Introduction

Salinity is a major abiotic stress for rice (*Oryza sativa* L.), often caused by excessive accumulation of Na⁺ in cells. Although Na⁺ is a beneficial element for some plants, such as C₄ species, high levels of cytosolic Na⁺ are toxic to rice; excess Na⁺ can destabilize membranes and proteins and negatively affect many cellular and physiological processes (Horie *et al.* 2012, Munns and Tester 2008, Negrão *et al.* 2011). Intracellular K⁺ deficiency and K⁺/Na⁺ imbalance, caused by Na⁺ over-accumulation, are strongly associated with salt sensitivity in plants (Horie *et al.* 2012, Munns and Tester 2008, Negrão *et al.* 2011). Control of Na⁺ accumulation and maintenance of a high K⁺/Na⁺ ratio in plants, especially in shoots, may be essential for improving plant salt tolerance (Hauser and Horie 2008, Horie *et al.* 2012, Ren *et al.* 2005).

In attempts to understand the genetic factors controlling Na⁺ and K⁺ concentrations, and the Na⁺/K⁺ ratio, quantitative trait loci (QTLs) associated with these parameters under salt stress have been identified using mapping populations in rice. As a result, more than 100 QTLs, distributed widely along all 12 rice chromosomes, have been identified. Among them, a few of the QTLs were mapped to the short arm of chromosome 1.

Using a population of F₈ recombinant inbred lines (RILs), derived from a cross between ‘Pokkali’ and ‘IR29’, Gregorio (1997) identified a major QTL (*saltol*) for K⁺ and Na⁺ absorption and the Na⁺/K⁺ absorption ratio, flanked by the AFLP markers P3/M9-8 and P1/M9-3 on chromosome 1, and this QTL explained 64.3–80.2% of the total phenotypic variation. Subsequently, Bonilla *et al.* (2002) re-mapped this QTL to a chromosomal region between the simple sequence repeat (SSR) markers RM23 and RM140, by integrating fragment length polymorphism (RFLP) and simple sequence length polymorphism markers onto the linkage map. Furthermore, Niones (2004) and Thomson *et al.* (2010) re-identified and fine mapped the *saltol* locus using the RILs, backcross lines, and near-isogenic lines (NILs) developed from a cross between ‘Pokkali’ and ‘IR29’. This QTL has now been confirmed to be located between the SSR markers RM578 and IM8854. These results will facilitate map-based cloning of the *saltol* locus.

Key Words: rice, salt tolerance, shoot K⁺ concentration, quantitative trait locus, fine mapping.
chromosome 1 in a region flanked by the RFLP markers RG811 and RZ276, using F8 and F9 RILs from a cross between the rice varieties ‘Co39’ and ‘Moroberekan’.

Beyond these major QTLs [saltol, SKCl], and those detected by Haq et al. (2010), there are several QTLs with lesser effects on shoot Na⁺ concentration (SNC), SKC, and the shoot Na⁺/K⁺ ratio (SNKR), which have been mapped to the short arm of chromosome 1 in rice, most in the same general region as saltol and SKCl (Koyama et al. 2001, Pandit et al. 2010, Zheng et al. 2015). Although it seems likely that these reported major and minor QTLs for the Na⁺ and K⁺ concentrations, and Na⁺/K⁺ ratio are located in adjacent or even the same chromosomal regions, it remains difficult to determine whether they are within the same locus, because most have only been mapped by a preliminary QTL analysis. Further fine mapping is required to confirm the (allelic) relationships among these loci.

Previously, we repeatedly mapped QTLs for SKC and SNC to the short arm of chromosome 1, using three F2 mapping populations constructed by crossing two salt-sensitive mutants (rss2 and rss4) and their wild-type cultivar ‘Nipponbare’ with an indica cultivar ‘Zhaiyeqing8’ (‘ZYQ8’; Deng et al. 2015b, Zhou et al. 2013). The QTLs, designated qSKC-1 and qSNC-1, are located between SSR markers RM283 and RM312, and explained 8.0–40.6% and 11.5–28.3% of the total phenotypic variation, respectively. For the two loci, the allele derived from ‘ZYQ8’ increased SKC but decreased SNC in each mapping population. Based on those results, we performed fine mapping of qSKC-1 using extreme individuals from the ‘Nipponbare’ × ‘ZYQ8’ and rss4 × ‘ZYQ8’ F2 populations in this study.

Materials and Methods

Plant materials

Two F2 populations were used. One (n = 506) was derived from the cross between ‘Nipponbare’ (Oryza sativa L. ssp. japonica) and ‘ZYQ8’ (Oryza sativa L. ssp. indica), from which 204 randomly chosen individuals were used for primary mapping. The other population (n = 890) was generated previously by crossing rss4 with ‘ZYQ8’ (Deng et al. 2015b). rss4 is a salt-sensitive mutant derived from ethyl methanesulfonate-treated ‘Nipponbare’, the decreased salt tolerance of which was conferred by a locus on chromosome 6 but not by qSKC-1 or qSNC-1 (Deng et al. 2015b).

Seeding cultivation and salt treatment

The methods of seedling cultivation and salt treatment were described previously (Deng et al. 2015a, 2015b, Zhou et al. 2013). Briefly, uniformly germinated seeds were sown into 96-well plates with removed well bottoms, which had been floating in a black container filled with distilled water for 5 days and were then transferred into Yoshida’s solution (Yoshida et al. 1976). Seedlings were cultured in an artificial growth chamber under 28/24°C day/night temperatures, 70% relative humidity, and a 14-h photoperiod with 800 μmol m⁻² s⁻¹ photosynthetically active radiation.

To analyze the salt tolerance of ‘Nipponbare’ and ‘ZYQ8’, 14-day-old seedlings were exposed to Yoshida’s solution containing 120 mM NaCl. After 8 days of treatment, six seedlings each were sampled to determine the ion concentrations, and approximately 48 seedlings each were transferred to Yoshida’s solution to recover for 10 days, after which the survival rates were determined. Five replicates were performed for each analysis.

To evaluate the salt tolerance of the F2 population derived from ‘Nipponbare’ × ‘ZYQ8’, 12–16 14-day-old seedlings from each F2:3 line and their parents were exposed to Yoshida’s solution containing 120 mM NaCl for 8 days, and then the shoots of each line were sampled and mixed to determine ion concentrations.

Progeny tests of the recombinants were performed using 20–24 F2:3 individuals from each F2 plant. After 8 days of exposure to 120 mM NaCl, the shoots of each F2:3 seedling were sampled separately for ion concentration analysis.

Determination of K⁺ and Na⁺ concentrations

The K⁺ and Na⁺ concentrations were measured as described previously (Deng et al. 2015a, 2015b, Zhou et al. 2013). Briefly, the samples were oven-dried for 3 days at 80°C and then extracted in 100 mM nitric acid for 2 h at 90°C after weighing. The K⁺ and Na⁺ concentrations of the extracts were quantified using inductively coupled plasma-optical emission spectrometry (ICP-OES; Perkin-Elmer, Norwalk, CT, USA).

Linkage map construction, QTL analysis

In total, 204 randomly chosen individuals from the ‘Nipponbare’ × ‘ZYQ8’ F2 population were genotyped using 14 SSR and three insertion/deletion (InDel) markers, and a linkage map of chromosome 1 was constructed from the genotype data using MAPMAKER/EXP 3.0 (Lander et al. 1987). The previously reported 184 individuals of the rss4 × ‘ZYQ8’ F2 population were genotyped using three InDel markers (IM7685, IM9146, and IM7719), and a linkage map was constructed using the 14 existing SSR markers supplemented with the three InDel markers.

SSR markers were obtained from Gramene (http://www.gramene.org/), and the InDel markers were developed based on genomic sequences by BLAST searching the sequences of ‘Nipponbare’ and ‘93-11’ (http://www.ncbi.nlm.nih.gov/). The procedures for SSR and InDel analysis, including DNA template preparation, polymerase chain reaction (PCR) amplification, and electrophoresis and silver staining of the PCR products, were described previously (Jing et al. 2007).

Composite interval mapping (CIM) using Windows QTL Cartographer 2.5 was used for QTL analysis. The significant logarithm of the odds (LOD) score for detection of QTLs was calculated based on 1,000 runs of randomly shuffling the trait values at an experiment-wise significance level of 0.05 (Churchill and Doerge 1994). In this study, the threshold values of LOD for SKC and SNC were 2.63 and 2.78,
Comparison of salt tolerance in ‘Nipponbare’ and ‘ZYQ8’

At the seedling stage, ‘ZYQ8’ showed significantly higher salt tolerance than did ‘Nipponbare’. After treatment with 120 mM NaCl for 8 days, most ‘Nipponbare’ plants were severely wilted and could not recover after salt removal, whereas ‘ZYQ8’ showed only slightly wilting, with >70% of the plants surviving (Fig. 1A, 1B). There was no significant difference in the Na\(^+\) or K\(^+\) concentration between shoots of ‘Nipponbare’ and ‘ZYQ8’ before salt treatment (Fig. 2A, 2B). By contrast, ‘ZYQ8’ accumulated significantly less Na\(^+\) and more K\(^+\) in shoots than ‘Nipponbare’ under salt stress (Fig. 2A, 2B). After exposure to NaCl stress for 8 days, the concentrations of Na\(^+\) and K\(^+\) in ‘ZYQ8’ were 0.74 and 0.89 μmol mg\(^{-1}\) DW (dry weight), respectively, whereas those in ‘Nipponbare’ were 1.34 and 0.73 μmol mg\(^{-1}\) DW, respectively (Fig. 2A, 2B).

Identification of the QTL responsible for salt tolerance on chromosome 1

In total, 204 randomly chosen individuals from the ‘Nipponbare’ × ‘ZYQ8’ F\(_2\) population were evaluated for salt tolerance and genotyped with 17 markers covering the whole of rice chromosome 1 for the QTL analysis. Both SKC and SNC in the F\(_2\) population showed continuous distributions after salt stress for 8 days (Fig. 3A, 3B). A major QTL involved in SKC was detected in the interval between the InDel markers IM7685 and IM9146, with IM7719 as the closest marker to the peak (Fig. 4A, Table 1). This QTL explained 23.2% of the phenotypic variance, and the additive effect of the ‘ZYQ8’-derived allele increased the SKC by 0.06. One QTL responsible for SNC was mapped to the region flanked by RM283 and IM9146, which explained 19.0% of the phenotypic variance and had an additive effect of 0.21 in terms of reducing the SNC associated with the...
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ZYQ8'-derived allele (Fig. 4A, Table 1). Both of the
QTLs were located between RM283 and RM580, where
qSKC-1 and qSNC-1 had been mapped in the F2
population of rss4 × ‘ZYQ8’ (Deng et al. 2015b).

To confirm the location of qSKC-1 and qSNC-1,
three additional InDel markers, IM7685, IM7719, and IM9146,
were used to genotype the 184 previously reported individ-
uals from the rss4 × ‘ZYQ8’ F2 population (Deng et al. 2015b).
As a result, qSKC-1 and qSNC-1 were detected
between IM7685 and IM9146, and between RM283 and
IM9146, respectively, consistent with the results of QTL
mapping in the ‘Nipponbare’ × ‘ZYQ8’ F2 population
(Fig. 4B, Table 1).

To assess the genetic effects of qSKC-1 and qSNC-1,
ANOVA was performed using the genotypes of the InDel
marker IM7719, the closest marker to both the qSKC-1 and
qSNC-1 loci in the ‘Nipponbare’ × ‘ZYQ8’ and rss4 × ‘ZYQ8’ F2 populations. In both populations, plants homo-
yzous for the ‘Nipponbare’ or rss4 genotype had a sig-
ificantly lower SKC than did those homozygous for the

Table 1. Quantitative trait loci (QTLs) for shoot K+ concentration (SKC) and shoot Na+ concentration (SNC) identified in two F2 populations by composite interval mapping on chromosome 1

| F2 population | Trait  | QTL     | Flanking markers | LOD score (%) | PVE | Additive effect |
|---------------|-------|---------|------------------|---------------|-----|----------------|
| ‘Nipp’ × ‘ZYQ8’ | SKC   | qSKC-1  | IM7685–IM9146    | 8.2           | 23.2| –0.06          |
|               | SNC   | qSNC-1  | RM283–IM9146     | 3.9           | 19.0| 0.21           |
| rss4 × ‘ZYQ8’ | SKC   | qSKC-1  | IM7685–IM9146    | 7.9           | 30.7| –0.06          |
|               | SNC   | qSNC-1  | RM283–IM9146     | 5.4           | 19.2| 0.17           |

‘Nipp’, ‘Nipponbare’; ‘ZYQ8’, ‘Zhaiyeqing 8’; LOD, logarithm of the odds; PVE, percentage of phenotypic variance explained.

a Positive values indicate that the allele increased the SKC or SNC score was derived from ‘Nipponbare’ or rss4, and negative one from ‘Zhaiyeqing 8’.

Fig. 4. Chromosomal positions of identified QTLs responsible for shoot K+ concentration (SKC) and shoot Na+ concentration (SNC) in two F2 populations. (A) Logarithm of the odds (LOD) score curves for the QTLs responsible for SKC and SNC in the ‘Nipponbare’ (‘Nipp’) × ‘Zhaiyeqing 8’ (‘ZYQ8’) F2 population. (B) LOD score curves for the QTL for SKC and SNC in the rss4 × ‘ZYQ8’ F2 population. The continuous and dashed black vertical lines indicate the LOD thresholds for the presence of a quantitative trait locus responsible for SNC and SKC, respectively.

Fig. 3. Frequency distribution of K+ and Na+ concentrations in shoots of the ‘Nipponbare’ (‘Nipp’) × ‘Zhaiyeqing 8’ (‘ZYQ8’) F2 population (n = 204) under 120 mM NaCl treatment for 8 days. (A) Shoot K+ concentration (SKC). (B) Shoot Na+ concentration (SNC).
‘ZYQ8’ genotype (Table 2). The heterozygous plants had SKC levels similar to those of the plants homozygous for the ‘ZYQ8’ genotype in the rss4 × ‘ZYQ8’ F2 populations; whereas they had significantly higher and lower SKC than plants homozygous for the ‘Nipponbare’ and ‘ZYQ8’ genotypes, respectively, in the ‘Nipponbare’ × ‘ZYQ8’ F2 population (Table 2). The genetic effects of qSNC-1 in the two F2 populations were similar to that of qSKC-1 in the rss4 × ‘ZYQ8’ F2 population, but not in the ‘Nipponbare’ × ‘ZYQ8’ F2 population. In both populations, plants homozygous for the ‘ZYQ8’ genotype had SNC levels similar to those of the heterozygous plants, and both of them had a significantly lower SNC than the plants homozygous for the ‘Nipponbare’ or rss4 genotype (Table 2).

**Fine mapping of qSKC-1**

Given that qSKC-1 was the only and main-effect QTL for SKC detected in three F2 populations ('Nipponbare' × ‘ZYQ8’, rss2 × ‘ZYQ8’ and rss4 × ‘ZYQ8’), qSKC-1, but not qSNC-1, was selected for fine mapping using the extreme sampling strategy. In total, 100 individuals with extremely low or high levels of SKC from the ‘Nipponbare’ × ‘ZYQ8’ F2 population, containing 506 individuals (including the previous 204 individuals), were selected for recombinant screening using IM7685 and IM9146. Ten and four recombinants were identified between IM7685 and qSKC-1, and between IM9146 and qSKC-1, respectively (Fig. 5, Table 3). Of these 14 recombinants, 9 and 5 had extremely low and extremely high SKC levels, respectively (Fig. 5, Table 3). To confirm the phenotypes of the 14 recombinants, progeny tests were performed on 20–24 F2,3 individuals from each recombinant F2 plant. All F2,3 individuals of the nine recombinants (group N1–N4) with high-extreme phenotypic values of SKC had fixed, high levels of SKC (Table 3). In addition, progenies from group N1–N4 generally had significantly higher levels of SNC and leaf damage than progenies from group N5–N8, although the progenies from some F2 recombinants (group N1, N4, N5 and N8) showed continuous distributions across a wide range of phenotypes in terms of SNC (Supplemental Table 1).

To further delimit the region encompassing the qSKC-1 locus, the InDel marker IM7719 and six additional SSR or InDel markers developed within the region between IM7685 and IM9146 were used to genotype the 14 recombinants (Supplemental Table 2). These analyses identified one qSKC-1 co-segregating marker (IM8748), along with single recombinant events between RM578 and qSKC-1 and between IM8854 and qSKC-1 (Fig. 5, Table 3). The recombinant events increased gradually at markers flanking RM578 and IM8854 (Fig. 5, Table 3). From these data, the qSKC-1 locus was determined to be within a 445-kb region between the markers RM578 and IM8854 (Fig. 5, Table 3).

Fine mapping of the qSKC-1 locus was also performed using the previously constructed rss4 × ‘ZYQ8’ F2 population (Deng et al. 2015b). In total, 120 individuals with extremely low or high levels of SKC from this F2 population of 890 individuals were selected for genotyping using IM7685 and IM9146. Ten recombinant events between

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**Table 2.** Shoot K+ concentration (SKC) and shoot Na+ concentration (SNC) in each group classified by genotypes of the nearest marker to qSKC-1 and qSNC-1 (IM7719) in two F2 populations

| F2 population | Trait | Mean (μmol mg−1 DW) |
|---------------|-------|---------------------|
|               |       | 1/1 | 1/2 | 2/2 |
| ‘Nipp’ × ‘ZYQ8’ | SKC   | 0.95e | 1.00b | 1.06a |
|               | SNC   | 1.38a | 1.24b | 1.17b |
| rss4 × ‘ZYQ8’  | SKC   | 0.69b | 0.75a | 0.78a |
|               | SNC   | 1.48a | 1.35b | 1.25b |

DW: dry weight; ‘Nipp’, ‘Nipponbare’; ‘ZYQ8’, ‘Zhaiyeqing 8’.

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**Fig. 5.** Physical map of the qSKC-1 locus. Numbers below the linkage map indicate the number of recombination events detected between the qSKC-1 locus and the respective markers in the ‘Nipponbare’ (‘Nipp’) × ‘Zhaiyeqing 8’ (‘ZYQ8’) and rss4 × ‘ZYQ8’ F2 populations. Arrows indicate the region encompassing qSKC-1.
Table 3. Genotypes of F$_2$ recombinants in two F$_2$ populations and the shoot K$^+$ concentration (SKC) of their F$_{2:3}$ progenies

| F$_2$ population | Group$^a$ | Markers and genotype$^b$ | SKC (μmol mg$^{-1}$ DW)$^c$ | Frequency distribution of SKC in F$_{2:3}$ plants (μmol mg$^{-1}$ DW)$^d$ | No.$^e$ |
|-----------------|-----------|--------------------------|-----------------------------|---------------------------------|--------|
|                 |           | IM7685 IM7719 IM7852 IM8284 RM578 IM8748 IM8854 IM8916 IM9146 | ≤0.60 0.61–0.70 0.71–0.80 0.81–0.90 0.91–1.00 1.01–1.10 ≥1.11 | | |
| ‘Nipp’ × ‘ZYQ8’ | N1        | H H H H N N N N N N | 0.71 | 2 26 53 10 | 4 |
|                 | N2        | H H H H H N N N N N | 0.72 | 1 21 19 5 | 2 |
|                 | N3        | H H H H H N N N N N | 0.74 | 7 13 2 | 1 |
|                 | N4        | N N N N N N N N H H | 0.73 | 19 24 | 4 |
|                 | N5        | H H H Z Z Z Z Z Z Z | 1.01 | 18 22 3 | 2 |
|                 | N6        | H H H H Z Z Z Z Z Z | 1.01 | 9 13 | 1 |
|                 | N7        | Z Z Z Z Z Z H H H | 0.99 | 1 8 10 1 | 1 |
|                 | N8        | Z Z Z Z Z Z Z Z Z | 1.02 | 13 11 | 1 |
|                 | ‘Nipp’ N1 | N N N N N N N N N | 0.78 | 2 7 5 | 1 |
|                 | ‘ZYQ8’ N2 | Z Z Z Z Z Z Z Z Z | 0.97 | 7 6 | 1 |
|                 | ‘Nipp’ R1 | H H H H R R R R R R | 0.71 | 4 21 17 2 | 2 |
|                 | ‘ZYQ8’ R2 | H H H H R R R R R R | 0.69 | 1 40 26 1 | 3 |
|                 | ‘Nipp’ R3 | H H H H H H R R R R | 0.73 | 1 13 26 2 | 2 |
|                 | ‘ZYQ8’ R4 | R R R R R R H H H | 0.70 | 12 9 | 1 |
|                 | ‘Nipp’ R5 | R R R R R R H H H | 0.68 | 1 8 10 1 | 1 |
|                 | ‘ZYQ8’ R6 | R R R R R R H H H | 0.70 | 12 10 | 1 |
|                 | ‘Nipp’ R7 | H H H H H H H H H | 0.99 | 2 22 20 | 2 |
|                 | ‘ZYQ8’ R8 | Z Z Z Z Z Z H H H | 1.02 | 7 12 1 | 1 |
|                 | ‘Nipp’ R9 | Z Z Z Z Z Z Z Z Z | 1.02 | 1 27 34 5 | 3 |
|                 | ‘ZYQ8’ R10| R R R R R R R R R R | 0.74 | 1 4 8 1 | 1 |

‘Nipp’, ‘Nipponbare’; ‘ZYQ8’, ‘Zhaiyeqing 8’.

$^a$ Group indicates genotype category.
$^b$ The genotype of the markers are represented by N (‘Nipponbare’ homozygous), Z (‘ZYQ8’ homozygous), H (heterozygous), and R (rss4), respectively.
$^c$ The value was calculated from the F$_{2:3}$ progenies of all F$_2$ recombinants with the same genotype category.
$^d$ The number in each range was calculated from F$_{2:3}$ progenies of all F$_2$ recombinants with the same genotype category.
$^e$ No. represents the number of F$_2$ recombinants.
IM7685 and qSKC-1 and six between IM9146 and qSKC-1 were identified (Fig. 5, Table 3). Of these 16 recombinants, nine (group R1–R5) and seven (group R6–R9) had extremely low and extremely high phenotypic values of SKC, respectively, which were confirmed in progeny tests using F2:3 individuals (Table 3). In addition, the progenies from most F2 recombinants with low-extreme phenotypic values of SKC except group R1, had generally low levels of SNC and leaf damage, and those with high-extreme phenotypic values of SKC except group R6, had generally high levels of SNC and leaf damage (Supplemental Table 1).

These 16 recombinants were used for genotyping employing the seven markers located between IM7685 and IM9146. Consequently, the qSKC-1 locus was delimited to the interval between RM578 and IM8854, with two recombinant events between RM578 and qSKC-1 and between IM8854 and qSKC-1, consistent with the results of the fine mapping using the ‘Nipponbare’ × ‘ZYQ8’ F2 population (Fig. 5, Table 3).

**Discussion**

Rice is a salt-sensitive crop, and its low salt tolerance is associated with excessive Na+ accumulation and the resulting K+ deficiency and K+/Na+ imbalance in plants (Horie et al. 2012, Munns and Tester 2008, Negrão et al. 2011). Recent genetic and molecular analyses of Na+ and K+ concentrations and the Na+/K+ ratio in rice under salt stress suggest that they are generally governed by polygenes. More than 100 QTLs for these physiological parameters of salt tolerance have been detected in rice, but only a few have been cloned or fine-mapped. In the present study, one major QTL (qSKC-1) for SKC was confirmed and delimited to a 445-kb region flanked by the markers RM578 and IM8854.

In previous studies, the QTLs for SKC and SNC (qSKC-1 and qSNC-1) were detected in the interval between SSR markers RM283 and RM312 on chromosome 1, using the F2 populations derived from crosses of rss2 × ‘ZYQ8’, ‘Nipponbare’ × ‘ZYQ8’, and rss4 × ‘ZYQ8’ (Deng et al. 2015b, Zhou et al. 2013). In the present study, based on the linkage maps constructed with 17 SSR and InDel markers using the reconstructed ‘Nipponbare’ × ‘ZYQ8’ F2 populations and the previously reported rss4 × ‘ZYQ8’ F2 population, the QTLs for SKC and SNC were mapped to the interval between IM7685 and IM9146, and between RM283 and IM9146, respectively. Further association analysis indicated that the marker IM7719 was significantly associated with SKC and SNC in both F2 populations. According to the linkage map of chromosome 1 (Fig. 4A), the markers IM7685, IM7719 and IM9146 are located between the markers RM283 and RM312. Therefore, both QTLs are located in the same chromosomal region as those identified previously (Deng et al. 2015b, Zhou et al. 2013). These results confirmed the presence of both qSKC-1 and qSNC-1 on chromosome 1. Nevertheless, it was noteworthy that the genetic effects of qSKC-1 in different F2 populations were not exactly the same. The heterozygotes had phenotypic values of SKC intermediate between those of the two homozygotes in the ‘Nipponbare’ × ‘ZYQ8’ F2 population that was constructed in this and previous studies, whereas they tended to have SKC levels similar to the plants homozygous for the ‘ZYQ8’ genotype in both rss4 × ‘ZYQ8’ and rss2 × ‘ZYQ8’ F2 populations (Table 2 and data not shown). rss4 was sensitive to salt stress and accumulated higher levels of Na+ but lower levels of K+ in shoots than the wild-type ‘Nipponbare’ under conditions of NaCl stress, which might be attributable to mutation of a single gene (rss4) located in the qSNC-6 QTL on chromosome 6 (Deng et al. 2015b). Difference in salt tolerance between rss4 and ‘Nipponbare’ results in different phenotypic values and frequency distribution pattern in terms of SNC and SKC in the rss4 × ‘ZYQ8’ and ‘Nipponbare’ × ‘ZYQ8’ F2 populations, and thus affects the phenotypic variability of SNC and SKC among the genotypes at the qSKC-1 locus.

Besides qSNC-1, another QTL for SNC (qSNC-2) has been mapped to the long arm of chromosome 2 using the rss4 × ‘ZYQ8’ F2 populations (Deng et al. 2015b). This QTL explained 22.8% of the phenotypic variance, and the allele derived from ‘ZYQ8’ increased the SNC. In this study, a genetic map of chromosome 2 was also constructed using the ‘Nipponbare’ × ‘ZYQ8’ F2 population, but no QTL for SNC or SKC was detected (data not shown), which was in accordance with the results for the QTL mapping using the rss2 × ‘ZYQ8’ F2 population (Zhou et al. 2013). These results suggest that qSNC-2 is unstable across different genetic backgrounds and environments. An additional study should be performed to identify the effect of this QTL on salt tolerance in rice.

For the two QTLs qSKC-1 and qSNC-1, the allele that increased SKC and decreased SNC was derived for ‘ZYQ8’ in all mapping populations (Deng et al. 2015b, Zhou et al. 2013). In previous studies, using a double haploid population derived from the cross between ‘ZYQ8’ and a japonica variety ‘Jinx17’, Gong et al. (1999) identified a major QTL (Std) associated with the number of survival days of the seedlings (SDS) under salt stress on the short arm of chromosome 1. Std was located between the markers RG612 and C131, and the allele from ‘ZYQ8’ at this locus increased the SDS. In comparison, qSKC-1 and qSNC-1 are located adjacent to the chromosome region encompassing Std. In rice, many studies have suggested that SDS is negatively correlated with SNC but positively correlated with SKC in shoots (Cheng et al. 2012, Qiu et al. 2015, Sun et al. 2007, Zang et al. 2008). Based on these results, qSKC-1, qSNC-1, and Std may be the same locus associated with salt tolerance in ‘ZYQ8’.
In previous studies, several major QTLs associated with SNC, SKC, and SNKR have been mapped to the short arm of rice chromosome 1. To compare the physical positions of these QTLs, we performed BLAST searches using the sequences of markers flanking each of these QTLs (Table 4). The QTL SKC1 identified by Lin et al. (2004) has been cloned by a map-based cloning approach; SKC1 encodes a HKT-type transporter (Os01g0307500) and is located at 11.46 Mb on chromosome 1. The saltol locus has been repeatedly identified and mapped to the chromosomal region between 10.71 and 15.12 Mb that encompasses the SKC1 locus. The QTL clusters for the K⁺ and Na⁺ concentrations and Na⁺/K⁺ ratio identified by Haq et al. (2010) and Pandit et al. (2010) were located in the region corresponding to 11.08–14.63 Mb and 10.08–17.52 Mb on chromosome 1, respectively. In our previous studies, the qSKC-1 locus was initially mapped between SSR markers RM283 (bp 8792648–8797365) and RM312 (bp 10063864–10063971) in two F₂ populations derived from crosses between rss2 and ‘ZYQ8’ and between ‘Nipponbare’ and ‘ZYQ8’; it was then remapped to the interval flanked by RM283 and RM580 (bp 9606674–9606895) using a F₂ population derived from a cross between rss4 and ‘ZYQ8’ (Supplemental Table 2; Deng et al. 2015b, Zhou et al. 2013). Based on these results, qSKC-1 is located in the region corresponding to 4.89–10.06 Mb on chromosome 1. In this study, qSKC-1 was mapped to the interval between IM7685 (bp 7685831–7685967) and IM9146 (bp 9146791–9146925) and was further delimited to a 445-kb region bracketed by the markers RM578 (bp 8411145–8411370) and IM8854 (bp 8855893–8856060), consistent with the results of our previous studies. In comparison, qSKC-1 was located ~3.0 Mb upstream from the SKC1 and saltol loci, as well as the QTL cluster identified by Haq et al. (2010). Thus, qSKC-1 appears to be a distinct locus from the previously reported major QTLs for salt tolerance. Moreover, it is difficult to determine whether qSKC-1 and the minor QTLs identified by Koyama et al. (2001) and Zheng et al. (2015) are located at the same locus or different tightly linked loci, because they were mapped preliminarily, and the chromosomal regions encompassing them were greater than 10 Mb.

Recently, Negrão et al. (2011) constructed a list of rice genes shown to be involved in salt tolerance by semi-quantitative PCR, real-time PCR, or Northern blotting. Among these candidate genes related to salt tolerance, five are located on the short arm of chromosome 1: OsDREB2A (Os01g0165000), OsLEA5 (Os01g0225600), OsNLT3 (Os01g0261200), SALT (Os01g0348900), and Os01g0355100. Two of these genes [OsLEA5 (bp 6922131–692278) and OsNLT3 (bp 8792648–8797365)] are located in chromosomal regions encompassing the qSKC-1 locus, based on the preliminary mapping, and OsNLT3 is just within the 445-kb interval where qSKC-1 was finally mapped. OsNLT3 encodes a member of the NAM, ATAF, and CUC (NAC) transcription factors, referred to as OsNAC8 or ONAC074 (Kim et al. 2010). The NAC transcription factors have been found to be involved in responses to salinity in many plants. In rice, several NAC transcription factor family genes, such as SNAC1, OsNAC5, OsNAC6 and ONAC045, are induced by salt stress, and overexpression of these genes enhances plant salt tolerance (Hu et al. 2006, Nakashima et al. 2007, Takasaki et al. 2010, Zheng et al. 2009). Several studies have found that the level of OsNLT3 transcripts was induced significantly by multiple abiotic stresses, including high salinity, drought, and cold and heat, although the physiological functions and molecular mechanisms of this gene have not been reported (Fang et al. 2008, Kim et al. 2010). These results suggest that OsNLT3 is an important candidate gene for qSKC-1. However, there are more than 60 other candidate genes annotated in the Rice Annotation Project Database (RAP-DB; http://rapdb.dna.affrc.go.jp/) within the 445-kb region encompassing the qSKC-1 locus, and the accurate identification of this locus is difficult at present.

Additional experiments are needed to map the qSKC-1

Table 4. Previously reported quantitative trait loci (QTLs) for K⁺ and Na⁺ concentrations and Na⁺/K⁺ ratio on rice chromosome 1

| QTL     | Trait                          | Parentage                 | Marker flanking         | Genomic position (bp) | References                        |
|---------|--------------------------------|----------------------------|-------------------------|-----------------------|-----------------------------------|
| SKC1    | SKC                            | Nona Bokra/Koshihikari     | C1211–S2139             | 11458955–11463442     | Lin et al. (2004), Ren et al. (2005) |
| saltol  | SNC, SKC, SNKR                 | Pokkali/IR29               | (Os01g0307500) P3/M9-8–P1/M9-3 | –                    | Gregorio (1997)                  |
|         |                                |                           | RM23–RM140               | 10705545–12302086     | Bonilla et al. (2002)             |
|         |                                |                           | RM1287–RM10825           | 10833939–13323350     | Thomson et al. (2010)            |
|         |                                |                           | RM1287–RM7075            | 10833939–15119967     | Alam et al. (2011)               |
|         | leaf Na⁺ concentration, leaf K⁺ concentration, Leaf K⁺/Na⁺ ratio | Co39/Moroberekan          | RG811–RZ276             | 11078494–14627907     | Haq et al. (2010)                |
| qNaSH-1.1, qKSH-1.1, qNa/KSH-1.1 | SNC, SKC, SNKR | CSR27/MI48 | RM294–HvSSR01-46 | 10077939–17516026 | Pandit et al. (2010)          |
| qNa/KSH-1.1 | Na⁺ uptake, SKC, SNKR | IR4630/IR15324             | Changbai10/Dongnong425  | RM580–RM9             | –                                 |
|         |                                |                           |                         | 9606674–23326243       | Koyama et al. (2001), Zheng et al. (2015) |

SKC: shoot K⁺ concentration; SNC, shoot Na⁺ concentration; SNKR: shoot Na⁺/K⁺ ratio.

a The genomic position (bp) was inferred by NCBI blast analysis of the sequence of markers flanking QTLs (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
locus to a more confined chromosomal region using larger mapping populations. Thus, a more precise physical map covering the qSKC-1 locus is currently being constructed using a larger ‘Nipponbare’ × ‘ZYQ8’ F2 population. Moreover, we are currently developing a set of NILs by backcrossing the recombinants with ‘Nipponbare’ followed by selfing to eliminate non-target chromosome regions. The constructed NILs will be used not only for further fine mapping of qSKC-1 but also to identify the relationship between qSKC-1 and qSNC-1. In this and previous studies, qSKC-1 and qSNC-1 have always been mapped to the same chromosomal region, and the allele that enhances salt tolerance at the two loci is derived from ‘ZYQ8’ in all mapping populations, suggesting that qSKC-1 and qSNC-1 may be present within the same locus. Based on the results of progeny tests, however, not all the progenies from F2 recombinants with low-extreme phenotypic values of SKC showed fixed, high levels of SNC, and not all those of with high-extreme phenotypic values of SKC showed fixed, low levels of SNC. One possibility is that there are more than one locus for SNC in the region encompassing qSKC-1 and qSNC-1, and the recombination events among these loci resulted in the inconsistency between SNC and SKC in progenies from some groups (such as groups N1, N8, R1 and R6). Moreover, some additional undetected QTLs for salt tolerance located in other genomic regions may also be involved in the regulation of SNC in individuals of groups N1, N8, R1 and R6, and the combined action of them and qSNC-1 are responsible for the moderate levels of SNC in these F2 plants and their progenies. The F2 individuals of groups N1, N8, R1 and R6 had extremely low or high phenotypic values in terms of SKC but not SNC, which were reliable for fine mapping of the qSKC-1 locus, but cannot be used for recombinant screening and progeny tests in fine mapping of qSNC-1, considering that it is difficult to exclude the influence of other loci for SNC. As a next step, additional experiments are required to confirm the relationship between qSKC-1 and qSNC-1, such as a comparison of Na+ and K+ concentrations in the NILs and functional analysis of the target qSKC-1 gene after its isolation using a map-based cloning strategy.

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