Theor Appl Genet (1993) 87:184–192

THEORETICAL AND APPLIED GENETICS

Mapping salt-tolerance genes in tomato (*Lycopersicon esculentum*) using trait-based marker analysis

M. R. Foolad, R. A. Jones

Department of Vegetable Crops, University of California, Davis, Davis, CA 95616-8746, USA

Received: 5 November 1992 / Accepted: 1 March 1993

Abstract. The germination responsiveness of an F_2 population derived from the cross *Lycopersicon esculentum* (UCT5) × *L. pennellii* (LA716) was evaluated for salt tolerance at two stress levels, 150 m*M* NaCl + 15 m*M* $CaCl_2$ and 200 m*M* NaCl + 20 m*M* $CaCl_2$. Individuals were selected at both tails of the response distribution. The salt-tolerant and salt-sensitive individuals were genotyped at 16 isozyme loci located on 9 of the 12 tomato chromosomes. In addition, an unselected (control) F_2 population was genotyped at the same marker loci, and gene frequencies were estimated in both selected and unselected populations. Trait-based marker analysis was effective in identifying genomic locations (quantitative trait loci, QTLs) affecting salt tolerance in the tomato. Three genomic locations marked by *Est-3* on chromosome 1, *Prx-7* on chromosome 3, and *6Pgdh-2* and *Pgi-1* on chromosome 12 showed significant positive effects, while 2 locations associated with *Got-2* on chromosome 7 and *Aps-2* on chromosome 8 showed significant negative effects. The identification of genomic locations with both positive and negative effects on this trait suggests the likelihood of recovering transgressive segregants in progeny derived from these parental lines. Similar genomic locations were identified when selection was made either for salt tolerance or salt sensitivity and at both salt-stress treatments. Comparable results were obtained in uni- and bidirectional selection experiments. However, when marker allele gene frequencies in a control population are unknown, bidirectional selection may be more efficient than unidirectional selection in identifying marker-QTL associations. Results from this study are discussed in relationship to the use of molecular markers in developing salt-tolerant tomatoes.

Communicated by G. Wenzel

Correspondence to: M. R. Foolad

Key words: Tomato – Salt tolerance – Seed germination – Isozyme markers – QTL mapping

Introduction

Salinity is a major cause of the loss of agricultural land and reduction in crop productivity (Epstein 1972). One way to alleviate the problem is the breeding of salt-tolerant genotypes that perform better than current sensitive varieties under moderate to high salinity stress. Evidence collected from many crop species including *Lycopersicon* suggests that salt tolerance is a stage-specific phenomenon (Greenway and Munns 1980; Maas 1987; Shannon 1985, Jones 1986a) and that the seed germination and early seedling growth stages are the most vulnerable to salinity stress (Cook 1979; Jones et al. 1988). Salinity stress greatly delays the onset, reduces the rate, and increases the dispersion of germination events in tomatoes (Jones et al. 1988). This sensitivity has important biological and applied significance. The costly operations of greenhouse seedling production and transplantation into the field have encouraged tomato producers to grow direct-seeded plants. Breeding tomato genotypes that can germinate rapidly and uniformly under salinity stress would ameliorate the need for seedling transplantations under saline conditions.

Salt tolerance in the tomato has been reported to be quantitatively inherited (Jones 1986a, 1987; Foolad and Jones 1991, 1992a, b). Direct selection under field conditions for quantitative traits is difficult because variable environmental factors adversely affect the precision and repeatability of such traits (Jones and Qualset 1984). A suggested approach to breeding for quantitative traits is the indirect selection for linked markers (Sax 1923; Thoday 1961; Thompson and Thoday 1979; Falconer 1981).

For an indirect selection marker to be useful in a breeding program, it has to exhibit both a heritability greater than the heritability of the trait itself and a significant genetic correlation with the trait (Falconer 1981). Molecular and biochemical marker loci closely linked to quantitative trait loci (QTLs) affecting the trait are good candidates (Tanksley and Rick 1980; Burr et al. 1983; Edwards et al. 1987; Stuber et al. 1987; Lander and Botstein 1989; Tanksley et al. 1989; Lande and Thompson 1990). However, the utility of marker-aided selections for quantitative traits remains largely speculative.

Two approaches have been proposed to determine linkage between marker loci and nearby QTLs. The first, *marker-based analysis* (Tanksley and Rick 1980; Burr et al. 1983), is based on differences in the mean value of a quantitative trait among "marker" genotypes in the F_2 or BC progeny of a cross between inbred lines; these differences are presumably generated by QTLs linked to the marker locus. The second approach, *trait-based analysis* (Stuber et al. 1980; Lebowitz et al. 1987; Lander and Botstein 1989), is based on changes in marker allele frequencies in selected lines derived from the F_2 of a cross between inbred lines or in the high and low phenotypic classes of an F_2 or BC population. The latter approach, employed in this study, is more useful when the analysis is aimed primarily at a single quantitative trait. This would include the analysis of polygenic resistance traits where only a part of the population survives following exposure to an environmental challenge/stress.

Among the tomato-related wild species, *L. pennellii* has shown tolerance to high salinity stress (Dehan and Tal 1978; Phills et al. 1979; Tal and Shannon 1983; Jones 1986a) and contains both numerous mapped alleles at electrophoretically detectable enzyme loci and DNA markers that differ from the cultivated type, *L. esculentum* (Tanksley and Rick 1980; Tanksley and Mutschler 1990). This marker system has been used to detect linkage between markers and loci determining qualitative and quantitative traits in tomato (Tanksley et al. 1982; Zamir and Tal 1987; Bournival et al. 1989; Martin et al. 1989; Kinzer et al. 1990). The objective of this study was to identify possible linkage associations between marker loci and genomic locations (QTLs) affecting salt tolerance during germination in an F_2 population of the interspecific cross between *L. esculentum* and *L. pennellii*. The effectiveness of trait-based marker analysis and the efficiency of selection strategies in identifying marker-QTL associations are compared.

Materials and methods

Plant materials

Crosses were made between *L. esculentum* cv 'UCT5' (pistillate parent) and *L. pennellii* (LA716), and F_2 seeds were generated by self-pollinating F_1 flowers and bulk-harvesting mature-ripened F_1 fruits. 'UCT5' is a horticulturally superior, multiple disease-resistant, advanced breeding line that is salt sensitive at all stages of plant development (Jones 1986b). 'LA716' is a self-compatible highly inbred line that easily hybridizes with *L. esculentum* and shows salinity tolerance characteristics (Tal and Shannon 1983; Jones 1986b). The two parents are homozygous and heteromorphic at more than 16 isozyme loci (Tanksley and Rick 1980).

Screening for salt tolerance at germination

To select the extreme phenotypic classes (i.e., high and low salt-tolerant individuals), the following experiments were conducted:

Experiment 1. Sterile germinating media (0.8% agar) containing 150 m*M* NaCl + 15 m*M* $CaCl_2$ was prepared in petri dishes. The water potential of the media was approximately −8.6 bars on a Wescor-5100 vapor pressure osmometer (Wescor, Logan, Utah). F_2 seeds were surface sterilized with 0.5% sodium hypochlorite solution for 10 min, rinsed with sterile distilled water several times, and briefly blotted. A total of 2500 seeds were aseptically plated on 25 petri dishes and incubated at 20° ± 0.5 °C in the dark. Germination response was scored visually as radicle protrusion at 6-h intervals for the first 15 days and at 12-h intervals for the next 20 days. Selection of the salt-tolerant phenotypic class included the 287 seeds (12.1% of the total viable seeds; see below) that germinated at or before 6.375 days after the initial imbibition and are referred to as the "tolerant class 1" (TC1). When we assume a normal phenotypic distribution, this proportion of selected individuals corresponds to a selection intensity of $i = 1.663$ (Falconer 1981). At the end of 35 days, approximately 60% of the seeds had germinated, and little additional germination occurred at this salt concentration. To further discriminate relative salt sensitivity among the ungerminated seeds, we transferred the seeds to a less concentrated salt medium (100 m*M* NaCl + 10 m*M* $CaCl_2$). Plates were maintained in the incubator and scored for germination every 12 h for an additional 15 days. At this time, 12.4% of the original seed population had failed to germinate. To distinguish whether the remaining ungerminated seeds were either nonviable or very salt sensitive, the seeds were transferred onto non-saline media. Within a few days after transfer, more than 50% of the seeds germinated. Approximately 5% of the total (126 seeds) remained ungerminated and were assumed to be initially nonviable (this proportion was comparable to germination tests under control conditions; data not shown). The seeds that germinated following transfer to the non-saline media (186 seeds; 7.8% of the total viable seeds) constituted the selected "sensitive class" (SC). This proportion corresponds to a selection intensity of $i = 1.867$.

Experiment 2. This experiment was designed to select for salt tolerance at a higher salt-stress intensity than the previous experiment. Media was prepared with 200 m*M* NaCl + 20 m*M* $CaCl_2$ and 0.8% agar, with a water potential of −11.0 bars. Germination responses at this high salt concentration were very slow; hence germination was scores on a daily basis. Thirteen days after plating, 97 of 1700 F_2 seeds (i.e., 6% of the potentially viable seeds) had germinated and were classified together as the selected "tolerant class 2" (TC2). The corresponding selection intensity for this proportion was $i = 1.985$.

To examine the salt responsiveness of the parents ('UCT5' and 'LA716'), we evaluated 500 seeds of each parental line for germination performance at each of the two salt-stress treatments. Germination response distribution of the parental lines and the selected F_2 populations were analyzed by survival anal-

ysis with life tables (Lee 1980; Scott and Jones 1982), and the mean time to 50% germination was estimated (Table 1).

Isozyme analysis

The selected "tolerant class 1", "tolerant class 2", and "sensitive class" individuals were transferred from the petri dishes into multipot trays, and after 3 weeks, into a hydroponic system. An unselected sample of 330 F_2 individuals (control) was also grown under similar conditions. Vigorously growing roots from the selected and unselected individuals were assayed using starch gel electrophoresis to determine plant genotypes at 16 isozyme loci. The isozyme loci scored (Table 2) were distributed on 9 of the 12 tomato chromosomes (Tanksley and Rick 1980) and were within about 20 centimorgans (cM) of covering nearly 41% of the tomato genome. Gel electrophoresis, enzyme extraction, and activity staining procedures were according to Tanksley and Orton (1983).

Statistical analysis of marker allele frequencies

Allele frequencies at the 16 enzyme loci were estimated for the selected and control populations using their respective genotype frequencies. The variance of the allele frequency was calculated as a binomial variance (Falconer 1981) as follows:

$$s^2 q = pq/2N,$$

where, p and q are the corresponding allele frequencies at a given isozyme locus and N is the number of individuals genotyped at that locus.

The allele frequency differences between a selected class and the control population (unidirectional selection) or between a sensitive and a tolerant class (bidirectional selection) were determined at individual marker loci. Standard error of the allele frequency difference at each isozyme locus was calculated as follows:

$$SE = (p_1 q_1/2N_1 + p_2 q_2/N_2)^{1/2},$$

where p_1 and q_1 are the allele frequencies at a locus and N_1 is the number of individuals in either the control population (in the case of unidirectional selection) or the sensitive class (in the case of bidirectional selection), and p_2 and q_2 are the allele frequencies at a locus in a tolerant phenotypic class and N_2 is the number of individuals genotype in this class. To test the statistical significance of the allele frequency differences between selected and control populations or between two selected classes, we compared the allele frequency difference at each isozyme locus with the corresponding *SE*; if the difference was equal or greater than three *SE*, it was considered to be significant.

Estimation of the standardized effects of the QTLs

Falconer (1981) has provided an approximate expression relating the selection intensity, i, with the coefficient of selection, s, acting on an individual QTL (i.e., $s = iD$, where $D = 2d/\sigma p$ is the standardized effect of the QTL, d is the difference between the two homozygotes at the QTL, and σp is the population phenotypic standard deviation). With further substitution for s (Falconer 1981, p 28), assuming no recombination between the marker and the QTL, the standardized effect of a QTL as a function of selection intensity and the difference in allelic fre-

Table 1. Days to 50% germination of the parental lines and F_2 populations grown under two salt-stress treatments of 150 m*M* NaCl + 15 m*M* $CaCl_2$ (salt 1) and 200 m*M* NaCl + 20 m*M* $CaCl_2$ (salt 2)

Genotype	Treatment	
	Salt 1	Salt 2
UCT5	17.37	>60.00[a]
LA716	5.16	13.45
F_2	11.50	>60.00[a]

[a] Did not reach 50% germination following 60 days of incubation

Table 2. Monogenic segregation of enzyme-coding genes in an unselected F_2 population derived from the interspecific cross *Lycopersicon esculentum* × *L. pennellii*

Locus	Chromosomal location	Genotype[a]			p[b]	χ^2 (1:2:1)	χ^2 (1:1)
		e/e	*e/p*	*p/p*			
Prx-1	1	63	156	111	0.573	14.94***	13.95***
Skdh-1	1	51	176	103	0.579	17.85***	16.39***
Est-3	1	81	175	72	0.486	1.84	0.49
Dia-2	1	69	181	80	0.517	3.84	0.74
Est-7	2	57	182	91	0.552	10.50**	7.00**
Prx-2	2	53	165	112	0.589	21.10***	21.10***
Prx-7	3	63	170	97	0.551	7.31*	7.00**
Tpi-2	4	60	182	88	0.542	8.25*	4.75*
Pgm-2	4	60	163	107	0.571	13.43**	13.38***
Got-2	7	70	158	102	0.548	6.80*	6.21*
Aps-2	7	71	175	84	0.520	2.24	1.02
Est-2	9	54	180	96	0.564	13.42**	10.68**
Prx-4	10	15	176	139	0.688	94.65***	93.21***
6Pgdh-2	12	50	171	109	0.589	21.53***	21.10***
Pgi-1	12	44	182	104	0.594	26.80***	23.28***
Aco-1	12	70	179	81	0.517	3.11	0.74

*, **, *** Significant at the 5%, 1%, and 0.1% probability levels, respectively

[a] *e/e*, Homozygous for *L. esculentum* alleles; *e/p*, heterozygous; *p/p*, homozygous for *L. pennellii* alleles

[b] p, Allele frequency for *L. pennellii*

quencies at a linked marker, resulting from a one-step, bidirectional selection in an F_2 population, can be estimated as:

$$D = 2\,\delta q/[iq(1-q)]$$

where δq is the difference in marker allele frequencies between the high (tolerant) and the low (sensitive) phenotypic classes; i is sum of the selection intensities (standardized selection differential) between the means of the tolerant and the sensitive classes and the population mean; and q is the allele frequency at the QTL-linked marker locus in the unselected population. Using this expression, we estimated the approximate standardized effect of the marker-linked QTL (i.e., the difference between the two homozygotes at a QTL in standard units) for the QTL-linked markers.

Results

Germination responses of the parental lines and selected F_2 populations

The salt-tolerant parent ('LA716') germinated significantly faster than the salt-sensitive parent (UCT5) under both salt-stress treatments. The difference between the parents was, however, larger under the higher than the lower salt-stress treatment (Table 1). The salt-sensitive parent did not obtain 50% germination following 2 months of incubation under the higher salt-stress treatment. Under the lower salt-stress treatment, seeds of the F_2 population germinated intermediate relative to the parents (Table 1). Under the higher salt-stress treatment, the F_2 population did not reach 50% germination by the end of the experiment, although it was very close.

Segregation of marker loci in unselected and selected F_2 populations

In the unselected population, segregation at 12 of the 16 marker loci showed significant deviations from both the expected Mendelian genotypic ratio of 1:2:1 and the expected allele frequency ratio of 1:1 (Table 2). In all 12 cases, there was an excess of *L. pennellii* alleles, predominantly *pennellii* homozygotes. The greatest distortion was observed at the *Prx-4* locus, where *esculentum* homozygotes were extremely infrequent. Similar segregation distortions were observed at most of the marker loci in the selected classes (Tables 3–5). However, selections for both high and low salt tolerance, and at both salt-stress intensities, consistently, and in most cases significantly, changed the allele frequencies at 6 marker loci when compared to the unselected population. The magnitude and direction of allele frequency changes varied with the selection conditions.

Marker allele frequency changes resulting from unidirectional selections

For the TC1 individuals, we were able to unambiguously score for all but the *Est-3* genotypes (Table 3). As a result of selection for salt tolerance at the intermediate salt-stress treatment (150 m*M* NaCl + 15 m*M* $CaCl_2$), the *L. pennellii* allele frequencies significantly increased at the *Prx-7*, *6Pgdh-2*, and *Pgi-1* loci and significantly decreased at the *Pgm-2* and *Got-2* loci (Table 6, column 1). The magnitude of changes in allele frequencies due to selection at these 5 loci were comparable.

Table 3. Monogenic segregation of enzyme-coding genes in the salt-tolerant group selected at 150 m*M* NaCl + 15 m*M* $CaCl_2$ (tolerant class 1)

Locus	Chromosomal location	Genotype[a]			p[b]	χ^2 (1:2:1)	χ^2 (1:1)
		e/e	*e/p*	*p/p*			
Prx-1	1	47	129	80	0.564	8.52*	8.51**
Skdh-1	1	44	139	70	0.551	7.81*	5.34*
Dia-2	1	41	124	84	0.586	14.85***	14.85***
Est-7	2	27	143	78	0.603	26.80***	12.52***
Prx-2	2	19	159	78	0.615	42.21***	27.20***
Prx-7	3	26	124	100	0.648	43.81***	26.56***
Tpi-2	4	70	134	49	0.458	4.37	3.49
Pgm-2	4	71	129	53	0.464	2.66	2.56
Got-2	7	79	125	46	0.434	8.71*	8.71**
Aps-2	8	68	112	68	0.500	2.32	0.00
Est-2	9	34	131	84	0.600	20.76***	20.08***
Prx-4	10	10	147	95	0.669	64.34***	57.34***
6Pgd-2	12	19	124	109	0.679	64.35***	64.28***
Pgi-1	12	17	124	115	0.688	71.44***	71.34***
Aco-1	12	46	131	72	0.552	6.03*	5.43*

*, **, *** Significant at the 5%, 1%, and 0.1% probability levels, respectively

[a] *e/e*, Homozygous for *L. esculentum* alleles; *e/p*, heterozygous; *p/p*, homozygous for *L. pennellii* alleles

[b] p, Allele frequency for *L. pennellii*

Table 4. Monogenic segregation of enzyme-coding genes in the salt-tolerant group selected at 200 m*M* NaCl + 20 m*M* $CaCl_2$ (tolerant class 2)

Locus	Chromosomal location	Genotype[a]			p[b]	χ^2 (1:2:1)	χ^2 (1:1)
		e/e	*e/p*	*p/p*			
Prx-1	1	9	19	25	0.651	13.91***	9.66**
Skdh-1	1	8	33	12	0.538	3.79	0.60
Est-3	1	6	26	21	0.642	8.51*	8.49**
Dia-2	1	15	20	18	0.528	3.53	0.34
Est-7	2	6	35	12	0.557	6.81*	1.36
Prx-2	2	6	31	16	0.594	5.30*	3.77
Prx-7	3	5	27	21	0.651	9.68**	9.66**
Tpi-2	4	9	31	13	0.538	2.13	0.60
Pgm-2	4	12	27	14	0.519	0.17	0.15
Got-2	7	19	31	3	0.349	11.19**	9.66**
Aps-2	8	33	17	3	0.217	40.77***	33.96***
Est-2	9	6	30	17	0.604	5.49	4.57*
Prx-4	10	2	34	17	0.641	12.74**	8.49**
6Pgdh-2	12	6	17	30	0.726	28.55***	21.74***
Pgi-1	12	8	14	31	0.717	31.75***	19.96***
Aco-1	12	10	30	13	0.528	1.26	0.34

*, **, *** Significant at the 5%, 1%, and 0.1% probability levels, respectively
[a] *e/e*, Homozygous for *L. esculentum* alleles; *e/p*, heterozygous; *p/p*, homozygous for *L. pennellii* alleles
[b] p, Allele frequency for *L. pennellii*

Table 5. Monogenic segregation of enzyme-coding genes in the salt-sensitive class

Locus	Chromosomal location	Genotype[a]			p[b]	χ^2 (1:2:1)	χ^2 (1:1)
		e/e	*e/p*	*p/p*			
Prx-1	1	25	93	57	0.591	12.39**	11.70***
Skdh-1	1	20	89	66	0.631	24.23***	24.18***
Est-3	1	51	65	14	0.358	21.06***	21.06***
Dia-2	1	34	103	38	0.511	5.67	0.18
Est-7	2	21	83	71	0.643	29.03***	28.57***
Prx-2	2	17	107	51	0.597	21.90***	13.21***
Prx-7	3	37	79	15	0.416	12.95**	7.39**
Tpi-2	4	30	98	47	0.549	6.21*	3.30
Pgm-2	4	24	107	44	0.557	13.26 **	4.57*
Got-2	7	18	65	92	0.711	74.15***	62.58***
Aps-2	8	18	102	55	0.606	20.45***	15.65***
Est-2	9	41	97	34	0.480	3.38	0.57
Prx-4	10	6	91	78	0.706	59.52***	59.25***
6Pgdh-2	12	34	101	40	0.517	4.58	0.41
Pgi-1	12	36	104	35	0.497	6.23*	0.01
Aco-1	12	43	85	47	0.511	0.33	0.09

*, **, *** Significant at the 5%, 1%, and 0.1% probability levels, respectively
[a] *e/e*, Homozygous for *L. esculentum* alleles; *e/p*, heterozygous; *p/p*, homozygous for *L. pennellii* alleles
[b] p, Allele frequency for *L. pennellii*

Selection for salt tolerance at the high salt-stress treatment (200 m*M* NaCl + 20 m*M* $CaCl_2$; TC2, Table 4) significantly increased the *L. pennellii* allele frequencies at the *Est-3, 6Pgdh-2,* and *Pgi-1* loci and also significantly decreased the *L. pennellii* allele frequencies at the *Got-2* and *Aps-2* loci (Table 6, column 2). Of these 5 loci 3 (*Got-2, 6Pgdh-2,* and *Pgi-1*) also showed significant allele frequency changes after selection at the intermediate salt-stress treatment (see previous section). The magnitude of allele frequency changes, however, were larger in the TC2 than in the TC1 (Table 6; compare columns 1 and 2).

Selection for the salt-sensitive individuals (SC, Table 5) significantly decreased *L. pennellii* allele frequencies at the *Est-3*, *Prx-7*, and *Pgi-1* loci and also significantly increased *L. pennellii* allele frequencies at the *Got-2* locus (Table 6, column 3). All of these enzyme markers also

Table 6. Differences in *Lycopersicon pennellii* allele frequencies when comparing selected and unselected F_2 populations. A negative sign indicates gene frequency changes in the opposite direction to the parental values

Locus	Selection scheme[a]				
	TC1-US	TC2-US	US-SC	TC1-SC	TC2-SC
Prx-1	−0.009	0.078	−0.018	−0.027	0.060
Skdh-1	−0.028	−0.041	−0.052	−0.080	−0.093
Est-3	NA[b]	0.156*	0.128*	NA	0.284*
Dia-2	0.069	0.011	−0.006	0.075	0.017
Est-7	0.051	0.005	−0.091	−0.040	−0.086
Prx-2	0.026	0.005	−0.008	0.021	−0.003
Prx-7	0.097*	0.100	0.135*	0.232*	0.235*
Tpi-2	−0.084	−0.004	−0.007	−0.091	−0.011
Pgm-2	−0.107*	−0.052	0.014	−0.093	−0.038
Got-2	−0.114*	−0.199*	−0.163*	−0.277*	−0.362*
Aps-2	−0.020	−0.303*	−0.086	−0.106*	−0.389*
Est-2	0.036	0.040	0.084	0.120*	0.124
Prx-4	−0.019	−0.047	−0.018	−0.037	−0.065
6Pgdh-2	0.090*	0.137*	0.072	0.162*	0.209*
Pgi-1	0.094*	0.123*	0.097*	0.191*	0.220*
Aco-1	0.035	0.011	0.006	0.041	0.017
SE	0.029	0.050	0.032	0.034	0.053

* Differences larger than three SE

[a] TC1, Tolerant class 1: salt-tolerant individuals selected at 150 m*M* NaCl + 15 m*M* $CaCl_2$; US, unselected population; TC2, tolerant class 2: salt-tolerant individuals selected at 200 m*M* NaCl + 20 m*M* $CaCl_2$; SC, sensitive class

[b] NA, Not available

Table 7. Approximate standardized effect of the marker-linked QTLs. A negative sign indicates presence of QTLs with effects in the opposite direction to the parental values

Locus	Selection scheme[a]	
	TC1-SC	TC2-SC
Est-3	NA[b]	0.59
Prx-7	0.53	0.49
Est-2	0.28	0.26
6Pgdh-2	0.38	0.45
Pgi-1	0.45	0.47
Got-2	−0.63	−0.76
Aps-2	−0.24	−0.81

[a] TC1, Tolerant class 1: salt-tolerant individuals selected at 150 m*M* NaCl + 15 m*M* $CaCl_2$; SC, sensitive class; TC2, tolerant class 2: salt-tolerant individuals selected at 200 m*M* NaCl + 20 m*M* $CaCl_2$

[b] NA, Not available

showed significant allele frequency changes after selection for salt tolerance.

Marker allele frequency changes resulting from bidirectional selection

Marker allele frequency differences between each of the tolerant classes and the sensitive class were calculated for all marker loci (Table 6, columns 4 and 5). The results of the bidirectional selection experiments were comparable to those of the unidirectional selection experiments. However, in addition to those marker loci that showed significant changes in allele frequencies upon unidirectional selections (i.e., *Est-3, Prx-7, Got-2, Aps-2, 6Pgdh-2,* and *Pgi-1*), *Est-2* on chromosome 9 also showed a significant allele frequency difference in a bidirectional selection (Table 6, column 4). As expected, the magnitude of allele frequency changes at these loci were larger in the bidirectional than the unidirectional selections.

Estimates of the standardized effect of the QTLs

Standardized effects of marker-linked QTLs were estimated for marker loci that showed significant allele frequency changes upon bidirectional selections (Table 7). Because selection intensities for all marker loci were the same, the estimated standardized effects of the linked QTLs were direct functions of the marker allele frequency differences between high and low phenotypic classes (see Materials and methods). The estimated standardized effects ranged from 0.24 to 0.63 phenotypic standard deviation at the lower salt treatment and 0.26 to 0.81 at the higher salt treatment (Table 7). With the exception of *Aps-2*, the estimated standardized effects of marker-linked QTLs were comparable at both salt-stress intensities (Table 7, compare columns 1 and 2). In estimating these values, we made the assumption of no recombination between the marker and the QTL. Violation of this assumption would cause the QTL effect to be underestimated.

Discussion

Identification of genomic locations with significant effects

Selection for a quantitative trait is expected to change the allele frequency of QTLs segregating for the trait. This means increasing the frequency of plus alleles (i.e., QTL alleles with positive effects on the trait) in the high class (e.g., salt tolerant) and also increasing the frequency of minus alleles in the low class (e.g., salt sensitive). If some marker loci are associated with the segregating QTLs (either directly due to pleiotropic effects or, more likely, due to linkage), marker allele frequencies will change in response to directional selection.

In this study, 6 of the isozyme loci examined (i.e., *Est-3, Prx-7, Got-2, Aps-2, 6Pgdh-2,* and *Pgi-1*) exhibited consistent, and in most cases significant, changes in allele frequencies when selection was applied in either or both directions and at two different salt-stress intensities (Table 6). Two of these markers, *6Pgdh-2* and *Pgi-1*, are only 16 map units apart on chromosome 12 and thus may be considered to have one genomic location. Therefore,

there are at least 5 genomic locations in these tomato lines with significant effects on the salt tolerance trait during germination. Of these, 3 contributed to the trait in a positive fashion and 2 in a negative fashion. The number of genomic locations identified represents a minimum estimate of the number of genes affecting this trait because the 16 markers examined cover only about 41% of the tomato genome within 20 cM limits. It is expected that by including other genetic markers (e.g., RFLPs and RAPDs) in the analysis, additional genomic locations with significant effects on the trait may be identified. The multiple genomic domains with significant effects detected in this study are consistent with previous suggestions that the salt-tolerance trait in the tomato is polygenically inherited (Foolad and Jones 1991, 1992b; Jones 1986b, 1987; Tal 1985).

The significant allele frequency changes observed at *Got-2* and *Aps-2* in the opposite direction to the parental values are intriguing. Their identification demonstrates the ability of marker analysis to uncover cryptic genetic variation that otherwise would have been masked by the large difference between the parents. Simultaneously, it raises questions concerning their origins. The salt-sensitive parent ('UCT5') used in this study has been bred for disease resistance characteristics mostly derived from *L. peruvianum*. Several accessions of this wild tomato species have exhibited salt-tolerance characteristics (Tal and Gavish 1973; Jones 1986b). It is tempting to speculate that during the breeding process, some salt-tolerance genes from *L. peruvianum* have been introgressed into the cultivated background through hitchhiking effects. The presence of both plus and minus QTLs in these tomato lines suggests a strong likelihood of being able to recover transgressive variants for this trait among segregating progeny.

The two markers *6Pgdh-2* and *Pgi-1*, closely linked together on chromosome 12, exhibited similar segregation patterns (Tables 2–5) and allele frequency changes in response to directional selections (Table 6). Whether each marker is linked to a separate QTL or whether there is a single QTL associated with both markers is unclear from the limited markers available.

The skewed segregations observed in this study did not interfere with the marker analysis because we did not assume normal Mendelian segregations when estimating the marker allele frequency changes due to selection. If a trait-based QTL analysis is based on a unidirectional selection, to determine the allele frequency changes it would first be necessary to obtain estimates of marker allele frequencies in an unselected (control) population unless marker loci segregate according to expected Mendelian ratios. In a bidirectional selection experiment, on the other hand, skewed segregation is counter-balanced by the divergent selection, thus obviating the need to analyze a control population.

Comparison between selection for high and low phenotypic classes

In most cases, isozyme markers that showed significant allele frequency changes upon selection for the high (tolerant) classes also showed similar results in response to selection for the low (sensitive) class (Table 6, compare columns 1 and 2 vs 3). This suggests a contribution of similar genomic locations to tolerance and sensitivity reactions and also the usefulness of marker analysis in detecting QTLs in both directions of selection. These results may find important practical applications. For example, for certain biotic and abiotic stress tolerances, due to technical reasons it may not be possible to collect data from one or the other selected phenotypic class. Under such conditions, data from only one selected class may be sufficient to detect segregating QTLs.

The magnitude of allele frequency changes due to selection were comparable in both directions (Table 6, compare columns 1 and 2 vs 3) even though relatively different selection pressures were applied in the opposite directions (see Materials and methods). To determine whether similar results would be obtained if equal selection pressures were applied in both directions, marker allele frequencies were recalculated for the TC1 and SC enforcing the same selection pressure (6%) as that applied for the TC2 (data not shown). The results indicated that except for *Aps-2*, allele frequency changes in both directions of selection were statistically similar. This suggests the occurrence of a symmetrical response to selection for this trait in these genetic materials and under the experimental conditions set forth in this study. If the experimental population exhibits a significant-asymmetrical response to opposite selections for a given trait, different results would be obtained in the high and low phenotypic classes (Falconer 1981). The results in this study may indicate that selection acted on the same subset of tolerance genes in both directions.

Comparison between uni- and bidirectional selection experiments

In the bidirectional selection experiment at the lower salt-stress treatment, in addition to the genomic locations that were identified upon unidirectional selections, a genomic location associated with the *Est-2* locus on chromosome 9 was also detected that had a significant effect on the trait (Table 6, column 4). The identification of this genomic location suggests that bidirectional selection has a greater potential than unidirectional selection to detect marker-QTL associations. However, when marker allele frequencies of a selected class are compared with expected Mendelian ratios with no standard errors, unidirectional selection may be more efficient. Under the latter condition, standard errors of marker allele frequency differences would be smaller in unidirectional than bidirectional selection experiments.

The observation that 6 of the 7 marker loci that showed significant allele frequency changes upon bidirectional selections also showed similar results in response to unidirectional selections (Table 6) suggests that QTLs associated with these 6 markers were possibly in close linkage with the markers and/or contributed major effects to the variation for this trait. The estimated values of the standardized effect of the QTLs linked to these markers were consistent with the latter conclusion, ranging from 0.24 to 0.63 phenotypic standard deviations at the lower salt treatment and from 0.45 to 0.81 at the higher salt treatment (Table 7). The genomic location associated with *Est-2*, on the other hand, reflects QTLs with smaller effects and/or loose linkage with the marker locus.

Comparison between the two salt-stress treatments

In most cases, markers that showed significant allele frequency changes after selection at the intermediate stress level (TC1-US) also showed similar results in response to selection at the high stress level (TC2-US) (Table 6). These results suggest that at least some of the genetic factors that facilitate rapid germination under the intermediate salt-stress level also contribute to improved responses under high salt-stress conditions. This is consistent with the results from heritability (Foolad and Jones 1991) and correlated response studies (M. R. Foolad et al., unpublished) for the same trait in the tomato. Allele frequency changes, however, were not similar at 2 of the marker loci: the *Pgm-2* locus showed significant allele frequency change only after selection for salt tolerance at the intermediate stress level, while the *Aps-2* locus showed significant allele frequency change after selection for salt tolerance at the high stress level (Table 6, columns 1 and 2); the allele frequency change at the *Aps-2* locus was also significant after bidirectional selections (Table 6, columns 4 and 5). These inconsistencies may be due to chance effects or they may arise if the QTLs associated with these markers are expressed only at specific stress levels.

Allele frequency changes were generally larger at the high stress level than at the intermediate one (Table 6, columns 1 and 2). Whether this was a result of a smaller population size, higher selection pressure, and/or a stronger expression of QTLs at the higher stress level is not immediately apparent. To eliminate the effect of selection pressure, the allozyme frequencies in the TC1 experiment were recalculated after enforcing the same selection pressure (6%) as that applied in the TC2 experiment (data not shown). The new allele frequencies indicated that except for the *Aps-2* locus the allele frequencies of the two classes were statistically similar. Further investigation is needed to determine whether QTLs associated with the *Aps-2* locus express only under high salt-stress conditions or if the inconsistency observed at this locus between the two salt-stress treatments was due to random genetic effects.

Marker-assisted selection for salt tolerance breeding in tomato

Results from this study and previous works (Tal 1985; Jones 1986a, 1987; Foolad and Jones 1991, 1992b) suggest that salt tolerance in tomato is polygenically (quantitatively) inherited. It has also been reported that tolerance at one stage of plant development is poorly correlated with tolerance at other developmental stages (Greenway and Munns 1980; Maas 1987; Shannon 1985; Jones 1986a). Specific developmental stages throughout the ontogeny of the plant should be evaluated separately for assessment of salt tolerance (Jones and Qualset 1984) and identification of molecular markers linked to tolerance components. Simultaneous introgression of genetic components contributing to the salt tolerance trait at different developmental stages into a single highly tolerant cultivar may be facilitated by marker-assisted selection. Currently, in our laboratory, investigations towards identifying additional molecular markers (Foolad et al. 1993) which show tight associations with salt tolerance characteristics at different stages of plant development in tomato are underway.

Acknowledgements. The authors wish to thank Dr. F. A. Bliss for his advice and critical reading of the manuscript. G. Romero and J. Bryce are also acknowledged for reviewing the manuscript. We also thank the California Tomato Research Board for financial assistance.

References

Bournival BL, Scott JW, Vallejos CE (1989) An isozyme marker for resistance to race 3 *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Theor Appl Genet 78:489–494

Burr B, Evola E, Burr FA, Beckmann JS (1983) The application of restriction fragment length polymorphism to plant breeding. In: Setlow JK, Hollaender A (eds) Genetic engineering principles and methods, vol 5. Plenum Press, New York, pp 45–59

Cook RE (1979) Patterns of juvenile morbidity and recruitment in plants. In: Solbrig OT, Jain S, Johnson GB, Raven PH (eds) Topics in plant population biology. Columbia Univ Press, New York, pp 207–301

Dehan K, Tal M (1978) Salt tolerance in the wild relatives of cultivated tomato: Response of *Solanum pennellii* to high salinity. Irrig Sci 1:71–76

Edwards MD, Stuber CW, Wendel JF (1987) Molecular marker-facilitated investigations of quantitative-trait loci in maize. I. Number, genomic distribution and types of gene action. Genetics 116:113–125

Epstein E (1972) Mineral nutrition of plants: principles and perspectives. Wiley, New York

Falconer DS (1981) Introduction to quantitative genetics, 2nd edn. Longman, New York

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

Foolad MR, Jones RA (1992a) Models to estimate maternally controlled genetic variation in quantitative seed characters. Theor Appl Genet 83:360–366

Foolad MR, Jones RA (1992b) 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

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

Jones RA (1986a) The development of salt-tolerant tomatoes: breeding strategies. Acta Hortic 190:101–114

Jones RA (1986b) High salt tolerance potential in *Lycopersicon* species during germination. Euphytica 35:575–582

Jones RA (1987) Genetic advances in salt tolerance. In: Nevins DJ, Jones RA (eds) Tomato biotechnology. Alan R. Liss, New York, pp 125–137

Jones RA, Qualset CO (1984) Breeding crops for environmental stress tolerance. In: Collins GB, Petolino JF (eds) Application of genetic engineering to crop improvement. Nijihoff/Junk Publ Dordrecht, pp 305–340

Jones RA, Hashim M, El-Beltagy AS (1988) Developmental responsiveness of salt-tolerant and salt-sensitive genotypes of *Lycopersicon*. In: Whitehead E, Hutchison F, Timmerman B, Varazy R (eds) Arid lands: today and tomorrow. Westview press, Boulder, Colo., pp 765–772

Kinzer SM, Schwager SJ, Mutschler MA (1990) Mapping of ripening-related or -specific cDNA clones of tomato (*Lycopersicon esculentum*). Theor Appl Genet 79:489–496

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 JS (1987) Trait-based analyses 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 Publ, Belmont, Calif.

Maas EV (1987) Salt tolerance of plants. In: Christie BR (ed) Handbook of plant science in agriculture, vol 2. CRC Press, Boca Raton, Fla., pp 57–75

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

Phills BR, Peck NH, McDonald GE, Robinson RW (1979) Differential responses of *Lycopersicon* and *Solanum* species to salinity. J Am Soc Hortic Sci 104:349–352

Sax K (1923) The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560

Scott SJ, Jones RA (1982) Low temperature seed germination of *Lycopersicon* species evaluated by survival analysis. Euphytica 31:869–883

Shannon MC (1985) Principles and strategies in breeding for higher salt tolerance. Plant Soil 89:227–241

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 traits. Crop Sci 27:639–648

Tal M (1985) Genetics of salt tolerance in higher plants: theoretical and practical considerations. Plant Soil 89:199–226

Tal M, Gavish V (1973) Salt tolerance in the wild relatives of the cultivated tomato: Water balance and absicic acid in *Lycopersicon esculentum* × *L. peruvianum* under low and high salinity. Aust J Agric Res 24:353–361

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, Mutschler MM (1990) Linkage map of the tomato (*Lycopersicon esculentum*) (2n = 24). In: O'Brian SJ (ed) Genetic maps; locus maps of complex genomes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 6.3–6.15

Tanksley SD, Orton TJ (1983) Isozymes in plant genetics and breeding. Elsevier, Amsterdam

Tanksley SD, Rick CM (1980) Isozyme gene linkage map of tomato: applications in genetics and breeding. Theor Appl Genet 57:161–170

Tanksley SD, Medina-Filho H, Rick CM (1982) Use of naturally-occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific cross of tomato. Heredity 49:11–25

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: New tools for an old science. Bio/Technology 7:257–264

Thoday JM (1961) Location of polygenes. Nature 191:368–370

Thompson JN, Thoday JM (1979) Quantitative genetic variation. Academic Press, New York

Zamir D, Tal M (1987) Genetic analysis of sodium, potassium and chloride ion content in *Lycopersicon*. Euphytica 36:187–191