000; Thornley 1998). RSR for the salt sensitive Opata85 was larger than that of the salt tolerant W7984. RSR was significantly positively correlated with salt injury index, indicating that RSR is a salt sensitive index in wheat.

According to Schreiner and Zozor (1998) chlorophyll content is an important salt tolerance index. The salt tolerant parent W7984 had a higher chlorophyll content than the salt sensitive parent Opata85. Chlorophyll content (CHLO) for the RILs was negatively correlated with salt injury index (SIISS) and the correlation was significant (–0.4567), indicating that CHLO can be used as a salt tolerance index in wheat.

Proline is one of the osmoprotectant molecules (osmolytes) which accumulates in many organisms, including bacteria, fungi, algae and plants in response to water stress and salinity (Csonka and Hanson 1991; Delauney and Verma 1993; Hanson and Hitz 1982). Correlations between proline accumulation and osmotic stress response indicated that proline plays an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity (Thomas et al. 1992; Delauney and Verma 1993; Ober and Sharp 1994; Serrano and Glaxiola 1994; Chiang and Dandekar 1995). The salt tolerant parent W7984 had a higher proline content than the salt sensitive parent Opata85. However, the correlation between PRO and salt tolerance index for the RIL was not significant. Hence we could not show an effect of PRO on salt response.

Co-located or tightly linked QTL

Overall, 47 QTL were mapped on 16 chromosomes. As many QTL clustered into single genetic intervals (Table 6), each cluster may represent a single locus, as has been noted previously for QTL for correlated traits (Paterson 1995; Veldbloom

Table 6 Co-located or tightly linked QTL related to salt tolerance

Chromosome	QTL	Interval	Trait
4D	*Qstgss-4D.2* *Qpdwg-4D.1* *Qrfwg-4D.1*	*Xbdo1081–Xfba177*	STGSS, PDWG, RFWG
3A	*Qstgss-3A.1* *Qrdwg-3A.1* *Qsii-3A.1*	*Xglk683–Xcdo460*	STGSS, RDWG, SIISS
3B	*Qpfws-3B.2* *Qpfwg-3B.2* *Qrdwg-3B.2* *Qpdws-3B.5* *Qsdws-3B.2* *Qsfws-3B.3*	*Xfbb168–Xbcd147*	PFWG, RDWG, SDWS, SFWS, PFWS, PDWS
6D	*Qsii-6D.5* *Qrfws-6D.2* *Qrdws-6D.2* *Qrsr-6D.8*	*Xfbb231.1–Xmwg916*	SII, RFWS, RDWS, RSR

et al. 1994). Thus, for example, salt tolerance index and both radicle and plumule biomass were correlated, and three QTL (*Qstgss-4D.2*, *Qpdwg-4D.1* and *Qrfwg-4D.1*) mapped to a common region on chromosome 4D. Positive alleles at all three were contributed by Opata85. The salt tolerance QTL in the interval *Xcdo1081–Xfbb226* appeared to be a major determinant at the germination stage. A similar pattern was observed for the six phenotypically correlated traits PFWS, PDWS, SFWS, SDWS, PFWG, and RDWG. Six QTL (*Qpfwg-3B.2*, *Qrdwg-3B.2, Qpfws-3B.2*, *Qpdws-3B.5*, *Qsfws-3B.3* and *Qsdws-3B.2)* co-located to the *Xfbb168-3B–Xbcd147-3B* interval with positive alleles contributed by W7984. Salt injury index QTL at the seedling stage were not correlated with those at the germination stage, but *Qsii-3A.1*, *Qrdwg-3A.1* and *Qstgss-3A.1* were clustered on chromosome 3A, all with positive alleles contributed by Opata85, suggesting a single salt tolerance locus or a QTL cluster effective during both the seedling and germination stages. The trait SIISS was correlated with RDWS, RFWS and RSR; the *Qrdws-6D.2*, *Qrfws-6D.2*, *Qsii-6D.5* and *Qrsr-6D.8* clustered on chromosome 6D, indicated a major salt tolerance complex, effective at the seedling stage.

Yoshiro and Kazuyoshi (1997) showed that the genetic basis of salt tolerance in barley at the germination stage differed from that at the seedling stage, with different QTL implicated at the two developmental stages. In the present study, we not only found distinct QTL controlling salt tolerance at the germination stage (on 4DL) and at the seedling stage (6DL), but also, there was at least two QTL (3B and 3A) effective at both stages. This may reflect a physiological difference between wheat and barley (Koyama et al. 2001). Quarrie et al. (2005) noted that homoeologous regions on chromosomes 5B and 5D harbor QTL for yield at the adult stage under salt stress, whereas Ellis et al. (2002) located salt tolerance-related QTL in barley effective at the adult stage on chromosomes 3H and 5H, and genetic control of barley shoot weight determined by a major factor on 5H. We also located salt tolerance-related QTL to homoeologous group 5, including *Qsii-5B.2* and *Qstgss-5A.3*. However, we have as yet no current evidence that these QTL are effective at the adult stage.

We identified four QTL (*Qsfws-2A.1*, *Qpdws-2A.1*, *Qpdws-2A.2*, and *Qrsr-2A.2*) mapping to various regions of chromosome 2A, but it was not possible to propose that any of these are identical with the locus determining leaf Na exclusion mapped on chromosome 2AL by Munns et al. (2002) and Lindsay et al. (2004). We uncovered many novel QTL, such as *Qrdwg-3A.1*, *Qsii-3A.1*, and *Qstgss-3A.1* clustered on chromosome 3A and controlling salt tolerance during the germination and the seedling stages; *Qrdwg-3B.2*, *Qpfwg-3B.2*, *Qpfws-3B.2*, *Qpdws-3B.5, Qsdws-*

3B.2 and *Qsfws-3B.3* all mapping to the interval *Xfbb168–Xbcd147* on chromosome 3B, with positive alleles contributed by W7984. The QTL corresponding to these various related traits were co-located in the same genetic interval, which appears to represent a major salt tolerance QTL. These QTL are suitable targets for wheat improvement via marker assisted selection in the immediate term, and possibly for map-based cloning in the longer term. It is currently impossible to determine whether the co-locating QTL represent a single locus or tightly linked loci. This can only be determined by fine-scale mapping.

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