

Genetic mapping of quantitative trait loci conditioning salt tolerance in wild soybean (*Glycine soja*) PI 483463

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Abstract Salt tolerance in soybean [*Glycine max* (L.) Merr.] is controlled by major quantitative trait loci (QTL) or single gene(s). Among soybean germplasm, wild soybean plant introduction PI 483463 was reported to have a single dominant gene for salt tolerance. The objective of this study was to genetically map the QTL in a recombinant inbred line (RIL) population derived from a cross between PI 483463 and Hutcheson. Simple sequence repeat (SSR) markers and universal soybean single nucleotide polymorphism (SNP) panel (the USLP 1.0) were utilized for molecular genotyping. The RILs were phenotyped in two independent tests in a greenhouse using a 1–5 scale visual rating method. The results showed that the salt tolerant QTL in PI 483463 was mapped to chromosome 3 in a genomic region between the Satt255 and

BARC-038333-10036 markers. The favorable allele inherited from PI 483463 conferred tolerance to salinity and had an additive effect on reducing leaf scorch. A subset of 66 iso-lines was developed from the F₃ families of the same cross and was used for genetic confirmation of the QTL. The integration of recombination events and the salt reaction data indicate that the QTL is located in the region of approximately a 658 kb segment between SSR03_1335 at nucleotide 40,505,992 and SSR03_1359 at nucleotide 41,164,735 on chromosome 3. This narrow region can facilitate further genomic research for salt tolerance in soybean including cloning salt tolerance genes.

Keywords Wild soybean · Salt tolerance · Mapping

Bo-Keun Ha and Tri D. Vuong contributed equally to this work.

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Introduction

Soil salinity is a major abiotic stress that adversely affects crop productivity and quality (Chinnusamy et al. 2005). The total world area of saline soils is over

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830 million ha, representing 26 % of the world's potentially arable land. About 20 % of irrigated land and over 2 % of non-irrigated land are affected by salinity (Lenis et al. 2011). The total saline area including sodic areas of North and South America is 15.8 and 129.0 mha, respectively (Rengasamy 2006). Over 85 % of world's soybean is produced in North and South America (Soystats 2012). Soybean [*Glycine max* (L.) Merr.] is considered a salt sensitive species (Lauchli 1984). Under salt stress, soybean yield was decreased by 20 % at 4.0 dS m⁻¹, and by 54 % at 6.7 dS m⁻¹ as much, respectively as 0 % at 0.8 dS m⁻¹ (Katerji et al. 2003). Thus, it is important to develop soybean cultivars with increased salt tolerance for stable production under saline conditions.

To find salt tolerance sources for breeding purpose, soybean scientists screened a larger number of soybean germplasm, including wild relatives. Their efforts and results were well summarized by Pathan et al. (2007). Abel (1969) reported that leaf scorch symptoms showing soybean injury are correlated well among genotypes, which exclude (tolerant) and those that do not exclude (intolerant) chloride from reaching the upper leaves. The exclusion of leaf chloride or salt tolerant trait was controlled by a single dominant gene *Ncl* from soybean line S-100. The *Ncl* gene was mapped to chromosome (Chr.) 3 corresponding to linkage group (LG) N by Lee et al. (2004) using 106 F_{2:5} recombinant inbred lines (RILs) derived from a cross between S-100 (salt tolerant) and cultivar Tokyo (salt sensitive). They found a major quantitative trait locus (QTL) linked to the SSR markers Sat_091, Satt237, and Satt339 on Chr. 3, which was associated with salt tolerance. Hamwieh and Xu (2008) also reported a major QTL in a wild soybean (*Glycine soja* Sieb. and Zucc.) accession JWS156-1. This salt-tolerant QTL accounted for 64.0 % of the total variation for salt tolerance and was located in the same genomic region on Chr. 3 as previously reported by Lee et al. (2004). Lately, Hamwieh et al. (2011) confirmed the genomic region for salt tolerance on Chr. 3 found by Hamwieh and Xu (2008) and Lee et al. (2004). Besides the major QTL on Chr. 3 as earlier reported, Chen et al. (2008) detected and mapped an additional QTL on Chr. 18 (LG G), flanked by the markers Sat_164 and Sat_358, when analyzing a new source of salt tolerance, cv. Kefeng No. 1.

Salt tolerance of some wild soybean accessions has been widely investigated. Results showed that there

was large genetic variability for the trait (Kao et al. 2006; Lenis et al. 2011; Pantalone et al. 1997). Furthermore, Luo et al. (2005) reported that salt tolerance mechanism was different between wild soybean and cultivated soybean. Salt tolerance in cultivated soybean was mainly due to prevention of Cl⁻ ion transport, and in wild soybean was mainly due to Na⁺ accumulation. This indicates that interspecific crosses between *G. max* and *G. soja* species offer the possibility leading to the improvement of salt tolerance in soybean. In an effort to evaluate soybean germplasm lines for salt tolerance, Lee et al. (2009) identified a wild soybean accession PI 483463 with high tolerance to salt. Subsequently, a genetic analysis showed that this PI had a single dominant gene, non-allelic to the previously identified gene in S-100 (Lee et al. 2004). The objective of this study was to genetically map the QTL in a RIL population derived from a cross of a salt tolerant wild soybean PI 483463 and a salt sensitive soybean cultivar Hutcheson.

Materials and methods

Population development

A wild soybean (*Glycine soja*) accession, PI483463, collected from Shanxi, China (GRIN, <http://www.ars-grin.gov/npgs/searchgrin.html>), was determined to have salt tolerance as compared to sensitive soybean cultivar Hutcheson (Lee et al. 2004) from greenhouse screening trials. PI 483463 has a single dominant gene for salt tolerance (Lee et al. 2009).

Identification and determination of salt tolerance in PI 483463 were performed as previously described (Lee et al. 2009). For developing an RIL mapping population, PI 483463 was crossed with a salt sensitive soybean cultivar Hutcheson in a greenhouse during the winter of 2005–2006 at the University of Missouri-Delta Research Center (UMDRC), Portageville, MO, USA. F₁ seeds were planted at the Lee Farm, UMDRC during the summer of 2006. Single pod decent method was applied to advance generations of this population from 2006 to winter of 2007–2008 in a greenhouse (winter) and soybean field (summer). In short, F₂ seeds were planted in a greenhouse during the winter of 2006–2007 to produce F_{2:3} seeds. Only 2–3 F_{2:3} seeds from each F₂ plant were planted in the field during the summer of 2007 to produce F_{2:4} seeds. Subsequently,

2–3 $F_{2.4}$ seeds from each F_3 plant were planted in a greenhouse during the winter of 2007–2008 to produce $F_{2.5}$ seeds. One hundred ninety $F_{2.5}$ RILs were each planted in the field in 10 seed hill plots at the Lee Farm, UMDRC, in the summer of 2008. Each hill was thinned to a single $F_{2.5}$ plant and $F_{2.6}$ seeds were harvested in the fall of 2008. A total of randomly selected 106 $F_{2.6}$ RILs were used to map QTL associated with salt tolerance in PI 483463.

For a confirmation study, we employed the same cross of PI 483463 and cv. Hutcheson and developed a subset of iso-lines. Briefly, under NaCl assays in the greenhouse, one $F_{2.3}$ line segregating for 3 tolerant:1 sensitive ratio was selected to develop iso-lines. Seeds of the selected $F_{2.3}$ line were subsequently planted in the field and F_3 plants were harvested individually. The iso-lines were advanced subsequent generations by the single-pod decent method in the field and a greenhouse as described above for RIL development. $F_{3.6}$ seeds were individually harvested from 131 $F_{3.5}$ plants in the fall of 2008. A total of 66 $F_{3.6}$ iso-lines were then used for the QTL confirmation study.

Salt reaction phenotyping

A plastic cone-tainer (PC) method (Lee et al. 2008) was used to determine salt reaction of 106 randomly selected lines out of 190 $F_{2.6}$ RILs and 66 iso-lines along with the parents. Seedlings were exposed to the salt solution (99.1 % table salt, Morton International, Inc. Chicago, IL, USA) in a randomized complete block design (RCDB) with two replications. Five soybean plants were evaluated in each replication. The phenotyping for tolerance was repeated to confirm the consistency of reactions to salinity of the parents and RILs. The phenotyping for Test 1, and Test 2 were conducted in a greenhouse at UMDRC at 2 week intervals during the 2008–2009 winter seasons. Phenotyping of the iso-lines, was conducted in a randomized complete block design (RCDB) with two replications.

Soybean lines were planted in 21-cm tall Ray Leach cone-tainers (Stuewe and Sons, Inc., Oregon, USA) filled with a sandy soil and placed in cone-tainer racks. At the V2–V3 growth stage (Fehr et al. 1971), racks of cone-tainers with five seedlings of parents, $F_{2.6}$ RILs, and iso-lines were placed in a plastic-lined wooden bed (Lee et al. 2008) with 100 mM NaCl solution. The bottom third of the cone-tainers were immersed in the salt solution to allow plant roots to take up Na^+ and

Cl^- . Individual soybean plants were rated for salt sensitivity or tolerance when the sensitive parent, cv. Hutcheson, showed severe leaf scorch (about 2 weeks after exposure to salt) or a four rating as described by Pantalone et al. (1997). Leaf scorch was scored using a 1–5 scale (1 = no apparent chlorosis; 2 = slight; 3 = moderate; 4 = severe chlorosis; and 5 = dead) (Lee et al. 2008).

DNA extraction

Five to seven seeds of each RIL and iso-line were grown in a greenhouse. Young trifoliolate leaves of V2 seedlings were collected for DNA extraction using the automated AutoGen 960 system and the CTAB protocol of the manufacturer (AutoGen Inc., Holliston, MA, USA) with minor modifications. Briefly, leaf tissue was freeze-dried and ground to fine powder and then mixed with CTAB extraction buffer. After a 1.5 h incubation period, chloroform was added to the suspension, followed by agitation and centrifugation. The aqueous layer was collected and treated with RNase enzyme. Following the DNA precipitation, DNA pellets were washed with 70 % ethanol and dissolved in TE (Tris–HCl–EDTA, pH 8.0) buffer. DNA concentration was quantified with a spectrophotometer (NanoDrop Technologies Inc., Centreville, DE, USA) and was normalized at 20 ng/ μl for polymerase chain reaction (PCR) amplification.

Single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) analysis

The universal soybean linkage panel 1.0 (the USLP 1.0) of 1,536 SNP loci, which was developed for the soybean genome (Hyten et al. 2008) and mapped onto the integrated molecular genetic linkage map, was used to genotype the RIL mapping population using the Illumina GoldenGate assays (Fan et al. 2006; Hyten et al. 2010). Briefly, genomic DNA samples of RILs were activated by biotinylation. Following PCR amplification with three oligonucleotide sequences (two allele-specific oligos and one locus-specific oligo) and oligonucleotide hybridization, washing was conducted to remove excess and mis-hybridized oligos. The resulting double-stranded PCR products were immobilized onto paramagnetic particles, followed by washing and denaturing. The released single-stranded DNAs were then hybridized to their

complementary bead type. Array imaging was performed using the Illumina BeadStation (Illumina, San Diego, CA, USA) to generate intensity data files. The allele calling for each SNP locus was subsequently performed using the BeadStudio 3.0 program (Illumina, San Diego, CA, USA). Each SNP locus was carefully checked for errors in the clusters of homozygous and heterozygous genotypes. Polymorphic SNP markers were then used to construct a genetic map as previously described by Vuong et al. (2010, 2011)

In addition to SNP markers of the USLP 1.0 panel, a subset of SSR markers that were mapped on Chr. 3 and associated with the genomic location of the known *Ncl* gene were selected and RILs were genotyped as described (Vuong et al. 2010, 2011). Polymorphic SSR markers were integrated into a SNPs-based genetic linkage map. These markers were also employed to genotype iso-lines in a confirmation study.

Data analysis and genetic mapping

In this study, a total 106 out of 190 RILs were used for genetic linkage analysis. The program JoinMap 3.0 (Van Ooijen and Voorrips 2001) was used to construct a genetic linkage map for 106 RILs using the Kosambi (1944) mapping function. A likelihood of odds (LOD) threshold score of 3.0 and a maximum genetic distance of 50 cM were utilized for initial linkage grouping of markers. For QTL mapping, a comprehensive analysis approach, including initial interval mapping (IM), cofactor selection, genome-wide permutation test (Churchill and Doerge 1994), and multi-QTL method (MQM) was performed with the program MapQTL 5.0 (van Ooijen and Voorrips 2001) to detect and map significant genomic region(s) associated with salt tolerance as previously described in details (Vuong et al. 2010, 2011). The proportion of the phenotypic variance explained by the QTL effects was estimated by MQM method at the QTL peaks. Additive (A) effects of significant QTL were estimated from an output of the program MapQTL 5.0. A multivariate ANOVA model in SAS (SAS Institute, Cary, NY, USA) was utilized to estimate the total phenotypic variation explained by the significant QTL.

A similar analysis method was also used to map SSR markers in iso-lines in order to confirm putative genomic interval(s) identified and associated with

tolerance to salinity. The chromosomes with LOD plots were created using the MapChart 2.2 program (Voorrips 2002) based on the outputs from JoinMap 3.0 and MapQTL 5.0.

Saturation of the QTL region with additional SSR markers

Once the QTL was positioned, additional SSR markers were used to fine map the genomic position of the QTL with some RILs showing different recombinant events in the target region. The primer sequences and location of new additional SSR markers were available from SoyBase (<http://soybase.org>; verified 12 March 2012). PCR was performed according to Song et al. (2010). The PCR products were analyzed on the LabChip GXII electrophoresis system with the HT DNA 1 k LabChip kit (Caliper LifeSciences, Hopkinton, MA, USA).

For salt reaction, five replications of each RIL (RIL 6, 14, 31, 67 and 149) and the parents were planted in a randomized complete block design in a greenhouse. Five soybean plants were evaluated in each replication. The phenotyping for salt reaction was conducted by the plastic container method (PC method) as mentioned above (Lee et al. 2008).

Results

Phenotypic variation in RIL and iso-line populations

To determine salt reaction, a total of 106 RILs derived from a PI 483463 × Hutcheson cross and their parents were phenotyped in two independent tests (Test 1 and Test 2) in a greenhouse using the PC method. The tolerant parent PI 483463 showed no apparent chlorosis in leaves (leaf scorch = 1 in two tests), while the sensitive parent Hutcheson showed severe chlorosis in leaves (leaf scorch = 5 in test 1 and 4.5 in test 2) as shown in Fig. S1 in supplementary and Fig. 1. The frequency distribution of the leaf scorch of the 106 RILs was continuous. However, based on leaf scorch score, genotypes with average leaf scorch scores of 1–2 were considered as tolerant (T), and genotypes with scores of 3–5 were rated as sensitive (S) (Lee et al. 2009), 42 RILs were rated tolerant and 50 RILs were rated sensitive. This fit a ratio of 1 tolerant to 1 sensitive ($\chi^2 = 0.695$, $P > 0.404$) for RILs.

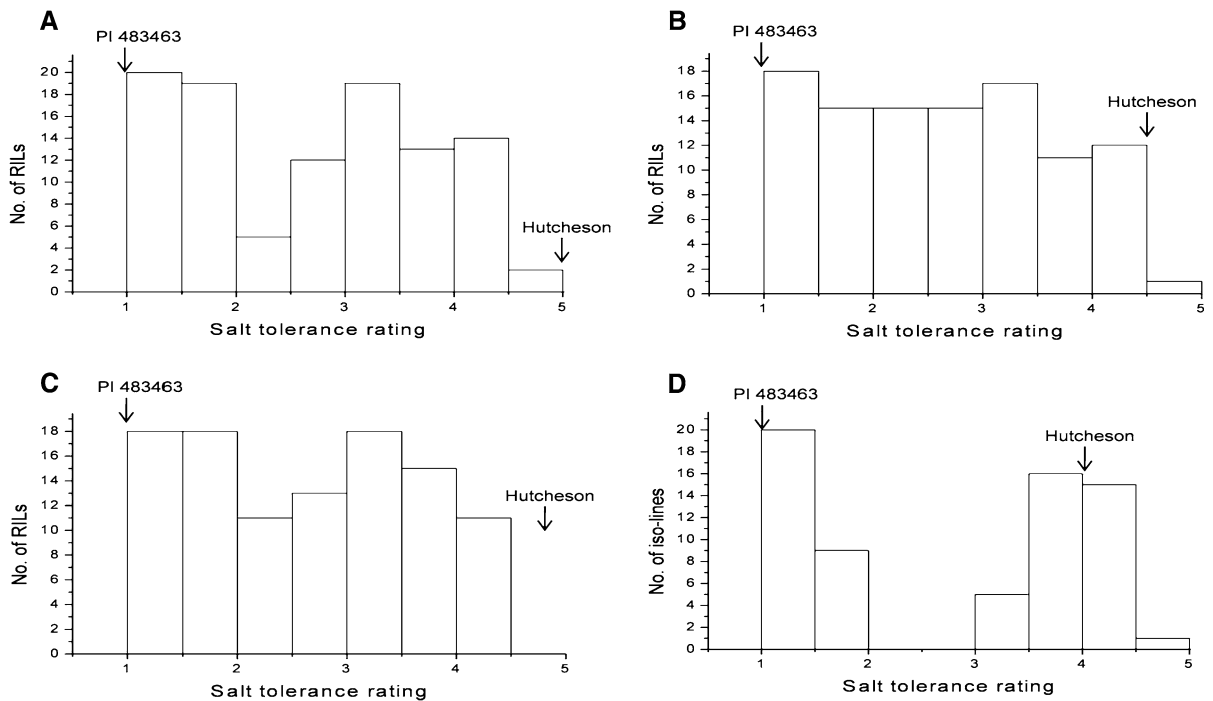


Fig. 1 Frequency distribution of salt tolerance rating in 106 RILs derived from a PI 483463 × Hutcheson cross. **a** Test 1 data, **b** Test 2 data, **c** combined data for test 1 and 2. **d** Frequency

distribution of salt tolerance rating in 66 iso-lines derived from a PI 483463 × Hutcheson cross

The leaf scorch scores ranged from 1.0 to 4.5 with a mean of 2.7 in test 1 and ranged from 1.0 to 4.7 with a mean of 2.6 in test 2. There was a significant positive correlation ($r = 0.82$, $P < 0.01$) between test 1 and test 2 in leaf scorch. There was no transgressive segregation for salt tolerance in the two independent tests.

The 66 iso-lines derived from the same cross segregated into 29 tolerant (less than 2 leaf scorch score) and 37 susceptible (more than 3 leaf scorch score) as shown in Fig. 1d which fit a ratio of 1 tolerant-to 1 sensitive ($\chi^2 = 0.969$, $P > 0.324$).

Mapping QTL for salt tolerance

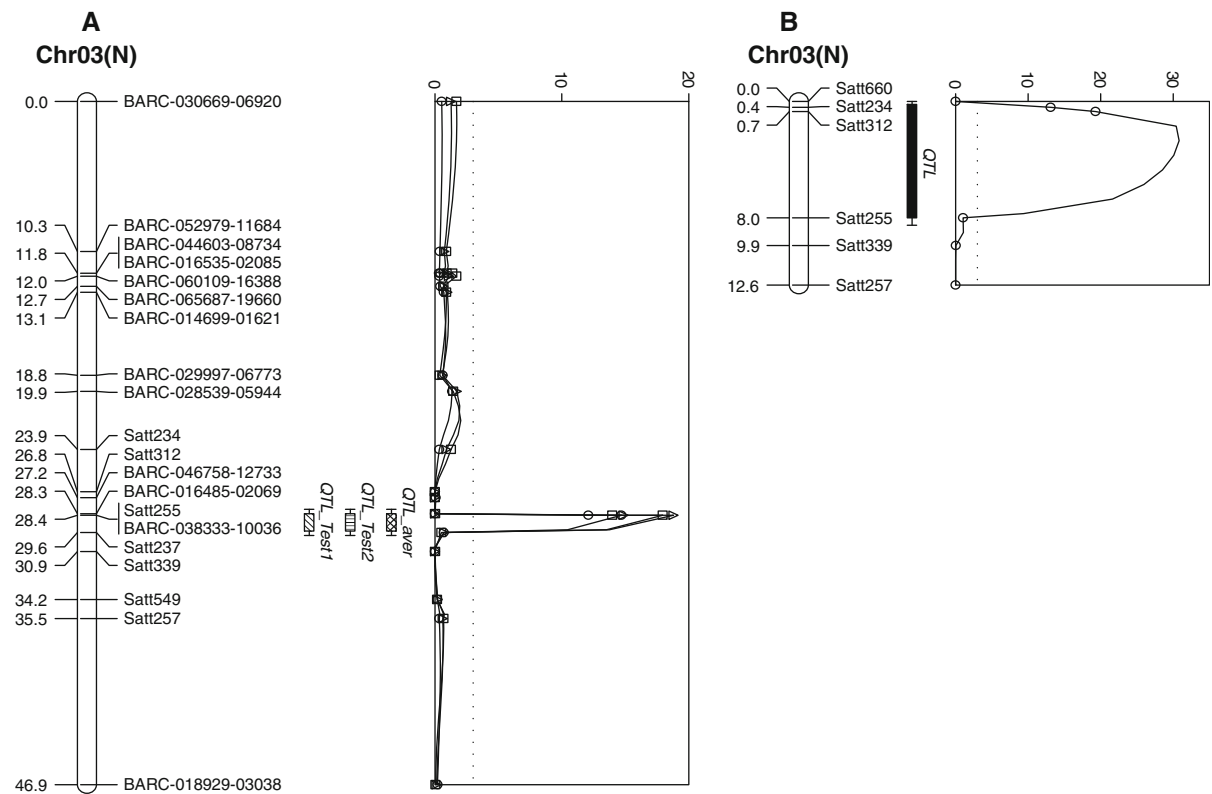
Among 1,536 SNP loci (the USLP 1.0) genotyped, 545 SNP markers (35.5 %) were polymorphic and utilized to construct a genetic linkage map. In addition, seven polymorphic SSR markers on Chr. 3 were integrated into a SNPs-based genetic linkage map. A molecular linkage map consisting of 545 SNPs and seven SSR markers was constructed, covering 2,153 cM across 20 chromosomes (genetic map not shown). The shortest linkage fragment had 23 markers on Chr. 14

covering 83.9 cM. The longest linkage fragment had 33 markers on Chr. 2 covering 144.0 cM. The average genetic distance between markers was 3.9 cM, varying from 0.5 to 36.2 cM. Overall, the marker order and distance between adjacent markers were similar to those on the soybean integrated linkage map of Hyten et al. (2010) and covered 93.8 % of soybean genome.

QTL analysis for salt tolerance was conducted by composite interval mapping using QTL Cartographer. Based on a genome-wide permutation test, LOD thresholds of 4.4, 3.4, and 3.9 ($P = 0.05$) in test 1, test 2, and combined data, respectively, were used to identify significant QTL. CIM analysis detected a significant QTL for salt tolerance in test 1, test 2, and combined data on Chr. 3 (LG N) (Table 1; Fig. 2). The QTL peaks overlapped in the genomic region flanked by Satt255 and BARC-038333-10036, with LOD scores of 17.9, 14.7, and 18.8 for test 1, test 2, and combined test data, respectively (Table 1). These QTL explained from 47.8 to 56.5 % of the total phenotypic variance of salt tolerance rating across tests. There were no putative QTLs related to salt tolerance on other soybean chromosomes except Chr. 3 in which the QTL were contributed by PI 483463.

Table 1 Marker intervals, LOD scores, R^2 values, and additive values of salt tolerance QTLs derived from a PI 483463 × Hutcheson cross

Salt reaction	Interval	QTL position (cM)	LOD	R^2 (%)	Additive effect
RILs					
Leaf scorch in test 1	Satt255—BARC-038333-10036	28.4	17.9	54.38	0.83
Leaf scorch in test 2	Satt255—BARC-038333-10036	28.4	14.7	47.8	0.70
Leaf scorch combined for tests 1 and 2	Satt255—BARC-038333-10036	28.4	18.8	56.5	0.76
Leaf scorch of iso-lines	Satt255–Satt312	0.7–8.0	30.8	94.8	1.27

**Fig. 2** Likelihood of odds (LOD) plots for Chr. 3 showing the location of QTL for salt tolerance using a LOD threshold of 3 (vertical dotted line). **a** These QTLs were mapped in RILs population derived from a cross between PI 483463 and

Hutcheson. QTL Test 1 from leaf scorch in Test 1, QTL Test 2 from in Test 2, QTL aver from leaf scorch combined for Tests 1 and 2. **b** This QTL was mapped in an iso-line population derived from the same cross between PI 483463 and Hutcheson

A subset of 66 iso-lines derived from the same cross was used to confirm salt tolerance QTL identified in the RIL mapping population. Six polymorphic SSR markers around the QTL region on Chr. 3 of the public consensus soybean map were used to genotype these iso-lines. Results of QTL analysis showed that the QTL was mapped to the Satt255–Satt312 interval, which is in the same genomic region as the QTL

identified from the RIL population (Table 1; Fig. 2). This QTL explained 94.8 % of the total phenotypic variance of salt tolerance rating.

Saturation of the QTL region

To further refine the genomic locations of recombinant events, SSR marker analyses and salt tolerance tests

were re-conducted with five RILs. We identified seven polymorphic SSR markers located between Satt255 and Satt312 and screened the RILs and two parents. Also, these RILs were re-phenotyped with five replications in a greenhouse.

Except for RIL 149, four RILs 6, 14, 31, and 67 showed salt tolerance (Fig. 3). RIL 67 showed salt tolerance but had the homozygous allele from the Hutcheson genome in the interval between Satt237 and SSR03_1335. However, RIL 31 had the homozygous allele from the Hutcheson genome in the interval between SSR03_1359 and Satt312 and showed salt tolerance. The integration of recombination events and the salt reaction data indicate that the QTL is located in the region of approximately 658 kb between SSR03_1335 at nucleotide 40,505,992 and SSR03_1359 at nucleotide 41,164,735 on Chr. 3.

Discussion

Soybean is one of the salt sensitive crops and its production is severely affected by soil salinity (Katerji et al. 2003). With efforts made the past several years, soybean researchers tried not only to understand the mechanism of salt reaction (Pathan et al. 2007), but also to identify QTL or gene(s) underlying salt tolerance in soybean. Abel (1969) initially reported that salt tolerance is controlled by single dominant gene, *Ncl*. Lee et al. (2004) mapped a major QTL conditioning salt tolerance in S-100 (*Glycine max*) around the markers Sat_091, Satt237, and Satt339 on Chr. 3, which was considered as the *Ncl* locus. Hamwieh and Xu (2008) also reported a major QTL in a wild soybean accession JWS156-1 that was located in the same genomic region on Chr. 3 as previously reported by Lee et al. (2004). Hamwieh et al. (2011) confirmed the genomic region of salt tolerance on Chr. 3 as previously studied (Hamwieh and Xu 2008; Lee et al. 2004) in a soybean genotype FT-Abyara. The salt tolerant FT-Abyara had no pedigree relationship with S-100 and was polymorphic to S-100 for SSR markers around the salt tolerant QTL. They concluded that salt tolerant gene of FT-Abyara may be not directly descended from S-100 or its descendent cultivars. In another study, a major QTL in cv. Kefeng No. 1 was detected and mapped on Chr. 18 (LG G), flanked by the markers Sat_164 and Sat_358 (Chen et al. 2008).

In this study, we have tried to detect and map a new salt tolerant QTL using a genetic background different from S-100. Previously, Lee et al. (2009) reported that a wild soybean (*Glycine soja* Sieb. and Zucc) PI 483463 had a different gene (*Ncl2*) from S-100 in allelism analysis. Using RIL and iso-line populations derived from the cross between PI 483463 and Hutcheson, a QTL was mapped to the genomic region of between markers Satt255 and BARC-038333-10036 on Chr. 3, which is in the same region of *Ncl* from S-100 (Fig. 2; Table 1). However, the location of the salt tolerant QTL in PI 483463 was narrowed down to the approximately 658 kb interval between SSR03_1335 at nucleotide 40,505,992 and SSR03_1359 at nucleotide 41,164,735 on Chr. 3 (Fig. 3).

It was speculated that S-100 and PI 483463 have different salt tolerance alleles or gene located at the same locus on Chr. 3 as reported by Lee et al. (2009). Based on the findings of this study, we cannot conclude that the tolerance genes from the two sources are different. Therefore, more studies are needed to determine if the tolerance genes from S-100 and PI 483463 are the same or different. In another test, PI 486463 and S-100 showed the same tolerant reaction after 18 days of salt treatment (Fig. S1); however, PI 486463 showed more salt tolerance than S-100 after 30 days of salt treatment. This suggests that the tolerance alleles from the two genotypes may be different. Segregating tolerant and susceptible genotypes for F₂ plants from S-100 × PI 483463 reported by Lee et al. (2009) may have differences in the degree of salt tolerance based on alleles derived from the two genotypes. Based on the gene annotation in the Soybean Genome Project, a 658-kb segment contained 80 annotated genes from the Williams 82 genome sequences (Schmutz et al. 2010). Among these, two genes (Glyma03g32890 and Glyma03g32900) encoding a sodium/hydrogen exchanger family were identified. Moreover, the gene (Glyma03g33240) encoding cation-transporting ATPase, and one gene (Glyma03g32850) encoding a heat shock protein 70 kDa were identified. First, plant Na⁺-H⁺ exchangers (NHXs) are tonoplast localized Na⁺/H⁺ antiporters regulating cytosolic Na⁺ concentration by vacuole sequestration (Phang et al. 2008). Over-expression of NHX antiporters have enhanced salt tolerance in Arabidopsis (Apse et al. 1999), cotton (Wu et al. 2004), rice (Fukuda et al. 2004), tomato (Zhang and Blumwald 2001), and wheat (Xue et al. 2004). In

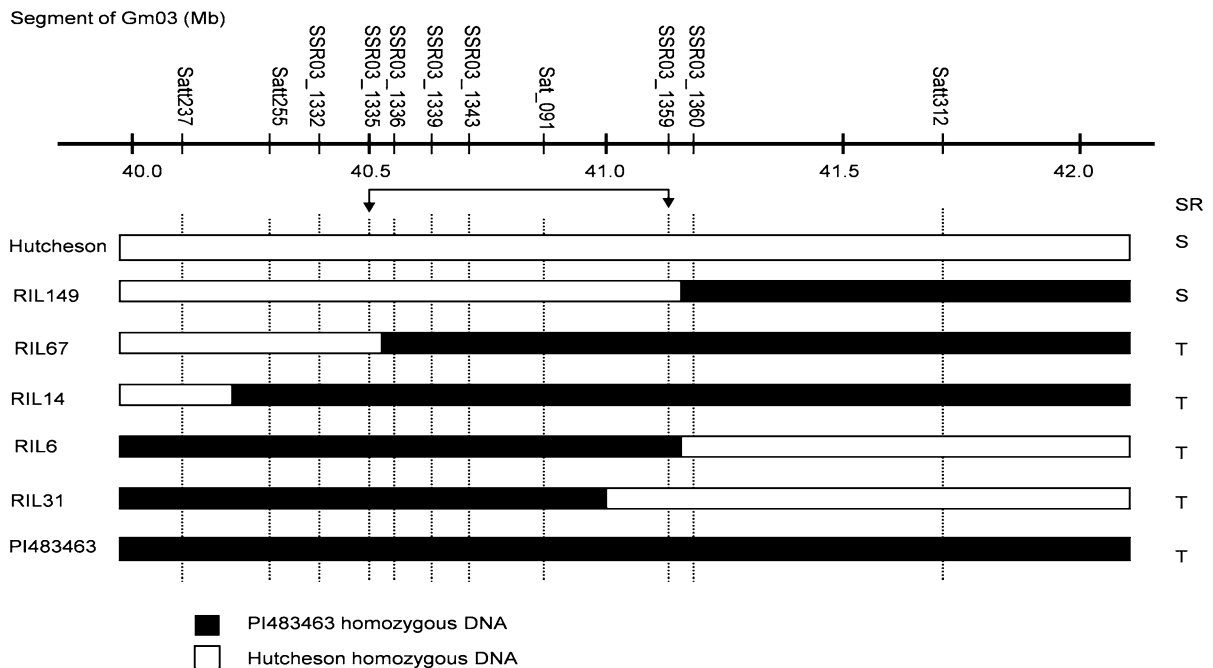


Fig. 3 Graphical genotypes of representative RILs from a PI483463 × Hutcheson cross in the QTL region on Chr. 3. SR indicates salt reaction of five RILs and parental lines used to refine the genomic region (*S* sensitive to salt and *T* tolerant to salt)

soybean, expression of the putative GmNHX1 gene located on soybean Chr. 20 was significantly induced by NaCl stress and overexpression of GmNHX1 in *Lotus corniculatus* decreased Na⁺ level and increased K⁺/Na⁺ ratio under NaCl stress (Li et al. 2006; Sun et al. 2006). In addition, Ca²⁺ plays an important role of the stress responsive signaling pathway and Ca²⁺-ATPase is considered as the major transporter for the efflux of cytoplasmic Ca²⁺ in plants (Phang et al. 2008). A mutation in Ca²⁺-ATPase located in the Golgi apparatus induced salt tolerance in *Saccharomyces cerevisiae* (Park et al. 2001). Soybean Ca²⁺-ATPase (SCA1) that is located in the plasma membrane was dramatically and rapidly induced by salt stress (Chung et al. 2000). Also, heat shock cognate 70 kDa (HSC70) are involved in many aspects of protein folding in normal physiological conditions as well as in biotic and abiotic stress conditions. HSC70 over-expression in transgenic lines showed a significantly higher root growth under salt stress condition in *Arabidopsis* (Cazalé et al. 2009).

The presence of these putative candidate genes were also confirmed in genome sequencing database of *Glycine soja* var. IT182932 (Kim et al. 2010). The

identities of four putative candidate genes between Williams 82 sequence and IT182932 sequence ranged from 93.1 % (Glyma03g32900) to 100 % (Glyma03g33240). However, the salt tolerance of these two genotypes (Williams 82 and IT182932) is unknown. Therefore, further studies are needed to determine the candidate genes underlying salt tolerance in PI 483463. Also, physiological studies for ion accumulation and differences in salt tolerance are needed to distinguish the salt tolerance levels between PI 483463 (*Glycine soja*) and S-100 (*Glycine max*).

In conclusion, our results revealed that salt tolerant QTL in PI 483463 was mapped in genomic region on Chr. 3, which is conserved between wild and cultivated soybeans. Also, we narrowed down an approximately a 658-kb segment with SSR markers for the salt tolerance allele in PI 483463. The salt tolerance QTL reported here will facilitate the genome research related to salt tolerance, including cloning the salt tolerance gene in soybean. DNA markers associated with the salt tolerance QTL in PI 483463 could be useful for marker-assisted selection in a soybean breeding program designed to improve salt tolerance.

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