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Salt tolerance in *Lycopersicon* species. V. Does genetic variability at quantitative trait loci affect their analysis?

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Abstract Salt tolerance was studied comparatively in three families derived from crosses between *Lycopersicon esculentum* Mill. and two related wild species [two accessions of *Lycopersicon pimpinellifolium* (Jusl.) Mill. and one accession of *Lycopersicon chesmannii* f. *minor* (Hook. f.) Mull.] by means of QTL analysis of fruit yield and earliness under conditions of salinity. From six polymorphic genomic regions involved in salt tolerance, three contained segregant salt-tolerant QTLs for the three families; two were found only in both families derived from *L. pimpinellifolium*; and one, involved in fruit number, was detected only in one of the *L. pimpinellifolium* families. Some differences regarding the effects of the wild alleles at orthologous QTLs were found. These effects were always negative in the *L. chesmannii* family. Comparing both *L. pimpinellifolium* families, the "wild" alleles at two out of nine common QTLs for fruit number and weight had effects with opposite directions, and the mode of gene action was clearly different at five of them. QTL analysis of earliness revealed the largest genotypic differences among families. Most drastic differences were found for the epistatic interactions in which all genomic regions containing QTLs were involved. These interactions between unlinked genes increased the range of variation of means, mainly upwards, as compared with genotypes at individual QTLs. Only one (affecting fruit weight) out of 27 interactions was detected in both *L. pimpinellifolium* families. Heterotic effects found for salt tolerance in one of the families can be explained by the presence of overdominant (or pseudo-overdominant) and dominant gene effects at QTLs controlling final fruit yield under conditions of salinity. Allelic variation at salt-tolerant QTLs exists, changing the additive and, mainly, the non-additive components of the genotypic value. Consequently, it may negatively affect the general applicability (or efficiency) of marker-assisted selection to improve salt tolerance in other segregant populations where QTLs were not studied. The use of more informative co-dominant markers, like microsatelites, might overcome these problems.

Key words Yield components · QTLs · MAS · Epistasis · Heterosis · Transgressive segregations

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Introduction

The lack of tolerant tomato accessions to high salinity concentrations within cultivated species forces breeders to use wild relatives to improve this quantitative trait (Jones 1986; Cuartero et al. 1992; Asins et al. 1993). Because genetic factors underlying quantitative trait expression can be studied individually through the intermediary of linked qualitative factors (Sax 1923), molecular markers should prove useful for identifying favorable factors in wild germ plasm, and for transferring them into cultivated species. The development of DNA markers and suitable statistical methods allow breeders to locate quantitative trait loci (QTLs) and estimate their genetic effects. An increasing number of QTLs involved in agronomically important traits have been detected using this approach (Tanksley 1993; Grandillo and Tanksley 1996). From the practical point of view, the main objective of these investigations is to apply marker-assisted selection (MAS) in the corresponding breeding programs. If a large fraction of additive genetic variance in a character can be explained using molecular markers, MAS can produce substantial increases in selection response (Lande 1992). MAS is especially useful in selection for characters that are difficult or expensive to measure. However, the cost of MAS is currently prohibitive for most commercial

applications. MAS could be more affordable if data on QTL analysis obtained in a segregant population could be used to apply MAS in others. There are only few reported studies using experimental data where MAS is compared with phenotypic selection. Stromberg et al. (1994) obtained similar responses in corn yield using either MAS or phenotypic selection, whereas MAS gave a larger selection response than phenotypic selection to improve tomato salt tolerance (Monforte et al. 1996). A possible explanation for the success of the latter authors might be that QTL detection and MAS were both carried out in the same segregant population, under the same environmental conditions.

Tomato is a model species for QTL-mapping analysis. Fruit-weight QTLs have been detected in several tomato interspecific crosses (Paterson et al. 1988, 1991; Bretó et al. 1994; Goldman et al. 1995; Grandillo and Tanksley 1996). Some of these QTLs have been mapped in similar chromosomal regions in crosses involving different wild parent donors, whereas others were detected in just one experiment. When polymorphic genomic regions containing QTLs are studied, differences in QTL detection among progenies involving different parental genotypes or species may be caused by several factors. The power of detection of a QTL depends on the population (backcross, F_2, near isogenic lines) under study, sampling size, degree of recombination, statistical methodology, environmental variance, etc. Moreover, the QTL allelic variation (additive variation), due to the presence of an allelic series with different gene effects on the trait, may also be the cause of differential detection; and this allelic variation may change the within- and between-loci interactions (non-additive variation). The effects of sampling sizes, experimental designs, statistical methods, etc., on the power of QTL detection have been recently reviewed (Carbonell and Asins 1996); however, the effects of QTL allelic variation, and the ensuing new interactions, have not yet been considered and may affect the efficiency of a generalized MAS scheme. The purpose of the present paper is to study these factors by comparing the analysis of salt-tolerant QTLs in three F_2 populations to infer how general the applicability of a MAS scheme can be in order to improve tomato salt tolerance.

Materials and methods

Plant material

F_2 populations were derived from three interspecific crosses between *Lycopersicon* spp.: cross A, *L. esculentum* cv "Madrigal" and *L. pimpinellifolium* line L1 (Bretó et al. 1994); cross B, *L. esculentum* var cerasiforme line E9 (a cherry tomato cultivar) and *L. pimpinellifolium* line L5 (Asíns et al. 1993); and cross C, line E9 and *L. chesmanni* line L2 (Asíns et al. 1993).

Plants were grown on sand in a greenhouse with both photoperiod (12-h light) and temperature (25 ± 10°C) control and irrigated with one-half Hoagland solution plus 171.1 mM of NaCl (conductivity 15 dS/m). The "A" family was analyzed as described by Bretó et al. (1994) using 15 plants from each parental and F_1 hybrid and 200 F_2 individuals. The "B" family consisted of 15 plants from each parental and F_1 hybrid and 150 F_2 plants, while the size of the "C" family was the same as the A family.

Trait analysis

Three yield components under salinity were studied for each plant: fruit Number (FN), total Fruit Weight (TW) and average Fruit Weight (FW) in grams, measured 9 weeks after plants started yielding. Additionally, earliness was taken into account because the time of exposure to salt treatment could be also related to salt tolerance. Earliness (EA) was measured as the duration of the vegetative cycle (from seed to the ripening date of the first fruit) in weeks relative to the ripening date of "Madrigal".

Non-additive and epistatic gene actions were estimated by using the contrasts described by Wrike and Weber (1986). A non-pooled *t*-test was employed for testing the significance of the contrasts.

Transgressive individuals were defined as those whose trait values exceeded the parental means by at least 2 standard deviations (SD). The proportion of individuals beyond 2 SD from the mean in a normal distribution is 0.0228 at each tail of the distribution. Theoretical and observed proportions of transgressive individuals were compared by a proportion contrast.

Molecular markers

ACO, EST, GOT, MDH, ME, PGI, PGM, SOD and TPI isozymic systems were screened in another tissue following the methods described by Bretó et al. (1993) to select polymorphic markers. Thirty two tomato cDNA clones, kindly provided by Dr. S. D. Tanksley, were used as probes for RFLP genotyping of the parental lines to select additional polymorphic markers. RFLP genotypes were determined using non-radioactive labelling methods as in Monforte et al. (1996).

F_2 individuals from the cross A were genotyped as in Monforte et al. (1996). All productive individuals were genotyped in the C F_2. RFLPs were scored only for plants with trait values higher or lower than 1 SD unit from the F_2 trait mean in the F_2 from cross B.

The effect of heterozygosity on the characters was analyzed by regressing each phenotypic value of the F_2 individuals on their percent heterozygosity for marker loci.

Linkage maps were obtained with the MAPMAKER 3.0 program (Lander et al. 1987), using a minimum LOD score of 3.0. Recombination fractions were transformed into centimorgans (cM) using the Kosambi mapping function (Kosambi 1944).

QTL analysis

Means of the quantitative trait within marker genotypes were compared by a *t*-test using non-pooled estimators of variance to detect associations between each marker locus and variation for the quantitative trait (Asíns and Carbonell 1988). Means were also used to estimate the additive effects (a) and dominance deviations (d) of QTLs (Edwards et al. 1987). The percent of phenotypic variance explained by the marker (R^2) was calculated as the ratio of the model sum of squares to the total sum of squares of the ANOVA using marker genotypes as factors. QTLs were also analyzed by simple interval mapping using MAPMAKER/QTL 1.1 (Lander and Botstein 1989) and LINKQTLF (Carbonell and Gerig 1991) computer programs and by composite interval mapping with the QTL Cartographer computer program (Zeng 1994). A QTL was declared to be linked to a marker, or located in an interval of markers, when

either the *t*-test was significant or the LOD score was greater than 0.985 or 1.17 in B, or A and the C F_2, respectively, according to the number of intervals and a total level of significance of 5%.

Epistatic interactions between markers were analyzed by a two-way analysis of variance for all pairwise combinations of the marker loci.

Results

Phenotype and marker analysis

Means, standard deviations, non-additivity and epistatic contrasts for the quantitative characters are shown in Table 1. Significant transgressive segregations were found only in A for earliness (EA), total weight (TW) and fruit number (FN). Important differences among families at any generation were clearly observed. Most striking differences refer to EA, ranging from early (L5) to extreme late ripening (L2). Moreover, within the C F_2, the plants that flowered were clearly classified into two groups differing by 20 weeks in ripening date. Almost all plants of *L. chesmannii* did not flower under salinity and only 52% of its F_2 plants yielded fruit, in spite of their good vegetative growth. Therefore, to avoid confounding factors (salinity and late flowering) in the QTL analysis of the C F_2, only the non-late (i.e. normal) flowering plants were included. Significant epistatic effects, but in opposite directions, were detected for this trait in the A and B families. Total weight showed significant non-additive effects in these families, although with different directions. Both *L. pimpinellifolium* parents had a high fruit number; however, F_1 and F_2 mean values from A were much higher than those from B. Non-additive and epistatic effects were significant and negative for this trait in the B family. The A family, the only one derived from a cultivar of *L. esculentum* with large-fruit size, showed the highest means for this trait.

Some markers did not show polymorphism in the three families. No genotypic differences were detected at any of the molecular marker loci between "Madrigal" and L9 of *L. esculentum* or between L1 and L5 of *L. pimpinellifolium*. The polymorphic marker loci and alleles presented by parental genotypes (species) are listed in Table 2. Deviations from the expected segregant ratio (1:2:1) were obtained for a few individual marker loci: TG48, TG63 and TG30 in A; TG180 and *Est4* in B; and TG23 and TG68 in C. Linkage maps were consistent among F_2s, but distances were smaller in the C F_2. Up to four genomic regions were suitable for QTL analysis by interval mapping.

Regression analysis of traits on the percentage of heterozygosity was only significant for total weight in B, but with low R^2 values ($P = 0.022$, $R^2 = 5.2\%$).

QTL analysis

Composite interval mapping gave the same results as simple interval mapping and marker-means comparison, so only the results from the latter analysis are shown in Table 3. The QTLs are identified by an abbreviation for the trait and the nearest significantly associated marker(s). The genotypic value of the homozygote for the "wild" allele (a), the dominance deviation of the heterozygote from the mean of the homozygotes (d), and the percentage of explained phenotypic variance at a single QTL (R^2) are also

Table 1 Means and standard deviations of the traits. NA, EP, Trans sup and Trans inf indicate significant non-additivity and epistatic effects, the proportion of transgressive individuals in the superior and inferior tail of the trait distribution respectively, at the probability level $* < 0.05$, $** < 0.01$, $*** < 0.0001$. (+) and (−) indicates the positive or negative directions of the effects.[#] Only one plant produced. Cross A is Madrigal × L1, cross B is E9 × L5 and cross C is E9 × L2 (see Materials and methods for more details)

Generation	Family	Earliness	Total weight	Fruit number	Fruit weight
Madrigal		0.00 ± 1.09	26.63 ± 11.6	1.33 ± 0.51	21.78 ± 11.2
E9		1.5 ± 1.18	6.42 ± 9.86	0.70 ± 1.00	10.32 ± 4.62
L1		1.33 ± 1.03	12.02 ± 2.51	22.00 ± 4.1	0.75 ± 0.1
L2[#]		20	1.1	3.00	0.37
L5		2.53 ± 0.96	15.30 ± 8.86	29.07 ± 14.19	0.51 ± 0.07
F_1	A	− 5.33 ± 0.52	49.35 ± 22.7	16.33 ± 7.06	3.78 ± 0.72
	B	0.14 ± 3.77	4.52 ± 3.79	2.33 ± 1.53	1.72 ± 0.68
	C	21.71 ± 1.44	27.95 ± 22.34	18.87 ± 12.69	1.48 ± 0.27
F_2	A	0.88 ± 4.00	30.09 ± 25.1	13.4 ± 10.3	3.17 ± 1.60
	B	2.35 ± 2.15	11.18 ± 10.41	6.01 ± 5.62	2.03 ± 0.98
	C	12.23 ± 12.71	8.82 ± 11.59	6.34 ± 8.47	1.64 ± 0.94
Contrasts					
NA	A	** (−)	** (+)		** (−)
	B		** (−)	** (−)	
EP	A	** (+)			** (−)
	B	** (−)	* (+)	* (−)	
Trans sup.	A	0.275***	0.21***	0.075**	
Trans inf.	A	0.175***	0.135***		
Trans sup.	B				
Trans inf.	B				

Table 2 Polymorphic marker loci and alleles presented by parental genotypes (species). Markers are defined in Material and methods and their chromosomal attachment is according to Tanksley et al. (1992). Capital letters indicate the restriction enzyme, the enzyme code is: A *Hae*III, E *Eco*RI, B *Bam*HI, H *Hin*dIII, D *Dra*I, X *Xba*I V *Eco*RV and the following number is the length of the polymorphic band in kilobase pairs (kb). Isozyme alleles were named as slow (s) and fast (f) bands. Bold markers could be screened in the three families

Chromosome	Marker	*L. esculentum*	*L. pimpinellifolium*	*L. chesmannii*
1	**TG24**	D2.7, E2.1, H9.6	D5.7, E2.1, H10	D5.0, E3.5, H10
	TG51	X9.5	X10	X9.5
	TG58	V20	V20	V4.0
2	**TG48**	H12	H10	H10
	TG180	E4.3, D2.5	E18, D1.8	E6.0, D1.8
3	**TG134**	B13	B25	B25
4	TG15	H2.3	H2.3	H2.5
	TG123	D2.7	D2.9	D2.7
	TG182	A9.0, B7.5	A8.5, B7.5	A11, B7.0
	TG339	V7	V7	V6.5
	Pgm2	s	s	f
5	**TG23**	A1.0, H7.7	A2.1, H4.7	A2.1, H7.7
7	TG20	H2.6	H2.6	H4
9	TG18	E2.3	E2.6	E2.3
10	TG43	E8.5	E11.9	E8.5
	TG63	H3.2	H2.4	H2.4
11	**TG30**	H4.9, V3.0	H4.0, V3.0	H4.9, V3.5
12	*Aco1*	s	f	s
	CD19	A8.0	A5.1	A8.0
	TG68	H5.6	H15	H15
	Est4	s	f	s
	TG28	B11.5	B11.5	B11.0

shown. The QTLs that could not be detected in a family because of the lack of marker polymorphism are indicated by (−). Only minor changes regarding the most likely positions of QTLs within intervals were found. A total of seven QTLs for EA, nine for TW, nine for FN and six for FW were detected, but not all of them were present in the three families. Some markers (*Est4*, TG30, TG43, TG134) and marker intervals (TG48–TG180, TG24–TG51, *Aco1*-TG68) showed a significant association with two or more traits. Few QTLs were detected in all three families, namely *tw-fn-fw*(*TG24–TG51*) and *fn-fw*(*TG48–TG180*), although the effect of the wild allele was not always in the same direction.

The direction of additive effects of yield-component QTLs agreed in general with the performance of the parents in the A family except for *fw*(*Aco1–TG68*). More exceptions to this rule appeared in the B family: namely *tw*(*TG123–TG182*), *twEst4*, *fn*(*Aco1–TG68*), *fnEst4*, *fn*(*TG123–TG182*), *fwEst4* and *fw*(*Aco1–TG68*). On the other hand, none of the alleles of the supposed salt-tolerant L2 line of *L. chesmannii* increased the value of any yield component under salinity in the present experiment. For EA, the direction of the effects of the wild alleles agreed with the parental values in families B and C, while these effects were opposite to those predicted at two out of four QTLs in A.

The proportion of phenotypic variance (R^2) explained by a QTL was usually below 10%. Because of the small sample size, only QTLs with relatively large effects could be detected in cross C ($>8\%$), whereas QTLs with as little effect as 2.86% were detected in the A cross. In spite of this, a relatively large number of QTLs were detected in family C.

Epistatic interactions between QTLs were studied only in the A and B families due to the small sample size of the C F_2. Significant epistatic interactions are summarized in Table 4 and were generally found only in families and traits where the contrasts for epistasis (see Table 1) were significant. With a few exceptions, the effect of epistatic interactions was to increase the range of variation of the means among genotypes for individual QTLs. Epistatic interactions between pairs of loci were very differentially distributed between families A and B. Every polymorphic marker locus associated with a QTL was involved in epistatic interactions affecting generally more than one trait. Epistatic interactions of some markers associated with EA affected FN and FW and vice versa; and epistatic interactions of some markers associated with FN or FW affected FW or FN, respectively. Only a common interaction (*Aco1*-TG43) for fruit weight was found between families A and B. Interestingly, the degree of dominance of alleles at *fw*(*Aco1–TG68*) in A (overdominance) is very different to that found in B (additive). These important differences in the within- and between-locus interactions must be due to allelic variation at the QTL.

Table 3 List of detected QTLs. All QTLs are shown in the second column and are named by the trait abbreviation and the nearby marker(s). *t* and LOD statistic values; additive effects (a); dominance deviation (d); (na = analysis not available because the marker was scored as dominant). The percentage of phenotypic variance explained by the markers (R^2) are calculated as explained in Materials and methods. (−) indicates lack of marker polymorphism in the population. LOD scores are specified where interval mapping analysis was possible

Trait	QTL	Madrigal × LI (Family A)						E9 × L5 (Family B)						E9 × L2 (Family C)					
		t	LOD	a	d	d/a	R^2	t	LOD	a	d	d/a	R^2	t	LOD	a	d	d/a	R^2
Earliness	*eaEst4*	2.27		0.98	0.04	0.04	2.86							–	–	–	–	–	–
	eaTG18	3		− 1.43	− 0.16	0.11	5.74							–	–	–	–	–	–
	eaTG23													3.06		1.91	− 0.27	0.14	14.04
	ea(TG24–TG51)	2.02	0.955	− 0.10	− 1.18	11.56	5.3	2.22	1.19	− 0.52	0.44	0.85	7.16						
	eaTG30							2.26		− 0.87	0.02	0.01	7.97						
	eaTG43							3.1		− 1.15	− 0.14	0.12	14.3	–	–	–	–	–	–
	ea(TG48–TG180)	2.67	0.697	0.94	0.86	0.91	3.7	2.17	1.6	− 0.37	1.23	3.27	13						
Total weight	*tw(Aco1–TG68)*	2.44	1.12	4.69	10.25	2.19	4.3												
	twEst4							2.18		− 2.68	− 0.84	0.31	3.55	–	–	–	–	–	–
	tw(TG182–Pgm2)	–	–	–	–	–	–	–	–	–	–	–	–	2.40	1.55	− 2.2	− 2.31	1.05	13.5
	twTG20	–	–	–	–	–	–	–	–	–	–	–	–	2.07		− 1.57	1.94	1.23	7.59
	tw(TG24–TG51)	3.14	1.88	3.65	15.35	4.21	7.4	2.47	1.1	3.99	− 0.82	0.20	6.92	2.15	1.4	− 1.87	2.8	1.78	12
	twTG43							2.89		4.41	2.80	0.63	8.2	–	–	–	–	–	–
	tw(TG48–TG180)							2.31	1.1	2.75	1.95	0.71	4.00	2.33	1.29	2.55	0.16	0.06	11
	tw(TG123–TG182)	2.9	1.61	8.48	8.80	1.02	6.0	2.88		− 1.92	5.61	2.91	7.85						
	twTG134													2.13		0.31	3.87	12.5	12.17
Fruit number	*fn(Aco1–TG68)*							1.96	1.03	− 1.73	− 0.18	0.10	4.8						
	fnEst4	2.25		0.84	3.55	4.24	3.14	2.56		− 1.47	0.87	0.58	4.72	–	–	–	–	–	–
	fnTG20	–	–	–	–	–	–	–	–	–	–	–	–	1.95		− 0.6	1.08	1.8	7.33
	fn(TG24–TG51)	3.4	2.8	5.69	1.41	0.25	13	2.42	1.37	1.45	1.83	1.26	6.30	1.41	0.98	− 0.01	1.52	51	8.7
	fnTG30							2.78		1.30	3.31	2.52	8.37						
	fnTG43	2.5		2.87	− 0.29	0.10	4.01	2.56		2.17	0.86	0.39	6.29	–	–	–	–	–	–
	fn(TG48–TG180)	2.5	0.98	3.13	2.49	0.80	3.8	2.52	1.40	1.74	0.86	0.49	5.00	2.36	1.09	− 0.77	1.03	1.6	9.4
	fn(TG123–TG182)	3.8	3.2	4.50	4.40	0.88	7.4	1.94		− 0.94	2.01	2.13	3.78						
	fnTG134							2.53		2.23	− 0.81	0.36	8.34	2.61		− 0.11	2.14	18.7	17.15
Fruit weight	*fw(Aco1–TG68)*	2.16	0.97	0.278	0.76	2.73	4.3	2.49	1.68	0.34	− 0.18	0.55	6.4						
	fwEst4							2.72		0.12	− 0.5	4.16	7.5	–	–	–	–	–	–
	fw(TG182–Pgm2)	–	–	–	–	–	–	–	–	–	–	–	–	3.62	3.22	− 0.62	0.12	0.2	25.8
	fw(TG24–TG51)	2.65	2.02	− 0.76	0.40	0.52	8.6	3.65	6.3	− 1.03	− 0.85	0.85	29.8	1.96	1.08	− 0.24	0.35	1.42	9.4
	fw(TG48–TG180)	2.99	3.15	− 1.53	− 1.34	0.88	36.6	2.9	1.99	− 0.43	− 0.49	1.13	14	2.02	0.92	− 0.32	− 0.21	0.66	8.3
	fwTG134	2.75		n.a.	n.a.	n.a.	4.04	3.21		− 0.61	− 0.21	0.34	19.3						

Table 4 Significant epistatic interactions between markers. Column R1 shows the range of means at the first marker involved in the significant interaction; R2, similarly for the second marker, and R(1 + 2) the range of means of the nine genotype classes of the interaction; n.s. the marker does not have significant effect on the trait, and n.d. no significant differences among the means of the two-locus genotypes. Bold markers are significantly associated with the trait, at least within a family

Cross	Trait	Interacting markers	R1	R2	R(1 + 2)
(A)					
Mad. × L1	Earliness	**TG18-TG23**	− 0.25–2.62	n.s.	− 1.67–5.00
		TG18-TG43	− 0.25–2.62	n.s.	− 1.22–4.10
		TG18-TG68	− 0.25–2.62	n.s.	− 2.14–5.25
		TG23-TG123	n.s.	n.s.	n.d.
		TG30-TG123	n.s.	n.s.	− 0.50–4.50
		TG48-TG51	n.s.	n.s.	− 2.08–5.00
		TG68-TG182	n.s.	n.s.	n.d.
	Fruit weight	*Aco1*-TG43	2.72–3.38	n.s.	2.17–5.22
		Aco1-TG23	2.72–3.38	n.s.	2.50–4.09
		TG48-TG23	2.91–3.89	n.s.	2.61–4.53
(B)					
E9 × L5	Earliness	**TG180**-TG63	− 3.55– − 0.85	n.s.	− 3.40–1.00
	Total weight	*Est4-Aco1*	9.49–14.83	n.s.	5.96–18.96
		TG134-*Aco1*	n.s.	n.s.	n.d.
		Est4-**TG180**	9.49–14.83	n.s.	6.51–34.24
		TG24-TG23	9.57–17.43	n.s.	6.89–29.64
		TG43-TG123	7.14–14.92	8.23–12.57	1.91–21.89
		TG63-**TG180**	n.s.	n.s.	n.d.
	Fruit number	*Est4*-TG180	4.58–9.78	n.s.	2.50–15.67
		TG43-TG18	4.39–8.96	n.s.	1.20–15.50
		TG43-TG123	4.39–8.96	4.85–7.80	1.83–12.71
	Fruit weight	*Aco1-Est4*	1.83–2.78	1.76–2.38	1.64–3.46
		Aoc1-TG43	1.83–2.78	n.s.	1.58–4.82
		Est4-**TG24**	1.76–2.38	1.91–2.71	1.62–4.26
		Est4-**TG68**	1.76–2.38	1.81–2.89	1.56–4.32
		TG24-TG30	1.89–2.61	n.s.	1.39–3.81
		TG24-TG48	1.89–2.61	1.88–2.91	1.77–4.31
		TG30-TG43	n.s.	n.s.	n.d.
		TG48-TG43	1.88–2.91	n.s.	1.31–3.70
		TG68-TG134	1.81–2.89	1.62–2.75	1.38–4.10

Discussion

Global differences among families

Knowledge gained from QTL mapping experiments is of great interest to breeders if the results are directly applicable to practical breeding programs. However, one important question in this context is whether, or not, results on QTL analysis from one population will apply to other experimental families. The data presented in this paper show that the number and location of QTLs involved in salt tolerance depends on the family. Families derived from the same interspecific cross, but different parental lines, having the same genotype for marker loci, showed more QTLs in common, although they differed in certain number of family specific QTLs, and in gene effects at common QTLs. This lack of differences at marker loci does not agree with the differences found for the quantitative traits.

The selection of suitable donors for salt tolerance in tomato is a critical step in the breeding program (Saranga et al. 1991; Cuartero et al. 1992; Asíns et al 1993). However, salt-tolerant genotypes do not behave similarly. Lines L1 and L5 of *L. pimpinellifolium* are salt tolerant in terms of total weight (TW) under salinity but they differ in the way they behave: with no reduction of fruit weight (FW) under salinity in L1 and by the high, non-decreasing fruit number (FN) in L5. Moreover, line L2 of *L. chesmannii* was declared to be salt tolerant because it yielded fruits at a conductivity of 30 dS/m, a level at which L1 and L5 did not yield fruit in a previous salt-tolerance screening experiment (Asíns et al. 1993). On the other hand, L2 yielded much fewer tomatoes under 15 dS/m than did L1 or L5. The present study has been carried out in a different greenhouse, although keeping all other environmental variables constant (temperature, photoperiod, amount and composition of nutrient solution, etc). At the end of the experiment (June 1996) we realized that the intensity of the sunlight, measured as photosynthetic photon flux density (PPFD), had been greatly reduced by the greenhouse plaques compared with the previous greenhouse (from 400 to 200 $\mu mol^{-2} s^{-1}$). A majority of

plants use environmental cues to regulate the transition to flowering. Any environmental variable exhibiting regular seasonal changes is a potential factor controlling the transition to flowering. The major factors are photoperiod, temperature and water availability, although some plants that do not require a particular photoperiod or temperature to flower are usually sensitive to irradiance. Additionally, there are strong interactions between these different factors, so that each factor can change the threshold value for the effectiveness of the others (Bernier et al. 1993). In Northern Europe, during the winter months, young tomato plants are raised in greenhouses, but no fruits are produced because of insufficient light conditions (Lindhout et al. 1994). Non-flowering problems in interspecific crosses involving *L. chesmannii* have been previously found in Spain (J. Cuartero, personal communication). *L. chesmannii* is endemic to the Galapagos Island, where skies are overcast and temperatures are mild during the time the species flourishes (Rick 1956); so, it is possible that irradiance interacting with water availability would determine the change from vegetative to reproductive development in this species. Some authors have defined *L. chesmannii* as low-salt tolerant (Saranga et al. 1991; Cuartero et al. 1992), whereas other considered it as salt tolerant (Rush and Epstein 1976; Läuchli 1986; Asíns et al. 1993). In the present study, line L2 from *L. chesmannii* hardly flowered at all under conditions of salinity but did under control conditions. Irradiance might be affecting its salt tolerance (in terms of fruit yield) by interfering with the onset of the reproductive development of the plant under conditions of salinity. It is noteworthy that its hybrid with *L. esculentum* overcame this problem, and was able to both flower and yield tomatoes under conditions of salinity, although only very late. Therefore, we think that L2 is a salt-tolerant genotype but very dependent on irradiance for its performance. This would explain the disagreement among results reported by different authors on its salt tolerance.

Important differences among F_1 families were found; most of them could not be explained by the differences among parental lines (Table 2). Thus, the A F_1 showed negative and positive heterotic effects for earliness and total weight (TW), respectively (i.e. favorable heterotic effects for both traits), while the B F_1 showed negative heterotic effects for TW and negative, non-additive effects for fruit number (FN). Comparing the TW and FN QTLs of A with those of B (Table 3) important differences in number, direction of the effect of the wild allele, degree of dominance, and the genotypic value of the heterozygote relative to those of both the homozygotes, are clearly observed. If fruit-weight QTLs of A and B are compared, differences at the *d* deviations are also found and they clearly enhance the performance of the A F_1.

Heterosis is an observed phenomenon that has been exploited by breeders to enhance the productivity of numerous crop and horticultural plants and its effects have been quantified in a wide variety of plant studies. However, the underlying genetic basis has not been satisfactorily explained despite many attempts to do so. Possible explanations include: dominance, true overdominance, pseudo-overdominance (i.e. nearby loci at which alleles having dominant or partially dominant advantageous effects are in repulsion linkage phase), and some types of epistasis, for instance the multiplicative interaction for complex traits which are the products of two or more characters (Schnell and Cockerham 1992). No epistatic interactions were detected for TW or FN in A. However, all TW QTLs in A showed overdominant (or pseudo-overdominant) and dominant gene effects; therefore, this might be the main cause of the positive heterosis found in this F_1 for TW. Similarly, Stuber et al. (1992), using a cross between two widely employed maize inbred lines, found that whenever a QTL for grain yield was detected it showed overdominance or pseudo-overdominance. How does one explain the poor performance of the B F_1?. When the dominance deviations *d* at the salt-tolerant QTLs are compared between the A and B F_2s, the number of QTLs showing a negative *d* is larger in B than in A. In fact, the contrast for non-additive effects was significant and negative for TW in B.

The phenomenon of transgressive segregation is very important from the evolutionary and breeding points of view given the ability of transgressive individuals to colonize new (extreme) habitats. Transgressive individuals were found for earliness, total weight, and, to a lesser extent, fruit number, in A, but for no trait in B. Transgressive segregations can be caused by the complementary actions of genes from the parentals, true- or pseudo-overdominance, and epistasis. From these non-exclusive causes, de Vicente and Tanksley (1993) found that the first one best explained their results. They also observed a significant correlation between phenotypic values and heterozygosis. The causes of transgressive segregation can be studied by analyzing the detected QTLs. Alleles at two EA QTLs in the A F_2 showed opposite effects from those predicted; therefore, transgressive segregation could be explained by complementary gene action at the QTLs involved, gathering all positive or all negative alleles within extreme genotypes. However, given that for this trait many more epistatic interactions were significant in the A F_2 compared to the B F_2, epistasis can not be ruled out. On the other hand, gene interactions at TW QTLs in the A cross are mostly overdominant and no epistatic interaction was found significant here; consequently, transgressive segregation would be best explained by true- or pseudo-overdominance at the TW QTLs in this family. Significantly, the family that showed favorable heterotic effects in F_1 for EA and TW also shows transgressive segregation in the F_2 for the same traits.

Comparative QTL analysis

The QTLs which we have mapped almost certainly do not comprise the entire set of genes which affect the trait under study but only a subset of genes, mainly because of the limited number of polymorphic markers scored through the genome. However, the development of salt-tolerant breeding lines from A by means of MAS based on this QTL analysis was very efficient compared to phenotypic selection (Monforte et al. 1996). Additionally, it is also clear that, using typical populations sizes (< 500 individuals), two or more polygenes closer than about 20 cM will usually appear as a single QTL because they can not be distinguished by segregation as separate genes (Tanksley 1993). Therefore, when we refer to a QTL, it does not necessarily mean a single locus, because we are most likely dealing with blocks of QTLs since most marker intervals are involved in the variation of more than the trait under study.

The detection of a QTL (or a block of QTLs) means that parental genotypes had alleles with different gene effects at this locus. From six polymorphic genomic regions involved in salt tolerance and earliness, only three contained segregant salt-tolerant QTLs in the three families (orthologous QTLs), two were only in both families derived from *L. pimpinellifolium*, and one, involved in fruit number, was detected only in one *L. pimpinellifolium* family. The wild allele for these orthologous QTLs always acts in the same direction, either increasing or decreasing the trait value, except for the C family where "chesmannii" alleles always produce a decreasing effect at all TW, FN and FW QTLs. Compared with the other families, a large number of QTLs show overdominance in C. QTL analysis in this family was carried out only in non-late-yielding plants, i.e. plants behaving like the cultivated species in the sense that they are not affected by irradiance to move towards reproductive development under conditions of salinity. The genes involved in this differential flowering behavior might be acting epistatically over the salt-tolerant QTLs modifying their actual gene effects; in fact, the late-yielding plants of this progeny were more salt-tolerant than the early yielding plants, causing a large dispersion of values relative to their means for TW and FN (Table 1). New ongoing experiments are focused to test this hypothesis.

From a total of 27 QTLs detected for all traits in polymorphic genomic regions of *L. pimpinellifolium* families, 15 were present in at least two families among the three studied here. Two of them affecting fruit weight [*fw*(*TG48–TG180*) and *fw*(*TG24–TG51*)] in the three families were located at similar positions in previous experiments (Paterson et al. 1988, 1991; Alpert et al. 1995). Alpert et al. (1995) identified a QTL at a similar position to *fw*(*TG48–TG180*). This QTL, which they named *fw2.2*, has major effects on fruit weight and accounts for 30–47% of the total phenotypic variance. *Fw*(*TG48–TG180*) also accounts for a large amount of phenotypic variance in the A family (36.6%) but its contribution is much smaller in the other two families. The differences in contribution and additive values among families for this QTL (higher in A than in both B or C) could be explained by the presence of a different "esculentum" allele at this QTL; in fact, B and C have the same female parent, *L. esculentum* var. "cerasiforme" E9 (a cherry tomato), as compared with "Madrigal", the large fruit cultivar used as the female parent in the A family. Alpert et al. (1995) concluded that variation at this locus differentiates the wild tomato species, *L. pimpinellifolium* and *L. pennelli* from the cultivated tomato and suggested that the domestication of the cultivated tomato involved a macromutation, or a major change, at this QTL. Our results show that the effect of both "esculentum" alleles is not the same under salinity. The effect of the allele from var. "cerasiforme" depends on the family (B or C) and is smaller than that from the cultivar "Madrigal" though both are larger than that of *L. pimpinellifolium* or *L. chesmannii*. Hence, instead of a major change, it seems to be the result of selection by man over specific genetic variability at this QTL. Conversely, the "cerasiforme" allele shows a higher contribution to fruit weight than that from cv "Madrigal" at *fw*(*TG24–TG51*). Therefore, *fw*(*TG48–TG180*) and *fw*(*TG24–TG51*) must be identified as orthologous QTLs with a high contribution to tomato fruit weight under conditions of salinity. None of the QTLs involved in EA have a similar location to those previously reported by Lindhout et al. (1994), although these authors used *L. pimpinellifolium* in the ancestry of one of the parental lines. An EA QTL (*eaTG48–TG180*) has a similar location to one recently found by Grandillo and Tanksley (1996).

Non-statistically detectable differences between allele effects, or QTL genotypes, in a family, compared with observed differences in another family, lead to differential QTL detection. Therefore, when polymorphism for marker loci exists, the differential detection of QTLs among families can be caused by allelic variation, although other causes may be involved, such as differences in the degree of genetic recombination and sampling error. Differences in the degree of recombination were found only in C, but did not increase the number of QTLs as compared with A or B, as would be expected; therefore, in our case, the differences in genetic recombination must affect only the precise genetic position of the QTL around the molecular markers and not the detection itself. Sampling errors may prevent the detection of QTLs with a low contribution to genotypic variance (Carbonell et al. 1992). Total weight under salinity is a very complex trait and is affected by fruit weight, fruit number, probably by earliness and other unmeasured traits, so most of the differences in the detection of TW QTLs might be due to sampling errors and/or other non-controllable factors rather than genetic variation. Thus, some QTLs for total

weight were detected in only one cross, but their related fruit-number QTLs were more commonly observed. In order to distinguish from sampling errors, only QTLs with a relatively high contribution ($R^2 \geqslant 4$) are now taken into account. Comparing the A and B families that are identical for the genetic markers scored, ten QTLs (three for EA, three for TW, three for FN, and one for FW) are segregating in one but not in the other family, which means that there exists genetic variation at these QTLs in spite of the apparent lack of variation at the corresponding marker loci. In these cases, and particularly for *fn*(*Aco1–TG68*), *fnTG30* and *fwEst4*, allelic QTL variation must be responsible for differential QTL detection. QTL allelic variation is even more evident when the 12 (2 + 2 + 5 + 4) QTLs that segregate simultaneously in both families are compared. In this case, the wild alleles at only nine of them show additive effects (a) in the same direction. Of these, only three show the same degree of dominance. Important differences in the within-locus gene interactions are found in four of them [*ea*(*TG24–TG51*), *tw*(*TG24–TG51*), *fw*(*Aco1–TG68*) and *fnEst4*] which show a clear overdominance in one family but not in the other. More dramatic differences were found for non-allelic gene interactions.

A considerable body of research in quantitative genetics suggests that epistatic interactions among loci at two-locus, three-locus and higher-order levels, often have major effects on adaptedness, especially in autogamous species, and have a considerable influence on phenotype (Spickett and Thoday 1966; Allard 1988; Pérez de la Vega et al. 1994). However, epistatic interactions did not show important effects previously in tomato and corn QTL mapping experiments (Paterson et al. 1991; Stuber et al. 1992; de Vicente and Tanksley 1993; Schön et al. 1994), although such effects have been reported in other species like barley (Thomas et al. 1995), soybean (Lark et al. 1995) and cowpea (Fatokun et al. 1992). We have found many epistatic interactions, especially for EA in the A cross and in the yield-related traits in the B cross. The study of epistatic interactions has revealed that the variation of a trait found at a QTL can be conditional to the presence of specific alleles at unlinked loci, so that epistatic interactions are influencing QTL detection, as happens in the case of *eaTG23* and *eaTG30* in the A family. Even though some QTLs were detected in more than one family, especially in A and B, the epistatic interactions were drastically different. Epistatic interactions are difficult to detect because large sample sizes are necessary and the statistical methods used to detect single QTLs can not be applied; therefore, the detection is more sensitive to sampling errors and environmental deviations. Given that the statistical methods developed for QTL analysis do not take into account allelic series at a QTL, nor are they powerful enough to detect epistatic interactions, it is not possible to establish the relative importance of the genetic background (epistatic interactions) versus QTL allelic variation in the comparison of families for QTL distribution. However, it is clear from the present results that both factors play important roles in the variation found for earliness and salt tolerance among families. Because of the weakness of the statistical methods, the analysis of near isogenic lines carrying different alleles of epistatic markers will provide information on the relative importance of these interactions, although more complex interactions (involving more than two loci) seem to be involved.

All these results strongly support the existence of important genotypic variation of QTLs that can decrease the efficiency, or even cause the failure, of a MAS scheme developed in a family where QTL analysis was not carried out, although the two families show the same genotype at all marker loci. If genetic markers were more closely linked to the QTLs (or located at the QTL), the variation at a QTL might be either shown by the marker or still remain unveiled due to the low "information content" of RFLPs. The allelic polymorphism at QTLs seems to be much higher than the allelic diversity at isozyme or RFLP loci, thus endangering the potential efficiency that MAS would provide in a breeding program. This conclusion completely agrees with the high mutability of polygenic characters (Sprague et al. 1960; Russell et al. 1963). Up to now, simple sequence repeat (SSR) markers are associated with the highest levels of polymorphism, a result of the high evolution rate of SSR loci (Morgante and Olivieri 1993), making them potentially the most informative class of markers. Therefore this new kind of co-dominant molecular marker could overcome these problems distinguishing all alleles at a QTL by parallel allelic variation at a linked associated marker locus.

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