Dynamic quantitative trait loci for salt stress components on chromosome 1 of rice

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Abstract. Rice varieties Co39 and Moroberekan differ for leaf Na^+ concentrations when grown at moderate salinity ($100–150$ mol m^{-3} NaCl; $10:1$ or $20:1$ Na^+ to Ca^{2+} ratio). Recombinant inbred lines (RILs) from a cross between them were used to map quantitative trait loci (QTL) under salt stress over several weeks. Two experiments (conducted with 170 and 96 RILs, and a linkage map of 126 RFLP markers) identified a major effect on QTL for leaf Na^+ concentration and $K^+:Na^+$ ratio on chromosome 1 in a region corresponding to $11.07–14.6$ Mbp. No leaf Cl^- QTL were detected. In a third experiment, leaves and sheaths were harvested after 7 and 21 days at 100 mol m^{-3} NaCl. The linkage map of chromosome 1 was improved by the addition of 28 microsatellite markers, which resolved distinct QTL for Na^+ and K^+ concentrations, and $K^+:Na^+$ ratio. After 7 days' stress, the most significant QTL were in the region of $11.56–12.66$ Mbp. The highest Na^+ concentrations were recorded in the sheaths. Na^+ concentration QTL were detected for leaves, but not for sheaths. After 21 days' stress, the region containing the most significant QTL extended to 11.07 Mbp in leaves and in sheaths. A QTL for the ratio of leaf Na^+ to sheath Na^+ concentrations was found at $11.39–12.39$ Mbp. These findings suggest that multiple genes in this region are involved in the response to salinity, and their impact is dynamic according to stress duration, and leaf age and type.

Additional keywords: ion accumulation, Nax1, Nax2, physiological response, salinity, *qSaltol*, *SalT*, *SKC1*.

Introduction

Of the 230 Mha of irrigated land worldwide, 19.5% (45 Mha) is salt-affected, much of which is in Asia where rice (*Oryza sativa* L.) is the main cereal crop (Ma *et al*. 2007). Salinity is a major constraint to the sustainability and expansion of rice cultivation in areas where rice production has not kept up with increasing demand from growing populations. In Pakistan, one million hectares of the rice growing area is salt-affected (Qureshi *et al*. 1991) and salinity accounts for crop yield reductions of 40–70% in these soils (Aslam *et al*. 1995). There is therefore an urgent need to develop high-yielding salt-resistant varieties to ensure the sustainability of agriculture in such regions (Witcombe *et al*. 2008).

Although halophytes can use Na^+ and Cl^- for internal osmotic adjustment to saline conditions (Flowers *et al*. 1986), many glycophytes survive saline conditions by excluding most of the salt from the transpiration stream, and therefore from the stems and leaves. The success of this depends on the extent to which salt influx into the plant can be tightly controlled. Too little accumulation can lead to poor osmotic adjustment, while too

much may result in overadjustment and in toxic concentrations of Na^+ or Cl^- in vital tissues. Physiological studies have shown that resistance to salinity damage in rice is associated with the ability of genotypes to exclude Na^+ from the shoots and maintain a high $K^+:Na^+$ ratio (Yeo and Flowers 1984; Yeo *et al*. 1990; Gregorio and Senadhira 1993; Asch *et al*. 2000; Bonilla *et al*. 2002) and that this ability is heritable (Yeo 1992; Garcia *et al*. 1997*a*). In saline conditions, the amount of salt reaching the shoots will depend on the transpirational demand and the ability to restrict the entry of salt into the various tissues of the root including, ultimately, the xylem. The greater salt susceptibility of rice compared with wheat has been attributed to less effective control of apoplastic bypass flow in rice. The bypass flow is that proportion of the transpiration stream (including any dissolved ions) that passes through an unregulated apoplastic connection rather than through a cell membrane. Such control is predominantly exercised at the rhizodermis and endodermis by the plasma membranes, particularly by the Casparian strip, a suberised layer in the walls of the endodermal cells that prevents apoplastic flow. Rice lines showing low Na^+ accumulation in

the shoots have lower rates of bypass flow (Yadav *et al*. 1996; Ranathunge *et al*. 2005*a*, 2005*b*; Krishnamurthy *et al*. 2009).

We investigated the physiology of salinity responses in different rice varieties that had salt applied either to the roots or to the leaves, and found that two of the varieties examined, Co39 and Moroberekan, did not differ in leaf Na^+ accumulation when saline solution was applied to the leaves, but Co39 had lower leaf Na^+ concentrations and a lower reduction in shoot fresh weight than Moroberekan when salt was applied to the roots (Akhtar 2002). A mapping population derived from these two varieties had been produced in International Rice Research Institute (IRRI) and used to detect quantitative trait loci (QTL) for drought responses (Champoux *et al*. 1995), days to flowering (Maheswaran *et al*. 2000) and blast resistance (Wang *et al*. 1994).

We report here on the detection of QTL in this population for leaf Na^+ and other traits in saline conditions across a range of leaf tissues over 3 weeks of stress. The results are compared with other reported QTL and genes for salinity responses in rice (Claes *et al*. 1990; Garcia *et al*. 1998; Gong *et al*. 1999; Prasad *et al*. 2000; Koyama *et al*. 2001; Lang *et al*. 2001*a*, 2001*b*, 2001*c*; Bonilla *et al*. 2002; Elahi *et al*. 2004; Lin *et al*. 2004; Walia *et al*. 2005; Lee *et al*. 2007; Mohammadi-Nejad *et al*. 2008). We discuss whether similar QTL, particularly *qSKC1* and *qSaltol*, identified in different populations could be due to the same or to different underlying genes.

Materials and methods

Plant material

F_8 and F_9 recombinant inbred lines (RILs) from a cross between the rice varieties Co39 (lowland *O. sativa indica*) and Moroberekan (West African upland *O. sativa japonica*) were used in these experiments. This cross was made in IRRI in 1988 and the population was derived from $15 F_1$ seeds that produced $\sim 300 F_2$ seeds. Single seed descent and a Rapid Generation Advance greenhouse (Vergara *et al*. 1982) were used to develop the mapping population, which consisted of 283 RILs in total (only 200 of these were available for the first two experiments). The population is skewed towards the Co39 alleles (80%) and the effect of this distribution on linkage analysis has been discussed previously (Manly 1994; Wang *et al*. 1994; Champoux *et al*. 1995).

Experimental work was carried out at Pen-y-Ffridd Research Station, Bangor University, UK. Seeds were sown in John Innes Compost No.1 in P-84 plug trays (Desch Plantpak, Malden, UK) until transplanting at >10 days into 20 plastic 2-dm^3 plant pots lined with horticultural fleece and filled with John Innes Number 2 compost (Lawrence and Newell 1939). Supplementary 400-W high pressure sodium vapour lamps were used to maintain a minimum photon flux density of photosynthetically active radiation (400–700 nm) of $350 \,\mu mol \, m^{-2} \, s^{-1}$ during the 16-h photoperiod. The minimum temperature was maintained at 25°C during the light period and 20°C during the dark period.

Experiment 1

Seeds were sown on 12 March 1999. The parental varieties (Co39 and Moroberekan; 10 replicates each) and single plants

of $170 F_8$ generation RILs were used (190 plants in total). Pots containing single transplanted plants were transferred to a flood bench system in a randomised layout for saline treatment.

The flood bench system consisted of 10 polypropylene tanks (80 cm × 56.25 cm × 32.5 cm) each containing 20 pots. Empty, inverted P-576 plug trays (Desch Plantpak) were placed under the pots in each tank to improve drainage. The tanks were on benches ~1 m high, and reservoirs (200 dm^{-3}) for irrigation were placed on the floor below. Submersible electric pumps were used to irrigate the tanks to the level of the soil surface once or twice a day (depending on transpirational demand) for at least 15 min. There were two water connections to each tank to circulate the solutions from the reservoir and act as a constant level device. Nutrients (Phostrogen plant food; Phostrogen, Corwen, Wales, UK) at $1 \, g \, dm^{-3}$ plus $0.5 \, cm^3 \, dm^{-3}$ micro-nutrients (Hoagland and Arnon 1950) plus $0.1 \, cm^3 \, dm^{-3}$ sodium silicate were supplied to the plants with the irrigation water. Salts (NaCl and $CaCl_2$) were added to the reservoirs to the required concentrations and the concentration in each reservoir was adjusted weekly to maintain constant electrical conductivity. Water lost by transpiration and evaporation was replaced to keep the reservoir volume at 200 dm^3.

Salt stress started 24 days after sowing (5 April 1999) and was increased in daily increments of 25 mol m^{-3} and 2.5 mol m^{-3} for NaCl and $CaCl_2$ respectively. The salinity reached 100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$ 27 days after sowing. The youngest fully expanded leaf from each plant was sampled for ion analysis after 14 days at this salinity (Day 41). The salt stress was increased from Day 41 by two further increments to reach 150 mol m^{-3} NaCl + 15 mol m^{-3} $CaCl_2$ on Day 43.

A salinity resistance score (SRS) for each plant was visually estimated from its general physical health, extent of leaf rolling and proportion of the green leaf lamina. A score of 0 was given to dead plants, 1 to plants with <10% green leaf, 2 to plants with 10–50% green leaf, 3 to plants with 50–90% green leaf, 4 to plants with >90% green leaf and some rolled leaves, and a score of 5 to plants showing no symptoms of injury and no leaf rolling. SRS1 was recorded after 36 days of salinity stress (Day 63) and SRS2 was recorded after 49 days stress (Day 76). Plants were finally harvested at 90 days, 63 days after salt stress was first imposed. Shoot fresh and dry weights were recorded.

Experiment 2

The two parental varieties and 96 F_9 generation RILs were used in this experiment. The RILs were selected according to the ion accumulation recorded in their leaf saps in Experiment 1. A third of the RILs were selected for high Na^+ and Cl^-, a third with low Na^+ and Cl^-, and a third were chosen for mid-range accumulation of these ions. Six replications for each RIL and parent variety were used (588 plants in total). Seeds were sown on 13 July 2000. Pots containing single transplanted plants were transferred to a flood bench system in a randomised layout for saline treatment.

The flood bench system and salt treatment were similar to Experiment 1, except that only one salinity level (150 + 15 mol m^{-3} NaCl and $CaCl_2$) was used. Salt stress was

started 24 days after sowing (6 August 2000) and full stress was reached 29 days after sowing. The salt and nutrient solutions were replaced on Day 39. The youngest fully expanded leaf from each plant was sampled for ion analysis after 15 days at this salinity (Day 44). SRS1 was recorded 17 days after reaching full salt stress (Day 46) and SRS2 was recorded after 34 days of full salt stress (Day 63). Plants were harvested for measurement of growth parameters after 47 days of salt stress (Day 76). The means of the six replicates of each RIL were calculated and used for QTL analysis.

Experiment 3

Seeds were sown on 28 June 2006. The parental varieties and 120 F_9 RILs (including some selected from previous experiments and some not previously used) were used, with three replicates consisting of two seedlings each: one was harvested after 7 days and the other after 21 days of salt stress. Pairs of uniform seedlings of each line were transplanted for the experiment (366 pairs of plants in total). On 23 July 2006, pots containing pairs of transplanted plants were transferred to a flood bench system, with 24 pots in each flood bench tank in a randomised layout for saline treatment.

Salt stress started 30 days after sowing, and was increased in daily increments of $25 \, \text{mol m}^{-3}$ and $1.25 \, \text{mol m}^{-3}$ for NaCl and $CaCl_2$ respectively. It was held at a final concentration of $100 \, \text{mol m}^{-3}$ NaCl + $5 \, \text{mol m}^{-3}$ $CaCl_2$ from 34 days after sowing. A Na^+ to Ca^{2+} ratio of 20 : 1 was maintained in the solution.

After 7 days of salt stress (Day 41), one of each pair of seedlings from all three replicates was harvested and four tissue samples were taken. These were (i) a newly emerging leaf, (ii) a fully expanded leaf, (iii) a lower or mature leaf and (iv) the combined leaf sheaths. Combined leaf sheaths were used because Experiment 3 was on a large scale and preparing individual leaf sheath samples would have taken too long. In an earlier experiment, individual leaf sheaths were dissected from the 'stems' and analysed separately.

Twenty-one days after reaching full salt stress (Day 55), one tiller of the second plant was harvested and two leaf samples were taken, one from the fully expanded leaf and one from the leaf sheath. No healthy emerging leaf tissue was available at this second harvest. The samples were washed briefly with distilled water, dried with tissue paper and stored in 1.5-cm^3 microcentrifuge tubes at $-20°C$ in the freezer for 1 week. Leaf sap was extracted from each sample as described below.

Sap extraction

The plant tissues were blotted dry with tissue paper and frozen at $-20°C$ for at least 24 h in 1.5-cm^3 polypropylene microcentrifuge tubes. After defrosting, the tissues were crushed with a sharp steel rod. Two pin holes were made in the base and cap of each tube. Tubes were placed inside a second microcentrifuge tube and centrifuged at $8000g$ for 10 min. The sap was collected into the lower tube and remaining tissue was left behind in the upper one. The sap was either analysed immediately or frozen for later analysis (Gorham *et al*. 1997).

Ion analysis

In the first two experiments, anions and cations were analysed with a Dionex ion chromatograph (Dionex 2000i; Dionex (UK) Ltd, Camberley, Surrey, UK) (Gorham and Bridges 1995). For anion analysis, $20 \, \text{mm}^3$ of sap sample was diluted in an auto-injector vial with $1.5 \, \text{cm}^3$ of anion eluant ($2.5 \, \text{mol m}^{-3}$ Na_2CO_3 + $2.4 \, \text{mol m}^{-3}$ $NaHCO_3$ in 2.5% propan2-ol) and analysed with an ion chromatograph (Dionex) fitted with an AS4A anion exchange column (at $50°C$), and an Anion Micro-Membrane Suppresser (Dionex). For cation analysis, $20 \, \text{mm}^3$ of sap was diluted in an auto-injector vial with $1.5 \, \text{cm}^3$ of cation eluant ($20 \, \text{mol m}^{-3}$ methane sulfonic acid in deionised water, $>18 \, \text{M}\Omega$) and analysed on a CS12 cation exchange column (Dionex) and a Self-Regenerating Cation Suppresser (Dionex) operated in auto-regeneration model. The column was heated to $50°C$. The system was automated by coupling to a Marathon auto-sampler (Spark-Holland, Emmen, The Netherlands) fitted with a 5-mm^3 PEEK sample loop (Rheodyne, Rohnert Park, CA, USA), and a CR5A plotting integrator (Shimadzu, Columbia, MA, USA). Standards for the Dionex ion chromatograph were prepared using a method similar to the sample preparation, but instead of leaf sap, the same amount ($20 \, \text{mm}^3$) of anion standard solution ($250 \, \text{mol m}^{-3}$ Cl^-, $100 \, \text{mol m}^{-3}$ NO_3^-, $100 \, \text{mol m}^{-3}$ $malate^{2-}$ and $100 \, \text{mol m}^{-3}$ SO_4^{2-}) was diluted with $1.5 \, \text{cm}^3$ of anion eluant. For cations, $20 \, \text{mm}^3$ of cation standard ($250 \, \text{mol m}^{-3}$ Na^+, $250 \, \text{mol m}^{-3}$ K^+, $100 \, \text{mol m}^{-3}$ Ca^{2+} and $100 \, \text{mol m}^{-3}$ Mg^{2+}) was diluted with $1.5 \, \text{cm}^3$ of cation eluant. In Experiment 3, cations in diluted sap samples were analysed with a Jenway PFP-7 flame photometer (Bibby Scientific Limited, Stone, UK).

Extraction and quantification of genomic DNA

Six-week-old plants sown on 28 June 2006 (three for each of 120 RILs used in Experiment 3 and three from each parental variety that were not transplanted from plug trays) were used for DNA extraction. DNA was extracted from 100 mg of the fresh leaf tissue from the three plants using DNeasy plant mini kits (Qiagen, Crawley, West Sussex, UK) according to the manufacturer's instructions. DNA was quantified using a PicoGreen dsDNA quantification kit (Molecular Probes, Leiden, The Netherlands) following the manufacturer's protocol. The fluorescence intensity was measured using a FluoStar Galaxy fluorescence microplate reader (BMG Laboratory-technologies Inc., Durham, NC, USA). DNA samples were diluted to a final concentration of $5–6 \, \text{ng mm}^{-3}$ for use in PCR.

Microsatellite markers

Simple sequence repeat (microsatellite) markers on rice chromosome 1 were selected from the Gramene (http://www.gramene.org/index.html, accessed 30 November 2009) database based on their map locations. A total of 56 SSR markers were used to test the polymorphism in the parents. The 28 SSRs that were polymorphic in the parents were used to genotype the 120 RILs. All PCR amplifications were performed in 16-mm^3 reaction volumes containing a final concentration of $1 \times$ ReadyMix (ABgene, Epsom, Surrey, UK), $3.0 \, \text{mol m}^{-3}$ $MgCl_2$, $0.25 \, \text{mol m}^{-3}$ of forward primer, $2.5 \, \text{mol m}^{-3}$ reverse

primer, 2.5 mol m^{-3} dye labelled primer and ~0.5 ng mm^{-3} genomic DNA. Genomic DNA was amplified by using the following PCR program: 94°C for 5 min, 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, followed by final extension at 72°C for 10 min. PCR products were separated using 2.5% Metaphor fine resolution agarose gel (SFR; NBS Biologicals, Huntingdon, Cambridgeshire, UK). For markers where the PCR products differed by less than 8 bp, a Beckman Wellred dye-labelled primer (Sigma-Aldrich, Dorset, UK) was included in the PCR reaction and PCR products were run on a CEQ8000 capillary sequencer (Beckman Coulter, High Wycombe, UK) to determine the size of the amplified fragments.

Map construction

The genotypic data for RFLP markers in 283 RILs were obtained from Brigitte Courtois (CIRAD, Montpellier, France). Linkage maps were generated using MapMaker/Exp. (version 3.0) computer software (Lander *et al*. 1987) and the Kosambi function was used. QTL mapping in Experiments 1 and 2 used a linkage map containing 126 RFLPs on all chromosomes and the marker locations were verified by comparison to other maps (McCouch *et al*. 1988; Tanksley *et al*. 1992). For Experiment 3, a new linkage map of chromosome 1 was constructed from genotypic data of 120 RILs with 28 SSRs and 16 RFLPs. The probe sequences of the 16 RFLPs located on chromosome 1 were searched on the Gramene database to determine their position and order on the physical map (Gramene Annotated Nipponbare Sequence 2006). cMap was used where probe sequences were not available. The order of RFLPs on chromosome 1 reported by Champoux *et al*. (1995) was confirmed but the orientation of the whole chromosome reported by Champoux *et al*. (1995) was inverted relative to the reference map of Causse *et al*. (1994) and the rice genome available through the Gramene database.

Statistical and QTL analysis

Trait data (mean values) for all RILs were recorded in Microsoft Excel. MINITAB (version 1.1) and SPSS (11.0) were used for statistical analysis. Skewed data were transformed (log and square root) before entry into the file for map construction using MAPMAKER/EXP (version 3.0b) and MAPMAKER/

QTL (version 1.1b). WinQTL Cartographer (version 2.5) (Wang *et al*. 2007) was used for QTL mapping. LOD scores ((logarithm (base 10) of odds ratio); Lander *et al*. 1987) were determined for each trait using 1000 permutations at 0.5% significance (Churchill and Doerge 1994). In Experiments 1 and 2, values of 1 + the calculated LOD threshold for each trait were used to detect QTL. In Experiment 3, the calculated LOD values were used as thresholds for the declaration of putative QTL, or 2.5 if the calculated value was lower than this figure. Only QTL detected on chromosome 1 are tabulated for Experiment 3.

Results

Leaf sap ion concentrations

In Experiment 1, Na$^+$ concentrations were significantly higher ($P \leq 0.001$) in Moroberekan than in Co39, and K$^+$ concentrations were lower ($P \leq 0.05$) (Table 1). The increases in monovalent ions were accompanied by decreases in Ca^{2+} and Mg^{2+} concentrations (data not shown). The differences in Cl$^-$ concentrations between Moroberekan and Co39 were not statistically significant. Mean values for visual SRS were lower after 49 days (SRS2) than at 36 days (SRS1), and were higher at both 36 and 49 days for Co39 than for Moroberekan, as were shoot fresh and dry weights. Broadly similar results were obtained in Experiments 2 and 3.

In the RILs, the distribution of leaf Na$^+$ concentrations (data for Experiment 3 are shown in Fig. S1, available as an Accessory Publication to this paper) and K$^+$ concentrations (data not shown) were highly skewed towards the lower end of the range in all experiments. Possible transgressive segregation (RIL values higher or lower than the parents) for leaf Na$^+$ concentrations was observed in all three experiments, but this may partly be a feature of measuring changing ion concentrations in leaves undergoing rapid dehydration as a result of osmotic imbalances between subcellular compartments.

In Experiment 3, after 7 days of full salt stress, the leaf sheaths had higher Na$^+$ concentrations than leaf laminae at any earlier developmental stage, and also had the lowest K$^+$ concentrations (Table 2). A similar pattern was observed after 21 days, although Na$^+$ concentrations in emerged leaf laminae and sheaths were

Table 1. Physiological and growth parameters of the parent varieties (Co39 and Moroberekan) in Experiment 1 (means of 10 replicates ± s.e.m.)

Parameter	NaCl (mol m^{-3})	CaCl$_2$ (mol m^{-3})	Days[A]	Co39	Moroberekan	P[B]	RILs
Leaf Na$^+$ (mol m^{-3})	100	10	14	4 ± 0	13 ± 2	***	8 ± 1
Leaf K+ (mol m^{-3})	100	10	14	267 ± 3	210 ± 5	***	283 ± 4
Leaf Cl$^-$ (mol m^{-3})	100	10	14	219 ± 13	191 ± 11	NS	231 ± 5
Leaf K$^+$ + Na$^+$ (mol m^{-3})	100	10	14	272 ± 3	223 ± 5	***	291 ± 4
SRS1[C]	100	10	36	3.2 ± 0.1	2.3 ± 0.2	**	2.7 ± 0.1
SRS2[C]	150	15	49	1.9 ± 0.2	0.7 ± 0.3	**	1.3 ± 0.1
Shoot fresh weight (g)	150	15	63	15.9 ± 0.7	5.7 ± 0.8	***	12.8 ± 0.3
Shoot DW (g)	150	15	63	6.5 ± 0.3	3.8 ± 0.4	***	6.9 ± 0.1
Shoot water (g g^{-1} DW)	150	15	63	1.5 ± 0.1	0.7 ± 0.4	NS	0.8 ± 0.0

[A]Days after reaching 100 mol m^{-3} NaCl. Add 3 for days after salt was first applied, or 27 for days from sowing.
[B]Probability of Moroberekan values being different from Co39 values. NS, non-significant; **$P \leq 0.01$; ***$P \leq 0.001$.
[C]Salinity resistance score (SRS) from 0 = dead to 5 = no visible damage. SRS1 and SRS2 were recorded 36 and 49 days after initiating the salt stress respectively.

Table 2.   Mean leaf sap ion concentrations for all RILs grown for 7 or 21 days at full salt stress ($100\,\mathrm{mol\,m^{-3}}$ NaCl$+5\,\mathrm{mol\,m^{-3}}$ CaCl$_2$; Experiment 3)

Time at full stress (days)	Part of leaf	K^+ $(\mathrm{mol\,m^{-3}})$	Na^+ $(\mathrm{mol\,m^{-3}})$	$K^+:Na^+$	K^++Na^+ $(\mathrm{mol\,m^{-3}})$
7	Emerging	277 ± 3	16 ± 1	29 ± 2	293 ± 3
7	Emerged	306 ± 3	9 ± 1	66 ± 5	315 ± 3
7	Mature	278 ± 3	6 ± 1	82 ± 5	283 ± 3
7	Sheath	251 ± 3	43 ± 2	8 ± 1	293 ± 3
21	Emerged	299 ± 4	28 ± 3	30 ± 3	327 ± 5
21	Sheath	255 ± 4	78 ± 4	5 ± 1	333 ± 3

higher than at 7 days. Na^+ concentrations were higher in the emerging leaves than in the older leaves, and the emerging leaves were among the first to show signs of damage under salt stress.

Leaf Cl^- concentrations were only measured in Experiment 1. No differences in leaf Cl^- were observed between the parental varieties, and the leaf Cl^- concentrations in the RILs were normally distributed. There were linear relationships between Cl^- and Na^+ concentrations in both parental varieties (data not shown), and concentrations of leaf Cl^- were considerably higher than Na^+ in both parents and the RILs. The $Na^+:Cl^-$ ratios were much higher in Moroberekan than in Co39 (Table 1). In the RILs, there was considerable variation in $Na^+:Cl^-$ ratios, but all values were above the $1:1$ $Na^+:Cl^-$ line. At $100\,\mathrm{mol\,m^{-3}}$ NaCl, however, the sum of Na^+ and K^+ concentrations significantly ($P \le 0.001$) exceeded the concentrations of Cl^- in the parents and in the mean of RILs (Table 1).

QTL analysis

In Experiment 1, QTL for leaf Na^+ concentration at 41 days (after 14 days at $100\,\mathrm{mol\,m^{-3}}$ NaCl$+10\,\mathrm{mol\,m^{-3}}$ CaCl$_2$) were identified on chromosomes 1, 4 and 12 (Table 3). Similar locations were identified for $K^+:Na^+$ ratio QTL. After reaching a final stress level of $150\,\mathrm{mol\,m^{-3}}$ NaCl$+15\,\mathrm{mol\,m^{-3}}$ CaCl$_2$, a QTL for shoot fresh weight was found on chromosome 12, while QTL for leaf water content were located on chromosomes 8, 9 and 11. In Experiment 2, at the higher stress level of $150\,\mathrm{mol\,m^{-3}}$ NaCl$+15\,\mathrm{mol\,m^{-3}}$ CaCl$_2$, a cluster of QTL for leaf Na^+, $K^+:Na^+$ ratio, plant score, shoot fresh weight and leaf water content was found in the region between markers RG811 and RZ276 on chromosome 1 (Table 3). Other QTL detected in Experiment 2 were for $K^+:Na^+$ ratio on chromosomes 4 and 11 (not shown).

In Experiment 3 after 7 days (Table 4) and 21 days (Table 5) at $100\,\mathrm{mol\,m^{-3}}$ NaCl$+10\,\mathrm{mol\,m^{-3}}$ CaCl$_2$ salt stress, QTL for Na^+ and K^+ concentration and the $K^+:Na^+$ ratio in the sap of leaves of different ages were found on chromosome 1. The majority of these QTL were in the region between the markers RM8095 and RM8115, corresponding to physical map positions of 11.23 to 12.66 Mbp.

After 7 days of stress, all the Na^+ accumulation QTL identified were in the region from 11.56 to 12.66 Mbp. Alleles from Moroberekan increased Na^+ concentration. A pattern of paired and tightly linked QTL peaks with LOD values dipping below 2.5 between the peaks was found in three ages of leaves: newly emerging leaves, fully expanded leaves and lower leaves (Table 4), but no QTL for Na^+ concentration were detected in the sap of the leaf sheath. After 7 days of stress, QTL for K^+

Table 3.   QTL detected in Experiments 1 (all chromosomes) and 2 (only QTL detected on chromosome 1)

Parameter	Chromosome	Peak (cM)	Peak LOD score	Threshold LOD score +1 range (cM)	Threshold LOD score +1	Peak R^2	Markers	Physical location of markers (Mbp)
				Experiment 1				
Na^+ ($\mathrm{mol\,m^{-3}}$ leaf sap) Day 41	1	28.4	7.8	20–40	3.8	0.201	RG811–RZ276	11.07–14.60
	1	33.0	8.7	20–40	3.8	0.173	RG811–RZ276	11.07–14.60
	4	6.0	5.9	1–12	3.8	0.145	RG190–RG449	8.59–17.69
	12	25.0	6.7	21–28	3.0	0.177	RZ397–RG9	5.76–17.98
	12	41.9	4.3	42–46	3.0	0.111	> RG869[A]	>7.73
$K^+:Na^+$ ratio Day 41	1	28.6	4.6	23–30	3.8	0.110	RG140–RG811	5.09–11.07
	1	33.3	5.3	30–35	3.8	0.110	RG811–RZ276	11.07–14.60
	4	5.6	7.5	0–13	3.8	0.155	RG190–RG449	8.59–17.69
	12	26.2	7.2	21–28	3.8	0.198	RZ397–RG9	5.76–17.98
Shoot fresh weight (g) Day 90	12	26.6	3.8	24–27	2.7	0.123	RG341–RG9	5.76–17.98
Leaf water (g g^{-1} DW) Day 90	8	91.0	5.8	84–91	3.9	0.161	RZ649–RG136	>26.98
	9	21.6	4.1	20–24	3.9	0.263	RZ206–RG358[A]	11.81–21.94
	11	49.0	4.5	45–67	3.9	0.137	CDO365–RG16	18.14–19.33
	11	64.1	5.6	45–67	3.9	0.255	RG16–RG1109	18.14–23.12
				Experiment 2				
Na ($\mathrm{mol\,m^{-3}}$ leaf sap)	1	33.0	4.7	28–35	3.8	0.137	RG811–RZ276	11.07–14.60
$K^+:Na^+$ ratio	1	33.9	7.7	26–44	3.8	0.228	RG811–RZ276	11.07–14.60
SRS1	1	34.9	5.2	32–38	4.2	0.200	RG811–RZ276	11.07–14.60
Shoot fresh weight (g) Day 76	1	33.0	4.7	32–34	4.2	0.210	RG811–RZ276	11.07–14.60
Leaf water content (g g^{-1} DW) Day 76	1	32.6	5.7	24–35	3.8	0.245	RG811–RZ276	11.07–14.60

[A]Few markers in this region.

Table 4. QTL for Na$^+$ and K$^+$ concentrations, and the K$^+$: Na$^+$ ratio in sap of different leaf tissues after 1 week exposure to 100 mol m^{-3} NaCl + 5 mol m^{-3} CaCl$_2$ in the Co39 × Moroberekan RIL population by composite interval mapping on chromosome 1 (Experiment 3)

Traits description		Flanking markers	LOD	Peak position (cM)	Range above threshold (cM)	Additive effect of the Moroberekan allele	Variation explained (%)	Physical position of flanking markers (Mbp)
Ion	Leaf age							
Na$^+$	Emerging	RM10776–RM493	5.41	91.2	90.8–94.0	0.880	20.71	12.21–12.26
		RM10793–RM8115	5.64	99.3	96.1–101.5	0.944	21.77	12.55–12.66
	Expanded	RM3412–RM10746	10.58	90.5[A]	86.8–94.0	0.382	34.74	11.56–11.72
		RM10793–RM8115	8.95	99.3	96.1–101.5	0.351	30.24	12.55–12.66
	Old	RM3412–RM10746	13.06	90.5	80.7–94.3	0.274	38.37	11.56–11.72
		RM10793–RM8115	10.22	99.3	95.0–101.5	0.261	31.50	12.55–12.66
K$^+$	Expanded	RM8095–K061	2.67	86.5	86.1–87.9	−30.491	10.17	11.23–11.36
	Old	K061–RM10720	2.73	87.4	86.5–88.6	−21.330	8.95	11.36–11.39
	Sheaths	RM1–RG140	2.82	21.0	19.7–23.9	−0.018	8.06	4.63–5.09
		RM8094–RM8095	2.69	80.0	80.0–80.0	0.021	8.49	11.23–11.23
		RM3412–RM10746	8.21	90.5	84.3–94.0	−0.044	26.51	11.56–11.72
		RM10793–RM8115	6.42	99.3	95.8–101.5	−0.038	21.21	12.55–12.66
K$^+$: Na$^+$	Emerging	RM10793–RM8115	6.06	99.3	96.1–101.5	−0.235	22.94	12.55–12.66
	Expanded	RM10776–RM493	11.52	91.2	81.5–94.3	−2.686	37.50	12.21–12.26
		RM10793–RM8115	10.93	99.3	95.8–101.5	−2.748	36.25	12.55–12.66
	Old	RM3412–RM10746	14.19	90.5	80.7–94.3	−3.111	40.39	11.56–11.72
		RM10793–RM8115	11.08	99.3	96.1–101.5	−2.784	33.32	12.55–12.66
	Sheaths	RMK061–RM10720	3.16	87.4	84.3–87.9	−0.268	12.11	11.36–11.39

[A]Highest of two peaks not resolved at the threshold.

Table 5. QTL for Na$^+$ and K$^+$ accumulation, and the K$^+$: Na$^+$ ratio in sap of different leaf tissues after 3 weeks exposure to 100 mol m^{-3} NaCl + 5 mol m^{-3} CaCl$_2$ in the Co39 × Moroberekan RIL population by composite interval mapping on chromosome 1 (data from Experiment 3)

Traits description		Flanking markers	LOD	Peak position (cM)	Range above threshold (cM)	Additive effect of the Moroberekan allele	Variation explained (%)	Physical position of flanking markers (Mbp)
Ion	Leaf age							
Na$^+$	Expanded	RG811–RM10710	3.38	74.1	73.2–74.7	−0.326	10.89	11.07–11.15
		RM3412–RM10746	9.83	89.5	82.5–94.0	0.374	24.39	11.56–11.72
		RM493–RM10782	5.20	97.0	95.8–97.9	0.363	20.78	12.26–12.38
		RG462–RG101	3.05	147.2	146.1–152.6	0.152	6.23	29.51–30.39
	Sheaths	RM1287–RM10699	2.84	64.6	63.6–66.1	−1.360	12.02	10.83–10.98
		RG811–RM10710	3.86	74.1	73.2–75.0	−1.923	15.17	11.07–11.15
		RM3412–RM10746	8.70	89.5	82.9–94.0	2.028	25.41	11.56–11.72
		RM493–RM10782	5.39	97.0	95.8–97.5	2.076	24.99	12.26–12.38
K$^+$	Expanded	RM10699–RG811	3.06	70.5	68.2–71.1	0.048	9.89	10.98–11.07
	Sheaths	RM6289–RM1344	4.39	44.1	38.2–49.7	−18.504	12.59	6.09–7.01
		RM10776–RM493	4.39	91.2	90.4–93.3	−24.566	12.42	12.21–12.26
		RM10793–RM8115	4.59	99.3	96.5–101.5	−24.845	12.94	12.55–12.66
K$^+$: Na$^+$	Expanded	RM1287–RM10699	4.63	66.6	62.5–69.0	1.669	16.90	10.83–10.98
		RG811–RM10710	5.06	74.1	72.2–79.7	2.075	15.76	11.07–11.15
		RM3412–RM10746	9.85	89.5	83.2–94.0	−2.167	26.84	11.56–11.72
		RM493–RM10782	7.83	97.0	95.4–98.3	−2.287	28.91	12.26–12.38
	Sheaths	RM1287–RM10699	3.98	64.6	62.5–68.2	0.218	15.61	10.38–10.98
		RG811–RM10710	5.39	74.1	72.9–77.5	0.312	19.98	11.07–11.15
		RM3412–RM10746	8.66	90.5	83.6–94.0	−0.338	24.85	11.56–11.72
		RM493–RM10782	6.26	97.0	95.8–97.9	−0.313	26.11	12.26–12.38
Sheath Na$^+$: leaf Na$^+$		RM10720–RM10782	5.99	95.3	95.8– 97.9	−0.313	18.70	11.39–12.39

concentration were detected in the fully expanded leaf, lower leaves and in the leaf sheath (Table 4). Moroberekan alleles reduced K$^+$ concentration for all these QTL except at 80 cM (corresponding to 11.23 Mbp) in sheaths. The two leaf sheath K$^+$ QTL with the highest LOD scores mapped to the same positions as the pairs of Na$^+$ QTL in all leaf ages. The other K$^+$ QTL mapped

to different positions, including regions around 86 cM, and had lower LOD scores. QTL for the $K^+ : Na^+$ ratio were detected in all four tissues after 7 days of stress (Table 4). In newly emerged, fully expanded and lower leaves, these loci were at the same map positions as the Na^+ QTL. In the leaf sheaths, the QTL for the $K^+ : Na^+$ ratio was in approximately the same position as the K^+ concentration QTL in the lower and fully expanded leaves. Moroberekan alleles reduced the $K^+ : Na^+$ ratio in all cases.

QTL for Na^+ concentration, K^+ concentration and the $K^+ : Na^+$ ratio were detected in both tissue types tested after 21 days at full salt stress (Table 5). For Na^+ and $K^+ : Na^+$ ratio, the QTL with the highest LOD scores were at ~90 cM (in the region between 11.56 and 12.66 Mbp, with tightly linked QTL peaks) and they acted in the same direction as QTL for the same traits detected after 7 days. However, for both traits in both tissues, additional smaller QTL were also detected around 64 and 74 cM, and these did not all act in the same direction. It is most noteworthy that at 74.1 cM, Moroberekan alleles decreased Na^+ concentration in both tissue types and increased $K^+ : Na^+$ ratios. Another Na^+ QTL was found at 147.2 cM, but this only accounted for a small part of the observed variation. In contrast to the results after 7 days of stress, four QTL for Na^+ concentration were found in the sap from the leaf sheaths. The strongest of these were in the same positions as the major QTL from the expanded leaves, at ~90 and 97 cM. The other two were at ~65 and 74 cM, and acted in the opposite direction, with the Moroberekan alleles decreasing leaf Na^+ concentrations. For K^+ in the leaf sheaths, a further QTL was detected at 44.1 cM. $K^+ : Na^+$ QTL were located at the same positions (~65, 74, 90 and 97 cM) in both the leaves and the sheaths. At both harvests, K^+ concentration QTL had the greatest additive effects (negative because alleles from Co39 contribute to increased K^+), although this was greatest in the leaves at the first harvest and in the sheaths at the second harvest.

After 21 days at full stress, but not after 7 days, there were differences between the parental lines in the ratio of sheath to leaf blade Na^+ concentrations (7.29 for Co39 and 1.92 for Moroberekan). QTL analysis of the RILs (Table 5) gave a single broad peak with an LOD score of 5.99 in the region between markers RM10720 and RM10782 (corresponding to 11.39 to 12.39 Mbp).

QTL analysis was also performed on data from Experiment 3 using the original RFLP data for the other chromosomes (data not shown). Minor QTL were found at the first harvest on chromosome 3 for emerging leaf and leaf sheath Na^+ concentrations near RZ284, and for K^+ concentrations in the emerging and expanded leaves between RG745 and RG227. $K^+ : Na^+$ ratio QTL with low LOD scores were associated with the distal region of chromosome 3 at both harvests in all but the expanding leaves. On chromosome 4, a minor Na^+ QTL was detected near RG864 in emerging leaves at the first harvest, and $K^+ : Na^+$ QTL were located in the same region in the emerging leaf at the first harvest and in leaf sheaths at both harvests.

Discussion

Comparison of QTL maps

The detection of QTL for components of salt stress (for Na^+, $K^+ : Na^+$ ratio, salt resistance score, shoot fresh weight and

water content) on chromosome 1 in Co 39 × Moroberekan RILs is in agreement with other studies (Singh *et al.* 2007; Table S1). However, studies that harvested a few days after application of salt (a common practice; see Table S2) may not necessarily give a comprehensive indication of the long-term consequences of salt stress. We mapped QTL pertaining to the responses of different tissues to salt stress over a 3-week period, and detected several QTL for Na^+ and K^+ concentration and the ratios between these ions. Our results suggest that the plants' responses to salt stress are dynamic, with different loci acting according to tissue type and duration of stress. Long-term stress may promote different physiological responses from those to short-term stress.

The pattern of QTL on chromosome 1 was different at 7 and 21 days of salt stress. After 7 days of stress, the QTL with the highest LOD scores were in the region 11.36 to 12.66 Mbp (Fig. 1), revealed as paired, tightly linked peaks for Na^+ and $K^+ : Na^+$ QTL. This double peak could be due to two separate genes that act independently to control monovalent cation discrimination (arrows 1 and 2 in Fig. 1). QTL analysis of the backcross lines derived from a cross between IR29 and Pokkali also revealed two peaks, one near RM140 and another near CP03970 (Bonilla *et al.* 2002; Niones 2004). These positions match the two clusters of QTL that we observed, making it unlikely that the gap between the two clusters is an artefact produced by the QTL analysis. In leaf sheaths, there were no QTL for Na^+ concentration or sheath and leaf blade Na^+ ratios after just 7 days; however, after 21 days of stress, four QTL were apparent for this trait. At 21 days the majority of QTL activity was in the region between 11.07 and 11.56 Mbp (arrows 2 and 3 in Fig. 1).

The positions of QTL for K^+ concentration in fully expanded and lower leaves were very close to the QTL for Na^+ concentration, indicating that the same chromosomal region is involved in the control of Na^+ and K^+ accumulation. The positions of the QTL for K^+ concentrations in the leaf sheaths were almost the same as for Na^+ concentrations in newly emerging, fully expanded and lower leaves, except for one QTL that was located at the top of chromosome 1 between 4.63 and 5.09 Mbp. Single genes (such as *Kna1*, *Nax1* and *Nax2*) are capable of reducing Na^+ concentrations and, at the same time, increasing K^+ concentrations.

Eight QTL for the $K^+ : Na^+$ ratio were identified in this study in different parts of the plants over the course of two sampling times after the imposition of salt stress. One QTL for $K^+ : Na^+$ ratio was detected in both the newly emerging leaf and the leaf sheath, whereas two were identified in the fully expanded leaf and the lower leaves under salt stress. They were in the same broad region as was involved in the control of Na^+ and K^+ concentrations.

Many of the QTL that we detected on chromosome 1 are in the same region as the large effect QTL reported previously. The QTL *qSKC1* was reported for shoot K^+ concentration in Nona Bokra × Koshihikari at 11.42 to 11.48 Mbp in hydroponic culture at 140 mol m^{-3} NaCl (Lin *et al.* 2004). *qSaltol* (for salt tolerance score) was mapped in a cross between the Pokkali and IR29 rice cultivars under saline hydroponics (12 dS m^{-1} for 16 days) and a QTL for low shoot Na^+ concentration was co-located with it (Bonilla *et al.* 2002). The *qSaltol* QTL covers a
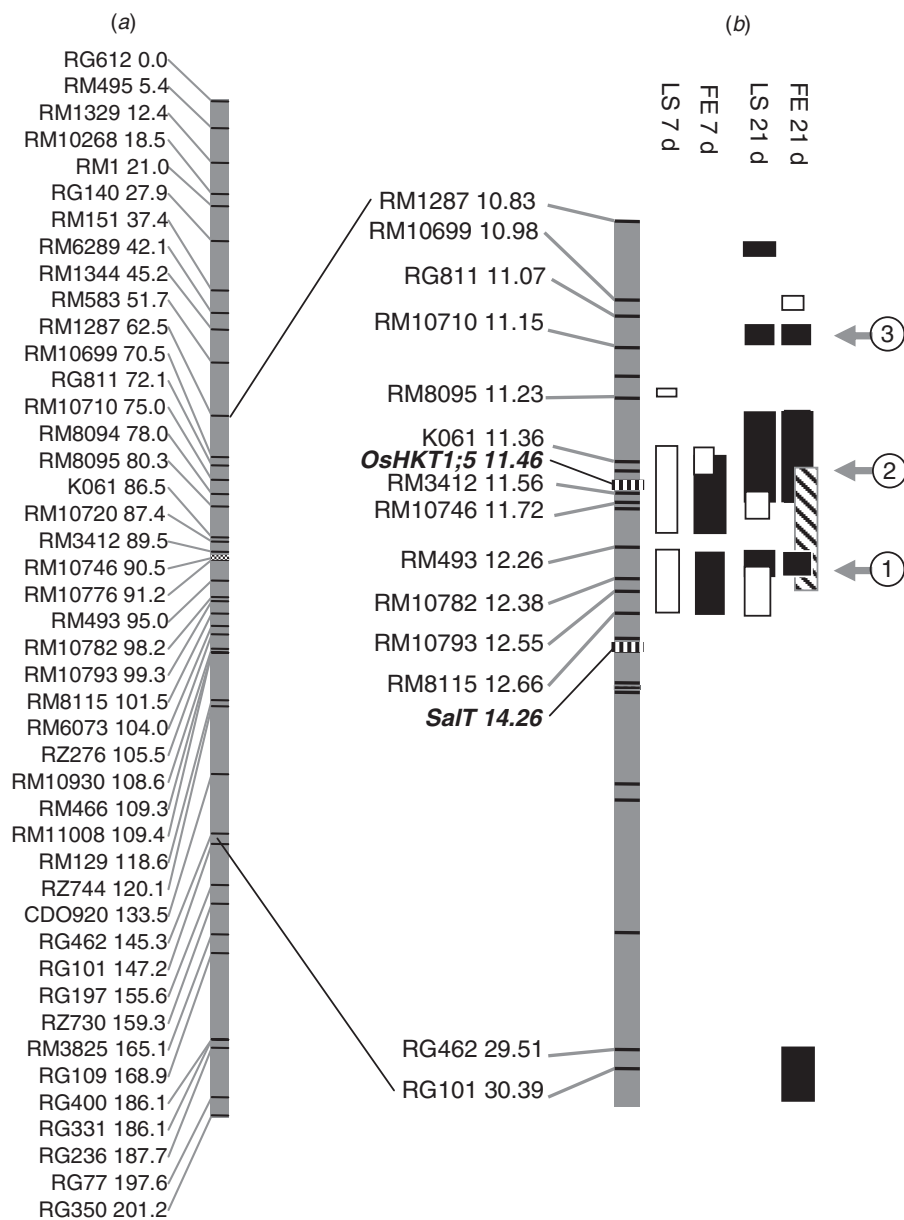
Fig. 1. (*a*) Chromosome 1 linkage map of Co39 × Moroberekan RILs with SSR and RFLP markers in cM distances. (*b*) Detail of the region of chromosome 1 corresponding to 10–30 Mbp where QTL for ion accumulation were detected under salt stress. QTL are shown for Na$^+$ accumulation (black) after 7 days and 21 days, and K$^+$ accumulation (white) after 7 and 21 days in fully expanded leaf tissue (FE) and leaf sheaths (LS) after exposure to 100 mol m^{-3} NaCl and 5 mol m^{-3} CaCl. A QTL for the ratio of sheath Na$^+$ to leaf Na$^+$ is shown (hashed). Numbered arrows indicate three QTL regions discussed in the text. The positions of *OSHKT1;5* and *SalT* are also shown.

much larger region (11.46–14.26 Mbp) and we resolved at least three separate QTL in the region; therefore further candidates may be responsible for *qSaltol*. Koyama *et al*. (2001) mapped three overlapping whole shoot QTL for sodium uptake to a large region of chromosome 1 in IR55178 using amplified fragment length polymorphisms (AFLPs). It is difficult to exactly compare the positions of these reported QTL with other maps because different markers types and plant materials were used.

Considering the synteny between wheat (*Triticum aestivum* L.) and rice chromosomes (Sorrells *et al*. 2003), there are two other places where one might look for genes controlling univalent cation accumulation. The first is the region of rice chromosome 3 that corresponds to the long arm of chromosome 4D of wheat where *Kna1* is located (Gorham *et al*. 1997), and the other is the long arm of rice chromosome 4 that corresponds to the *Nax1* region of wheat chromosome

2A^{m}L (Huang *et al.* 2006, 2008; Byrt *et al.* 2007). Although minor QTL were found on chromosomes 3 (Experiment 3) and 4 (Experiments 1 and 3) for Na$^+$ and K$^+$ accumulation, they were inconsistent between experiments, tissues and times, and at different locations on the chromosomes. This does not exclude the possibility that these loci may show variation in other cultivars, but for Co39 and Moroberekan, the major QTL were located on chromosome 1.

Physiology of salt tolerance in rice

The experiments reported here were designed specifically to examine ion accumulation, rather than salt resistance *per se*. In our experiments, the deleterious effects of salinity on overall plant appearance and growth seemed to be greater in Moroberekan than in Co39. Analysis of biomass data in saline conditions is appropriate in an agricultural context, but scientific interpretation is difficult because of the combined effects of vigour and salt resistance. Under non-saline conditions, Moroberekan had more vigorous vegetative growth than Co39 (data not shown), but under salinity, it had a greater reduction in shoot biomass.

Experiments using the apoplastic tracer dye 8-hydroxy-l,3,6-pyrenetrisulfonic acid (PTS) as an indicator of bypass flow (using the method of Yeo *et al.* 1987) showed that bypass flow could not account for the observed differences in Na$^+$ concentrations between Co39 and Moroberekan (Akhtar 2002).

While high tissue salt concentrations can lead to cellular dehydration, particularly if the salt is concentrated in the apoplast (Oertli 1968), it is probable that part of the difference between the parental lines in measured Na$^+$ and Cl$^-$ ion concentrations was caused by greater leaf dehydration in Moroberekan. Dehydration was earlier shown to be almost entirely responsible for an apparent increase in leaf K$^+$ concentrations (Akhtar 2002).

Osmotic adjustment, in the narrow sense of an increase in solute concentration on a dry weight basis, was achieved mainly through accumulation of Na$^+$ and Cl$^-$. Unsuccessful osmotic adjustment, in the sense that too many salt ions were accumulated, was shown here by the damage observed in the youngest emerging leaf laminae and by experimental evidence supporting the Oertli hypothesis in rice (Flowers *et al.* 1991; Akhtar 2002). We were not able to measure the water contents in the small samples of emerging leaf lamina and cannot, therefore, estimate to what extent tissue dehydration contributed to the apparent increase in Na$^+$ concentrations in the emerging leaves. Our experience in other (more salt-resistant) cereals has been that young tissues are protected from the accumulation of high concentrations of salts.

We found that Na$^+$ concentrations in the sap of leaf sheaths under both levels of salt stress were significantly higher than in emerging, fully expanded and mature leaves. Our results agree with Yeo and Flowers (1982), Matsushita and Matoh (1991) and Mitsuya *et al.* (2002) for rice; Davenport *et al.* (2005), James *et al.* (2006) and Huang *et al.* (2006) for wheat; Netondo *et al.* (2004) for *Sorghum* spp. and Colmer *et al.* (1995) for a wheat × *Thinopyrum* sp. amphiploid. Villalta *et al.* (2008) reported higher Na$^+$ in the stems than the leaves of tomato (*Lycopersicon esculentum* Mill.) under salinity. Villalta *et al.*

(2008) also suggested the presence of a mechanism at the leaf shoot interface to restrict the entry of Na$^+$ to the leaf and to help its re-translocation from leaves to stem. Jeschke (1984) concluded that protecting young leaves by maintaining low salt concentration was one of the most important salt tolerance mechanisms in plants.

It should be noted that the treatment conditions were somewhat different between our experiments and some of those reported by others (Table S2). The importance of standard protocols for phenotyping rice plants subjected to saline conditions was discussed by Singh *et al.* (2007). This is particularly important in terms of the ameliorating effects of both supplementary silicate and Ca^{2+} in hydroponic experiments. In soils, there are thought to be sufficient concentrations of these ions to prevent the massive influx of salts that are seen in their absence in hydroponics, particularly at high external salt concentrations (Grattan and Grieve 1999). Another factor that can affect the physiological responses to salt is the separation of the toxic effects of high salt accumulation from the purely osmotic effect of suddenly exposing plant roots to high concentrations of salt. Unfortunately such osmotic shocks are all too often a feature of experiments on responses of plants to salinity (Flowers 2004) unless stress is imposed gradually (Flowers *et al.* 2000; Koyama *et al.* 2001). Both of these physiological 'mistakes' feature in many of the reported QTL studies of salt responses in rice (Table S2).

Chloride is not always measured in salinity experiments, but it is the single most abundant ion. We did not detect QTL for Cl$^-$ concentration in leaf sap in the Moroberekan × Co39 population (this trait was tested in Experiment 1 only), but regulation of Cl$^-$ channels in rice has been reported by Diedhiou and Golldack (2006). It may be that there is sufficient variation in Cl$^-$ concentrations in different rice varieties to develop mapping populations to screen specifically for this trait, as suggested by the finding of several Cl$^-$ QTL in a population derived from the *O. sativa indica* varieties CSR27 and MI-48 (Ammar 2004, as described in Singh *et al.* 2007). This is important because it is not certain whether the chloride or sodium ion is most toxic.

Candidate genes for response to salinity

SalT is an EST linked to RZ276 (Causse *et al.* 1994), the expression levels of which match Na$^+$ concentration (Claes *et al.* 1990). The *SalT* protein contains a lectin-like domain, but its physiological function is unknown. Salt-induced expression of *SalT* has been characterised by Sahi *et al.* (2006) in a subtractive library between two salt-tolerant cultivars, CSR 27 and Pokkali. A gene corresponding to the *SalT* EST was upregulated in IR29 under stress (Walia *et al.* 2005). Dehydration, osmotic stress and ABA treatment also induced expression of *SalT*, which accumulates initially in the leaf sheaths in older plants, but in the leaves of seedlings (Garcia *et al.* 1997*b*, 1998). We did not detect any QTL for salt stress components that overlap *SalT* (Fig. 1), suggesting that *SalT* does not explain differences in Na$^+$ concentrations between Co39 and Moroberekan.

Saltol was identified as a QTL in Pokkali × IR29 RILs (see the review by Singh *et al.* 2007). Walia *et al.* (2005) carried out

global gene expression analysis under saline conditions (whole shoots were sampled after 8 days at a moderate stress level of 7.4 dS m^{-1}) in IR29 (susceptible) and FL478 (resistant) cultivars and reported expression of a candidate gene (*Os01g21060*) for *qSaltol* at 11.46 Mbp. Within the *qSaltol* region, a gene for the QTL q*SKC1* in the Nona Bokra × Koshihikari rice mapping population (Lin *et al.* 2004) was identified as *OsHKT8* by Ren *et al.* (2005), now renamed *OsHKT1;5* (Platten *et al.* 2006). It codes for a transporter that unloads Na$^+$ from the xylem (Ren *et al.* 2005; Huang *et al.* 2008). According to Byrt *et al.* (2007), it exhibits several physiological similarities to the non-syntenic wheat *HKT1;5* genes *Nax2* and *Kna1*, including the absence of influence on sheath to blade Na$^+$ concentration ratios. The location of *OsHKT1;5*, at 11.46 Mbp on chromosome 1 (Fig. 1) does, however, coincide with the QTL region reported here (Fig. 1; black and white bars). We observed a QTL in the same region that appears to control sheath to leaf blade Na$^+$ ratios (Fig. 1; hatched bar). Either the characterisation of *HKT1;5* genes as not affecting sheath to blade Na$^+$ concentrations does not apply in our population or else the QTL effect is determined by a different gene. Neither Ren *et al.* (2005) nor Gorham *et al.* (1990) actually measured sheath Na$^+$ concentrations in their papers on *SKC1* and *Kna1* respectively.

Nax1 has 80% sequence homology with *OsHKT1;4* (*OsHKT7*) on rice chromosome 4, and would be a candidate gene for the QTL that we found on this chromosome. The physical map location of *OsHKT1;4* does not, however, correspond with the QTL position (based on the limited number of RFLP markers that can be placed on the physical map of chromosome 4).

Conclusions

We detected numerous QTL for salt stress responses in the 1.83 Mbp region that has previously been shown in other populations to contain the QTL *qSKC1* (Lin *et al.* 2004) and *qSaltol* (Bonilla *et al.* 2002). Our results support the hypothesis that several different genes are likely to be contained within the *qSaltol* region. It has been suggested that more than 5% of the rice genome codes for membrane transport proteins, and that many of these have not been characterised (Amrutha *et al.* 2007). More than 900 genes have been annotated in the region containing *qSaltol* (Kumari *et al.* 2009) and the expression of many rice genes is influenced by salt stress in different rice genotypes (Walia *et al.* 2005; Sahi *et al.* 2006; Walia *et al.* 2007). There are also many other sodium and potassium transporters that may be involved in controlling leaf Na$^+$ concentrations (e.g. Munns and Tester 2008). Further fine mapping in near isogenic lines is required to dissect the QTL fully. Expression studies that test long-duration stress treatments with sampling of specific leaf types are now required to identify which genes contribute to the rice plant's response to salt, and which of them can be combined in breeding of varieties better adapted to saline and sodic soils.

Acknowledgements

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