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Conserved salt tolerance quantitative trait locus (QTL) in wild and cultivated soybeans

Aladdin Hamwieh[1,2] and Donghe Xu*[1]

[1] *Japan International Research Center for Agricultural Science (JIRCAS)*, 1-1 Ohwashi, Tsukuba, Ibaraki 305-8686, Japan
[2] *International Center for Agricultural Research in the Dry Areas (ICARDA)*, P.O. Box 5466, Aleppo, Syria

In the present study, we investigated salt tolerance heredity in wild soybean, *Glycine soja* Sieb & Zucc., and compared the salt tolerance quantitative trait locus (QTL) of *G. soja* with that of *Glycine max* (L.) Merr. An F_2 population (n = 225) derived from a cross between the salt sensitive soybean cultivar Jackson (PI548657) and a salt-tolerant wild soybean accession (JWS156-1) was used. Evaluation of salt tolerance in the seedling stage was carried out in hydroponic culture with half-strength Hoagland and Arnon nutrient solution containing 120 mM NaCl. Visual ratings of symptoms based on leaf scorching and chlorophyll content (SPAD value) were taken for each plant 20 days after salt treatment. Both traits showed continuous distribution; however, salt-tolerant plants (*i.e.* plants with a high salt tolerance rating (STR) and SPAD value) were predominant. QTL analysis revealed a major salt-tolerant QTL with a large dominant effect, which accounted for 68.7% of the total variance of the STR scale, on the soybean linkage group N. Our results indicated that the salt tolerance QTL confers a large dominant effect over salt sensitivity and that the salt tolerance QTL is conserved in both wild and cultivated soybeans.

Key Words: Salt tolerance, QTL, *Glycine soja*.

Introduction

The genus *Glycine* subgenus *Soja* includes two species: *Glycine max* (L.) Merr. (cultivated soybean) and *Glycine soja* Sieb & Zucc. (wild soybean). *G. soja* is the wild progenitor of *G. max*, which was domesticated in China (Hymowitz 1970) and is mainly distributed in East Asia areas such as China, Korea, and Japan. DNA marker analyses revealed that *G. soja* shows greater genetic diversity than *G. max* (Maughan *et al*. 1995, Xu *et al*. 2002, Xu and Gai 2003). Recently, several favorable traits, such as tolerance to dehydration (Chen *et al*. 2006), high lutein content (Kanamaru *et al*. 2006), seed protein electrophoretic variants (Fukuda *et al*. 2005), and yield and yield components (Li *et al.* 2008) have been identified in wild soybean, and demonstrated the usefulness of the wild soybean to improve cultivated soybean.

Salinity is one of the major abiotic stress factors, which restricts plant growth and yield, and is a major threat to agriculture sustainability. It has been estimated that 20% of the irrigated land in the world is presently affected by salinity (Yamaguchi and Blumwald 2005). Salt stress is reported to inhibit soybean germination and plant growth (Abel and MacKenzie 1964, Wang and Shannon 1999), nodule formation (Singleton and Bohlool 1984), and seed yield (Parker *et al.* 1983). Chloride is a major toxic element in soybean salt stress (Abel and MacKenzie 1964, Parker *et al*. 1983). Yang and Blanchar (1993) reported that average grain yields of Cl accumulator cultivars were significantly decreased by 16% on the addition of Cl, while the grain yields of Cl excluder cultivars were not significantly decreased. Ten of 15 cultivars were identified as salt-tolerant by Parker *et al*. (1983). They found that the average Cl content in the leaves of susceptible cultivars was 18 times more than that found in the leaves of tolerant cultivars, and that the susceptible cultivars had 37% less yields than the tolerant cultivars. Yang and Blanchar (1993) reported that of 60 soybean cultivars selected from five different maturity groups, 19 cultivars were Cl excluders. Shao *et al*. (1986) evaluated approximately 2,000 soybean varieties for salt tolerance and found that six varieties were highly salt-tolerant throughout the growing season.

Abel (1969) reported that Cl exclusion is controlled by a single dominant gene, *Ncl*., however, the genomic location of the *Ncl* gene has not been determined. Lee *et al*. (2004) evaluated 106 $F_{2:5}$ recombinant inbred lines (RIL) derived from a cross between soybean cultivars S-100 (salt-tolerant) and Tokyo (salt-sensitive) for salt tolerance in a saline field and a greenhouse. They identified one major quantitative trait locus (QTL) associated with salt tolerance around SSR markers Sat-091, Satt339, and Satt237 on linkage group N, and assumed that the major QTL might be the *Ncl* locus reported by Abel (1969).

A number of researchers have evaluated *G. soja*, wild soybean, as a source of useful genes. To obtain highly salt tolerant plant materials, we screened more than 300 wild

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*Corresponding author (e-mail: xudh@jircas.affrc.go.jp)

soybean germplasms, of which one wild accession (JWS156-1) from the Kinki area of Japan, showed the highest salt tolerance (unpublished data). Since little is known about the heredity of salt tolerance in wild soybean, we aimed (1) to study the heredity of salt tolerance in wild soybean and (2) to compare the salt-tolerance QTL identified in the present study with the QTL reported previously in cultivated soybean.

Materials and Methods

Plant materials

A total of 225 F_2 plants derived from a cross between the cultivated soybean cultivar *Glycine max* (L.) Merr. cv. Jackson (PI548657) and the salt-tolerant wild soybean accession JWS156-1 were used in this study. JWS156-1 was selected after screening a large number of wild soybean germplasms, which were provided by the National BioResource Project-Legume Base (http://www.legumebase.agr.miyazaki-u.ac.jp/index.jsp), for salt tolerance. Jackson is a salt-sensitive variety and has previously been used as a salt-sensitive cultivar in several studies (Abel and MacKenzie 1964, Abel 1969, Luo *et al*. 2005).

Evaluation of salt tolerance

Salt tolerance evaluation of the 225 F_2 plants and two parents was performed under controlled conditions in a greenhouse in the Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan. The seeds were sown on September 13, 2007. Five days after germination, the seedlings were transferred into a plastic container ($150 \times 75 \times 20$ cm) filled with nutrient solution. The seedlings were supported by Styrofoam plates ($90 \times 60 \times 3$ cm) with 64 holes of 2.5 cm diameter. The distance between the holes was 8.5×4.5 cm. The plants in the holes were supported by a small sponge bar to keep the roots suspended in the solution.

Half-strength Hoagland and Arnon (Asagawa 1985) nutrient solution was used. The composition of the full-strength nutrient solution is: KNO_3 (211.3 g/l), $Ca(NO_3)_2 \cdot 4H_2O$ (188.9 g/l), $NH_4H_2PO_4$ (23 g/l), $MgSO_4 \cdot 7H_2O$ (98.6 g/l), EDTA-Fe (22.621 g/l), $MnCl_2 \cdot 4H_2O$ (1.801 g/l), H_3BO_3 (2.86 g/l), $ZnSO_4 \cdot 7H_2O$ (0.22 g/l), $CuSO_4 \cdot 5H_2O$ (0.079 g/l), and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.037 g/l). The nutrient solution was constantly supplemented by a air stream with an air pump to keep the nutrient solution fully saturated with oxygen. The greenhouse temperature was maintained at 25 ± 2°C. The ambient light in the greenhouse was supplemented by high pressure sodium light for 14 hr/day.

Two weeks after transplantation, the plants were treated with nutrient solution containing 60 mM of NaCl. After three days, NaCl concentration was increased to 120 mM. The nutrient solution was replaced every three days during treatment. The pH of the nutrient solution was checked every day and was maintained at 6.0–6.5.

Each F_2 plant and its parents were rated for visual salt tolerance based on leaf scorching at 20 days after salt treatment, when the salt-sensitive parent Jackson clearly showed salt toxic symptoms. The rating scales ranged from 1 (complete death) to 5 (normal healthy leaves). Each plant was evaluated independently by 2 people to minimize human error. Moreover, plant chlorophyll content was measured using a chlorophyll meter spectrophotometer (Konica Minolta SPAD-502). This meter measures a numerical SPAD value, which is proportional to the chlorophyll content in the leaves.

QTL analysis

DNA was extracted from each F_2 plant according to a small-scale CTAB method. Briefly, fresh leaf material was frozen in liquid nitrogen and ground into fine powder, and subsequently transferred to a 2 ml centrifuge tube with 1 ml pre-heated CTAB extraction buffer (2% CTAB, 0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA). The suspension was incubated at 65°C for 50 min. After incubation, 700 µl chloroform-isoamyl alcohol (24:1) was added to the tube, and then the suspension was gently mixed for 10 min. Centrifugation was performed at 12,000 rpm for 20 min at room temperature and the supernatant was transferred to a new tube. DNA was precipitated by adding 2/3 volume of cold iso-propanol. The precipitated DNA was collected by centrifugation and washed with a washing buffer (75% ethanol, 200 mM sodium acetate) for 10 min. After air-drying for about 20 min, DNA was dissolved in 150 µl of 1 × TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

SSR markers from a genomic region on linkage group N, where a major QTL was detected by Lee *et al.* (2004), were tested for polymorphism between the two parents and 7 polymorphic SSR markers were subsequently identified. Sequence information of the SSR markers was based on the study by Song *et al*. (2004). PCR amplification reactions were performed in a final volume of 20 µl in the presence of 10 ng of template DNA, 10 pmol of each primer, 0.2 mM of each deoxynucleotide, 1 × PCR buffer, and 0.5 units *Taq* polymerase (*Taq*™, TaKaRa Bio Inc.). PCR was carried out for 35 cycles with 15 s at 94°C, 20 s at 55°C and 30 s at 72°C, followed by a final extension step of 7 min at 72°C. Amplified products were separated on an 8% polyacrylamide gel and stained by ethidium bromide. The band pattern was visualized on a Typhoon 9410 Fluorescent Imager (Amersham Biosciences).

Mapping was performed using JoinMap 4.0® (Van Ooijen 2004) software. Loci were assigned to linkage groups based on an LOD score >3 and recombination frequency <0.45. Map distances (cM) were calculated using Kosambi's mapping function. QTL analysis was carried out using the QTL IciMapping software that provided an improved statistical method for QTL mapping, known as called inclusive composite interval mapping (ICIM) (Li *et al.* 2007).

Results

The wild soybean accession JWS156-1 showed higher salt

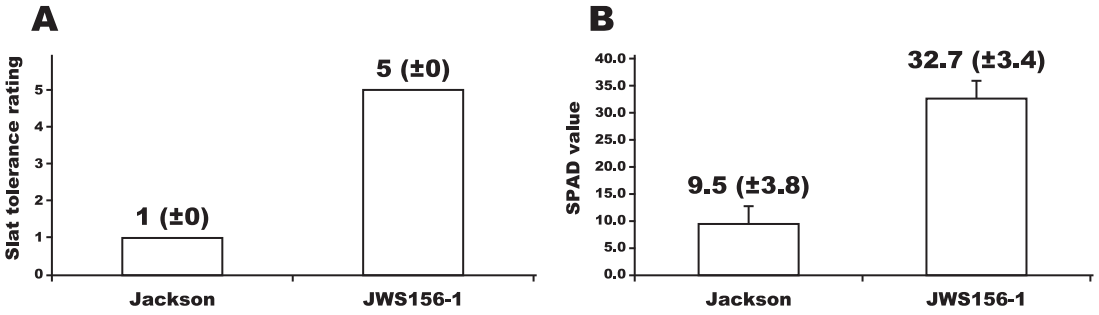

Fig. 1. Comparison of salt-tolerance ratings (STRs) (A) and plant leaf SPAD values (B) between soybean cultivar Jackson and wild soybean accession JWS156-1. Data are shown as the means ± SD (n = 6). Salt-tolerance rating (STR) values ranged from 1 (complete death) to 5 (normal healthy leaves). The SPAD value is proportional to the chlorophyll content in the leaves.

tolerance than the cultivated soybean cultivar Jackson. The STR value of all six replications of JWS156-1 was 5, while that of six replications of Jackson was 1 (Fig. 1-A). The SPAD value of JWS156-1 was significantly higher than that of Jackson (Fig. 1-B). The salt-tolerance performance of the six replications of the two parents also verified the efficiency and reliability of the evaluation method employed in the present study for identification of soybean genotypes with different salt tolerances.

The frequency distribution of the STR and SPAD values of the 225 F_2 plants and their parents is shown in Fig. 2. Both traits showed continuous distribution; however, salt-tolerant plants (i.e. plants with high STR and SPAD value) were predominant. The STR and SPAD values showed significant correlation (r = 0.8423**). QTL analysis revealed a major salt-tolerant QTL on linkage group N where a salt-tolerance QTL had been previously reported in the cultivated soybean (Lee *et al*. 2004). This QTL accounted for 68.7% of the total variance of the STR scale and 49.6% of the total variance of the SPAD values (Table 1 and Fig. 3).

Rating values were also used to develop two phenotypic classifications: salt-sensitive (STR values 1–2.5) and salt-tolerant (STR values 2.5–5). Of the 225 F_2 plants, 173 were classified as salt-tolerant and 52 as salt-sensitive. The segregation fitted a 3:1 ratio ($\chi^2 = 0.4281$, P = 0.5129) and suggested that the observed salt tolerance might be controlled by a single dominant gene.

The SSR marker Satt339 was mapped in the interval region of the salt-tolerance QTL identified in the present study (Fig. 3). In the group of F_2 plants that were homozygous for the JWS156-1 allele (BB) at locus *Satt339*, the average STR was 4.70 ± 0.61. This value was significantly higher than that of the F_2 plants homozygous for the Jackson allele (AA) (1.74 ± 1.05), indicating that the wild parent JWS156-1 contributed to salt tolerance. In the group of plants that were heterozygous (genotype AB) at locus *Satt339*, the average STR was 4.45 ± 0.84. This value was slightly lower than that of plants homozygous for the JWS156-1 allele (BB); however, it was significantly higher than that of plants homozygous for the Jackson allele (AA); therefore, the JWS156-1 allele is partially dominant over the Jackson allele. The SPAD value gave the same result (Table 2).

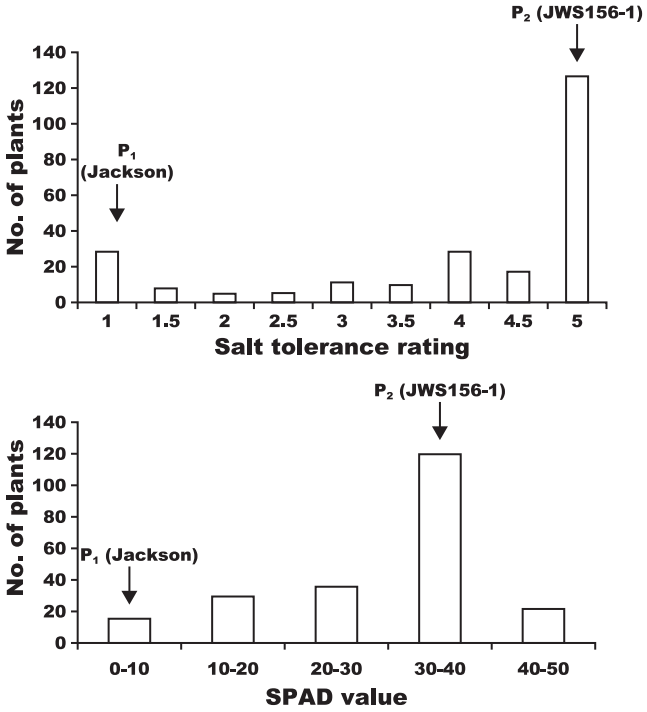

Fig. 2. Frequency distribution of salt-tolerance rating (STR) and plant leaf SPAD values of the 225 F_2 plants derived from a cross between the soybean cultivar Jackson and wild soybean accession JWS156-1. Values of parents are shown by arrows.

Discussion

Lee *et al*. (2004) estimated the most likely position of salt-tolerance QTL to be in the interval region between SSR markers Sat_091 and Satt237 using a mapping population derived from two cultivated soybean cultivars. In our experiment, the Sat-091 marker was monomorphic; however, marker Satt339 and its neighbor, marker Satt237, was located in the QTL interval region, suggesting that the salt-tolerance QTL detected in the inter-specific mapping population was the same QTL that was identified in the cultivated soybean. This can be attributed to the fact that the cultivated soybean inherited the salt-tolerant gene from wild soybean during the domestication process. Wang *et al*. (2004) tried to identify

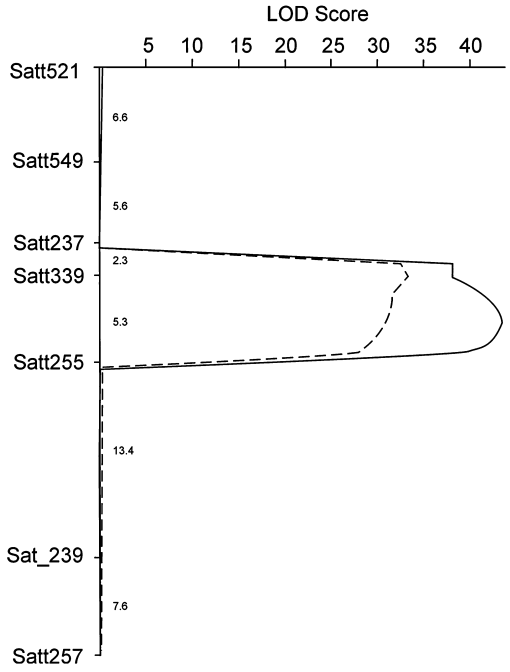

Fig. 3. QTL LOD score plots for salt-tolerance rating (STR) (solid line) and plant leaf SPAD values (dotted line) of the 225 F_2 plants derived from a cross between the soybean cultivar Jackson and wild soybean accession JWS156-1.

new QTLs for the yield by using interspecific soybean backcross populations; however, their results showed that of the 16 significant QTLs identified for yield, maturity, plant height, and lodging, only 2 minor QTLs on linkage group E had not been previously identified in cultivated soybean. They concluded that most of the useful QTLs from wild soybean, such as high protein, SCN resistance, and yield, were already present in cultivated soybean. This was not surprising since wild soybean is the progenitor of cultivated soybean and, as suggested by Liu *et al*. (2007), some useful genes from wild soybean have been repeatedly introduced into cultivated soybean and have been retained in a variety of cultivated soybean landraces.

Salt tolerance in plants is considered a complex trait (Foolad 2004, Flowers 2004). DNA marker analysis has enabled tagging of salt-resistance genes (or QTLs) in many plant species, such as wheat, tomato, *Arabidopsis* (Ma *et al*. 2007, Foolad and Chen 1999, Quesada *et al.* 2002). In rice, the salt-tolerant QTL *SKC1* involved in regulating K^+/Na^+ homeostasis under salt tolerance has been isolated by map-based cloning (Ren *et al*. 2005), showing the usefulness and competency of QTL analysis for understanding complex agronomy traits, such as salt tolerance. Accurate evaluation of soybean salt tolerance in a field is difficult because the distribution of salt concentration is uneven in the field. This makes DNA marker-assisted selection (MAS) particularly useful for improving salt tolerance in a breeding program. Lee *et al*. (2004) indicated that SSR markers associated with salt tolerance (such as Satt237 and Sat-091) could be used for marker-assisted selection. The present study further confirmed the association between SSR markers and salt tolerance in the wild soybean. Moreover, we also found that SSR marker alleles (Satt255, Sat-091 and Satt339) in wild soybean JWS156-1 were different from those of the soybean cultivar S-100 (data not shown). This result suggested that salt tolerance was not necessarily always associated with the S-100 allele reported by Lee *et al*. (2004). In order to use the markers as selection markers in a soybean breeding program, verification of the relationship between specific SSR-marker alleles and salt tolerance is needed.

Based on pedigree tracing, Lee *et al*. (2004) assumed that the QTL was likely to be the *Ncl* locus initially reported by Abel (1969). The present study confirmed the salt-tolerance QTL and revealed that the tolerance allele had a large dominant effect on the sensitive allele, and that the observed salt tolerance was most likely controlled by a single partially dominant gene. This result further supported the QTL detected in both wild and cultivated soybeans corresponding to the *Ncl* locus.

Table 1. QTLs for salt-tolerance rating (STR) and plant leaf SPAD value observed in a F_2 population derived from a cross between soybean (*G. mas*) cultivar Jackson and wild soybean (*G. soja*) accession JWS156-1

Trait	Nearest marker[a]	LOD	PVE (%)[a]	Additive[b]	Dominance[c]
STR	Satt339	43.4	68.7	−1.5	1.4
SPAD value	Satt339	33.4	49.6	−9.8	7.7

[a] Percentage of variance explained by the QTL.
[b] Additive effect of alleles of Jackson.
[c] Estimated dominance effect.

Table 2. Salt-tolerance rating (STR) and SPAD values for F_2 plants with Jackson (AA), JWS156-1 (BB), and heterozygous (AB) genotypes at the *Satt339* locus. The F_2 population was derived from a cross between soybean (*G. mas*) cultivar Jackson and wild soybean (*G. soja*) accession JWS156-1

Genotype	No. of plants (n)	Salt-tolerance rating (STR) Mean ± SD	SPAD value Mean ± SD
AA (Jackson type)	39	1.74 ± 1.05**	14.22 ± 8.09**
AB (Heterozygous type)	111	4.45 ± 0.84*	32.76 ± 6.65*
BB (JWS156-1 type)	65	4.70 ± 0.61	35.16 ± 6.91

* and **: Significant differences (T-test) at 5% and 1% levels when compared with plants having wild soybean JWS156-1 genotype (BB).

Although the salt-tolerance QTLs detected in wild and cultivated soybeans were mapped to the same genomic region, we have no evidence to state that salt tolerance in wild and cultivated soybeans is controlled by the same gene. Luo *et al*. (2005) studied the mechanism of salt tolerance for wild and cultivated soybeans. They observed that under salt stress, the salt tolerance of *G. soja* accessions was due to their successful withholding of Na^+ in roots and stems to decrease its content in leaves. Contrastingly, the salt tolerance of cultivated soybean was mainly due to the successful withholding of Cl^- in roots and stems to decrease its content in leaves. Further studies addressing the salt tolerance mechanisms of QTLs from wild and cultivated soybean are needed.

In conclusion, our results revealed that the major salt-tolerance QTL on linkage group N has a large dominant effect. This QTL is conserved in wild and cultivated soybeans. Markers Satt237, Satt339 and Satt255, which are closely associated with the salt-tolerance QTL, could be used for MAS in a soybean breeding program to improve salt tolerance.

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