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A major QTL conditioning salt tolerance in S-100 soybean and descendent cultivars

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Abstract Deployment of salt tolerant cultivars is an effective approach to minimize yield loss in a saline soil. In soybean, *Glycine max* (L.) Merr., substantial genetic variation exists for salt response. However, breeding for salt tolerance is hampered because no economically viable screening method has been developed for practical breeding. To facilitate the development of an effective screening method for salt tolerance in soybean, the present study was conducted to determine the heritability of salt tolerance and to identify associated quantitative trait loci (QTL). $F_{2:5}$ lines from the cross of 'S-100' (salt tolerant) × 'Tokyo' (salt sensitive) were evaluated in a saline field in Hyde County, N.C., USA, in 1999 and in a greenhouse located in Raleigh, N.C., USA, in 2001. S-100 and Tokyo are ancestors of popular soybean cultivars released for the southern USA. The visual salt tolerance ratings of the $F_{2:5}$ lines ranged from 0 (complete death) to 5 (normal healthy appearance). The entry-mean heritability for salt tolerance was 0.85, 0.48, and 0.57 in the field (four replications), greenhouse (two replications), and combined environments, respectively. The genotypic correlation between field and greenhouse ratings was 0.55, indicating reasonably good agreement between the two screening environments. To identify QTL associated with salt tolerance, each line was characterized with RFLP markers and an initial QTL single-factor analysis was completed. These results were used to identify genomic regions associated with the trait and to saturate the selected genomic regions with SSR markers to improve mapping precision. Subsequently, a major QTL for salt tolerance was discovered near the Sat_091 SSR marker on linkage group (LG) N, accounting for 41, 60, and 79% of the total genetic variation for salt tolerance in the field, greenhouse, and combined environments, respectively. The QTL allele associated with tolerance was derived from S-100. Pedigree tracking was used to examine the association between the salt tolerance QTL and flanking SSR marker alleles in U.S. cultivars descended from S-100 or Tokyo through 60 years of breeding. The presence of alleles from S-100 at the Sat_091 and Satt237 marker loci was always associated with salt tolerance in descendants. Alleles from Tokyo for these same markers were generally associated with salt sensitivity in descendent cultivars. The strong relationship between the SSR marker alleles and salt tolerance suggests that these markers could be used for marker-assisted selection in commercial breeding.

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Introduction

As agricultural land is increasingly salinized through inefficient fertilizer practices, salt-water intrusion, and use of poor quality irrigation water, development of salt tolerant cultivars becomes increasingly important as a means of combating salt-related yield losses (Abel and MacKenzie 1964; Epstein 1985; Maftoun and Sheibany 1979; Parker et al. 1983; Srivastava and Hemantaranjan 1998; Walthall et al. 1993; Winicov 1998). Many quantitative trait loci (QTL) have been identified which

condition plant responses to salt stress, including traits such as vegetative growth, plant survival, and ion transport and selectivity, which eventually affect fruit yield and weight (Ellis et al. 2002; Flowers et al. 2000; Foolad and Chen 1999; Koyama et al. 2001). Foolad et al. (1997) reported that eight genomic regions were identified on seven chromosomes bearing genes associated with salt tolerance in tomato (*Lycopersicum esculentum*) by using restriction fragment length polymorphism (RFLP) markers. Both the salt tolerant and relatively more salt sensitive tomato parents contributed favorable alleles at QTL on different chromosomes. Breto et al. (1994) identified six markers on six chromosomes associated with fruit traits in tomato under saline conditions. In rice (*Oryza sativa*), a total of 16 amplified fragment length polymorphism (AFLP) markers on four chromosomes were associated with ion concentration in the shoot (Flowers et al. 2000).

In soybean [*Glycine max* (L.) Merr.], salinity stress inhibits seed germination and seedling growth, reduces nodulation, and decreases biomass accumulation and seed yield (Abel and MacKenzie 1964; Essa 2002; Katerji et al. 1998; Saraj et al. 1998; Singleton and Bohlool 1984; Wang and Shannon 1999). These effects are induced by osmotically mediated interference with water and nutrient uptake (Brady and Weill 2002). Salinity stress can also cause severe leaf chlorosis, leaf bleaching and necrosis (known as leaf scorching), and ultimately plant death (Abel 1969; Parker et al. 1983, 1987). These more acute salinity stress symptoms are induced by chloride accumulation in the leaf (Abel and MacKenzie 1964; Yang and Blanchar 1993). Adverse physiological effects of chloride accumulation include decreased photosynthesis and formation of superoxide radicals, which cause membrane damage (Marschner 1995). Chloride is a major element in salts derived from fertilizer and sea water (Abel 1969; Parker et al. 1983).

Soybean is classified as a salt-sensitive glycophyte (Launchli 1984), and, thus far, no genotypic variation in response to the deleterious osmotic effects of salt has been reported. However, many soybean genotypes are resistant to the 'non-osmotic' acutely toxic effects of chloride per se. In four independent studies, 33 of 66, 6 of 16, 19 of 60, and 10 of 257 U.S. cultivars and breeding lines were identified as resistant to chloride, based on visual leaf-scorching ratings and/or reduced chloride levels in the leaf (Parker et al. 1983, 1986; Shao et al. 1995; Yang and Blanchar 1993). Xu et al. (1999) reported that eight Chinese landraces of soybean had high levels of salt tolerance. Some accessions of the wild progenitor of soybean (*G. soja* Sieb. and Zucc.), and the more distantly related perennial accessions have also been classified as chloride excluders or as salt resistant (Li et al. 2000; Pantalone et al. 1997; Wang et al. 1997; Zhang et al. 1999). Six randomly amplified polymorphism DNA (RAPD) markers were reported to be associated with salt tolerance in *G. soja*, but the genomic location of these markers was not ascertained (Zhang et al. 1999).

Reduced leaf scorching symptoms correlated well with reduced leaf chloride levels in many of the above studies, leading to the commonly used description of resistant types as chloride excluders. Chloride exclusion is controlled by a single dominant allele (*Ncl*) that was identified in the landmark cultivar 'Lee', released in 1953 for the southern USA (Abel 1969). Lee was derived from the cross of two major ancestors of southern U.S. cultivars, 'CNS' and 'S-100' (Carter et al. 2004). Recent field trials have demonstrated that S-100 is the source of the major chloride exclusion allele in Lee and that CNS is salt sensitive (T.E. Carter Jr., unpublished data). The genomic location of the *Ncl* allele has not been reported.

Microsatellites or simple sequence repeat (SSR) markers are highly polymorphic in soybean and currently applicable technologies associated with the marker (fluorescent labeling, multiplexing PCR products, and automated allele sizing) allow their high-throughput application (Diwan and Cregan 1997; Mitchell et al. 1997). As a result, DNA markers have been increasingly integrated into soybean improvement programs. Marker-assisted breeding has been proposed to accelerate the development of abiotic stress-tolerant cultivars (Cushman and Bohnert 2000; Khush 1999; Winicov 1998). There are few commercial examples of marker-assisted breeding for abiotic stress tolerance in soybean to date, however.

Although as many as 20% of soybean cultivars released for the southern USA have economic levels of salt tolerance, no economically viable screening method for salt tolerance has been developed for practical breeding use. As a result, salt tolerance determinations are made typically only at the time of cultivar release (Shannon and Carter 2003). For these reasons, salt tolerance may be a particularly good candidate for adaptation to marker-assisted breeding. A prerequisite for such a breeding effort is knowledge of the genomic location of the major gene which reportedly conditions salt tolerance in soybean (Abel 1969). To attain this knowledge, the objectives of this study were to: (1) estimate the heritability of salt tolerance in a population derived from the cross of S-100 (salt-tolerant) by Tokyo (salt-sensitive), (2) use RFLP and SSR markers to identify the major QTL associated with salt tolerance, (3) use SSR markers closely linked to salt-tolerant QTL to track the transmission of marker alleles from ancestor S-100 and Tokyo to descendent southern cultivars, and associate these alleles with the salt tolerance of the descendent cultivars.

Materials and methods

Phenotypic evaluation of salt tolerance

Genotypic materials

A total of 106 F_2-derived lines from the population S-100 × Tokyo were selected for phenotypic evaluation of salt tolerance. The S-100 × Tokyo population has been evaluated previously to discover an allele for water use

efficiency in S-100 (Mian et al. 1998). The 106 F_2-derived lines, plus Tokyo (salt sensitive), S-100 (salt tolerant), 'Lee 74' (salt tolerant cultivar near isogenic to Lee, and derivative of S-100; Abel 1969) and 'Essex' (salt sensitive; Yang and Blanchar 1993) were evaluated visually for leaf chlorosis and scorching in a naturally saline field site at Hyde County, N.C., USA, in 1999 and in a greenhouse at Raleigh, N.C., USA, in 2001. The F_2-derived lines were tested in the $F_{2:5}$ and later generations of inbreeding to minimize dominance effects on phenotypic ratings.

Field experiment

The 'on farm' field site provided by farmer Ronnie Swindell was selected because it is less than 1 m elevation above sea level, less than 1 km from the brackish waters of the Pamlico Sound, and has been repeatedly subjected to salt water flooding as a result of storm tides induced by hurricanes which commonly strike the Atlantic Coast. Prior to planting, the loamy Argent-series soil (fine, mixed, thermic, Typic, Endoaqualfs) was evaluated for salinity levels using a soluble salt index and received a score of 80, indicating that salt levels were sufficiently high to cause plant injury or death in soybean (D. Hardy 2003, personal communication, North Carolina Department of Agriculture). Additionally, white crusts of salt were occasionally observed on the soil surface, which is the typical characteristic of saline soil having an electrical conductivity ≥4.0 dS m^{-1} (U.S. Salinity Laboratory 1954). The soybean lines for the field experiment were planted on 7 June 1999. The experimental unit consisted of a single 2-m row, with approximately 20 plants/m after emergence. Plots were rated visually for leaf chlorosis and scorching on 18 and 29 August. Mean scores over the two dates were used in all subsequent analyses (Table 1). Visual ratings were scored on a 0 to 5 scale (0, all plants dead; 3, plants with light green or chlorotic appearance; and 5, plants with normal green leaves). The experimental design was a randomized complete block with four replicates.

Greenhouse experiment

Plants were grown in 27 cm diameter pots filled to a depth of 20 cm with builder's grade washed sand. Ten seed per pot were sown on 11 September 2001. At day 7 after emergence, plants were thinned to six per pot. Six genotypes germinated poorly and were discarded from the study. Nutrient solution was applied daily. At 14 days after emergence, 100 mM NaCl was added to the nutrient solution and this modified saline nutrient solution was applied daily for the remainder of the experiment. Application of saline nutrient solution to sand media causes salt accumulation over time and substantially increases the effective salt concentration in the root zone. To minimize this effect and maintain a stable salt concentration in the pots, the following protocol was adopted at 14 days after emergence. Each morning the pots were flushed with 1 liter of water to leach excess salts accumulated in the sand media. After draining for approximately 1 h, each pot received 500 ml of the saline nutrient solution. The photoperiod was extended to 18 h using incandescent lighting at the initiation of the experiment to prevent flowering.

Table 1 Mean scores for visual salt tolerance ratings, rating ranges, and heritability of F_2-derived lines in the field, greenhouse, and combined over environments. The scoring scale ratings range from 0, representing plant death, to 5, representing green leaves and a healthy appearance. The progeny F_2-derived lines are from the hybridization of the salt-tolerant parent S-100 and the salt-sensitive parent Tokyo. The estimation of the entry-mean heritability (ratio of genotypic to phenotypic variance) is based on the mean of four and two replications in the field and greenhouse, respectively

Source	Rating		
	Field	Greenhouse	Combined
Parents			
S-100	3.1	5.0	4.1
Tokyo	0.1	1.0	0.6
Controls			
Lee 74 (tolerant)	3.8	4.3	4.0
Essex (sensitive)	0.7	2.2	1.4
Progeny mean	1.7	2.4	2.0
Progeny range	0.0–5.0	0.2–5.0	0.1–4.9
LSD (0.05)	1.41	2.39	2.16
Heritability	(0.85)	(0.48)	(0.57)

The macronutrient composition of the nutrient solution was a modification of that employed by McClure and Israel (1979), and the micronutrient composition was a modification of that described by Ahmed and Evans (1960). The composition of the nutrient solution was: 4 mM $CaSO_4{\cdot}2H_2O$, 0.5 mM KH_2PO_4, 3 mM KNO_3, 2 mM $MgSO_4{\cdot}7H_2O$, 18 μM $FeSO_4{\cdot}7H_2O$, 18.9 μM KCl, 9.3 μM H_3BO_3, 0.9 μM $MnSO_4{\cdot}H_2O$, 0.9 μM $ZnSO_4{\cdot}7H_2O$, 0.18 μM $CuSO_4{\cdot}5H_2O$, and 0.18 μM $(NH_4)_6Mo_7O_{24}$. The pH of the solution was maintained at 6.2 by adding 1.0 M KOH. The electrical conductivity for the 100 mM NaCl nutrient solution was approximately 10.0 dS m^{-1} as determined using a 250A conductivity meter (Orion, Boston, Mass., USA).

Visual ratings of leaf scorching and chlorosis were taken 45 day after emergence (Table 1). The rating scale used in the greenhouse experiment was the same as for the field, with the exception that each plant in the pot was individually rated and then averaged to produce a final score for each pot. The experimental design was a randomized complete block with two replicates.

Statistical analysis of phenotypic data

All phenotypic data were analyzed using the PROC GLM procedure (SAS Institute 2001). Environment, replication, and genotype were considered random effects. Analysis of variance was computed for each environment individually and then combined over environments (Table 2). Geno-

type, genotype × environment, and error variance components (σ^2_g, σ^2_{ge}, and σ^2_e) were calculated from these analyses based on expected mean squares. Entry-mean heritability (h^2) of salt ratings within each test environment was defined as $h^2=\sigma^2_g/(\sigma^2_g+\sigma^2_e/r)$, where r is the number of replications (Falconer 1976). Entry-mean heritability over environments was defined as $h^2=\sigma^2_g/(\sigma^2_g+\sigma^2_{ge}/l+\sigma^2_e/lr)$, where l is the number of environments, and calculated as [(mean square progeny − mean square progeny × environment)/mean square progeny]. Dominance variance was assumed to be negligible in the calculation of h^2 because of the inbred nature of the population ($F_{2:5}$). The genetic correlation (r_g) between the field and greenhouse environment was calculated as described by Eisen and Saxton (1983) and Campbell and Carter (1990).

RFLP and SSR marker data collection

DNA from 10 to 12 plants from each of the $F_{2:5}$ lines and the two parents was extracted from the first trifoliolate leaves grown in the absence of salt stress in the greenhouse using the modified CTAB (Hexadecyltrimethylammonium bromide) procedure of Keim et al. (1988). Data for 116 polymorphic RFLP markers were collected using Southern blotting and hybridization as described previously (Mian et al. 1998). For the 32 SSR markers, PCR reactions were prepared based on the protocol by Diwan and Cregan (1997) with slight modifications as follows. The primers were labeled with fluorescent dyes of 6-FAM, NED, or HEX. The PCR reactions were performed in a dual 384-well or a 96-well GeneAmp PCR System 9700 (PE-ABI, Foster City, Calif., USA). A loading sample for each lane of a gel was prepared with loading mixture (2.0 μl of deionized formamide, 2.0 μl of loading buffer, 0.3 μl of Genescan Rox-500) and 1–2 μl of the PCR products. After the loading sample was denatured at 95°C for 5 min, the sample (~1.0 μl/lane with micro syringes) was run on a 4.8% (w/v) polyacrylamide gel in 12-cm plates with 1× TBE (Tris base, boric acid, and EDTA) running buffer using an ABI PRISM 377 DNA Sequencer (PE-ABI) at 750 V for approximately 2 h. The marker fragments were analyzed with GeneScan and scored with Genotyper software (PE-ABI).

Linkage map and QTL analysis

An initial genetic linkage map was constructed with the RFLP marker data using the Kosambi map function of Gmendel (Holloway and Knapp 1993; Kosambi 1944). The association of the RFLP markers with field ratings for salt tolerance was assessed by single-factor analysis of variance (SF-ANOVA) using the PROC GLM procedure (SAS Institute 2001). The RFLP markers significantly ($P<0.05$) associated with field salt tolerance were used as the basis to select an additional 32 linked SSR markers from soybean genetic linkage map for further population screening, and more detailed QTL analysis. These SSR markers were known to map within 20 cM of the identified RFLP markers, based on the consensus public soybean genetic linkage map (Cregan et al. 1999). The SSR markers were incorporated into the linkage analysis of the population and then subjected to SF-ANOVA. The SSR markers identified as significantly ($P\leq0.01$, Table 3) associated with salt tolerance via SF-ANOVA were then incorporated into a two-stage multiple regression analysis using the PROC REG procedure (SAS Institute 2001). In the first stage, each linkage group was analyzed separately to identify all SSR markers which could be retained ($P\leq0.001$) in a model for that particular linkage group (Table 4). In the second stage, all markers identified in

Table 2 Analysis of variance of the salt tolerance ratings for the field, greenhouse, and combined environments. The scoring scale ratings range from 0, representing plant death, to 5, representing green leaves and a healthy appearance. The progeny F_2-derived lines are from the hybridization of the salt-tolerant parent S-100 and the salt-sensitive parent Tokyo. Tolerant Lee 74 and sensitive Essex were used as control cultivars

Source	Environment					
	Field		Greenhouse		Combined	
	DF	Mean square (rating^2)	DF	Mean square (rating^2)	DF	Mean square (rating^2)
Environment					1	113.86*
Replication	3	70.65**	1	0.06	3	66.44**
Genotype	109	6.67**	103	3.00**	103	5.99*
Progeny	105	6.52**	99	2.88**	99	5.64**
Parent	1	13.50**	1	15.84**	1	29.23**
Control	1	23.51**	1	6.09**	1	23.91**
Genotype × environment					103	2.36**
Progeny × environment					99	2.43**
Parent × environment					1	0.58
Control × environment					1	0.81
Error	285	1.00	104	1.46	375	1.11

*p=0.05; **p=0.01

Table 3 DNA markers associated with visual salt tolerance ratings in the field, greenhouse, and combined environments based on single factor analysis of variance ($P \leq 0.01$). *S/S* Homozygous allele from S-100, *S/T* alleles from S-100 and Tokyo, *T/T* homozygous allele from Tokyo. The scoring scale ratings range from 0, representing plant death, to 5, representing green leaves and a healthy appearance

Linkage group	Markers	Field (rating)				Greenhouse (rating)				Combined (rating)			
		R^2 (%)	S/S	S/T	T/T	R^2 (%)	S/S	S/T	T/T	R^2 (%)	S/S	S/T	T/T
L	Cr354a	23	2.5	1.2	2.6	–	–	–	–	16	2.6	1.8	2.7
L	Satt166	9	1.9	1.4	2.4	–	–	–	–	6	2.2	1.9	2.5
N	Satt339	29	2.7	2.0	0.6	22	3.4	2.4	1.7	37	3.0	2.3	1.2
N	Satt237	32	2.7	2.0	0.6	22	3.4	2.5	1.7	38	3.0	2.3	1.1
N	Sat_091	35	2.6	2.1	0.6	29	3.5	2.5	1.6	45	3.1	2.3	1.1
N	Cr354b	25	2.7	1.9	0.7	16	3.0	2.6	1.6	29	2.8	2.3	1.1

stage 1 were incorporated into a multiple regression analysis across linkage groups (Table 4). Map Manager QTX (MMQ) was used to determine the likeliest position of the QTL retained in the final multiple regression analysis (Meer et al. 2002). The analysis employed the default values with various options including intercross for crossing type, linkage criterion at P=0.05, and Kosambi map function. The MMQ generally calculates the likelihood ratio statistic (LRS) for the association of the trait with an individual marker locus, which is converted to the LOD (log 10 of the odds ratio) by dividing the LRS value by 4.61 (Meer et al. 2002; Tanksley et al. 1989). To establish empirical significance thresholds for significance of the identified QTL, a permutation test was conducted for each rating of salt tolerance from the different environments in 1 cM steps for 1,000 permutations and the highly significant threshold (P=0.001) was used to determine the existence of a QTL.

Pedigree tracking

A total of 27 soybean cultivars descended from the U.S. soybean ancestors S-100 or Tokyo were screened for their SSR alleles at two marker loci which were closely linked to a major QTL for salt tolerance. Salt tolerance ratings for these descendent cultivars were obtained from field observations in Hyde County, N.C., USA (the same field location where the present QTL mapping study was conducted, T.E. Carter 2003, unpublished data), from Parker et al. (1986), or from the USDA Germplasm Resource Information Network (USDA GRIN). Pedigrees were obtained from Bernard et al. (1988) and from cultivar release notices.

Results

Phenotypic variation for salt tolerance

Visual ratings of salt tolerance for the F_2-derived lines ranged from a rating of 0 (complete death) to 5 (normal green leaves with healthy appearance) in both field and greenhouse environments (Fig. 1 and Table 1). The phenotypic correlation of genotypic means and the genotypic correlation between the field and greenhouse were 0.39 and 0.55, respectively (significant at $P \leq 0.01$). In both environments, S-100 exhibited superior salinity tolerance to both Tokyo and the salt-sensitive control cultivar Essex (Table 1). The salt-tolerant control cultivar Lee 74, derived from S-100, also exhibited significantly higher salt tolerance than did Tokyo or Essex. The heritability of salt tolerance based on entry means was 0.85, 0.48, and 0.57 in the field (four replications), greenhouse (two replications), and combined environments, respectively.

Analysis of variance indicated a significant ($P \leq 0.01$) genotype × environment (G × E) interaction for the F_2-derived lines, but not for parents and control genotypes (Table 2). In both environments, significant differences among genotypes were present for salt tolerance. Although there was a significant G × E interaction, genotype differences among the progeny were evident when the G ×

Table 4 DNA markers associated with visual salt tolerance ratings in the field, greenhouse, and combined over environments based on multiple regression analysis ($P \leq 0.001$) within and among linkage groups. The multiple regression analysis among linkage groups included all the significant markers based on the single-factor analysis of variance ($P \leq 0.001$)

Environment	Marker	LG	SF-ANOVA		Multiple regression		Positive allele
			R^2 (%)	P-value	R^2 (%) within LG	R^2 (%) among LGs	
Field	Sat_091	N	35	<0.0001	30	30	S-100
	CR354b	N	25	<0.0001	3	–	S-100
Greenhouse	Sat_091	N	29	<0.0001	29	29	S-100
Combined	Sat_091	N	45	<0.0001	42	44	S-100

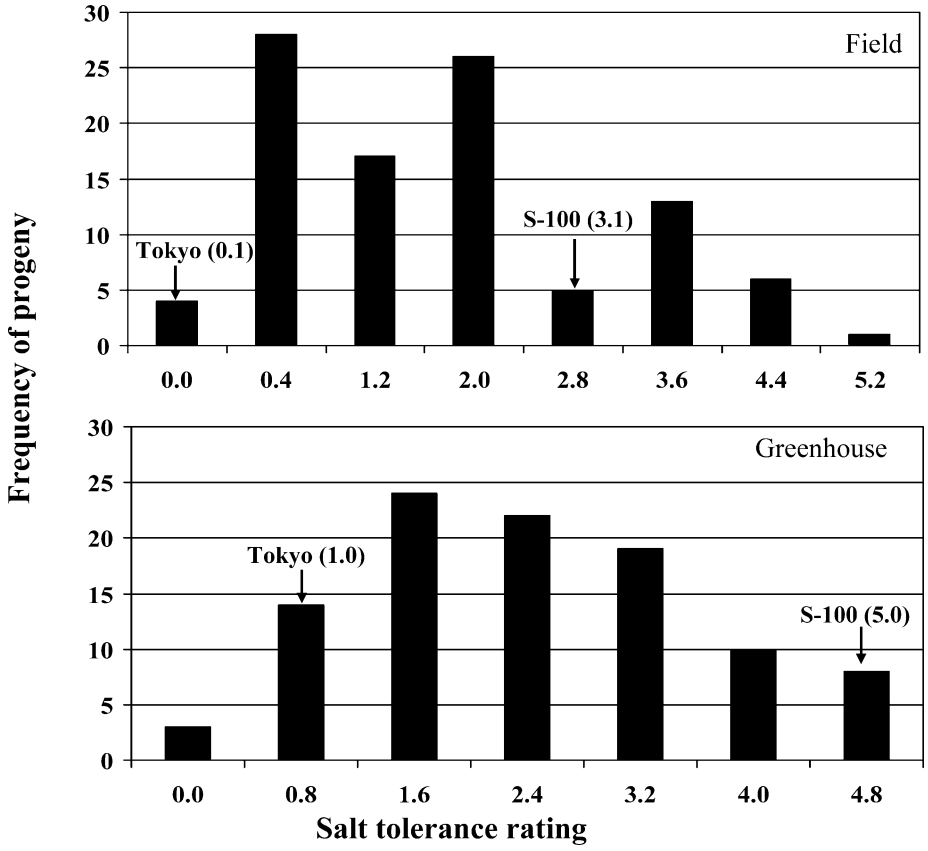

Fig. 1 Salt tolerance ratings of S-100, Tokyo, and the distribution of 100 F_2-derived progeny grown in the field (*top*) and greenhouse (*bottom*) environments

E mean square was used as the error term for progeny in the *F*-test.

Genetic map and QTL analysis

Visual ratings of salt tolerance from the field study were associated with RFLP markers on linkage groups L and N ($P \leq 0.05$). These results were used to select an additional 32 SSR markers for more detailed linkage analysis of the S-100 × Tokyo population. The linkage map of the population, which included the 32 SSR markers, indicated that there was close agreement between the order and map placement of SSR markers in comparison to previously published public genetic linkage map in soybean (Cregan et al. 1999).

The SF-ANOVA identified DNA markers putatively associated with salt tolerance ($P \leq 0.01$) on LG-L in the field and combined over environments, and LG-N in the field, greenhouse, and combined over environments (Table 3). Multiple regression analyses within linkage group and across linkage groups indicated that Sat_091 on LG-N was the most important marker associated with salt tolerance (Table 4). When the marker and phenotypic data were analyzed with Map Manager QTX, the QTL for salt tolerance was closely linked to the markers on LG-N identified by SF-ANOVA (Fig. 2). The most likely position of the major QTL on LG-N was in the 3.6 cM interval between SSR markers Satt237 and Sat_091 based on the field, greenhouse, and combined environments. The LOD scores for the QTL were 9.4, 7.2, and 12.9 for the field, greenhouse, and combined environments, indicating the existence of the QTL at $P=0.001$ (Fig. 2). At its most likely position in the interval Satt237-Sat_091, the QTL accounted for 35, 29, and 45% of the total phenotypic variation in visual salt ratings in the field, greenhouse, and combined environments, respectively.

Pedigree tracking

Based on the QTL identified with the S-100 × Tokyo mapping population, the descendents of U.S. ancestors S-100 and Tokyo were assayed with the Sat_091 and Satt237 markers, which were the SSR markers most closely linked to the major gene for salt tolerance (Fig. 3 and Table 5). Descendent U.S. cultivars and breeding lines from these two ancestors which had known phenotypic salt responses were represented as gray (salt-tolerant) or white (salt-sensitive) in the figure, with intermediate tolerance represented as brick-pattern background (Fig. 3).

For the Sat_091 marker, Tokyo possessed a 153-bp band and S-100 a 159-bp band. Other band sizes detected at the Sat_091 locus were 194 and 199 bp. For the Satt237 marker, the Tokyo and S-100 alleles were 252 and 240 bp, respectively, and other bands detected were 237, 274, 275, 278, and 280 bp. All descendants that had the same allele sizes as S-100 at the Sat_091 and Satt237 loci appeared to be salt tolerant (11 out of the 13 tolerant descendants). Other tolerant lineages of 'Hill', 'Dyer', and 'Forrest' had

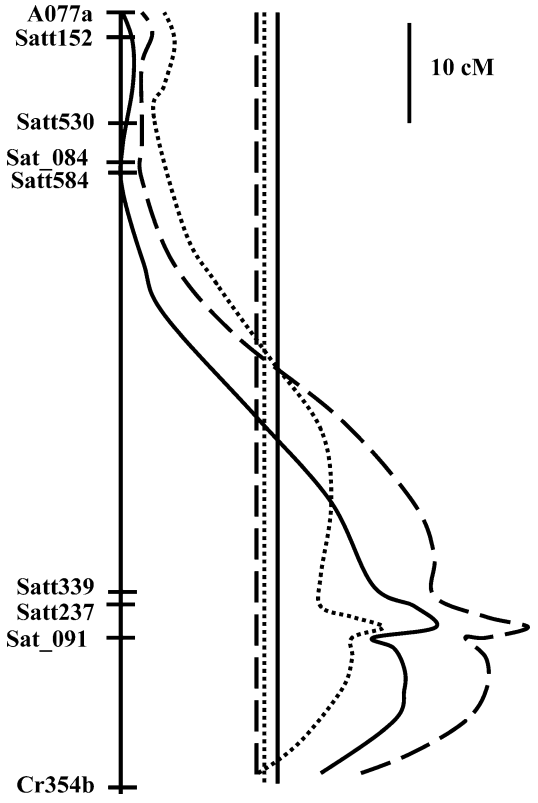

Fig. 2 Linkage map and the QTL likelihood plots indicating salt tolerance QTL estimated in the field (*solid line*), greenhouse (*dotted line*), and combined environments (*dashed line*) on LG-N. The *horizontal lines* in this figure indicate highly significant threshold levels (P=0.001) based on permutation tests, corresponding to LOD scores=4.7, 3.9, and 3.7 in the field (*solid line*), greenhouse (*dotted line*), and combined environments (*dashed line*), respectively

allele sizes of 159, 194, or 199 at Sat_091 and 274 and 278 bp at Satt237 (Table 5 and Fig. 3). It is likely that the salt tolerance and SSR marker alleles in these three cultivars are derived from an ancestor other than S-100 (i.e. 'Dunfield'). All descendants that had the same allele sizes as Tokyo at the Sat_091 and Satt237 loci appeared to be salt sensitive (11 out of 12 sensitive descendants). Descendents that had the 159 bp band at the Sat_091 locus, and the 280 bp band at the Satt237 locus were found to be intermediate ('Cook' and 'Young') or sensitive ('Davis') (Table 5 and Fig. 3). These alleles were probably derived from ancestors other than Tokyo or S-100 (i.e. 'Ralsoy' or 'Roanoke').

Discussion

The superior salt tolerance of S-100 was expressed in both the field and greenhouse environments (Fig. 1). Salt tolerance identified in the progeny of S-100 × Tokyo was also expressed reasonably well in both field and greenhouse environments (the genotypic correlation was 0.55), indicating that the two environments can be used effectively for salt tolerance screening. The higher heritability in the field (0.85) than in the greenhouse environment (0.48) indicated that differences among the F_2-derived lines were more consistently expressed in the field, which was related to the increased replication number, larger experimental unit size (field row plot versus greenhouse pot), and greater uniformity of the field site in comparison to the greenhouse.

The results from SF-ANOVA, multiple regression analyses, and interval mapping demonstrated the existence of a major QTL on LG-N for salt tolerance. This QTL, flanked by markers Satt237 and Sat_091, accounted for 41, 60, and 79% of the total genetic variation (percentage phenotypic variation explained by the QTL divided by the heritability) for the field, greenhouse, and combined data, respectively. The identification of this major QTL in two

Table 5 Salt tolerance rating of ancestral cultivars derived from S-100 or Tokyo and their allele size (bp) at the Sat_091 and Satt237 loci. Salt tolerance ratings are from T.E. Carter Jr. (unpublished data, 2003) (*C*), USDA GRIN (*G*), or Parker et al. (1986) (*P*). Ratings by T.E. Carter were based on scales of tolerant (>3.0), intermediate (2.5–3.0), and sensitive (<2.5). Note that based on recent data collected in the same field used for this mapping population, the USDA Genetic Resource Information Network (GRIN) ratings for CNS and Hill are incorrect and that CNS is salt sensitive and Hill is salt tolerant. *T* Tolerant, *I* intermediate, *S* sensitive

Ancestor	Rating	Allele size (bp)	
		Sat_091	Satt237
S-100	$T^{C, G}$	159	240
Centennial	$T^{C, P}$	159	240
Cook	I^{C}	159	275/280
D49-2491	T^{C}	159	240
Dillon	T^{C}	159	240
Forrest	T^{C}	199	274/278
Gordon	T^{P}	159	240
Haskell	T^{C}	159	240
Hill	T^{C}, S^{G}	194/199	274/278
Hutton	T^{P}	159	240
Johnston	T^{C}	159	240
Lee	$T^{C, G, P}$	159	240
Manokin	T^{C}	159	240
Wright	T^{P}	159	240
Young	I^{C}	159	275/280
Tokyo	$S^{C, G}$	153	252
Benning	S^{C}	153	237
Bragg	$S^{C, P}$	153	252
Braxton	S^{P}	153	252
CNS	S^{C}, T^{G}	159	280
Cobb	S^{P}	153	252
Davis	$S^{C, P}$	159	275/280
Essex	S^{C}	153	237/252
GaSoy 17	$S^{C, P}$	153	252
Haberlandt	S^{G}	153	252
Hutcheson	S^{C}	153	237
Jackson	$S^{C, G, P}$	153	252
Ogden	$S^{C, G}$	153	252
Palmetto	$S^{C, G}$	153	252
Volstate	S^{G}	153	252

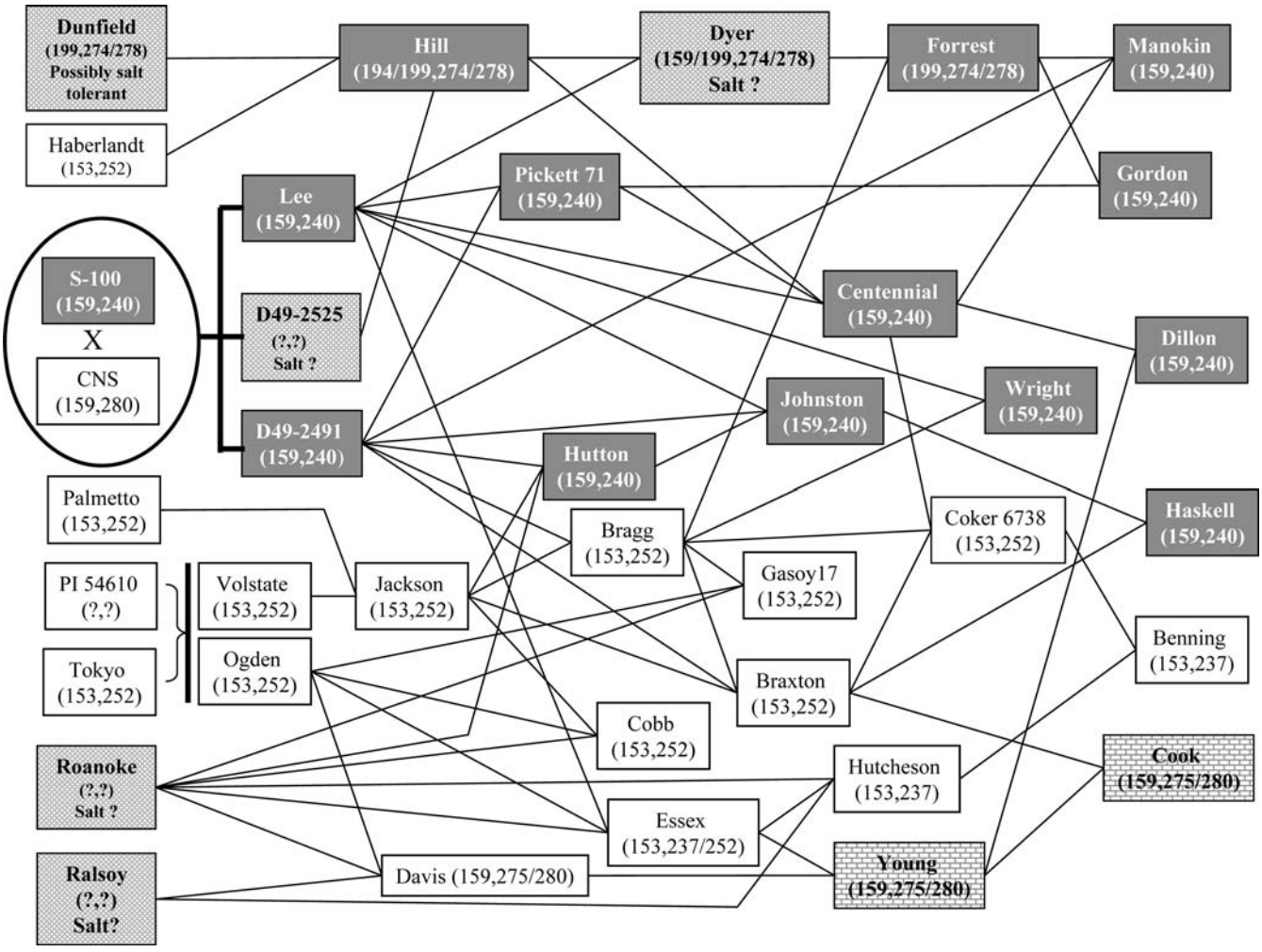

Fig. 3 Partial pedigrees of cultivars derived from S-100 or Tokyo indicating their salt tolerance, *gray* (salt-tolerant), *white* (salt-sensitive), and *brick-pattern* (intermediate), and allele sizes in base pairs (bp) at Sat_091 and Satt237 loci. Accessions having a *checker-pattern* background have an unknown salt reaction. In most cases, *connecting lines* represent lineages with intermediate breeding steps rather than direct crosses, and indicate the probable source of the SSR marker alleles

contrasting environments indicates a consistent expression of a salt tolerance QTL in this soybean population.

Pedigree tracking was used to examine post hoc the association between salt tolerance and SSR markers alleles in U.S. cultivars descended from S-100 over the past 60 years. The purpose was to gain insight into the robustness and durability of these markers as predictors of salt tolerance in practical breeding. The SSR marker (Satt237 and Sat_091) alleles from S-100 which flank the major tolerance QTL were consistently associated with salt tolerance in descendent cultivars and breeding lines. Salt tolerant 'Manokin', 'Gordon', 'Wright', 'Pickett 71', 'D49-2491' and Lee were all derived from both soybean ancestors CNS and S-100 and have the S-100 bands at the two marker loci (159 bp at Sat_091 and 240 bp at Satt237). There was no clear evidence that the linkage between salt tolerance and the SSR markers was broken through generations of breeding. Similarly, the allele in salt-sensitive 'Braxton', 'GaSoy 17', 'Bragg', 'Cobb', 'Jackson', and 'Volstate' lineage appears to be inherited from the ancestor Tokyo, and all have a 153-bp band at Sat_091 and the 252 bp band at Satt237.

Our data indicate that the major QTL on LG-N is likely to be the *Ncl* locus initially reported by Abel (1969). This assumption is based on the results that show S-100 is the source of the salt tolerant allele at the major LG-N QTL in the cultivar Lee. In addition, it appears this major QTL (*Ncl*) was present in most salt tolerant descendants of Lee or its salt tolerant sister line, D49-2491. The consistency of these results suggests the SSR markers identified in this study could be used in marker-assisted selection for salt tolerance in practical breeding. Currently, greenhouse or field screening to identify salt tolerance is more expensive and requires more time to complete than do assays for SSR markers.

Recent greenhouse studies (T.E. Carter 2003, unpublished data) suggest that several ancestors of U.S. soybean cultivars are salt tolerant. Thus, there is a possibility that additional salt tolerance alleles may exist in U.S. breeding populations or that additional SSR marker alleles may be associated with the *Ncl* salt tolerance allele. The pedigree

tracking of the salt tolerant reaction and SSR alleles for the cultivars Hill, Dyer and Forrest suggest this possibility (Fig. 3). The relationship between marker alleles and salt tolerance should be verified within a specific breeding program prior to routine application.

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