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RFLP mapping of QTLs conferring salt tolerance during the vegetative stage in tomato

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Abstract Quantitative trait loci (QTLs) contributing to salt tolerance during the vegetative stage in tomato were investigated using an interspecific backcross between a salt-sensitive *Lycopersicon esculentum* breeding line (NC84173, maternal and recurrent parent) and a salt-tolerant *Lycopersicon pimpinellifolium* accession (LA722). One hundred and nineteen BC_1 individuals were genotyped for 151 RFLP markers and a linkage map was constructed. The parental lines and 119 BC_1S_1 families (self-pollinated progeny of the BC_1 individuals) were evaluated for salt tolerance in aerated saline-solution cultures with the salt concentration gradually raised to 700 mM NaCl + 70 mM $CaCl_2$ (equivalent to an electrical conductivity of approximately 64 dS/m and a water potential of approximately − 35.2 bars). The two parental lines were distinctly different in salt tolerance: 80% of the LA722 plants versus 25% of the NC84173 plants survived for at least 2 weeks after the final salt concentration was reached. The BC_1S_1 population exhibited a continuous variation, typical of quantitative traits, with the survival rate of the BC_1S_1 families ranging from 9% to 94% with a mean of 51%. Two QTL mapping techniques, interval mapping (using MAPMAKER/QTL) and single-marker analysis (using QGENE), were used to identify QTLs. The results of both methods were similar and five QTLs were identified on chromosomes 1 (two QTLs), 3, 5 and 9. Each QTL accounted for between 5.7% and 17.7%, with the combined effects (of all five QTLs) exceeding 46%, of the total phenotypic variation. All QTLs had the positive QTL alleles from the salt-tolerant parent. Across QTLs, the effects were mainly additive in nature. Digenic epistatic interactions were evident among several QTL-linked and QTL-unlinked markers. The overall results indicate that tomato salt tolerance during the vegetative stage could be improved by marker-assisted selection using interspecific variation.

Key words *Lycopersicon esculentum* · *L. pimpinellifolium* · Salt tolerance · Vegetative growth · Restriction fragment length polymorphism (RFLP) · Quantitative trait loci (QTLs)

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Introduction

Salinity is a major environmental constraint to crop production in many regions of the world. Regardless of the cause (ion toxicity, water deficit or nutrient ion imbalance), high salinity in the root zone severely impedes normal plant growth and development, resulting in reduced crop productivity. It is estimated that worldwide more than 13% of the cultivated lands and approximately 33% of irrigated agricultural lands are afflicted by high salinity (Epstein et al. 1980; Flowers et al. 1986). This estimate fails to account for the additional lands that are not considered to be agricultural due to their very high salt concentrations. This would include areas along the seashores in temperate parts of the world and millions of hectares of desert lands in Africa, the Middle East, Asia, and North America. Such lands could be agriculturally productive if more salt-tolerant species or cultivars were available. Moreover, the salinized areas are increasing at a rate of 10% annually; low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices are among the major contributors to the increasing soil salinity (Syversten et al. 1989; Kalaji and Pietkiewica 1993). Most crop species are sensitive to low to moderate levels of salinity (Maas 1986). The cultivated tomato, *Lycopersicon esculentum*, is considered moderately sensitive to salt-stress.

A significant approach to minimizing the deleterious effects of soil salinity entails biological strategies focused upon the exploitation or development of plants capable of tolerating high levels of salt. Significant progress in breeding for salt tolerance is dependent upon an understanding of the genetic and epigenetic control of associated traits. Although considerable information is presently available regarding the physiological and metabolic aspects of salt responsiveness (Levitt 1980; Lauchli and Epstein 1990), research devoted to the characterization of the genetic control of salt tolerance has been very limited (Foolad and Jones 1991; Dvorak et al. 1992; Foolad 1996 a, 1997; Monforte et al. 1997). Insufficient genetic knowledge of tolerance traits has severely restricted breeding efforts for improved salt tolerance of cultivated crops.

Plant response to salt-stress is complex and appears to be controlled by more than one gene and is high ly influenced by environmental variation (Yeo and Flowers 1986; Foolad 1996 b; Foolad et al. 1997). Direct selection in field sites is difficult because uncontrollable environmental factors adversely affect the precision and repeatability of such trials. Furthermore, salt tolerance is a developmentally regulated, stage-specific phenomenon. Tolerance at one stage of plant development is poorly correlated with tolerance at other developmental stages (Greenway and Munns 1980; Shannon 1985; Lauchli and Epstein 1990; Johnson et al. 1992; Foolad and Lin 1997 a). Partitioning of salt tolerance into component traits related to specific ontogenic stages and the identification of the number and magnitude of effects of genes which affect tolerance would contribute to a better understanding of the genetic control of this trait and, hence, facilitate the rapid development of salt-tolerant plants.

Each developmental stage (which may be considered as a separate trait) may require a different screening procedure, and simultaneous or sequential screening may be impractical or impossible. A suggested approach has been the identification and utilization of indirect selection markers which are genetically linked with the trait(s) of interest (Sax 1923; Thoday 1961; Thompson and Thoday 1979). For a selection marker to be useful in a breeding program, it has to exhibit an heritability greater than the heritability of the trait itself and a significant genetic correlation with the trait, and also to be economically cost effective. Molecular marker loci closely linked to quantitative trait loci (QTLs) are strong candidates (Lander and Botstein 1989; Tanksley et al. 1989; Lande and Thompson 1990), and have been used extensively to identify and map QTLs associated with complex traits, including yield, stress tolerance/resistance and nutritional qualities (Martin et al. 1989; Paterson et al. 1991; Stuber et al. 1992; Martin et al. 1993; Foolad et al. 1997). Molecular markers tightly linked to genes of interest make it possible to screen simultaneously for several traits without the need to apply various screening procedures to the population.

Most commercial cultivars of tomato are moderately sensitive to salt-stress at all stages of plant development (Jones 1986 a; Maas 1986; Foolad and Lin 1997 b). However, genetic resources for salt tolerance have been identified within the related wild species and primitive cultivars of tomato (Tal and Shannon 1983; Jones 1986 b; Foolad and Jones 1991; Foolad 1996 a, 1997; Foolad and Lin 1997b). These genetic resources offer prospects for the development of tomato cultivars with a greater tolerance to salt-stress. However, the complexity of the trait, insufficient genetic knowledge of tolerance components, lack of efficient selection criteria, and difficulties in the identification and transfer of tolerance genes from unadapted germplasm into the cultivated background have all severely limited the progress in salt tolerance-breeding in tomato. During an extensive evaluation of tomato germplasm for salt tolerance we identified an accession (LA722) within *Lycopersicon pimpinellifolium*, a closely-related, red-fruited wild species of tomato, which exhibited high salt tolerance during seed germination (Foolad and Lin 1997 b; Foolad et al. 1998 b), the vegetative stage and reproduction (MR Foolad, unpublished data). Here we report on the use of BC_1 and BC_1S_1 progeny of a cross between this accession and a relatively salt-sensitive tomato breeding line (NC84173) and the identification of QTLs for salt tolerance during the vegetative stage in tomato.

Materials and methods

Plant materials

An interspecific cross was made between a *Lycopersicon esculentum* Mill. breeding line (NC84173) and a *L. pimpinellifolium* (Jusl.) Mill. accession (LA722). NC84173 is a horticulturally superior, advanced breeding line (R. Gardner, University of North Carolina, USA) which is relatively salt-sensitive whereas LA722 is a self-compatible accession which has been identified as salt-tolerant (see the Results and discussion section). A single F_1 hybrid plant was backcrossed to NC84173 (pistillate parent) and BC_1 seeds were produced. One hundred and nineteen BC_1 individuals were grown to maturity in the greenhouse and self-pollinated to produce BC_1S_1 seed. The BC_1 population was used for the RFLP analysis and map construction whilst the BC_1S_1 population (consisting of 119 BC_1S_1 families) was used for the phenotypic evaluation.

Salt treatment and trait evaluation

Seeds of the parental lines and BC_1S_1 families were surface sterilized with a 0.5% NaOCl solution (10% regular bleach), rinsed with deionized water and sown in multi-cell trays containing a soil-less mix (1/3 each of peat moss, perlite and vermiculite). Two-week-old seedlings were transferred to hydroponic tanks after their roots were washed to remove the growing media. Each tank ($148 \times 118 \times 30$ cm) contained 135 liters of half-strength modified Hoagland solution (Epstein 1972) and accommodated 96 or 97 plants. Eight plants of

each of the two parental lines and of the 119 BC_1S_1 families (a total of 968 plants) were used which were completely randomized in ten tanks. Seedlings were grown in hydroponic solutions under greenhouse conditions with average day/night temperatures of approximately 28/18°C and a photon flux density of 400 ± 50 μmol/m²s. The hydroponic solutions were continuously and vigorously aerated. The first increment of salts at 50 mM NaCl and 5 mM $CaCl_2$ was added 4 days after transplanting and additional increments of the salts were added every 2 days to achieve a final concentration of 700 mM NaCl + 70 mM $CaCl_2$ (equivalent to an electrical conductivity of approximately 64 dS/m and a water potential of approximately − 35.2 bars). This final salt concentration was chosen because it provided the best discrimination in salt tolerance between the parental lines and among the BC_1S_1 families. The electrical conductivity and pH of the solutions were monitored periodically and the solutions were replenished every 2 days with distilled water to replace that lost by evapotranspiration. The experiment was then identically repeated for a second time; thus 16 plants from each of the parental lines and BC_1S_1 families (a total of 1928 plants) were evaluated for salt tolerance.

Plants were evaluated for survival under salt-stress 14 days after the final salt concentration was reached (approximately 8-week-old plants). Each plant was evaluated for survival using a scale of 0–4, with 0 indicating a dead plant and 4 indicating a healthy plant with no obvious symptoms of salt damage. The survival value of each family was determined as the average of the survival values of individuals within the family. The data were then transformed into percentage survival (i.e., values were multiplied by 25) and used for QTL mapping.

RFLP analysis, map construction and QTL mapping

RFLP analysis and map construction

Nuclear DNA was extracted from approximately 10 g of leaf tissue from each of the parental lines and the 119 BC_1 individuals using standard protocols for tomato as described elsewhere (Bernatzky and Tanksley 1986; Foolad et al. 1993). Genomic DNAs were digested with six restriction enzymes, including *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*1 and *Xba*1, following the manufacturer's instructions, and subjected to gel electrophoresis. Genomic blots were prepared and hybridized with 151 DNA probes detecting polymorphism between the two parents including 132 random genomic or cDNA clones (obtained from Dr. Steven Tanksley, Cornell University, Ithaca, N.Y., USA), 17 germination-related cDNAs (obtained from Dr. Kent Bradford, University of California, Davis, Calif., USA) and cDNAs of two known potassium-transporter genes (obtained from Dr. Leon Kochian, Cornell University, Ithaca, N.Y., USA). Probes were labeled with ^{32}P-dCTP by primer extension (Feinberg and Vogelstein 1983). Agarose-gel electrophoresis, Southern blotting, hybridizations and autoradiography were as described elsewhere (Foolad et al. 1993, 1995). A genetic linkage map was constructed with 151 RFLP (restriction fragment length polymorphism) markers (Chen and Foolad 1998) and used for QTL mapping.

QTL mapping

Two analytical approaches were employed to identify putative QTLs and estimate their phenotypic effects. First, interval analysis using MAPMAKER/QTL computer program v. 1.1 (Lincoln et al. 1992): this analysis was used to identify marker intervals on the tomato chromosomes that contained QTLs. In the present study, QTLs which were identified with a LOD score (Lander and Botstein 1989) threshold of 2.2 ($P \leq 0.001$ in single-marker analysis) were referred to as major QTLs, and those with a LOD score threshold of less than 2.2, but greater than 1.2 ($P \leq 0.01$ in single-marker analyses), were referred to as minor QTLs. The LOD scores obtained from MAPMAKER/QTL were used to construct QTL-likelihood plots (Lander and Botstein 1989; Paterson et al. 1991) of detected QTLs using Microsoft Excel v. 5.0 for Macintosh. The MAPMAKER/QTL program was also employed to obtain estimates of the percentage of the total phenotypic variation explained (PVE) by each QTL. The multi-locus model from MAPMAKER/QTL program was used to estimate the percentage of phenotypic variation accounted for by the various combinations of significant QTLs.

The second approach employed for QTL mapping was single-marker analysis using the QGENE computer program (Nelson 1997): this program was used to determine the association between individual marker loci and putative QTLs. This program uses marker-genotype groups as class variables for the detection of linkage between markers and putative QTLs. The QGENE program was also used to test for pairwise epistatic interactions among QTL-linked and QTL-unlinked markers.

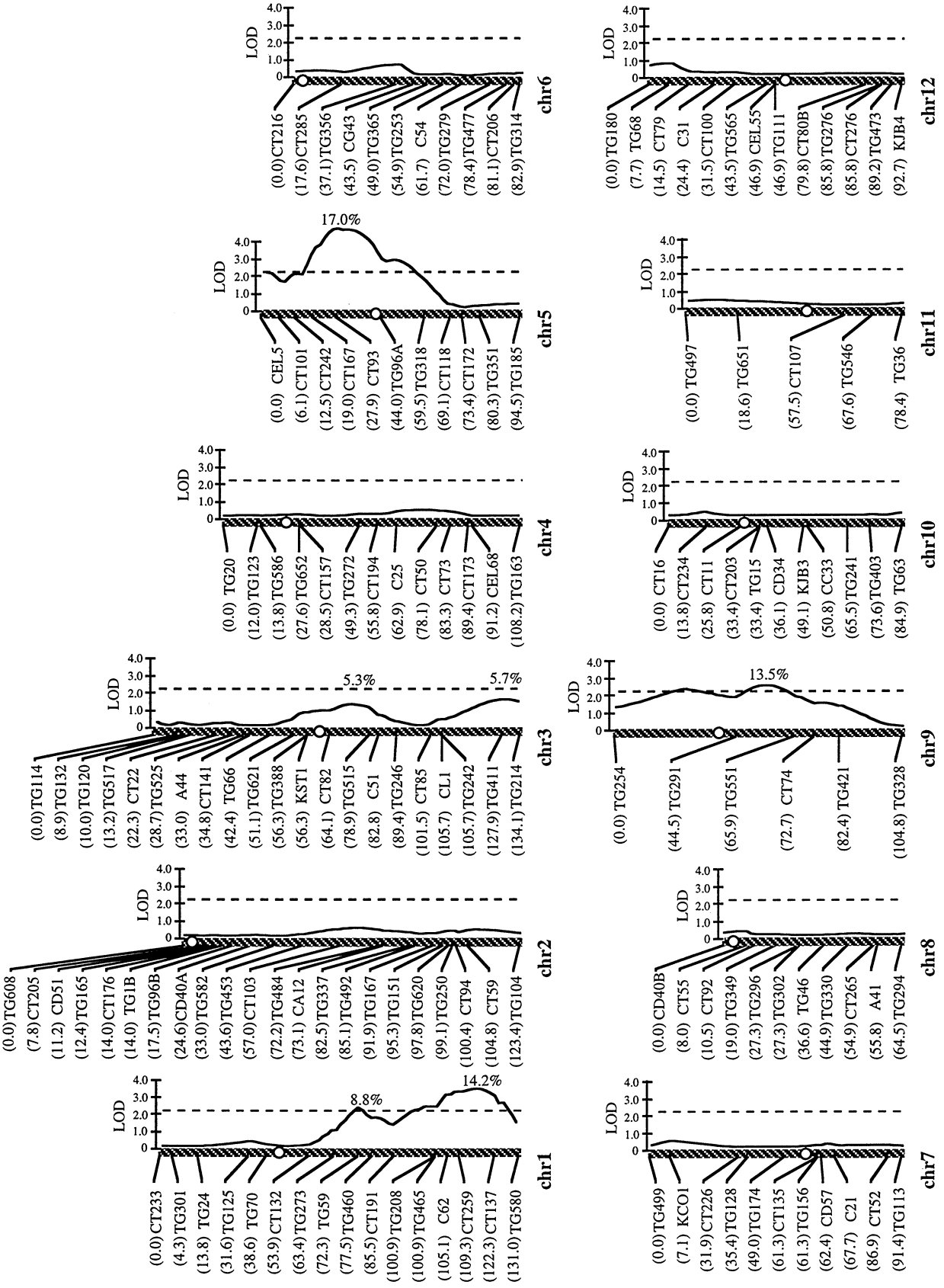
Results and discussion

Genetic linkage map

A total of 151 RFLP markers were scored for each of the 119 BC_1 plants and a genetic linkage map was constructed as described elsewhere (Chen and Foolad 1998). The linkage map spanned approximately 1192 cM of the tomato genome with an average distance between markers of 7.9 cM (Fig. 1).

Plant response to salt stress

Under the low to moderate levels ($\leq$300 mM) of salt-stress the growth of tomato plants was retarded, compared to the unstressed plants (data not shown). As the level of salt concentration was further increased, leaf chlorosis and wilting started to appear. Plants of the cultivated parent (NC84173) were more severely stressed than plants of the wild parent (LA722), and there was considerable variation in the BC_1S_1 population. Some plants started collapsing when the salt concentration reached approximately 500 mM of NaCl. However, the salt level was gradually increased to a concentration of 700 mM NaCl + 70 mM $CaCl_2$ before plants were evaluated for final survival. (Based on our experience, NC84173 exhibits less salt sensitivity than many commercial cultivars of tomato and this necessitated the use of a rather high concentration of salt to discriminate among plants.) Plants were evaluated for survival on the 14th-day after the final salt concentration was reached. At this time, approximately 80% of the LA722 plants and 25% of the NC84173 plants survived; among the BC_1S_1 families, the survival value ranged from 9% to 94% with a mean of 51.4% (see Fig. 2). The variation for survival in the BC_1S_1 population was continuous, typical of quantitative traits, and was skewed towards the salt-tolerant parent (LA722). This skewness indicates the involvement of genes with a significant dominant effect on salt


LOD
4.0
3.0
2.0
1.0
0
chr1
(0.0) CT233
(4.3) TG301
(13.8) TG24
(31.6) TG125
(38.6) TG70
(53.9) CT132
(63.4) TG273
(72.3) TG59
(77.5) TG460
(85.5) CT191
(100.9) TG208
(100.9) TG465
(105.1) C62
(109.3) CT259
(122.3) CT137
(131.0) TG580
8.8%
14.2%
chr2
(0.0) TG608
(7.8) CT205
(11.2) CD51
(12.4) TG165
(14.0) CT176
(14.0) TG1B
(17.5) TG96B
(24.6) CD40A
(33.0) TG582
(43.6) TG453
(57.0) CT103
(72.2) TG484
(73.1) CA12
(82.5) TG337
(85.1) TG492
(91.9) TG167
(95.3) TG151
(97.8) TG620
(99.1) TG250
(100.4) CT94
(104.8) CT59
(123.4) TG104
chr3
(0.0) TG114
(8.9) TG132
(10.0) TG120
(13.2) TG517
(22.3) CT22
(28.7) TG525
(33.0) A44
(34.8) CT141
(42.4) TG66
(51.1) TG621
(56.3) TG388
(56.3) KST1
(64.1) CT82
(78.9) TG515
(82.8) C51
(89.4) TG246
(101.5) CT85
(105.7) CL1
(105.7) TG242
(127.9) TG411
(134.1) TG214
5.3%
5.7%
chr4
(0.0) TG20
(12.0) TG123
(13.8) TG586
(27.6) TG652
(28.5) CT157
(49.3) TG272
(55.8) CT194
(62.9) C25
(78.1) CT50
(83.3) CT73
(89.4) CT173
(91.2) CEL68
(108.2) TG163
chr5
(0.0) CEL5
(6.1) CT101
(12.5) CT242
(19.0) CT167
(27.9) CT93
(44.0) TG96A
(59.5) TG318
(69.1) CT118
(73.4) CT172
(80.3) TG351
(94.5) TG185
17.0%
chr6
(0.0) CT216
(17.6) CT285
(37.1) TG356
(43.5) CG43
(49.0) TG365
(54.9) TG253
(61.7) C54
(72.0) TG279
(78.4) TG477
(81.1) CT206
(82.9) TG314
chr7
(0.0) TG499
(7.1) KCO1
(31.9) CT226
(35.4) TG128
(49.0) TG174
(61.3) CT135
(61.3) TG156
(62.4) CD57
(67.7) C21
(86.9) CT52
(91.4) TG113
chr8
(0.0) CD40B
(8.0) CT55
(10.5) CT92
(19.0) TG349
(27.3) TG296
(27.3) TG302
(36.6) TG46
(44.9) TG330
(54.9) CT265
(55.8) A41
(64.5) TG294
chr9
(0.0) TG254
(44.5) TG291
(65.9) TG551
(72.7) CT74
(82.4) TG421
(104.8) TG328
13.5%
chr10
(0.0) CT16
(13.8) CT234
(25.8) CT11
(33.4) CT203
(33.4) TG15
(36.1) CD34
(49.1) KJB3
(50.8) CC33
(65.5) TG241
(73.6) TG403
(84.9) TG63
chr11
(0.0) TG497
(18.6) TG651
(57.5) CT107
(67.6) TG546
(78.4) TG36
chr12
(0.0) TG180
(7.7) TG68
(14.5) CT79
(24.4) C31
(31.5) CT100
(43.5) TG565
(46.9) CEL55
(46.9) TG111
(79.8) CT80B
(85.8) TG276
(85.8) CT276
(89.2) TG473
(92.7) KJB4

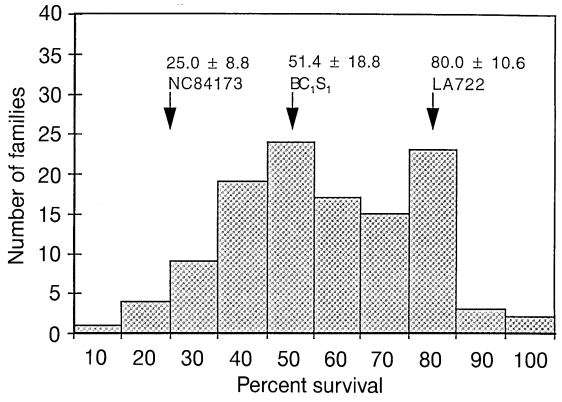


Fig. 2 Frequency distribution for tomato salt tolerance during the vegetative stage in a BC_1S_1 population of *L. esculentum* (NC84173) × *L. pimpinellifolium* (LA722) grown in aerated saline hydroponic solutions under greenhouse conditions. Means of the parental lines and BC_1S_1 population (± SE) are shown by *arrows*

tolerance during the vegetative stage in tomato, consistent with our previous results (Foolad 1996 a).

Identification of QTLs conferring salt tolerance

The survival data from each replication, as well as pooled data over replicates, were analyzed separately for the identification of QTLs. The results were almost identical across replications and, thus, only the QTL results based on pooled data are presented and discussed.

Interval mapping

Interval mapping identified four major (LOD ≥ 2.2 and $P \leq 0.001$) QTLs and one minor (LOD ≥ 1.2 and $P \leq 0.01$) QTL for salt tolerance; the major QTLs were located on chromosomes 1 (two QTLs), 5 and 9, and the minor QTL was located on lower end of chromosome 3 (Table 1, Fig. 1). The QTL with largest effect was identified in the interval CT167–CT93 on chromosome 5 (PVE = 17.7%, LOD = 4.3) and the QTL with smallest effect was identified in the interval TG411–TG214 on chromosome 3 (PVE = 5.7%, LOD = 1.3) (Fig. 1). Multilocus analysis indicated that the five QTLs together accounted for 46.4% of the total phenotypic variation for this trait.

The desirable QTL alleles (for salt tolerance) were all contributed from the salt-tolerant wild parent (LA722). However, one minor QTL with a LOD = 1.1 (just below the threshold level) and a PVE = 5.3% was identified on chromosome 3 in the interval CT82–TG515, for which the desirable QTL allele was contributed from the cultivated parent (data not shown in Table 1, but see Fig. 1). Interestingly, the chromosomal position of this QTL was approximately 60 cM from another QTL (with LOD = 1.3) on this chromosome for which the desirable QTL allele was contributed from the salt-tolerant parent (Fig. 1). The identification of a minor QTL for salt tolerance in the cultivated parent (NC84173) was not unexpected because NC84173, although salt-sensitive compared to LA722, exhibits some level of salt tolerance compared to many other accessions and lines of the cultivated species (M. R. Foolad, unpublished data). However, the significance of the two QTLs with small LOD scores on chromosome 3 is unclear.

On chromosome 1, two adjacent QTL-likelihood peaks were identified with LODs of 2.3 (interval TG59–TG460) and 3.5 (interval CT259–CT137) and individual PVE values of 8.8% and 14.2%, respectively; the two peaks were approximately 43 cM apart (Fig. 1). The presence of multiple nearby likelihood peaks on this chromosome, however, does not necessarily mean the presence of multiple genomic regions on this chromosome with significant effects on the trait (Lander and Botstein 1989; Paterson et al. 1991). Multilocus analysis using MAPMAKER/QTL indicated that the combined effects of the two adjacent likelihood peaks on chromosome 1 was 17.5%, significantly less than the sum of the individual effects of the two QTLs (PVE = 23.0%), and only slightly greater than the individual effects of the interval CT259–CT137 alone (14.2%). This lack of additivity may be due to the presence of colinearity effects between the adjacent QTLs or else to the fact that only a single QTL is present on this chromosome for salt tolerance.

Single-marker analysis

The results of the single-marker analysis were generally similar to the results of the interval mapping in that the same five genomic regions were identified with significant effects on salt tolerance. The QTL-linked RFLP markers that were identified by single-marker analysis are listed in Table 1. The single-marker analysis identified marker-linked QTLs on chromosomes 1, 5, and 9 with $P < 0.001$, and on chromosome 3 with $P < 0.01$ (Table 2). In addition, similar to interval mapping, the single-marker analysis identified (at $P < 0.05$) a QTL on chromosome 3 linked to TG515 for which the desirable allele (for salt tolerance) was contributed from the

Fig. 1 An RFLP linkage map of tomato chromosomes constructed based on a BC_1 population of a cross between *L. esculentum* (NC84173) and *L. pimpinellifolium* (LA722). The names of the markers and their map positions (in cM based on the Kosambi function) are shown at the *left* of the chromosomes. The *open circle* on each chromosome indicates the approximate location of the centromere. The LOD score plots at the *right* of the chromosomes indicate the most likely positions of QTLs for salt tolerance; the *height* of the LOD curve indicates the strength of the evidence (log 10 of the odd ratio) for the presence of a QTL at each location; the *dashed vertical lines* at a height of 2.2 indicate the stringent threshold that the LOD score must cross to allow the presence of a QTL to be inferred (see the text). The maximum-likelihood position of the QTL(s) is the *highest point* on the curve

Table 1 QTLs detected for salt tolerance during vegetative growth (survival under high salt-stress) based on interval mapping and single-marker analysis in a BC_1S_1 population of an interspecific cross between *L. esculentum* (NC84173) and *L. pimpinellifolium* (LA722). For the single-marker analysis, the most closely associated molecular-marker locus is indicated by *. LOD = log-likelihood; PVP = percent phenotypic variation explained; *E* = *L. esculentum* allele; *PM* = *L. pimpinellifolium* allele; phenotypic effect = difference between the *E*/*PM* and *E*/*E* in percentage survival

Interval	Chrom.	Interval length (cM)	Interval mapping			Single-marker analysis				
			LOD	PVE	Phenotypic effect	*P* value	R^2 %	*E*/*E* % survival	*E*/*PM* % survival	Phenotypic effect
TG59*–TG460	1	5.2	2.3	8.8	11.3	0.0003	11.1	44.6	57.5	12.9
CT259*–CT137	1	13.0	3.5	14.2	14.4	0.0002	11.3	45.1	57.9	12.8
TG411*–TG214	3	6.2	1.3	5.7	8.7	0.0090	5.9	47.6	56.8	9.2
CT167–CT93*	5	8.9	4.3	17.7	15.8	< 0.0001	16.4	44.6	59.9	15.3
TG291–TG551*	9	21.4	2.5	13.5	13.9	0.0010	6.9	47.5	57.5	10.0

Table 2 Bi-locus analysis of QTLs affecting salt tolerance during vegetative growth in a BC_1S_1 population derived from an interspecific cross between *L. esculentum* (NC84173) and *L. pimpinellifolium* (LA722). LOD = log-likelihood; PVP = percent phenotypic variation explained

Bi-locus QTL model	Chromosomes	LOD		PVE	
		Bi-locus model	Sum of the QTLs	Bi-locus model	Sum of the QTLs
TG59–TG460/CT259–CT137	1, 1	4.6	5.8	17.5	23.0
TG59–TG460/CT82–TG515	1, 3	3.1	3.4	12.1	15.1
TG59–TG460/TG411–TG214	1, 3	4.3	3.4	17.2	15.5
TG59–TG460/CT167–CT93	1, 5	7.1	6.6	25.7	25.8
TG59–TG460/TG291–TG551	1, 9	4.5	4.8	18.8	22.3
CT259–CT137/CT82–TG515	1, 3	5.1	4.6	21.1	19.5
CT259–CT137/TG411–TG214	1, 3	5.3	4.8	21.4	19.9
CT259–CT137/CT167–CT93	1, 5	7.8	7.8	29.0	31.2
CT259–CT137/TG291–TG551	1, 9	5.4	6.0	22.9	27.7
CT82–TG515/TG411–TG214	3, 3	3.0	2.4	14.5	11.0
CT82–TG515/CT167–CT93	3, 5	5.3	5.4	21.1	22.3
CT82–TG515/TG291–TG551	3, 9	3.4	3.6	17.1	18.8
TG411–TG214/CT167–CT93	3, 5	5.3	5.6	20.9	22.7
TG411–TG214/TG291–TG551	3, 9	3.4	3.8	16.7	19.2
Ct167–CT93/TG291–TG551	5, 9	6.9	6.8	27.9	30.5

Table 3 Pairwise epistatic interactions between genomic regions for salt tolerance during vegetative growth in a BC_1S_1 population derived from an interspecific cross between *L. esculentum* (NC84173) and *L. pimpinellifolium* (LA722). Markers in bold face are QTL-linked

Marker interval	Chromosomes	*F* value	*EPM*/*EPM*[a] % survival	*EE*/*EPM* % survival	*EPM*/*EE* % survival	*EE*/*EE* % survival
CT259–TG460	1–1	12.56	56.3	58.0	62.2	39.3
TG59–CT22	1–3	10.66	52.7	51.6	63.4	39.0
CT137–CT93	1–5	10.53	60.8	58.7	54.9	32.3
CT59–TG302	1–8	17.32	42.5	63.5	55.4	49.0
TG24–**TG291**	1–9	12.55	62.0	51.9	40.8	54.0
TG151–TG302	2–8	22.53	41.7	64.4	56.2	47.0
TG411–CT216	3–6	11.28	52.7	57.1	59.8	41.0
TG96A–CT172	5–5	11.77	60.0	32.9	51.2	50.9
TG96A–TG330	5–8	21.06	66.6	41.5	49.6	54.2

[a] *E* = *L. esculentum* allele; *PM* = *L. pimpinellifolium* allele

salt-sensitive parent (data not shown in Table 1). Furthermore, the single-marker analysis identified only one location (linked to marker TG551) on chromosome 9 associated with salt tolerance. The overall results indicated comparable efficacy of the interval mapping and single-marker analysis in identifying marker-linked QTLs.

The individual and combined effects of QTLs

Bi-locus and multi-locus analyses indicated that the QTLs identified on different chromosomes were mostly independent of each other and their effects were generally additive. Table 2 displays the combined (simultaneous) effects as well as the sum of the individual effects

of all possible pairwise combinations of the identified QTLs. In most cases, the combined effects of QTLs which were located on different chromosomes were similar to the sum of their individual effects (Table 2). This observation indicates that the QTLs identified on different chromosomes were probably independent of each other and affected salt tolerance through different physiological processes. This observation also indicates the lack of significant epistatic interaction effects between QTLs for most pair-wise combinations (see the next section on epistasis). The multi-locus analysis also supported the suggestion of independency of the QTLs on different chromosomes. For example, the combination of QTLs on chromosomes 1 (interval CT259–CT137), 3 (interval TG411–TG214), 5 (interval CT167–CT93), and 9 (interval TG291–TG551) could together account for 43.2% of the total phenotypic variation, slightly less than the sum of their individual effects (50.4%). The overall results indicate that the transfer of these four QTLs to the cultivated tomato background is expected to significantly improve tomato salt tolerance during the vegetative stage.

Epistatic interactions

Pairwise epistatic interactions between all markers (a total of 11 325 interactions) were examined ($P \leq 0.002$, $F \geq 10.00$) using QGENE. A total of 34 two-locus epistatic interactions were identified, which could be grouped into nine distinct between-region interactions (each region containing one or more marker locus). The identified epistatic interactions were of three types: (1) interactions between QTL-linked regions, of which two interactions were identified; (2) interactions between QTL-linked and QTL-unlinked regions, of which six interactions were identified; and (3) interactions between QTL-unlinked regions, of which only one was identified (Table 3).

Of the nine between-region epistatic interactions, the most significant one ($F = 22.53$) was between two QTL-unlinked regions, one on chromosome 2 (linked to TG151) and the other on chromosome 8 (linked to TG302) (Table 3). Interestingly, neither of these two regions were found to have significant independent effect on salt tolerance (see Fig. 1). Among the interactions which were identified between QTL-linked regions and QTL-unlinked regions, the interaction between the QTL-linked marker TG96A on chromosome 5 and the QTL-unlinked marker TG330 on chromosome 8 was the most significant one ($F = 21.06$). The same genomic region on chromosome 8 also exhibited interaction with a QTL-linked region on chromosome 1 (linked to CT59). The results clearly indicated the positive effect of a region on chromosome 8 on salt tolerance, an effect which was manifested only when epistatic interactions were examined. Of the two epistatic interactions which were identified between QTL-linked regions, one occurred between two regions on chromosome 1 ($F = 12.56$) and the other between regions on chromosomes 1 and 5 ($F = 10.53$). The relatively small number of interactions identified (only two) between QTL-linked regions was in agreement with the results of the bi-locus QTL analysis (described in the previous section) which demonstrated that most of the identified QTLs exerted their effects independent of each other and were nearly additive when in combination. For the remaining four epistatic interaction effects, which were identified between QTL-linked and QTL-unlinked regions, the F values barely exceeded the significant threshold level of 10 (Table 3).

The number (percentage) of significant two-locus interactions ($34/11325 \cong 0.003$) was smaller than the percentage expected to occur by chance; thus, it is possible that some or all of the identified interactions were due to chance events. Further investigations, including the use of larger populations or the construction of isogenic lines, are necessary to determine the exact nature of these interactions. For breeding purposes, however, QTLs which do not require epistatic interactions are most desirable.

Number, magnitude of effects, and nature of QTLs

The results of this study indicate the presence of five QTLs with differential effects on the total phenotypic variation; four QTLs were identified with major effects (PVE > 10%) and one QTL was identified with a minor effect (PVE = 5.7%) (Table 1). The combined effects of the major QTLs could account for 43.2% of the total phenotypic variation and, when the minor QTL on chromosome 3 (TG411–TG214) was added, a total of 46.4% of the total phenotypic variation could be accounted for. It is likely that there were more QTLs with minor effects which were not detected in this study, and could possibly be detected if a larger population was used. In addition, recessive QTLs contributed from the donor parent can not be detected in BC_1/BC_1S_1 populations. Furthermore, some of the remaining variation could be due to epistatic interactions among QTL-linked and unlinked markers (as described in the previous section). The results support the hypothesis that quantitative traits are often controlled by the effects of a few major QTLs which act in concert with a number of smaller-effect QTLs (Lande and Thompson 1990; Paterson et al. 1991; deVicente and Tanksley 1993). The number of QTLs affecting a quantitative trait has a significant bearing on the applicability of marker-assisted selection for the improvement of the trait. In this study, the finding that only a few major QTLs affected tomato salt tolerance during the vegetative stage indicates that a rapid response to directional selection for this trait is expected.

All of the identified QTLs with a LOD > 1.2 and a $P < 0.01$ had the desirable QTL alleles from the

salt-tolerant parent (LA722), consistent with the parental phenotypes. However, one location (CT82–TG515 on chromosome 3) was identified at a LOD = 1.1 (slightly less than the threshold level) and with effects in the opposite direction to the parental phenotypes. Although the effect of this QTL was quite small (PVE = 5.3%), its finding demonstrates the ability of the marker analysis to uncover cryptic genetic variation that otherwise would have been masked by the large difference between the parents.

Implications for crop improvement

During its evolution and the domestication process, the cultivated tomato has undergone various genetic "bottlenecks" imposed by selection and the extreme inbreeding of limited collections, particularly in Europe and North America (Rick 1976). These events have resulted in the depletion of genetic variability within *L. esculentum* (Miller and Tanksley 1990; Foolad et al. 1993). For example, very little variation for salt tolerance has been identified within the tomato cultigen (Tal and Shannon 1983; Jones 1986 a). Fortunately, the related wild species of tomato are a rich source of useful genes and desirable traits, and are potentially useful for the improvement of the cultivated tomato through hybridization and selection. For example, genetic sources of salt tolerance were previously identified within some of the distantly related, green-fruited wild species of tomato such as *Lycopersicon chilense*, *Lycopersicon pennellii* and *Lycopersicon peruvianum* (Rick 1973; Tal and Shannon 1983). Exploitation of gene resources within these wild species in breeding programs, however, is not without inherent difficulties. The existence of barriers (such as incompatibility reactions) in the initial crosses and early backcross generations, the reduced viability and sterility of the hybrid, as well as segregation distortion and reduced recombination in the segregating generations, have all been well-documented (Rick 1962, 1983; Foolad 1996 c). In addition, most of these wild donor materials are deficient for many of the horticultural traits required in modern cultivated systems. Thus, upon interspecific hybridization the task becomes more one of eliminating the great bulk of undesirable genes introduced from the wild donor rather than incorporating genes of interest into a more desirable genetic background. A series of backcrosses to the cultivated recurrent parent, alternated with selfed generations, are required before the desired combinations of parental characteristics can be selected. This procedure, commonly referred to as "prebreeding", is costly and time-consuming. In the present study, the identification of a source of salt tolerance in *L. pimpinellifolium* is significant because this species is genetically the closest wild species of tomato and its use in breeding programs would reduce the time and effort required to recover the genome of the cultivated tomato, compared to the use of the other, less-related, wild species of tomato. The ease of handling of the progeny of the cross between *L. esculentum* and *L. pimpinellifolium*, together with the knowledge of the genomic location of genes (QTLs) contributing to salt tolerance, can facilitate more rapid incorporation of salt tolerance-characteristics into modern cultivars of tomato.

This study has revealed three regions on chromosomes 1, 5 and 9 of the *L. pimpinellifolium* parent with a major effect (PVE = 43.2%) on tomato salt tolerance during the vegetative stage. Previously, we determined that the same *L. pimpinellifolium* accession was also both salt-tolerant and cold-tolerant during seed germination (Foolad and Lin 1997 b; Foolad et al. 1998 a, b) and had several desirable fruit-quality characteristics for which marker-linked QTLs were revealed (unpublished data). Marker-assisted selection may facilitate the simultaneous transfer of genes (QTLs) for these desirable traits from LA722 into modern cultivars of tomato, providing opportunities to rapidly developing cultivars with enhanced characteristics. However, the real value of the identified QTLs has to be tested in different genetic backgrounds and environments, and in combination with other desirable horticultural characteristics.

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