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RFLP mapping of QTLs conferring salt tolerance during germination in an interspecific cross of tomato

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Abstract Most cultivars of tomato (*Lycopersicon esculentum*) are sensitive to salinity during seed germination and at later stages. Genetic resources for salt tolerance have been identified within the related wild species of tomato. The purpose of the present study was to identify quantitative trait loci (QTLs) for salt tolerance during germination in an inbred backcross (BC_1S_1) population of an interspecific cross between a salt-sensitive tomato 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 restriction fragment length polymorphism (RFLP) markers and a genetic linkage map was constructed. The parental lines and 119 BC_1S_1 families (self-pollinated progeny of 119 BC_1 individuals) were evaluated for germination at an intermediate salt-stress level (150 mM NaCl + 15 mM $CaCl_2$, water potential approximately −850 kPa). Germination was scored visually as radicle protrusion at 8-h intervals for 28 consecutive days. Germination response was analyzed by survival analysis and the time to 25, 50, and 75% germination was determined. In addition, a germination index (GI) was calculated as the weighted mean of the time from imbibition to germination for each family/line. Interval mapping, single-marker analysis and distributional extreme analysis, were used to identify QTLs and the results of all three mapping methods were generally similar. Seven chromosomal locations with significant effects on salt tolerance were identified. The *L. pimpinellifolium* accession had favorable QTL alleles at six locations. The percentage of phenotypic variation explained (PVE) by individual QTLs ranged from 6.5 to 15.6%. Multilocus analysis indicated that the cumulative action of all significant QTLs accounted for 44.5% of the total phenotypic variance. A total of 12 pairwise epistatic interactions were identified, including four between QTL-linked and QTL-unlinked regions and eight between QTL-unlinked regions. Transgressive phenotypes were observed in the direction of salt sensitivity. The graphical genotyping indicated a high correspondence between the phenotypes of the extreme families and their QTL genotypes. The results indicate that tomato salt tolerance during germination can be improved by marker-assisted selection using interspecific variation.

Key words *Lycopersicon esculentum* · *L. pimpinellifolium* · Salt tolerance · Restriction fragment length polymorphism (RFLP) · Quantitative trait loci (QTLs) · Seed germination · Molecular markers · Graphical genotyping

Introduction

Most commercial cultivars of tomato (*Lycopersicon esculentum*) are sensitive to salinity stress during all stages of plant development, including seed germination and emergence, seedling survival and growth, and vegetative growth and reproduction (Maas 1986; Asins et al. 1993; Foolad and Lin 1997a). Under salt-stress, during germination, for example, many seeds do not germinate or else germinate so sporadically that plants grow differentially, delaying plant establishment and leading to variability in crop maturation. During later stages, plant growth and development are very limited and, as a result, flowers and fruits are not produced.

Genetic resources for salt tolerance have been identified within the related wild species and primitive

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cultivars of tomato (Tal and Shannon 1983; Asins et al. 1993; Foolad 1996 a; Foolad and Lin 1997 a). These resources are potentially useful for the development of salt-tolerant tomato cultivars. However, limited progress has been made in salt-tolerance breeding in tomato. This is in part due to the complexity of the trait, insufficient genetic knowledge of tolerance components, lack of efficient selection criteria, difficulties in the identification and transfer of tolerance genes from unadapted germplasm into the cultivated background, and limited breeding efforts.

In many crop plants, including tomato, salt tolerance has been identified as a developmentally regulated, stage-specific phenomenon, with tolerance at one stage of plant development being poorly correlated with tolerance at other developmental stages (Greenway and Munns 1980; Johnson et al. 1992; Foolad and Lin 1997 b). In addition, at each stage of plant development, salt tolerance appears to be controlled by more than one gene and to be highly influenced by environmental factors (Foolad 1996 a, b, 1997; Foolad et al. 1997). A partitioning of salt tolerance into components related to specific ontogenetic stages, and the identification of the number and magnitude of gene effects, would contribute to a better understanding of the genetic control of this trait and, hence, may facilitate the rapid development of salt-tolerant plants.

During the past several decades Mendelian genetic theories have been extremely useful for discerning the genetic basis of simply inherited traits which exhibit discrete variation; such traits are often controlled by one or a few genes and their expressions are minimally affected by environmental variation. Biometrical genetic models, on the other hand, have been remarkably useful for describing the inheritance of traits which exhibit continuous (quantitative) variation; these traits are often controlled by more than one gene and/or their expressions are highly influenced by environmental variation. However, biometrical genetic protocols have been less efficient in discerning the precise genetic basis of quantitative traits and, hence, have contributed minimally to the genetic improvement of plants.

During the past two decades, the use of molecular-marker technology and quantitative trait locus (QTL) mapping has contributed to a better understanding of the genetic basis of many agriculturally and biologically important quantitative traits such as yield (Stuber et al. 1987), resistance/tolerance to biotic and abiotic stresses (Martin et al. 1989; Foolad et al. 1997), and nutritional quality in numerous crop species (Paterson et al. 1991). Among many recent discoveries have been the identification of the number and chromosomal location of genes which affect quantitative traits and the determination of the individual and combined effects of quantitative trait loci (QTLs). In addition, molecular-marker technology has allowed the identification of QTLs with "major" or "minor" effects, the detection of intralocus and interlocus (epistasis) interactions, and a better understanding of important genetic phenomena such as heterosis, transgressive segregation and genotype × environment interactions (Stuber et al. 1992; deVicente and Tanksley 1993; Zhang et al. 1994; Li et al. 1997).

In a previous investigation, we used an F_2 population of an interspecific cross between a salt-sensitive tomato cultivar (UCT5) and a salt-tolerant accession of *Lycopersicon pennellii* (LA716) and identified eight genomic regions with significant effects on salt tolerance during tomato seed germination (Foolad et al. 1997). *L. pennellii*, however, is a distantly related, green-fruited wild species of tomato which is cross compatible with the cultivated tomato only as the male parent, and the interspecific progeny exhibit extensive segregation distortion, incompatibility (incongruity) and sterility, in addition to numerous other undesirable horticultural characteristics. Furthermore, LA716 is salt tolerant only during seed germination and not at later stages of plant development. Recently, however, we identified an accession (LA722) within a more closely related tomato wild species, *Lycopersicon pimpinellifolium*, which exhibits high salt tolerance during seed germination (Foolad and Lin 1997 a), vegetative growth and reproduction, and displays numerous other desirable horticultural characteristics such as high fruit quality and disease resistance (M. R. Foolad, unpublished data). Accessions of *L. pimpinellifolium* produce red fruits and hybridize reciprocally with the cultivated tomato, and the interspecific progeny do not exhibit any morphological, physiological, or genetic disorders (Rick 1983). Among the wild species of tomato, *L. pimpinellifolium* is the only one for which natural introgression with *L. esculentum* has been demonstrated (Rick 1958), and it has been frequently considered as a useful source of germplasm for the breeding of the cultivated tomato (e.g., Rick 1983; Grandillo and Tanksley 1996; Tanksley et al. 1996). The identification and genetic characterization of useful sources of salt tolerance within this species could significantly contribute to the development of salt-tolerant tomato cultivars. The present study was carried out to examine the genetic basis of salt tolerance during germination in LA722 by identifying QTLs for this trait. The results provided an opportunity to examine whether similar genomic regions were responsible for salt tolerance during germination in LA722 and LA716, accessions of two distantly related wild species, *L. pimpinellifolium* and *L. pennellii*, respectively.

Materials and methods

Plant materials

Highly inbred sources of NC84173 (*L. esculentum* Mill.) and LA722 (*L. pimpinellifolium* Jusl.) were hybridized and F_1 progeny produced. NC84173 is a multiple disease-resistant, advanced breeding line

(R. Gardner, University of North Carolina, Fletcher, N.C., USA) which is salt sensitive during germination; LA722 is a self-compatible accession which is highly salt tolerant during germination (Foolad and Lin 1997 a). 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, self-pollinated, and produced BC_1S_1 seed. The BC_1 population was used for the RFLP analysis and map construction and the BC_1S_1 population was used for the phenotypic evaluation and QTL analysis.

RFLP analysis and map construction

Nuclear DNA was extracted from approximately 10 g of leaf tissue from each of the parental lines and 119 BC_1 individuals, using standard protocols for tomato (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, and subjected to gel electrophoresis. Genomic blots were prepared and hybridized with 151 DNA probes detecting polymorphism between the two parents (Chen and Foolad 1998), 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). A genetic linkage map was constructed using 151 restriction fragment length polymorphism (RFLP) markers (Chen and Foolad 1998) and employed for the QTL mapping.

Trait evaluation

Twenty milliliters of sterile germination medium (0.8% agar) containing 150 mM of NaCl + 15 mM of $CaCl_2$ were added to each Petri plate. The water potential of the saline medium was approximately -850 kPa, as measured on a Wescor-5100 vapor-pressure osmometer (Wescor, Logan, Utah). Seeds of the parents and 119 BC_1S_1 families were surface-sterilized with 0.5% sodium hypochlorite solution for 10 min, rinsed with sterile distilled water several times, and briefly blotted. Four-hundred seeds of each of the two parental lines and 160 seeds of each of the BC_1S_1 families were sown on germination media under aseptic conditions. Each Petri plate (15-cm diameter) contained 80 seeds and was considered as one replication. Petri plates were randomized in a precision incubator (Model 815, GCA Corporation) maintained dark at 20 ± 0.5°C. Germination response was scored visually as radicle protrusion at 8-h intervals for 28 consecutive days. The experiment was repeated for a second time using identical conditions. Seed viability for each seed lot was estimated by conducting a seed-germination test on agar media with no salt added (control treatment).

Statistical analysis

Analysis of phenotypic data

Germination response was analyzed by survival analysis with life tables (Lee 1980). This is a nonparametric (distribution-free) procedure that measures time responses (e.g., time to a percentile germination) and provides several descriptive statistics for response over time. The number of seeds exposed to risk (potentially able to germinate), the number germinated, and the number withdrawn (i.e., censored observations due to contamination or lack of germination of viable seeds by the end of experiment) were assessed. The time in days to 25% (T25), 50% (T50), and 75% (T75) germination was calculated for each replication (Petri plate) and pooled over replications. These germination criteria were used for a comparison among genotypes (parental lines and BC_1S_1 families) and for QTL analysis. Pairwise correlation coefficients among the various germination percentiles were determined using Pearson correlation analysis (Steel and Torrie 1980). In addition to germination percentiles, a germination index (GI) was calculated for each of the parental lines and BC_1S_1 families, using the following formula:

$$GI = (\Sigma T_i N_i)/S,$$

where T_i is the time (in days) between seed imbibition and germination, N_i is the number of seeds germinated on day i, and S is the total number of seeds germinated. GI is a weighted mean of the time from imbibition to germination, and thus is a measure of germination mean. A small GI value indicates faster germination. This germination criterion, however, only accounts for seeds that germinated by the end of the experiment.

When calculating the time to germination, seeds that germinated within an interval were presumed to have germinated at the midpoint of that interval. Seeds that failed to germinate in the control treatment were assumed to be non-viable. Above this percentage, seeds that failed to germinate in the salt-stress treatment were included in the analysis as right-censored observations (i.e., seeds that were viable but failed to germinate by the last observation time). Hence, the sample size for each replication was the number of viable seeds, not the number of seeds that were sown or germinated.

QTL analysis

Three analytical approaches were used to identify putative QTLs and estimate their phenotypic effects. First, interval analysis using MAPMARKER/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. The LOD scores obtained from MAPMARKER/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 MAPMARKER/QTL program was also used to obtain estimates of the percentage of the total phenotypic variation explained (PVE) by each QTL (which is nearly equivalent to the R^2 values obtained from single-marker analysis for cases when a QTL mapped close to a specific marker). The multi-locus model from the MAPMARKER/QTL program was used to estimate the percentage of phenotypic variation accounted for by various combinations of significant QTLs.

The second approach employed for QTL mapping was the 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 markers, between QTL-linked and QTL-unlinked markers and among QTL-unlinked markers. The distribution of the recurrent parent (L. *esculentum*) genotype in the BC_1 population and the graphical genotypes (Young and Tanksley 1989) of BC_1 individuals were also determined by this program.

The third approach to identify marker-linked QTLs was the analysis of distributional extremes. In this method, the 12 fastest-germinating families (approx. 10% of the total) and 11 slowest-germinating families were selected as the extreme tails of the BC_1S_1 population. Within each of the selected classes, the marker-allele frequencies were calculated at all of the 151 marker loci. Marker-allele frequency differences ($p_T - p_S$) between the selected salt-tolerant

and salt-sensitive progeny families were estimated for each marker locus, where p_T is the frequency of the ith allele at the kth marker locus among the salt-tolerant families and p_S is the frequency of the ith allele at the kth marker locus among the salt-sensitive families. A trait-based marker analysis (TBA), also known as "selective genotyping", which measures differences in marker-allele frequencies between selected classes, was used to identify marker-linked QTLs (Lebowitz et al. 1987; Lander and Botstein 1989; Darvasi and Soller 1992; Foolad et al. 1997). Allele frequency differences were found to be significant when $p_T - p_S \geqslant 2\sigma_p$, where $\sigma_p = (p_T q_T/2N_T + p_S q_S/2N_S)^{1/2}$ is the standard error of the difference between marker-allele frequencies, N_T is the number of salt-tolerant families, and N_S is the number of salt-sensitive families (Foolad et al. 1997); this test provides a confidence interval of more than 95% (Steel and Torrie 1980; Foolad et al. 1997). At each marker locus, a significant allele-frequency difference between the salt-tolerant and salt-sensitive classes was inferred as an association of the marker locus with a putative QTL(s) affecting salt tolerance (Stuber et al. 1980; Lebowitz et al. 1987; Lander and Botstein 1989; Foolad et al. 1997).

Results and discussion

Genetic map

A total of 151 RFLP markers were scored for each of the 119 BC_1 plants and a genetic linkage map was constructed (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). Because of the low level of marker polymorphism between the two parents, three gaps larger than 30 cM were left on chromosomes 9, 11, and 12 (Fig. 1). The length of this map, however, is comparable with that of the high-density RFLP map of tomato (Tanksley et al. 1992); the order of the markers in the two maps is also in good agreement, although there are some differences in the distribution of recombination along the chromosomes (Chen and Foolad 1998).

Germination responses of the parental lines and BC_1S_1 families

Seed of the *L. pimpinellifolium accession* (LA722) germinated significantly more rapidly than seed of NC84173, as determined either by the germination percentiles or the germination index (Table 1); this indicates a greater salt tolerance of LA722 during germination, consistent with the results of previous investigations (Foolad and Lin 1997 a). Seed of the BC_1S_1 families germinated intermediate between the two parents although, as expected, they were more similar to the slow-germinating recurrent parent (Table 1; Fig. 2). These results are in agreement with those of several previous studies on salt tolerance during germination in tomato which indicated the involvement of genes with additive effects on this seed trait (Foolad and Jones 1991, 1992; Foolad 1996 b). In the BC_1S_1 population, the segregation for germination time under salt-stress was obvious (Fig. 2) and the germination standard deviations were much larger than those for the parental lines (Table 1). The BC_1S_1 population exhibited a continuous variation for germination under salt-stress, typical of quantitative traits (Fig. 2).

Across BC_1S_1 families, there were highly significant correlations among the various germination criteria, including T25, T50, T75 and the germination index (Table 2). In addition, the results of QTL analyses were similar when different germination criteria were employed (see below). Therefore, only the results based on the time to 50% germination (T50) are presented and discussed. In the BC_1S_1 population, T50 ranged from 4.67 to >28 days (a few families did not reach 50% germination by the end of the experiments); no family germinated as fast or faster than the fast-germinating donor parent (LA722), whereas 10.6% of the families germinated significantly more slowly than the slow-germinating recurrent parent (this includes families that did not reach T50 by 28 days).

Identification of QTLs conferring salt tolerance

Interval mapping

The interval-mapping analysis identified four QTL-likelihood peaks on chromosomes 1, 9, and 12 with LOD scores greater than 2.0 and individual effects of 11.5–15.6% of the total phenotypic variation (PVE), and three QTL-likelihood peaks on chromosomes 2, 5, and 7 with LOD scores greater than 1.2 and individual effects of 6.5–8.5% PVE (Table 3); the QTL likelihood plots for peaks with LODs >2.0 are depicted in Fig. 3. All identified QTL intervals except CT52–TG113 on chromosome 7 (LOD = 1.52, PVE = 7.2%) had the favorable QTL alleles from the fast-germinating donor parent (LA722).

The QTLs with largest effects were identified on chromosome 1; on this chromosome, two QTL peaks were identified with LODs of 2.66 and 3.53 and individual PVE values of 12.6 and 15.6%, respectively (Table 3, Fig. 3). The presence of multiple nearby likelihood peaks on this chromosome, however, does not necessarily mean the presence of multiple genomic

Fig. 1 A restriction fragment length polymorphism (RFLP) linkage map of tomato chromosomes constructed based on a BC_1 population of a cross between *L. esculentum* (NC84173; pistillate and recurrent parent) and *L. pimpinellifolium* (LA722). The *number* identifying the chromosome is shown at the *top* of each chromosome. The names of the markers are listed at the *right* of chromosomes. *Numbers on the left* are centiMorgan (cM) distances (Kosambi function) between adjacent markers. The *boxes* on chromosomes 1, 2, 5, 7, 9, and 12 indicate the approximate location of QTLs detected for salt tolerance during germination in the BC_1S_1 population

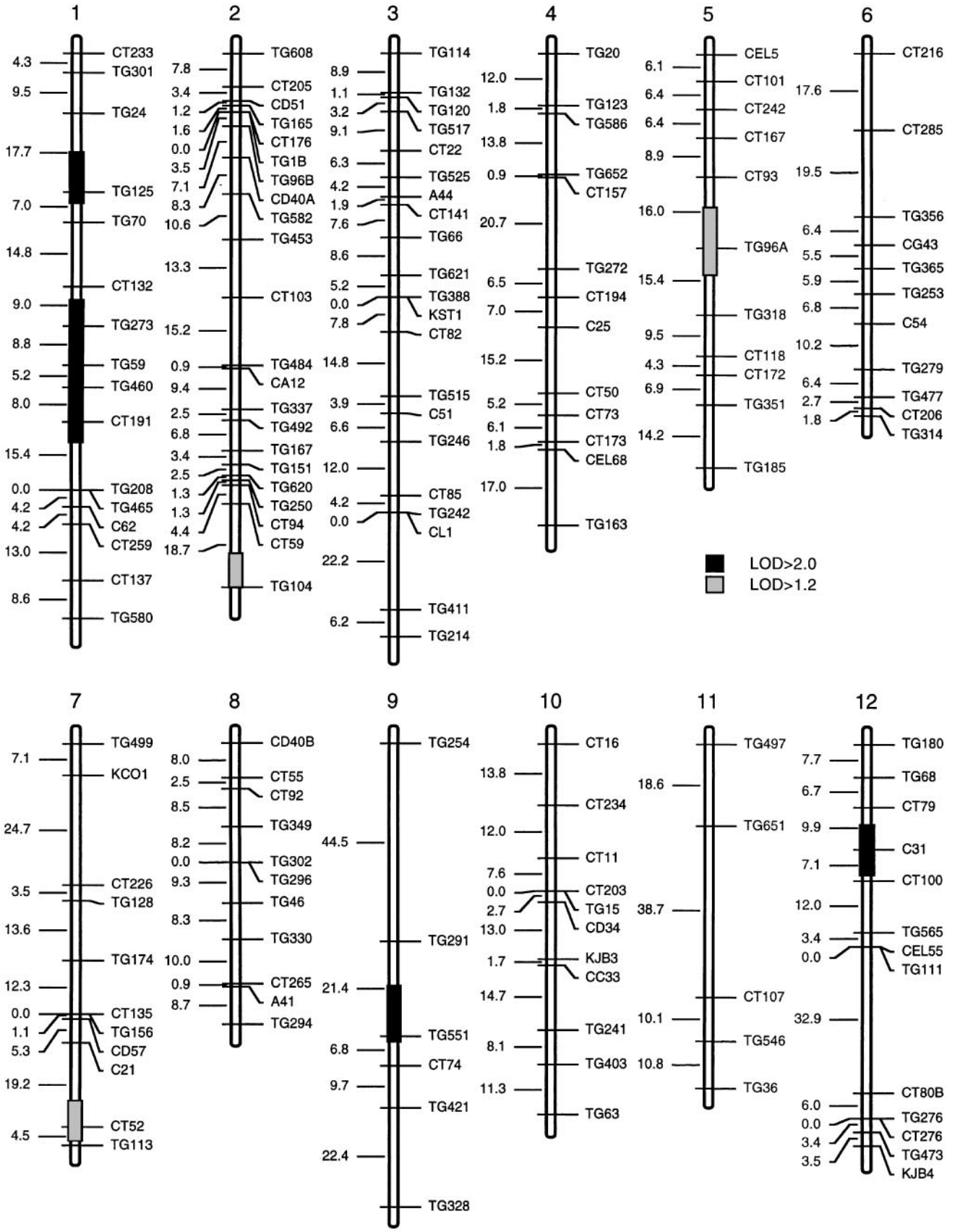
1
4.3 CT233
TG301
9.5 TG24
17.7 TG125
7.0 TG70
14.8 CT132
9.0 TG273
8.8 TG59
5.2 TG460
8.0 CT191
15.4 0.0 TG208
4.2 TG465
4.2 C62
13.0 CT259
CT137
8.6 TG580
2
7.8 TG608
3.4 CT205
1.2 CD51
1.6 TG165
0.0 CT176
3.5 TG1B
7.1 TG96B
8.3 CD40A
10.6 TG582
TG453
13.3 CT103
15.2 0.9 TG484
9.4 CA12
2.5 TG337
6.8 TG492
3.4 TG167
2.5 TG151
1.3 TG620
1.3 TG250
4.4 CT94
18.7 CT59
TG104
3
8.9 TG114
1.1 TG132
3.2 TG120
9.1 TG517
CT22
6.3 TG525
4.2 A44
1.9 CT141
7.6 TG66
8.6 TG621
5.2 TG388
0.0 KST1
7.8 CT82
14.8 TG515
3.9 C51
6.6 TG246
12.0 CT85
4.2 TG242
0.0 CL1
22.2 TG411
6.2 TG214
4
12.0 TG20
1.8 TG123
TG586
13.8 TG652
0.9 CT157
20.7 TG272
6.5 CT194
7.0 C25
15.2 CT50
5.2 CT73
6.1 CT173
1.8 CEL68
17.0 TG163
5
6.1 CEL5
CT101
6.4 CT242
6.4 CT167
8.9 CT93
16.0 TG96A
15.4 TG318
9.5 CT118
4.3 CT172
6.9 TG351
14.2 TG185
LOD>2.0
LOD>1.2
6
17.6 CT216
CT285
19.5 TG356
6.4 CG43
5.5 TG365
5.9 TG253
6.8 C54
10.2 TG279
6.4 TG477
2.7 CT206
1.8 TG314
7
7.1 TG499
KCO1
24.7 CT226
3.5 TG128
13.6 TG174
12.3 0.0 CT135
1.1 TG156
5.3 CD57
C21
19.2 CT52
4.5 TG113
8
8.0 CD40B
2.5 CT55
CT92
8.5 TG349
8.2 0.0 TG302
9.3 TG296
TG46
8.3 TG330
10.0 0.9 CT265
8.7 A41
TG294
9
TG254
44.5 TG291
21.4 TG551
6.8 CT74
9.7 TG421
22.4 TG328
10
CT16
13.8 CT234
12.0 CT11
7.6 0.0 CT203
2.7 TG15
CD34
13.0 KJB3
1.7 CC33
14.7 TG241
8.1 TG403
11.3 TG63
11
18.6 TG497
TG651
38.7 CT107
10.1 TG546
10.8 TG36
12
7.7 TG180
TG68
6.7 CT79
9.9 C31
7.1 CT100
12.0 TG565
3.4 CEL55
0.0 TG111
32.9 CT80B
6.0 TG276
0.0 CT276
3.4 TG473
3.5 KJB4

Table 1 Germination responses (days ± SD) of the parental lines and BC_1S_1 population of the cross *L. esculentum* (NC84173) × *L. pimpinellifolium* (LA722) evaluated under salt-stress

Genotype	Germination response[a]				Final germination (%)[b]
	T25	T50	T75	GI	
NC84173	7.24 ± 0.56	10.04 ± 0.67	16.42 ± 1.78	9.57 ± 0.84	86
LA722	2.96 ± 0.28	3.50 ± 0.14	4.46 ± 0.34	3.71 ± 0.10	99
BC_1S_1	5.93 ± 1.18	7.92 ± 2.40	12.91 ± 6.23	7.73 ± 2.12	90

[a] Time to 25% (T25), 50% (T50), or 75% (T75) germination and germination index (GI)
[b] Percentage of viable seeds that germinated by the end of 28 days under salt-stress

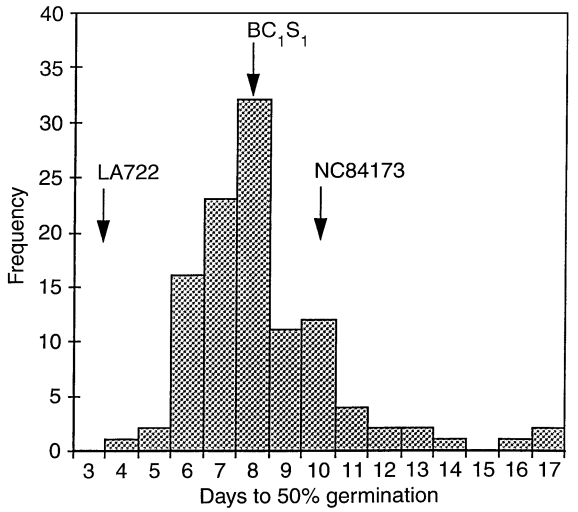

Fig. 2 Frequency distribution for the time to 50% germination (T50) under salt-stress in the BC_1S_1 population. Means of the parental lines and BC_1S_1 population are shown by *arrows*

Table 2 Pairwise phenotypic correlations between the time to 25% (T25), 50% (T50), and 75% (T75) germination and germination index (GI) under salt-stress in the BC_1S_1 population

Germination response	Germination response		
	T50	T75	GI
T25	0.86**	0.83**	0.85**
T50		0.83**	0.82**
T75			0.70**

** Significant at the $P < 0.01$

regions on this chromosome with significant effect (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 this chromosome was 17.3%, slightly higher than the individual effects of the interval TG273–TG59 alone (15.6%). This may be due to the presence of colinearity effects among the adjacent QTLs or because only a single genomic region is present on this chromosome with a significant effect on this trait. Furthermore, it cannot be determined from this study whether there is a single QTL or multiple QTLs at each interval (i.e., genomic region). The resolution of QTL mapping might be improved by adding more genetic markers and using larger populations to allow an examination of more recombinant types.

Multilocus analysis indicated that the QTLs identified on different chromosomes were, for the most part, independent of each other and that their effects were generally additive. For example, the major QTLs on chromosomes 1 (interval TG273–TG59, PVE = 15.6%), 9 (TG291–TG551, PVE = 12.4%), and 12 (CT31–CT100, PVE = 11.5%) could together account for 35.6% of the total phenotypic variation; similarly, the minor QTLs on chromosomes 2 (CT59–TG104, PVE = 6.5%), 5 (TG96A–TG318, PVE = 8.5%), and 7 (CT52–TG113, PVE = 7.2%) could together account for 20.9% of the total phenotypic variation. However, when both major and minor QTLs were considered simultaneously, their combined effects were less than the complete additive and could together account for 44.5% of the total phenotypic variation. This may be due to "less-than-additive epistatic interactions" among QTLs (Eshed and Zamir 1996), or to the fact that some of the QTLs affect this trait through similar (or identical) developmental processes. It is tempting to speculate that perhaps some of the minor QTLs are redundant forms of the major QTLs and, thus, in the presence of the major QTLs the effects of minor QTLs are diminished. However, further investigations are needed to determine the genetic relationships among the major and minor QTLs identified in this study.

Single-marker analysis

The results of the single-marker analysis were generally similar to the results of interval mapping in that the same seven genomic locations were identified with significant effects on the trait. The QTL-linked RFLP markers that were identified by single-marker analysis are listed in Table 3. The single-marker analysis identified marker-linked QTLs on chromosomes 1, 5, 7, and 12 with $P < 0.01$ and on chromosomes 2 and 9 with $P < 0.05$ (Table 3). The results indicate a comparable efficiency of the interval mapping and single-marker analysis in identifying marker-linked QTLs.

Table 3 QTLs detected for salt tolerance during germination based on interval mapping and single-marker analysis in the BC_1S_1 population. For the single-marker analysis, the most closely associated molecular marker locus is indicated by *. LOD = log-likelihood; PVP = percent phenotypic variation explained; phenotypic effect = difference between the E/PM and E/E in days. Parentheses (in the interval column) indicate QTLs with effects opposite to those expected from the parental means

Interval	Chrom.	Interval length (cM)	Interval mapping			Single-marker analysis				
			LOD	PVE	Phenotypic effect	*P* value	R^2 %	E/E[a] mean	E/PM mean	E/PM-E/E
TG125*–TG70	1	7.0	2.66	12.6	−0.92	0.0010	10.86	7.83	6.97	−0.86
TG273*–TG59	1	8.8	3.53	15.6	−1.03	0.0003	13.58	7.94	6.95	−0.99
CT59–TG104*	2	18.7	1.20	6.5	−0.67	0.0473	6.4	7.73	6.99	−0.74
TG96A*–TG318	5	16.0	1.52	8.5	−0.75	0.0053	8.3	7.76	7.00	−0.76
(CT52*–CT113)	7	4.5	1.52	7.2	0.69	0.0084	7.2	6.96	7.65	0.69
TG291–TG551*	9	21.4	2.01	12.4	−0.91	0.0131	6.3	7.70	7.04	−0.66
C31*–CT100	12	7.1	2.40	11.5	−0.89	0.0005	12.41	7.93	6.99	−0.74

[a] E/E = homozygous for *L. esculentum* alleles; E/PM = heterozygous

Distributional-extreme analysis

The results of distributional-extreme analysis were generally consistent with the results of interval mapping and single-marker analysis. Monogenic segregation of RFLP markers which were identified to be QTL-linked by interval mapping and/or single-marker analysis are displayed in Table 4 for the selected salt-tolerant and salt-sensitive classes; for these markers, the allele-frequency differences between the extreme classes are also shown in the table. The results indicated presence of large allele frequency differences between the extreme classes for these markers, suggesting their association with QTLs affecting salt tolerance during germination in tomato. However, in most cases the marker-allele frequency differences were only close to the significant level and were significant ($P < 0.05$) only for markers on chromosome 1 (Table 4). The observation that no other marker locus in this population exhibited a marker-allele frequency difference as large as that observed for any of the markers in Table 4, indicates that the latter markers were most likely linked to QTLs affecting salt tolerance.

Factors affecting the efficiency of distributional extreme analysis (selective genotyping) in detecting marker-linked QTLs were described elsewhere (Foolad et al. 1997). In the present study, the most likely reason for the reduced effectiveness of this analysis in detecting marker-linked QTLs, compared to the interval mapping and single-marker analysis, was the use of a rather small population and, consequently, the resulting large standard errors for testing allele-frequency differences between extreme classes (Table 4).

Comparison of the QTL results with our previous study

There were some similarities and differences between the results of the present study and those of our previous study in which an F_2 population of an interspecific cross between *L. esculentum* (UCT5) and *L. pennellii* (LA716) was used to identify QTLs contributing to salt tolerance during germination in tomato (Foolad et al. 1997). First, in both studies, a similar number of genomic regions were identified with significant effects on this trait. Second, in both studies, chromosomes 1, 9, and 12 contained favorable QTL alleles from the corresponding salt-tolerant wild parents while chromosome 7 contained favorable QTL alleles from the corresponding salt-sensitive cultivated parents. On these chromosomes, however, the locations of the identified QTLs in the two studies were not necessarily identical; a limited number of shared markers between the two studies precluded a precise comparison of the positions of the QTLs. Third, and in contrast, the previous study identified favorable QTLs on chromosome 2 of the sensitive parent and chromosome 3 of the tolerant parent, whereas the present study identified favorable QTLs on chromosomes 2 and 5 of the tolerant parent. Overall, the results indicate that some of the QTLs for salt tolerance during germination in tomato are conserved across species, but some other QTLs seem to be species-specific. For practical purposes, however, the results of the present study would be more useful because *L. pimpinellifolium* is a preferred species for gene introgression, compared to *L. pennellii*. The *L. pimpinellifolium* accession (LA722) used in this study exhibits a number of other desirable horticultural characteristics which could be incorporated into commercial tomato cultivars through marker-assisted selection and breeding. Currently, we are identifying QTLs for several other horticultural traits in LA722, including salt tolerance during vegetative growth and reproduction.

Number, magnitude of effects, and nature of QTLs

The results of this study indicate the presence of QTLs with differential effects on salt tolerance; a few QTLs

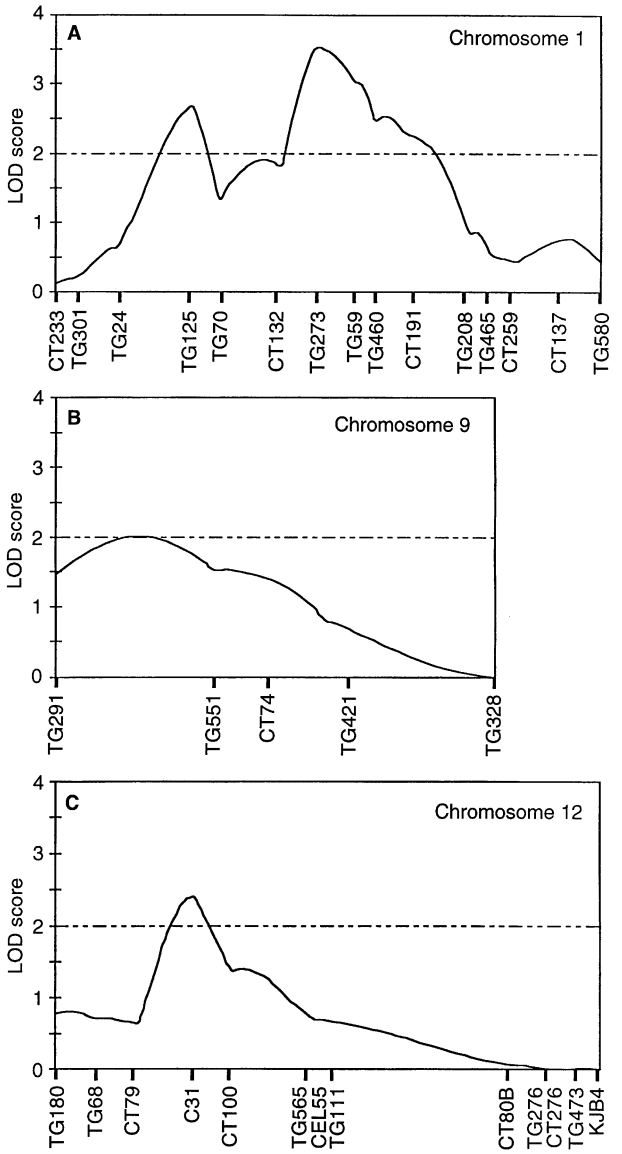

Fig. 3 QTL likelihood plots indicating LOD scores for tomato seed germination under salt-stress along chromosomes 1 (**A**), 9 (**B**), and 12 (**C**). The order of the marker loci is presented along the abscissa; the map distance between adjacent markers can be found in Fig. 1. The height of the curve indicates the strength of the evidence (log 10 of the odd ratio) for the presence of a QTL at each location. The maximum-likelihood position of the QTL(s) is the highest point(s) on the curve

were identified with considerable effects (PVE $> 10\%$) and a few others were identified with smaller effects (PVE $< 10\%$) (Table 3). The three intervals containing QTLs with large effects, TG273–TG59, TG291–TG551, and CT31–CT100 on chromosomes 1, 9, and 12, respectively, together could account for 35.6% of the total phenotypic variation. When the minor QTLs were added, a total of 44.5% 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. In addition, some of the remaining variation could be due to epistatic interactions among QTL-linked and/or unlinked markers (see below). 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); this is consistent with the results of several other investigations (e.g., 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 that quantitative trait. The identification of a few genomic locations with large effects on tomato salt tolerance during germination indicates that a rapid response to directional selection for this trait is expected, which is consistent with previous research (Foolad 1996 b; Foolad and Lin 1997 b).

As expected, most of the identified QTLs had the favorable alleles from the salt-tolerant parent (LA722). However, the QTL detected on chromosome 7 had the favorable allele from the salt-sensitive parent. This finding demonstrates the ability of marker analysis to uncover cryptic genetic variation that otherwise would have been masked by the large difference between the parents. Such an observation is not uncommon and has been reported for many other traits and in various species (e.g., Weller et al. 1988; deVicente and Tanksley 1993). The presence of favorable QTL alleles in both parents suggests a strong likelihood for recovering transgressive segregants and provides a source of new alleles for plant breeding.

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 31 two-locus epistatic interactions [equivalent to 12 between-region interactions (each region containing one or more marker)] were identified. The identified interactions were of two types: interactions between QTL-linked regions and QTL-unlinked regions, of which four interactions were identified; and interactions between QTL-unlinked regions, of which eight interactions were identified (Table 5). Some general comments can be made about these interactions: (1) in most cases, the F values barely exceeded the threshold level of 10 and were always less than 14 (Table 5); (2) the F values were generally larger for the interactions involving QTL-linked regions (see Table 5); (3) for many of the interactions involving QTL-unlinked regions, the contributing regions exhibited some independent effects, although the effects were statistically not significant; and (4) the percentage of significant two-locus interactions [either based on all interactions

Table 4 Monogenic segregation of QTL-linked RFLP markers on tomato chromosomes for the selected salt-tolerant and salt-sensitive classes. A negative sign of the allele frequency difference between the tolerant and sensitive classes ($p_T - p_S$) indicates allele-frequency changes in the opposite direction to the parental phenotype

Genetic marker	Tolerant class			Sensitive class			Difference	
	E/E[a]	E/PM	p_T[b]	E/E	E/PM	p_S[b]	$p_T - p_S$	σ_p[c]
Chromosome 1								
TG125	0	14	0.500	6	5	0.227	0.273*	0.130
CT132	1	13	0.464	7	4	0.182	0.282*	0.125
TG273	1	13	0.464	7	4	0.182	0.282*	0.125
TG59	1	13	0.464	7	4	0.182	0.282*	0.125
TG460	2	12	0.429	7	4	0.182	0.247	0.125
CT191	1	12	0.462	8	3	0.136	0.326*	0.122
Chromosome 2								
TG104	2	10	0.416	6	3	0.167	0.249	0.133
Chromosome 5								
TG96A	3	11	0.392	6	4	0.200	0.192	0.128
Chromosome 7								
CT52	10	4	0.143	3	8	0.364	−0.221	0.122
TG113	10	4	0.143	3	8	0.364	−0.221	0.122
Chromosome 9								
TG551	2	12	0.429	7	4	0.182	0.247	0.125
CT74	2	12	0.429	7	4	0.182	0.247	0.125
Chromosome 12								
C31	2	12	0.429	7	4	0.182	0.247	0.125
CT100	2	12	0.429	6	5	0.227	0.201	0.129

* Significant at $P < 0.05$
[a] E/E = homozygous for *L. esculentum* alleles; E/PM = heterozygous
[b] *L. pimpinellifolium* allele frequency among the tolerant (p_T) and sensitive progeny (p_S)
[c] Standard error of the difference between allele frequencies

Table 5 Two-way epistatic interactions between markers for salt tolerance during germination in the BC_1S_1 population

Marker 1			Marker 2			*F* value	EPM/EPM[a] T50[b]	EE/EPM T50	EPM/EE T50	EE/EE T50
QTL-linked	QTL-unlinked	Chromosome	QTL-linked	QTL-unlinked	Chromosome					
TG273		1		CT92	8	13.14	6.93	8.66	6.97	7.13
TG113		7		CT92	8	13.95	8.45	6.85	6.93	7.18
TG125		1		CT141	3	10.76	6.40	8.06	7.51	7.59
TG551		9		TG582	2	10.43	7.29	6.73	7.34	8.40
	CT82	3		TG63	10	12.72	6.79	7.85	7.84	7.07
	TG242	3		TG296	8	10.74	7.99	6.91	6.87	7.45
	TG123	4		CT11	10	12.03	6.76	7.70	7.85	6.99
	CT216	6		TG294	8	10.45	7.17	7.48	7.94	6.53
	KCO1	7		TG328	9	10.73	7.87	6.68	7.17	7.72
	TG330	8		A41	8	10.11	7.4	9.47	7.77	7.00
	TG254	9		TG546	11	10.00	6.44	7.73	7.70	7.39
	CT234	10		CD34	10	13.07	6.96	8.67	8.17	7.29

[a] E/E = homozygous for *L. esculentum* alleles; E/PM = heterozygous
[b] Time to 50% germination

($31/11325 \cong 0.003$) or only interactions between unlinked markers ($20/11325 \cong 0.002$)] was even smaller than the percentage expected to occur by chance; thus, it is possible that some of these interactions were due to chance events. Further investigations, including the use of a larger population or the construction of isogenic lines with various combinations of these markers, are necessary to determine the exact nature of these interactions. Several previous investigations indicated very limited or no interactions among QTLs (Stuber et al. 1992; deVicente and Tanksley 1993; Grandillo and Tanksley 1996) whereas others suggested the presence of significant epistatic interactions among QTL-linked or -unlinked markers (Cocherham and Zeng 1996;

Eshed and Zamir 1996; Li et al. 1997). For breeding purposes, QTLs which do not require epistatic interactions are more desirable.

Relationship between phenotypes and QTL genotypes and the prospect for marker-assisted selection

The correspondence between the phenotypes and QTL genotypes of the BC_1S_1 families was determined by graphical genotyping (Young and Tanksley 1989) using QGENE. The results indicated a very high correspondence between the phenotypes and QTL genotypes. For example, the graphical genotypes of the three most-rapidly germinating (salt-tolerant) and the three most-slowly germinating (salt-sensitive) BC_1S_1 families for chromosomes 1, 9, and 12 are displayed in Fig. 4. The three salt-tolerant families (Fig. 4, *top*) had the best-possible QTL compositions (i.e., had favorable QTL alleles from the salt-tolerant parent) and the three salt-sensitive families had often the opposite (i.e., negative) QTL alleles (Fig. 4, *bottom*). These results strongly indicate that the identified QTLs in this study were genuine and, thus, could be used in marker-assisted selection (MAS) and breeding for improved salt tolerance during germination in tomato. Further, the results of the graphical genotyping indicate the power of marker analysis in the precise identification of genomic locations with significant effects on quantitative traits and, thus, the predictive value of QTL genotyping.

The present study identified five genomic regions in the wild genotype with significant positive effects on salt tolerance during germination in tomato. Introgression of this small number of genomic regions by MAS into the cultivated background is feasible, providing opportunities to rapidly develop tomato cultivars with enhanced salt tolerance during germination. These five regions, however, together could account for only

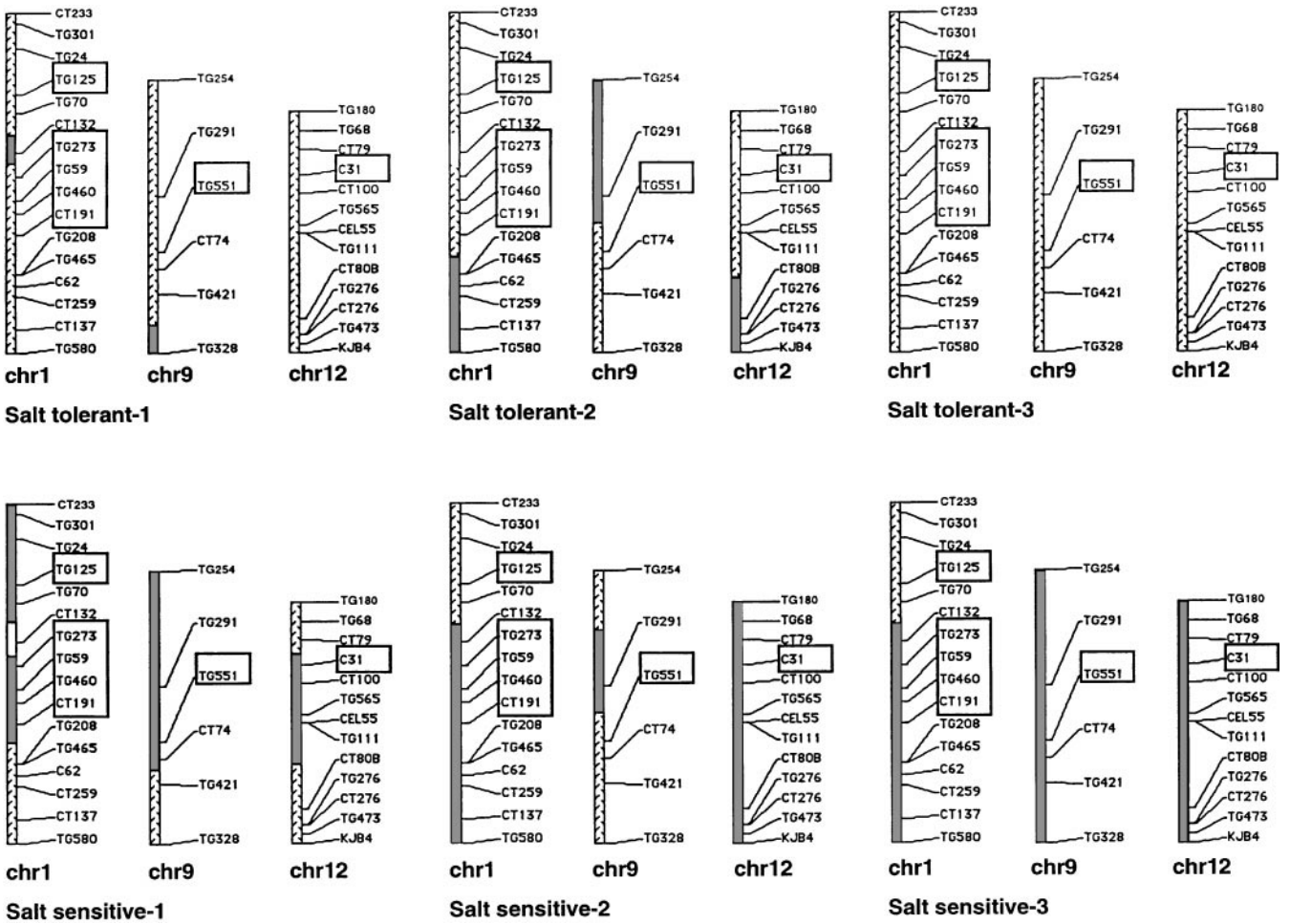

Fig. 4 Chromosomal constitution of the parental BC_1 individuals of the three fastest germinating (salt-tolerant) and three slowest germinating (salt-sensitive) BC_1S_1 families for chromosomes 1, 9, and 12. The *diagonal hatching area* indicates the presence of heterozygous genotypes at the marker loci and the *dark gray area* indicates homozygous recurrent parent genotypes. The names of the markers are listed at the *right* of chromosomes. The enclosed (*boxed*) markers indicate the approximate location of QTLs contributing to salt tolerance during germination in this population. It is apparent that the fast-germinating families had the favorable QTL alleles (for rapid germination under salt-stress) and the slow-germinating families had the opposite QTL alleles

40.3% of the total phenotypic variation. It is likely that there were more genomic regions (probably with smaller effects) in this wild germplasm which were not detected in this study. However, as the number of genomic regions to be transferred increases, the utility of MAS becomes less clear, as larger and larger populations would be necessary to identify the favorable QTL combinations. An alternative approach would be to incorporate only major QTLs, but use more than one gene resource in the subsequent deployment of useful genes. This approach may be more rational in most breeding programs, as more than one germplasm is often utilized during the life of a breeding project.

Conclusion

Three methods of marker-QTL analysis were effective in identifying QTLs with significant effects on salt tolerance during germination in tomato. It appears that this seed trait in tomato is controlled by a few major QTLs which act in concert with a number of smaller effect QTLs; the identified QTLs together could account for 44.5% of the total phenotypic variation, indicating that additional unidentified QTLs with presumably smaller effects might be involved. As expected, most of the favorable QTL alleles were contributed from the salt-tolerant parent. There was a high correspondence between the phenotype and QTL genotype of the progeny, indicating the predictive value of QTL genotyping, and confirming that the identified QTLs were genuine. The results indicate that the prospect for using marker-assisted selection to improve tomato seed germination under salt-stress is good.

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References

Asins MJ, Breto MP, Carbonell EA (1993) Salt tolerance in *Lycopersicon* species. II. Genetic effects and a search for associated traits. Theor Appl Genet 86: 769–774

Bernatzky R, Tanksley SD (1986) Majority of random cDNA clones correspond to single loci in the tomato genome. Mol Gen Genet 203: 8–12

Chen FQ, Foolad MR (1998) A molecular linkage map of tomato based on an interspecific cross between *Lycopersicon esculentum* and *L. pimpinellifolium* and its comparison with other molecular maps of tomato. Genome (in press)

Cocherham CC, Zeng ZB (1996) Design III with marker loci. Genetics 143: 1437–1456

Darvasi A, Soller M (1992) Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theor Appl Genet 85: 353–359

deVicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 585–596

Eshed Y, Zamir D (1996) Less-than-additive epistatic interactions of quantitative trait loci in tomato. Genetics 143: 1807–1817

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling fragments to high specific activity. Anal Biochem 132: 6–13

Foolad MR (1996 a) Genetic analysis of salt tolerance during vegetative growth in tomato, *Lycopersicon esculentum* Mill. Plant Breed 115: 245–250

Foolad MR (1996 b) Response to selection for salt tolerance during germination in tomato seed derived from P.I. 174263. J Am Soc Hort Sci 121: 1006–1011

Foolad MR (1997) Genetic basis of physiological traits related to salt tolerance in tomato, *Lycopersicon esculentum* Mill. Plant Breed 116: 53–58

Foolad MR, Jones RA (1991) Genetic analysis of salt tolerance during germination in *Lycopersicon*. Theor Appl Genet 81: 321–326

Foolad MR, Lin GY (1997 a) Genetic potential for salt tolerance during germination in Lycopersicon species. HortScience 32: 296–300

Foolad MR, Lin GY (1997 b) Absence of a relationship between salt tolerance during germination and vegetative growth in tomato. Plant Breed 116: 363–367

Foolad MR, Jones RA (1992) Parent-offspring regression estimates of heritability for salt tolerance during germination in tomato. Crop Sci 32: 439–442

Foolad MR, Jones RA, Rodriguez RL (1993) RAPD markers for constructing intraspecific tomato genetic maps. Plant Cell Rep 12: 293–297

Foolad MR, Stoltz T, Dervinis C, Rodriguez RL, Jones RA (1997) Mapping QTLs conferring salt tolerance during germination in tomato by selective genotyping. Mol Breed 3: 269–277

Grandillo S, Tanksley SD (1996) QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium*. Thoer Appl Genet 92: 935–951

Greenway H, Munns R (1980) Mechanism of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31: 149–190

Johnson DW, Smith SE, Dobrenz AK (1992) Genetic and phenotypic relationships in response to NaCl at different developmental stages in alfalfa. Theor Appl Genet 83: 833–838

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124: 743–756

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199

Lebowitz RJ, Soller M, Beckmann M (1987) Trait-based analysis for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor Appl Genet 73: 556–562

Lee E (1980) Statistical methods for survival data analysis, Wadworth, Lifetime Learning Publications, Belmont, California

Li ZK, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain-yield components in rice (*Oryza sativa* L.). Genetics 145: 453–465

Lincoln S, Daly M, Lander E (1992) Constructing genetic maps with MAPMAKER/EXP 3.0. 3rd Whitehead Institute Technical Report

Maas EV (1986) Salt tolerance of plants. Appl Agric Res 1: 12–26

Martin B, Nienhuis J, King G (1989) Restriction fragment length polymorphisms associated with water-use efficiency in tomato. Science 243: 1725–1728

Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3: 239–245

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127: 181–197

Rick CM (1958) The role of natural hybridization in the derivation of cultivated tomatoes in western South America. Econ Bot 12:346–367

Rick CM (1983) The potential of exotic germplasm for tomato improvement. In: Vasil IK, Scowcroft WR, Freys, KJ (eds) Plant improvement and somatic cell genetics. Academic Press, New York, pp 1–28

Steel RGD, Torrie JH (1980) Principles and procedures of statistics, 2nd edn. McGraw-Hill, New York

Stuber CW, Moll RH, Goodman MM, Schaffer H, Weir BS (1980) Allozyme frequency changes associated with selection for increased grain yield in maize. Genetics 95:225–236

Stuber CW, Edward MD, Wendel JF (1987) Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component trais. Crop Sci 27:639–648

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Tal M, Shannon MC (1983) Salt tolerance in the wild relatives of the cultivated tomato: responses of *Lycopersicon esculentum*, *L. cheesmanii*, *L. peruvianum*, *Solanum pennellii* and F_1 hybrids to high salinity. Aust J Plant Physiol 10:109–117

Tanksley SD, Ganal MW, Prince JP, Vicente MCd, Bonierbale MW, Broun P, Fulton TM, giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92:213–224

Weller JI, Soller M, Brody T (1988) Linkage analysis of quantitative traits in an interspecific cross of tomato (*Lycopersicon esculentum* × *Lycopersicon pimpinellifolium*) by means of genetic markers. Genetics 118:329–339

Young ND, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77:95–101

Zhang Q, Gao YJ, Yang SH, Ragab RA, Maroof MAS, Li ZB (1994) A diallel analysis of heterosis in elite hybrid rice based on RFLPs and microsatellites. Theor Appl Genet 89:185–192