Theor Appl Genet (1999) 98: 593–601

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Salt tolerance in Lycopersicon spp. VII. Pleiotropic action of genes controlling earliness on fruit yield

Received: 30 June 1998 / Accepted: 31 August 1998

Abstract The change from vegetative to reproductive development (earliness) in *Lycopersicon chesmannii* line L2 was delayed for 20 weeks when compared to other *Lycopersicon* species under greenhouse conditions. The interspecific hybrid of *L. chesmannii* L2 and *L. esculentum* E9, a cherry tomato cultivar, also showed this delay in reproductive development. The distribution of this character in the F_2-derived population showed a bimodal shape, plants could be scored easily as "early" or "late" in two nutrient conditions (optimum and high salinity). A QTL with major effects on earliness was detected in salinity, which explained 35.6% of the phenotypic variation. The effect of this QTL greatly diminished under control conditions, indicating differences in the genetic control of earliness between treatments. ACC synthase or phytochrome B2 are the products of candidate genes for such a major QTL. Other QTLs with minor effects, and epistatic interactions, are also involved in earliness under both conditions. A "late" F_2 subpopulation yielded twice as much as an "early" F_2; conversely, "early" plants were taller than "late" plants, regardless of the treatment. QTL analysis, carried out in both subpopulations, showed that yield differences may be explained by *chesmannii* alleles showing negative additive effects at some QTLs only in the "early" subpopulation. The effect of population subdivision on QTL analysis was investigated by computer simulations to show sample-size or random effects; thus, important pleiotropic or regulatory effects of genes controlling earliness on yield that affect QTL analysis, have been reveiled. Therefore alleles controlling earliness in *L. chesmannii* have to be taken into account for a more efficient utilization of the genetic resources of this species.

Key words QTLs · Epistasis · Genetic resources · Plant height · Regulatory genes

Communicated by P. M. A. Tigerstedt

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Introduction

Wild germplasm represents the major reservoir of genetic variation for crop species. Unfortunately, utilization of this genetic potential is not an easy task and germplasm banks are still under-utilized and under-developed. The application of molecular-marker technologies allows an easier development of these genetic resources in the pre-breeding process. The introgression of genomic regions from wild germplasm into an elite cultivar usually produces a decrease of agronomic merits; thus, the interactions of the introgressed genes with the cultivar background generally reduce the expected favorable effects (Doubley et al. 1995). In tomato, wild germplasm is being used with the aid of molecular markers to improve agronomic traits such as soluble solids (Azanza et al. 1994; Chetelat et al. 1995), earliness (Lindhout et al. 1994), fruit color (Grandillo and Tanksley 1996), yield (Tanksley et al. 1996), and salt tolerance (Monforte et al. 1996).

In a previous study, we compared quantitative trait loci (QTLs) involved in the "salt tolerance" of three families derived from crosses of tomato cultivars and wild species (Monforte et al. 1997a, b). Two of the families had the same tomato cultivar as the recipient (*Lycopersicon esculentum* line E9, a cherry tomato cultivar) and two different donor genotypes: *Lycopersicon pimpinellifolium* line L5 and *Lycopersicon chesmannii* line L2. One of the objectives was to study the effect of the genetic background on QTL detection. *L. chesmannii* and its hybrid with E9 were extremely late, and

F_2 plants were clearly classified into two subpopulations differing by 20 weeks in "ripening time" (earliness, EA). In this previous study, only non-late (i.e. normal) plants were included in the QTL analysis of salt tolerance because late plants had been over-exposed for an additional 20 weeks to salt treatment and, therefore, this could be a confounding factor in the evaluation of "salt tolerance". It was found (Monforte et al. 1997 b) that *chesmannii* alleles always showed negative effects at the detected salt tolerance QTLs (i.e. *L. chesmannii* is not a suitable donor of salt tolerance). Unexpectedly, "late" ripening plants yielded more fruits than "early" plants of the same family, either in the presence or the absence of salt. Several questions arose from this finding: (1) which genes are involved in the delay of ripening?, (2) why do "late" plants yield more tomatoes than "early" plants, especially under salinity, and (3) how do the answers to the previous questions affect the management of genetic resources of *L. chesmannii* to introgress salt tolerance or fruit yield QTLs into tomato cultivars?

In order to answer these questions, in the F_2 family derived from *L. chesmannii* L2 we have carried out: (1) a QTL analysis of earliness, yield and plant height, (2) a comparative QTL analysis between "early" and "late" subpopulations, and (3) an investigation by computer simulation of the effect of random population subdivision on QTL analysis. Two nutrient conditions, optimum and high salinity, were considered in each case.

Materials and methods

Plant materials

Plants belong to the C family described in Monforte et al. (1997 a, b). They were derived from a cross between *L. esculentum* Line E9 and *L. chesmannii* Line L2 and consisted of 30 plants from each parental and F_1 hybrid and 400 F_2 plants derived by the self-pollination of a single F_1 plant. Seeds were germinated in March 1996 and plants were grown in a greenhouse with a $25 \pm 10^{\circ}C$ temperature control. Plants were divided into two populations of equal size and grown under two treatments. Control plants were cultured in individual pots filled with peat plus sand and irrigated with tap water (approximately 2 dS/m). For the salt treatment, plants were grown on sand and irrigated with a one-half Hoagland solution plus 171.1 mM NaCl (conductivity 15 dS/m). Not all plants produced fruits; the final number of individuals analyzed were 103 for the saline treatment and 178 for the control.

Traits

Three yield components were studied in each plant: fruit number (FN), total fruit weight (TW) and average fruit weight (FW) in grams, measured 9 weeks after plants started yielding. Earliness (EA) was measured as the length of the vegetative cycle (from seed to the ripening date of the first fruit) in weeks relative to the ripening date of the first plant that yielded mature fruits. Height (HE) of the plant in cm was recorded at the sixth week of treatment and the internodal distance (ID) in cm between the 9th and 10th leaf was measured when plants were 3- months old.

Genotyping

Molecular markers (isozymes and RFLPs) were analyzed on every productive F_2 plant (Monforte et al. 1996, 1997 a). We included in this study a second RFLP detected with the TG182 probe. It was named TG182b and is linked to the TG23 marker on chromosome 5.

Sampling simulations

One-hundred replicates of simulated data were obtained by randomly splitting the total F_2 experimental populations into two samples of size equal to the "early" and "late" subdivisions in each growing condition (51 and 52 for the saline population, and 101 and 77 for the control).

Data analysis

Means and standard errors were calculated for each trait within the two developmental classes: "late" and "early". EA was analyzed by two approaches: it was considered as a categorical ("early" or "late"), or as a continuous, variable (measured in weeks). Putative QTLs affecting earliness were identified by either linkage analysis, using MAPMAKER (Lander et al. 1987), or by single-marker mapping analysis, as in Bretó et al. (1994). The Bonferroni correction was used in order to have an overall significance level of 0.05. Epistatic interactions affecting EA and FN were analyzed by two-way ANOVA for pairwise combinations of significant marker loci.

QTL analysis of yield and vegetative traits was carried out as described in Monforte et al. (1997 a) in the whole population, in both subpopulations including the "early" plants or the "late" plants, and in the simulated subpopulations. The effect of EA, measured as a categorical variable, on yield or vegetative traits was studied by one-way ANOVA using earliness classes ("late" or "early") as the classification factor.

Results

Earliness

Plants could be classified into two groups or subpopulations: "early" plants (EA ranging from 1–10 weeks), which yielded fruits from June to September, and "late" plants (earliness above 20 weeks), which yielded fruits from November to February. Parental genotypes clearly differed in this trait: *L. chesmannii* was extremely late and *L. esculentum* cerasiforme E9 was mostly "early"; this behavior was unaffected by the saline treatment. EA of the F_2 plants ranged from very early to extremely late, showing a bimodal distribution in both growing conditions (Fig. 1) with an important proportion of transgressive segregants at both tails of the distribution.

Markers associated with EA, and estimates of the genetic effects of the putative QTLs, are presented in Table 1, along with the significant epistatic interactions. As expected from the bimodal distribution, a QTL with major effects (*eaTG23–TG182b*) was

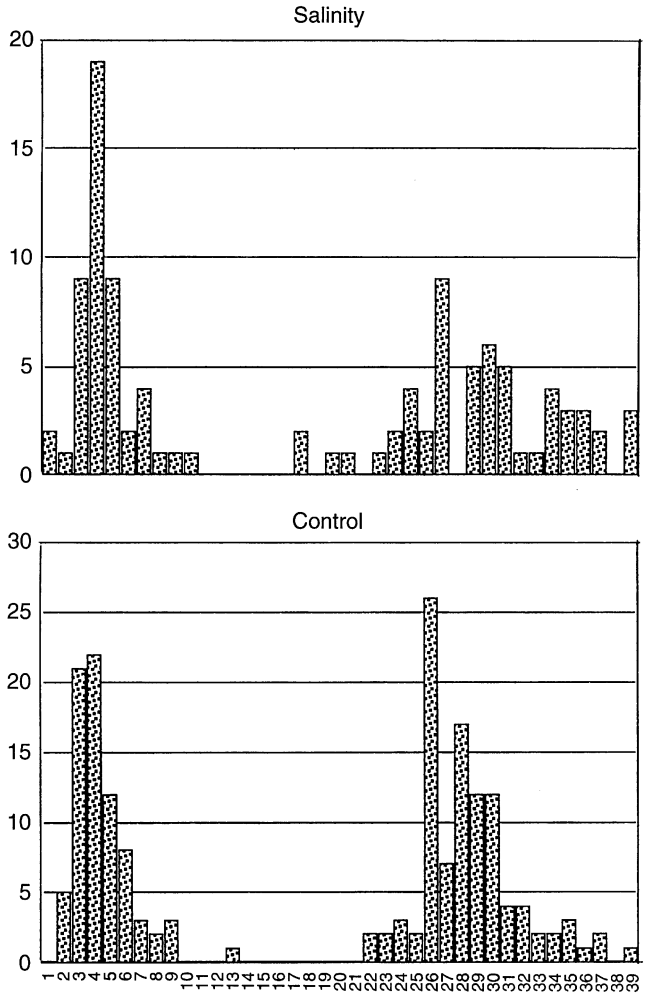

Fig. 1 Frequency distribution for earliness of F_2 plants under control and salt treatments (in weeks)

detected in salinity, although its effect was less important under control conditions.

Under salt treatment, a closer relationship between the distribution of EA and the segregation at marker TG23 was observed: homozygous plants for the *esculentum* alleles at this marker were "early", whereas homozygous plants for the *chesmannii* alleles at this marker were "late". In fact, when EA was recorded as a categorical variable (i.e. "early" versus "late"), linkage analysis showed a putative unique locus affecting EA linked to TG23 with a LOD score of 8.2 and a distance of 32 cM. At least a second genetic locus linked to the TG68 marker was also involved in EA, its effect being more evident when plants were heterozygotes at the TG23–TG182b marker interval. An epistatic interaction between TG28 and TG68 was significant for EA under salinity. Epistatic interactions at both conditions have important contributions to the phenotypic variation and present only one common marker locus (TG68).

Table 1 Markers, intervals (in boxes), and epistatic interactions associated with earliness. Estimates of additive effects (*a*), dominance deviations (*d*) and their contribution to the total variability of the trait in % (R^2)

Marker	Earliness		
	a	*d*	R^2
	Control		
TG23	4.68	2.65	9.5
TG68 × TG339			7.4
	Salinity		
TG23	9.52	5.02	35.6
TG182b	7.71	1.85	24.6
TG68	6.03	− 1.08	11.5
TG68 × TG28			9.54

Pleiotropic effects of genes controlling earliness on yield and vegetative traits

Means and standard errors of yield and vegetative traits of "early" and "late" subpopulations under both treatments were computed (Table 2). Early plants were taller than late plants, especially under salinity, but the most important differences were observed in TW and FN; "late" plants yielded twice as much as "early" plants. Differences in FW were not significant. Trait differences between "late" and "early" subpopulations were observed in both treatments. In order to investigate whether or not these trait variations had a genetic basis, QTL analysis within "late" and "early" subpopulations and the F_2 plants as a whole was studied for both treatments (Tables 3–5). A set of QTLs, mostly associated with the interval TG23–TG182b (where at least one important gene controlling EA is located), was detected only in one subpopulation or else showed a change in the direction of the additive effects. The *chesmannii* alleles were associated with a positive increase in the trait value of "late" plants, whereas they reduced the trait value of "early" plants (*tw–fnTG23–TG182b*) under control conditions. Three epistatic interactions involving a common genomic interval controlling EA (TG23 and TG182b) contribute importantly to the variation of FN under salinity.

In order to check if these differences in QTL detection between EA subpopulations were due to sampling size, an investigation of the effect of random population subdivision on QTL analysis was carried out by computer simulation for each trait and treatment. Three general situations occurred.

(1) Depending on how low or high the contribution of the QTL to the trait, a small or large proportion of subpopulations will present a significant effect at this QTL, respectively. The means for *a*, *d* and R^2 will be close to those obtained for the global F_2.

Table 2 Basic statistics of fruit weight (FW), number of fruits (NF), total fruit weight (TW), height (HE) and internodal distance (ID). *P* is the significance level (in %) for the significant mean comparisons between both earliness classes

Trait	Control					Salinity				
	Early		Late			Early		Late		
	Means	SE	Means	SE	*P*	Means	SE	Means	SE	*P*
FW	2.61	0.25	2.13	1.02		1.62	0.15	1.64	0.12	
NF	4.35	0.41	11.07	0.83	0.01	3.57	0.37	8.98	1.55	0.16
TW	10.02	1.04	17.76	1.24	0.01	5.73	0.79	11.69	2.01	0.91
HE	45.16	1.00	41.39	0.97	0.82	76.92	1.87	64.35	2.36	0.01
ID	4.21	0.16	4.02	0.13		6.46	0.24	5.6	0.44	1.47

(2) If a QTL is detected in less than 5% of the simulated populations, or is not detected at all but is detected in the "late" or the "early" subpopulations, we conclude that its detection is in fact an effect of the earliness subdivision.
(3) If the mean for *a* over significant simulations for a QTL is very different from that observed at either the "late" or the "early" subpopulations it must again be due to the subdivision into earliness classes.

Situation 1 is explained as random fluctuations due to sampling size. Situations 2 and 3 have to be interpreted as pleiotropic or regulatory effects of genes governing earliness.

Statistics for *a*, *d* and R^2 have been included in Tables 3–5 (in italics) for simulated subdivisions (if higher than 5 reps. were significant) where a marker locus was found associated to the trait in the "late" and "early" subpopulations. It is clear that some QTLs are not detected just by random subsampling, namely *fnTG23* (in control and salinity), *fwPgm2–TG15* (in salinity), *twTG23–TG182b* (in control), *idTG15* (in control) and *heTG48–TG180* (in control). Therefore their differential detection, when both EA subpopulations are compared, can not be explained by the random subsampling but by the actual specific subdivision into earliness classes, which is a proof at the QTL level of the pleiotropic effects of the genes involved in EA on the quantitative traits studied.

Discussion

The distribution of earliness in the F_2 population showed a clear bimodal shape under both salt and control treatments, indicating the segregation of a gene with major effects. This conforms with earliness in segregating populations of rapesed cultivars (Ferreira et al. 1995), and with heading date for rice (Li et al. 1995).

Differences in earliness between tomato cultivars and wild relatives usually ranges from several days to a few weeks (Lindhout et al. 1994; Grandillo and Tanksley 1996), so the late fruit yield of *L. chesmannii* L2 (origin Galapagos islands) has to be considered exceptional within the genus *Lycopersicon*. Earliness at ripening date is closely related to flowering date (i.e. the duration of the vegetative cycle). During the development of recombinant inbred lines from the family under study, it was observed that the delay in flowering date is followed by a delay in ripening date. However, the bimodal distribution of both traits is only found when RILs are grown under greenhouse conditions (data not shown). Therefore, sunlight filtering through the greenhouse plaques is the major cause of the extreme delay in flowering.

QTL analysis in the salt-treated population confirmed the segregation of a major QTL influencing earliness linked to the marker TG23 (Table 1), which we have called *eaTG23–TG182b*. When plants are homozygous at this QTL, it determines their EA class; whereas when they are heterozygous, the action of a second QTL (*eaTG68*) becomes more evident. Other loci with minor effects (as suggested by the significant epistatic interaction between the TG68 and TG28 markers) must also influence earliness. De Vicente and Tanksley (1993) also detected earliness QTLs around TG23 and TG68 markers in a cross involving an accession of *L. pennelli*. The estimated additive effects at these QTLs were negative and their contribution quite similar to what we have found under control conditions but much smaller than in the case of salinity. Therefore, these QTLs might be orthologous and differences could be explained by allelic variability at these QTLs and the great influence of salinity and sun-light conditions on the contribution of *chesmannii* alleles. TG23 is close to CHS3 (chalcone synthase, involved in flavonoid biosynthesis), PHYB2 (phytochrome B2; Van Tuinen et al. 1997) and even ACC4 (ACC synthase). The analysis of RILs will allow a fine mapping of the region and, additionally, to test more thoroughly the effects of epistatic interactions and environmental conditions.

By including computer simulations we have been able to check the repeatability of QTL detection across

Table 3 Markers, intervals (in boxes), and epistatic interactions associated with fruit number and total weight in control and salinity for the F_2 population as a whole (All) and for each earliness subpopulation (Late or Early); *a*, *d* and R^2 as in Table 1. The results of random subsampling simulations are in italics in the row immediately below if more that five of them resulted in a significant association with the trait

Marker	Fruit Number																
	All			Late							Early						
	a	*d*	R^2	NSS	*a*	SD	*d*	SD	R^2	SD	NSS	*a*	SD	*d*	SD	R^2	SD
Control																	
TG23					4.07		1.75		9.8			− 2.21		− 0.55		20.6	
											7	*3.8*	*0.13*	*− 1.22*	*0.97*	*12.4*	*2*
TG134				6	*3.86*	*0.34*	*− 2*	*1.55*	*15.4*	*2*							
Salinity																	
TG23												− 0.75		− 0.59		2.5	
TG28 × TG23			19.38														
TG48 × TG23			11.02														
TG134 × TG182b			14.81														
Markers	Total Weight																
	All			Late							Early						
	a	*d*	R^2	NSS	*a*	SD	*d*	SD	R^2	SD	NSS	*a*	SD	*d*	SD	R^2	SD
Control																	
TG48	− 5.23	1.51	9.5		− 6		1.33		11.7			− 4.23		1.95		10.3	
				90	*− 5.27*	*0.77*	*1.71*	*1.45*	*10.4*	*3*	*72*	*− 5.84*	*0.95*	*1.52*	*1.68*	*12.8*	*4*
TG180	− 3.45	− 2.66	7.2														
				27	*− 4.86*	*0.77*	*− 2.04*	*2.01*	*12.4*	*4*	*26*	*− 4.37*	*0.74*	*− 2.2*	*1.08*	*10.2*	*3*
TG23												− 5.66		− 1.3		20.6	
TG182b												− 6.96		− 3.96		35.9	

Table 4 Similar information as in Table 3 except for Fruit Weight

Marker	Fruit Weight																
	All			Late							Early						
	a	*d*	R^2	NSS	*a*	SD	*d*	SD	R^2	SD	NSS	*a*	SD	*d*	SD	R^2	SD
Control																	
TG48	− 0.69	− 0.34	23.9		− 0.66		− 0.32		27			− 0.73		− 0.37		21.9	
				98	*0.7*	*0.13*	*0.33*	*0.14*	*25.2*	*2.1*	*100*	*− 0.68*	*0.09*	*− 0.36*	*0.1*	*24.5*	*5*
TG180	− 0.86	− 0.46	18		− 0.62		− 0.39		30.3			− 1.19		− 0.66		18.4	
				91	*− 0.9*	*0.18*	*− 0.4*	*0.22*	*24.9*	*9*	*100*	*− 0.88*	*0.13*	*− 0.43*	*0.16*	*22.1*	*7*
TG134	− 0.76	− 0.08	10.6		− 0.76		− 0.08		10.6			− 1.19		− 0.66		18.4	
				43	*− 0.66*	*0.18*	*0.08*	*0.26*	*16.9*	*4*	*47*	*− 0.69*	*0.18*	*0.01*	*0.2*	*14.8*	*3*
Salinity																	
TG48	− 0.44	− 0.02	10		− 0.51		0.05		15.7								
				44	*− 0.55*	*0.08*	*− 0.03*	*0.17*	*17.2*	*5*	*24*	*− 0.56*	*0.12*	*− 0.07*	*0.19*	*18*	*7*
Pgm2	− 0.49	0.08	10									− 74		0.15		21.3	
				15	*− 0.69*	*0.12*	*0.13*	*0.23*	*22.1*	*6*	*18*	*− 0.75*	*0.15*	*0.22*	*0.25*	*22.1*	*6*
TG182												− 0.5		0.35		15.1	
TG339	− 0.36	0.33	10.8									− 0.5		0.4		16.2	
				28	*− 0.49*	*0.08*	*0.27*	*0.2*	*15.9*	*4*	*22*	*− 0.5*	*0.08*	*0.24*	*0.19*	*15.9*	*4*
TG15	− 0.45	− 0.02	10.3									− 0.51		− 0.02		13.1	
				9	*− 0.65*	*0.1*	*0.1*	*0.38*	*20.9*	*6*	*8*	*− 0.61*	*0.11*	*− 0.07*	*0.19*	*20.7*	*5*
TG28	− 0.21	0.34	5.2														
TG134				*6*	*− 0.55*	*0.04*	*0.19*	*0.23*	*15*	*2*	*11*	*− 0.51*	*0.09*	*0.32*	*0.21*	*13.9*	*2*

Table 5 Similar information as in Table 3 except for vegetative traits

Marker	Height																
	All			Late							Early						
	a	*d*	R^2	NSS	*a*	SD	*d*	SD	R^2	SD	NSS	*a*	SD	*d*	SD	R^2	SD
Control																	
TG182	4.12	0.31	8								5.08		− 1.63		16.3		
				32	*5.5*	*0.72*	*0.52*	*2.05*	*15.3*	*4.1*	*51*	*4.89*	*0.62*	*0.09*	*1.33*	*11.8*	*3.1*
TG339	5.3	− 0.39	12.9		4.7		0.35		9.1			5.31		− 0.85		17.8	
				69	*5.75*	*0.92*	*0.07*	*1.62*	*17*	*4.8*	*84*	*5.3*	*0.74*	*− 0.61*	*1.37*	*14.4*	*4*
TG15	4.55	− 0.83	11.7														
				28	*6.31*	*1.00*	*− 0.75*	*2.43*	*23.1*	*6.4*	*50*	*5.38*	*0.7*	*− 0.89*	*1.31*	*16.2*	*3.5*
TG48	− 3.8	− 2	10.7		− 4.31		− 2.91		14.4								
				44	*− 4.86*	*0.8*	*− 1.42*	*1.68*	*15.7*	*5.2*	*73*	*− 4.44*	*0.67*	*− 1.78*	*1.3*	*14.1*	*3.3*
TG180	− 3.48	1.34	6.4		− 4.07		1.78		9.9								
				31	*− 4.62*	*0.72*	*1.92*	*1.54*	*11.9*	*3.2*	*60*	*− 4.17*	*0.53*	*1.43*	*1.34*	*9.9*	*2.1*
Markers	Internodal distance																
	All			Late							Early						
	a	*d*	R^2	NSS	*a*	SD	*d*	SD	R^2	SD	NSS	*a*	SD	*d*	SD	R^2	SD
Control																	
TG48	− 0.54	− 0.16	10.2		− 0.54		− 0.22		10.6			− 0.55		− 0.07		9.9	
				51	*− 0.67*	*0.11*	*− 0.21*	*0.22*	*15.1*	*5.1*	*67*	*− 0.59*	*0.09*	*− 0.26*	*0.17*	*12.5*	*2.9*
TG180	− 0.48	− 0.22	8.2		− 0.5		− 0.05		8.4								
				18	*− 0.74*	*0.11*	*− 0.08*	*0.24*	*15.7*	*4.5*	*45*	*− 0.68*	*0.09*	*− 0.11*	*0.17*	*13.4*	*2.6*
TG15					0.59		0.05		18.7								
Salinity																	
TG28	− 0.63	0.03	7.3		− 0.87		− 0.05		14.1								
				8	*− 0.92*	*0.08*	*− 0.28*	*0.48*	*17.5*	*2.8*	*19*	*− 0.96*	*0.12*	*− 0.08*	*0.38*	*17.7*	*3.4*

different samples of the same population, whether salt-treated or not. Little information is presently available on the repeatability of QTL identification across different samples and environments. A number of confounding factors, such as different sets of markers, sources of parental lines, type of progeny, different sets of environments and particularly sampling of progeny, have been reported as possible causes of inconsistency in QTL detection (Beavis 1994). Based on simulation experiments the latter author provided indications that these inconsistencies may occur because of a small number of progeny. By contrast, experimental data suggest repeatedly that this is seldom the case, the more frequent situation being the presence of one or two major QTLs that explain a large part of the variability, and a number of minor loci accounting for smaller amounts of the phenotypic variance (Beavis 1994). The analysis of simulations confirms the differences found for QTL detection, comparing presence versus absence of salt or "early" versus "late" experimental subpopulations. All QTLs detected in the global F_2 are also detected in more than 5% of the subsamples, except for *fwTG28* (in salinity). Given that the latter QTL shows a low contribution in the F_2, its detection must be affected by the reduction of sampling size in simulations. On the other hand, there are two cases, *fnTG134* (in control) and *fwTG134* (in salinity), that are detected in more than 5% of the random subpopulations (6–11%) but not in any experimental one. This could be due to spurious detection, i.e. by chance there is a difference in performance between some individuals differing in genotype at the marker locus. In the process of sampling most of the chance associations are broken up, due to the fact that at each new simulated sample a different subset of the population is represented. Thus, we have seen that most estimates in the global populations (especially for *a*) are close to their means over significant samples.

"Late" subpopulations yielded more fruits and grew less than the "early" subpopulations. Yield differences were particularly surprising under salt treatment because late-yielding plants had been exposed longer to the stress agent; that is, "late" F_2 plants showed a higher salt tolerance than "early" F_2 plants. A QTL analysis comparison of "early" and "late" subpopulations suggests that differences in yield (in control), were caused by changes in the genetic effects at QTL alleles (*fn–twTG23–TG182b*) (Table 3). A positive additive effect of the *chesmannii* allele at *fnTG23* was observed in the "late" subpopulation whereas this effect is negative in the "early" subpopulation, making this QTL undetected in the F_2 as a whole. The random subsampling simulated by computer shows that this situation can not be explained by the process of subsampling but rather by the specific classification of plants into earliness classes; i.e. it should be interpreted as a pleiotropic (regulatory) effect of the genes controlling earliness on the yield-QTL alleles. We have shown in Table 2 that there is an association or phenotypic correlation between earliness and FN, TW, HE and ID. The genetic cause of such a correlation is chiefly pleiotropy, which is a common property of major genes, but has not frequently been considered in quantitative genetics. Linkage is also a cause of transient correlation, particularly in populations derived from crosses between divergent genotypes, as in our study. This might be the case for *fnTG23* and *eaTG23–TG182b* but, even so, this earliness QTL, or another one, is causing the additive value of the *chesmannii* allele at *fnTG23* to change drastically from minus 2.21, in the "early" subpopulation, to plus 4.07, in the late subpopulation. Increased yield in salinity for the "late" subpopulation, i.e. salt tolerance, is explained, at least in part, by the appearance of epistatic interactions with important contributions (11–19.4%). These important epistatic interactions involving TG23-TG182b that affect FN in salinity re-inforces the idea of pleiotropy through epistasis. A model to explain the trait differences between the two subpopulations can be suggested given that most yield QTLs suffering pleiotropic effects show negative additive effects for the *chesmannii* alleles in the "early" subpopulation,. The genetic effects of *chesmannii* alleles at yield QTLs would be negative compared to the esculentum alleles unless the switch to reproductive development was promoted by chesmannii alleles at earliness QTLs. It is as if the factor induced by the esculentum alleles at earliness QTLs would not activate the *chesmannii* alleles of yield QTLs. Hence, the yield of populations derived from this cross will depend greatly on the pleiotropic or regulatory effects of genes governing earliness on yield-QTL alleles. This is not the first time that pleiotropic effects have been attributed to earliness genes; for example, on plant height in rice (Li et al. 1995) and on fruit weight in tomato (Banerjee and Kalloo 1989; Kemble and Gardner 1992; Lindhout et al. 1994). We have found no association between earliness and FW, but the *chesmannii* allele at *fwPgm2–TG339* is only detected, and with negative effect, in the "early" subpopulation for salinity. However, it is important to point out that, in these studies, other wild species are involved and the location of earliness genes is different from that in our experiment.

Jiang and Zeng (1995) have developed a statistical model to test pleiotropy using the composite interval-mapping procedure. This test is based on the hypothesis of equality of the positions for the QTL having an effect on trait 1 and the QTL affecting trait 2 relative to the flanking markers. From the functional point of view, pleiotropy is here interpreted as an enzyme coded by a gene at the QTL that is involved in two different metabolic pathways (traits). In our study, the pleiotropic effects resemble more the regulatory action of a gene controlling earliness on the expression of other genes involved in yield and height.

These pleiotropic effects of earliness genes on yield and vegetative traits have important consequences for the management of the genetic resources of *L. chesmannii*

germplasm. *L. chesmannii* has been proposed as a salt tolerance donor by several authors (Rush and Epstein 1981; Jones 1986; Läuchli 1986; Mahmond et al. 1986; Asíns et al. 1993), whereas other authors consider it as low salt-tolerant (Shanon et al. 1987; Cuartero et al. 1992; Saranga et al. 1992). We showed previously that *L. chesmannii* contains QTLs involved in salt tolerance not detected in *L. pimpinellifolium* (Monforte et al. 1997 b), and have now shown that the gene effects depend greatly on the genotype at the loci governing earliness. Considering the F_2 population as a whole, some QTLs, such as *fnTG23* in control or *fwTG23–TG182b* in salinity, would not have been detected. Even worse, if we had studied only the "early" population, we would have concluded that *L. chesmannii* is not suitable for improving the salt tolerance of tomato cultivars. Therefore, we suggest taking into account the presence of pleiotropic effects of the genes controlling earliness in order to achieve a more efficient utilization of the *L. chesmannii* germplasm.

Acknowledgments This work was supported in part by grants from Conselleria de Cultura, Educació i Ciència (A.J.M.), CICYT (E.A.C.) and INIA (M.J.A.). The experiments comply with current Spanish law

References

Asíns MJ, Bretó MP, Carbonell EA (1993) Salt tolerance in *Lycopersicon*. I. Character definition and changes in gene expression. Theor Appl Genet 86: 737–743

Azanza F, Young TE, Kim D, Tanksley D, Juvik JA (1994) Characterization of the effect of introgressed segments Theor Appl Genet 87: 965–972

Banerjee MK, Kallo (1989) The inheritance of earliness and fruit weight in crosses between cultivated tomatoes and two wild species of *Lycopersicon*. Plant Breed 102: 148–152

Beavis WD (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. In: Proc 49th Annual Corn and Sorghum Industry Res Confe ASTA, Washington, D.C.

Bretó MP, Asíns MJ, Carbonell EA (1994) Salt tolerance in *Lycopersicon* species. III. Detection of QTLs by means of molecular markers. Theor Appl Genet 88: 395–401

Chetelat RT, DeVerna JW, Bennet AB (1995) Introgression into tomato (*Lycopersicon esculentum*) of the *L. chmielewskii* sucrose accumulator gene (*sucr*) controlling fruit sugar composition. Theor Appl Genet 91: 327–333

Cuartero J, Yeo AR, Flowers TJ (1992) Selection of donors for salt tolerance in tomato using physiological traits. New Phytol 121: 63–69

De Vicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 584–596

Doubley J, Stec A, Gustus C (1995) T*eosinte branched* and the origin of maize: evidence for epistasis and evolution of dominance. Genetics 141: 333–346

Ferreira ME, Satagopan J, Yandell BS, Williams PH, Osborn TG (1995) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor Appl Genet 90: 727–732

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

Jiang C, Zeng Z (1995) Multiple trait analysis for genetic mapping of quantitative trait loci. Genetics 140: 1111–1127

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

Kemble JM, Gardner RG (1992) Inheritance of shortened fruit maturation in the cherry tomato Cornell 871213–1 and its relation to fruit size and other components of earliness. J Am Soc Hort Sci 117: 646–650

Lander E, Green P, Abrahamson J, Barlow A, Daley, Lincoln S, Newburg (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181

Läuchli A (1986) Responses and adaptations of crops to salinity. Acta Hort 190: 243–246

Li ZK, Pinson SRM, Stansel JW, Park WD (1995) Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.) Theor Appl Genet 91: 374–381

Lindhout P, Van Heusden S, Pet G, Van Ooijen JW, Sandbrink H, Ververk R, Vrielink R, Zabel P (1994) Perspectives of molecular marker-assisted breeding for earliness in tomato. Euphytica 79: 279–286

Mahmoud MH, El-Beltagy AS, Helal RM, Maksoud MA (1986) Tomato variety evaluation selection for salt tolerance. Acta Hort 190: 559–566

Monforte AJ, Asíns MJ, Carbonell EA (1996) Salt tolerance in *Lycopersicon* species. IV. High efficiency of marker-assisted selection to obtain salt-tolerant breeding lines. Theor Appl Genet 93: 765–772

Monforte AJ, Asíns MJ, Carbonell EA (1997 a) Salt tolerance in *Lycopersicon* species. V. Does genetic variability at quantitative trait loci affect their analysis? Theor Appl Genet 95: 284–293

Monforte AJ, Asíns MJ, Carbonell EA (1997b) Salt tolerance in *Lycopersicon* species. VI. Genotype by salinity interaction in quantitative trait loci detection: constitutive and response QTLs. Theor Appl Genet 95: 706–713

Rush DW, Epstein E (1981) Breeding and selection for salt tolerance by the incorporation of wild germplasm into a domestic tomato. J Am Soc Hort Sci 106: 699–704

Saranga Y, Cabanes A, Zamir D, Marani A, Rudich J (1992) Breeding tomatoes for salt tolerance: inheritance of salt tolerance and related traits in interspecific populations. Theor Appl Genet 84: 390–396

Shanon MC, Gronwald JW, Tal M (1987) Effect of salinity on growth and accumalation of organic and inorganic ions in cultivated and wild tomato species. J Am Soc Hort Sci 112: 416–423

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

Van Tuinen A, Cordonnier-Pratt MM, Pratt L, Verkerk R, Zabel P, Koornneef M (1997) The mapping of phytochrome genes and photomorphogenic mutants of tomato. Theor Appl Genet 94: 115–122