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Salt tolerance in *Lycopersicon* species. III. Detection of quantitative trait loci by means of molecular markers

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Abstract A segregating population derived from a cross between *L. esculentum* cv Madrigal and a line of *L. pimpinellifolium* was used to identify genetic markers linked to QTLs involved in salinity tolerance in terms of yield, under a conductivity of 15 dS/m (171.1 mM NaCl). Six markers resulted, associated with QTLs affecting average fruit weight, fruit number and total weight under salinity. One of them, *Aco-1*, behaves reversely to the expectation from parental means; this and other features make it a promising target to obtain salt-tolerant tomatoes. Epistatic interactions were also found, thus affecting the criteria for marker-assisted selection. Although only 41% of the loci assayed were polymorphic, a high efficiency in identifying QTLs was achieved, since 43% of the marker loci are linked to QTLs for the trait under study.

Key words Salt tolerance · *Lycopersicon* · Yield · QTLs · Molecular markers · MAS · Epistasis

Introduction

Salinity is a major problem affecting an ever-increasing area throughout the world; saline stress affects the agricultural productivity and the survival of many crops. Few cultivated species can be considered as moderately salt tolerant; this is the case of tomato (Maas and Hoffman 1977). However, the levels of salinity found in many regions are too high to allow for adequate growth and production of tomato plants; for this reason, attempts to improve salt tolerance of tomato have always involved wild relatives of the cultivated species (Rush and Epstein 1981; Sacher et al. 1982; Shannon 1984; Cuartero et al. 1992; Saranga et al. 1992). Moreover, Cheesman (1988) re-examined the mechanisms involved in the overall response to salt and emphasized the high number of metabolical and physiological factors implicated in this character.

Unfortunately, in spite of considerable years of research, there are still no examples of crop cultivars that have proven commercially successful in severe saline ecosystems. The main reasons for this failure are the need for a wild species as the parent donor and the polygenic inheritance of the trait: therefore, traditional breeding schemes are unsuitable. The alternative is to use indirect selection for a genetically-correlated trait which is either easier to evaluate or has a much higher heritability than the desired one. Up to now, no such a character has been found that correlates with yield under strong salinity (Saranga et al. 1992; Asíns et al. 1993b). Nowadays, molecular markers linked to loci affecting a trait of interest can be used for indirect selection. Moreover, the availability of detailed linkage maps of molecular markers makes it possible to dissect quantitative traits into discrete genetic factors, called QTLs – quantitative trait loci – by Gelderman (1975). When a high-enough number of markers are scored in a family segregating for a given polygenic trait, accurate estimates of genic effects and QTL locations can be obtained by means of the interval-mapping methodology (Lander and Botstein 1989; Carbonell et al. 1992). Molecular markers have been used to study several quantitative traits including cold tolerance (Vallejos and Tanksley 1983), water use efficiency (Martin et al. 1989), insect resistance (Nienhius et al. 1987), and fruit weight and quality parameters, such as soluble solids content and pH, as well as some vegetative growth traits (Osborn et al. 1987; Paterson et al. 1988, 1991; Tanksley and Hewitt 1988, Weller et al. 1988) in tomato and traits related to yield and vegetative development in maize (Kahler and Wehrhahn 1986; Beavis et al. 1991; Edwards et al. 1992). In all these cases it has been possible to detect genes controlling the character of interest. In tomatoes, most of these studies involve progenies derived from interspecific hybrids where the parental species are distantly related, which increases the number of

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polymorphic markers to be screened. However, for breeding purposes, the donor species should be as closely related to the cultivated species as possible.

In previous studies, a line of *L. pimpinellifolium* (B. Juss.) Miller was selected as the salt-tolerant donor given its reduced decrease in yield under a salinity level of 15 dS/m (Asíns et al. 1993a) and its close relationship with the cultigen (Bretó et al. 1993). In order to develop a marker-assisted selection scheme in the breeding program, the objectives of the present study are: (1) to identify molecular markers linked to QTLs controlling tomato yield under salinity in an F_2 family derived from a cross of *L. esculentum* Miller by its closest related wild species *L. pimpinellifolium*, and (2) to study the effects of the individual QTLs and their epistatic interactions.

Materials and Methods

A segregating F_2 population of 206 plants obtained by selfing an interspecific hybrid from a cross between *L. esculentum* cv Madrigal and the salt-tolerant donor *L. pimpinellifolium* Line 1 (Asíns et al. 1993a) was studied for performance under saline conditions in terms of yield (as defined by Asíns et al. 1993b). Plants were grown on sand and irrigated with one-half Hoagland solution plus 171.1 mM NaCl, thus reaching a conductivity of 15 dS/m (Asíns et al. 1993a). Three yield components were evaluated for each individual plant: fruit number (FN), average fruit weight (FW) and total fruit weight (TW); all of them were measured at 9 weeks after plants started producing fruit.

In a search for polymorphic markers differing in the parental lines, 20 isozymic loci, 24 tomato genomic DNA clones, and two RAPDs were screened. All but the RAPDs are markers at known mapping positions.

ACO, EST, GDH, GOT, MDH, ME, PGI, PGM, SOD and TPI isozyme systems were screened in anther tissue, following the methods described by Bretó et al. (1993). Plant DNA was extracted according to the procedure of Dellaporta et al. (1983). For RFLP analysis, DNA was digested to completion with the enzymes *Eco*RI, *Dra*I, *Bam*HI or *Hin*dIII, using 15 µg of DNA and 30 units of the restriction enzyme, for 2 h at 37 °C. The genomic DNA digests were separated by electrophoresis in 0.8% agarose in TAE (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA.Na_2), and Southern-blotted to Immobilon-N filters (Southern 1975); after allowing the transfer to proceed for 20–24 h, the membranes were given a 15-min wash in 2 × SSC (300 mM NaCl, 30 mM sodium citrate), dried at room temperature for 30 min, and the DNA was fixed to the membranes in a vacuum oven at 80 °C for 2 h. Twenty-four tomato genomic DNA clones, kindly provided by Dr. S. D. Tanksley, were used as probes. Inserts were excised from plasmids, but whole restriction reactions were labelled by means of the random primer method (Feinberg and Vogelstein 1983). Probes were separated from unincorpored nucleotides through several washes with Ultrafree-MC filter units (UFC3LGC00, Millipore). Southern blots were left in 5 × SSPE (1 × SSPE is 180 mM Nacl, 10 mM NaH_2PO_4, 1 mM EDTA) for 15 min, and later prehybridized (5–8 h) and then hybridized overnight at 42 °C in a solution containing 50% Formamide, 5 × SSPE, 0.5% SDS, 5 × Denhardt's solution and 500 µg/ml of sheared single-stranded salmon sperm DNA; for hybridization, 2×10^6 cpm of the probe were added per ml of prehybridization solution. Membranes were washed at 65 °C in the following solutions: twice for 5 min in 2 × SSPE; twice for 20 min in 2 × SSPE, 0.1% SDS; twice for 20 min in 0.1 × SSPE, 0.1% SDS, and twice for 20 min in 0.1 × SSPE, 1% SDS. Autoradiographies were performed at −70 °C, for 2–3 days, using X-ray film (X-OMAT, Kodak) and intensifier screens. For reprobing, the blots were denatured (in 0.4 M NaOH) and neutralized (in 0.1 × SSPE, 0.1% SDS, 0.2 M Tris-Cl, pH 7.5) for 30 min each wash, at 45 °C. The PCR reaction mixture was 10 mM Tris-Cl, pH 9.0, 50 mM KCl, 3 mM $MgCl_2$, 100 µM each of dNTP, 0.2 µM primer (OPA01, from Operon Technologies, Inc.), 300 ng of genomic DNA and 0.5 U of *Taq* polymerase; when mixed, it was overlaid with 50 µl of paraffin. An automatic thermal cycler was programmed for 5 min at 94 °C, 45 cycles of 1 min at 94 °C, 2 min at 44 °C and 2 min at 72 °C for each cycle, and a final extension step of 10 min at 72 °C. Reaction products were resolved on a 1.4% agarose gel in TAE.

Chi-square goodness-of-fit statistics were calculated for the expected Mendelian segregation ratios of each marker, and two-way contingency tables tested for independent assortment of RAPDs and isozyme or RFLP markers.

A QTL was declared associated with a marker locus when the means of the quantitative trait in each genotypic class of the marker are significantly different by a t-test, using non-pooled estimates of variance (Asins and Carbonell 1988). Two-way analysis of variance for all pairwise combinations of the marker loci showing association with plant yield parameters under salinity with the rest of the loci was used for epistatic interactions.

The effect of heterozygosity on FW, FN and TW was estimated by regressing each phenotypic value of the F_2 individuals on their percent heterozygosity using all markers with codominant loci (H_1). For FN a regression on the percent heterozygosity using only those markers statistically associated with this character (H_2) was also calculated.

Results

Only two isozyme loci (*Aco-1* and *Est-4*), ten probes (denoted by TG followed by a number) and two RAPDs, among the ten bands obtained when using OPA01 as primer (PD2 and PD3), were polymorphic in the parental lines. Table 1 shows their chromosomal location, when known, the Rf of isozymes and the size of the DNA bands, the restriction enzyme used for the RFLP analysis, the observed frequencies of genotypic classes in the F_2 population and the chi-square probability for anomalous segregation, when significant. Three loci showed deviation from the expected Mendelian segregation ratios. The test of linkage among PD2 or PD3 and the rest of the markers did not result in significant evidence for association.

Figure 1 shows the segregation of some of the markers in the F_2 population. Except for TG 68, TG 134 and the RAPDs, three genotypes can be distinguished. For RAPDs, three polymorphic bands were evident, their size being 1 200, 650 and 500 kb. All individuals showing the 650-kb band also had the 1200-kb band and vice versa, so we will consider them as the same marker (PD3) and the 500-kb band as a different one (PD2), both segregating for presence vs absence.

Several QTLs related to salt tolerance were detected (Table 2); six markers showed some degree of association with the components of yield: three of them with average fruit weight (FW), four with fruit number (FN), and one with total weight (TW) which also has the highest computed t value for FN (TG 123). The TG 48 marker locus is related to both FW and FN, suggesting the existence of a pleiotropic effect at this genomic location, but in opposite directions: the genotype *EE* at this locus (homozygous for the *E* allele, derived from the cultivated species, *L. esculentum*) increases FW and re-

Table 1 Data from the polymorphic loci. Genotypic frequencies are: *EE*:*EP*:*PP* for codominant loci, *EE*:*EP*,*PP* for PD3, and *EE*,*EP*:*PP* for the rest (see Fig. 1 for codes). Significance levels refer to the chi-square probability of non-Mendelian segregation when significant

Marker	Chromosome location	Rf or Size		Restriction enzyme	Observed frequencies	Significance level
		Madrigal	Line 1			
Aco-1	12	36	40		45:91:57	
Est-4	12	75	78		35:102:54	
TG 18	9	2350	3370/2860	*Eco*RI	38:113:48	
TG 23	5	7700	4725	*Hin*dIII	38:111:48	
TG 24	1	2690/2300	5775	*Dra*I	60:87:51	
TG 30	11	4950	4000	*Hin*dIII	43:85:70	0.0035
TG 43	10	9400/3400	13800/3400	*Eco*RI	41:92:59	
TG 48	2	1700	3200	*Dra*I	39:88:70	0.0026
TG 63	10	2650	2350	*Dra*I	65:85:44	0.0233
TG 68	12	5630		*Hin*dIII	131:49	
TG 123	4	2750/2380	2970/2380	*Dra*I	55:89:54	
TG 134	3	13450		*Bam*HI	152:47	
PD2		500			146:53	
PD3			1200		51:148	

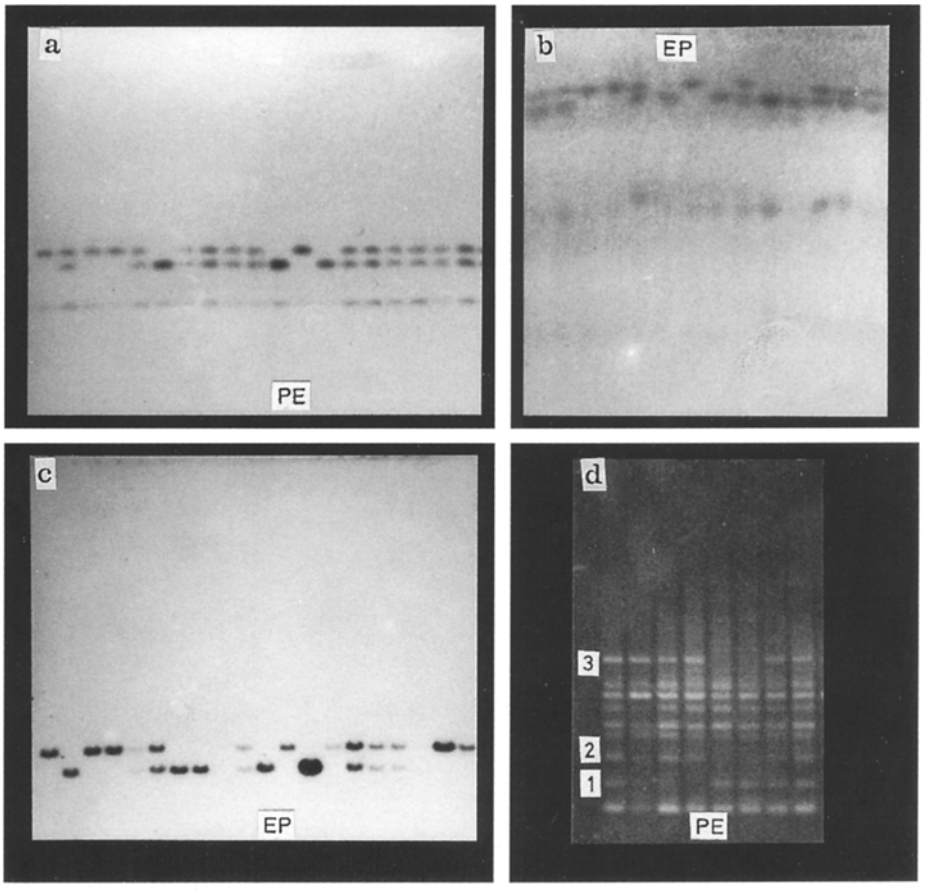


Fig. 1 a–d Examples of molecular markers segregating in the F2 population. **a)** ACO-1 allozymes (upper region). **b)** EST-1 allozymes. **c)** Autorradiograph of a Southern blot hybridized with TG 63 probe. **d)** Ethidium bromide stained gel showing the products of the PCR reaction using the primer OPA01: 1, 2 and 3 are the RAPDs screened: band 1 (500 Kb) is named PD2, and bands 2 and 3 (650 and 1200 Kb) correspond to PD3. "E" and "P" indicate the profiles of the cultivated and the wild line, respectively

duces FN. For markers linked to QTLs affecting FN, the F_2 plants with the *P* allele at the marker loci (derived from Line 1, of *L. pimpinellifolium*), at single or double dose, produced more fruits under salt treatment than plants homozygous for the *E* allele. This effect occurs in three out of the four markers. Heterozygous plants for the other marker, TG 43, have an intermediate mean for FN. For markers associated with QTLs affecting FW, plants homozygous for the *P* allele at loci TG 48 and TG 134 yielded smaller fruits; conversely, for *Aco-1*,

Table 2 Marker loci associated to QTLs for yield under salinity. NS means no significant differences between genotypic classes concerning each yield related parameter. When significant, the genotypes (see Fig. 1 for codes) are ordered regarding to their means, and the computed t-statistics for the difference between homozygous classes is also shown (except for TG 134 marker locus, where the statistics refer to presence vs absence of the *E* allele)

Marker	Total weight	Fruit number	Fruit weight
Aco-1	NS	NS	*PP, EP* > *EE* −2
Est-4	NS	NS	NS
TG 18	NS	NS	NS
TG 23	NS	NS	NS
TG 24	NS	*PP, EP* > *EE* −3.4	NS
TG 30	NS	NS	NS
TG 43	NS	*PP* ≥ *EP* ≥ *EE* −2.5	NS
TG 48	NS	*PP, EP* > *EE* −2.5	*EE* > *EP, PP* 2.9
TG 63	NS	NS	NS
TG 68	NS	NS	NS
TG 123	*PP, EP* > *EE* −2.9	*PP, EP* > *EE* −3.8	NS
TG 134	NS	NS	*EE, EP* > *PP* 3.4
PD2	NS	NS	NS
PD3	NS	NS	NS

plants with the *P* allele had higher FW values. Loci affecting FW showed dominance effects.

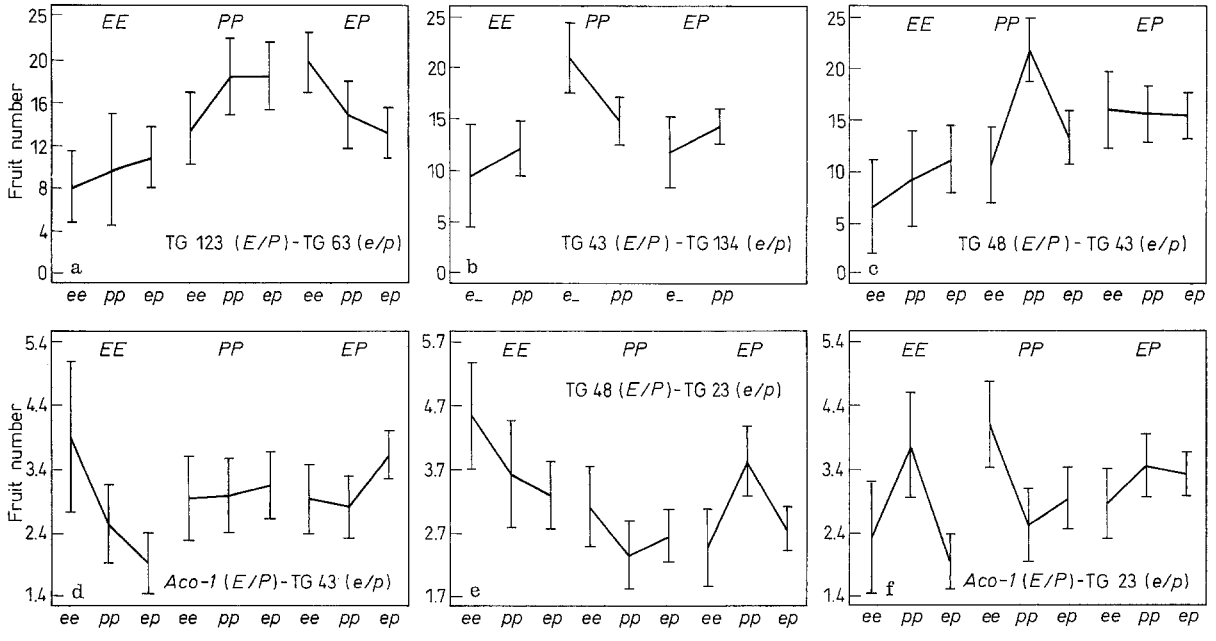
The six epistatic interactions that resulted in significance are shown in Fig. 2a–f. Three of them affect FW, and other three affect FN: (a) The presence of the *P* allele at the TG 63 locus (not found to be related to yield under salinity) decreases FN when plants are heterozygous at the TG 123 locus, but not when they are homozygous. (b) Plants homozygous for the *P* allele at both TG 43 and TG 134 (the latter is related to a QTL for FW) suffer a significant decrease in FN. (c, d) The TG 43 locus (related to a QTL for FN) interacts with TG 48 in the determination of FN and with *Aco-1* on FW. In case c, FN shows a maximum in plants homozygous for the *P* allele at both loci. *PP* plants at the *Aco-1* locus are not affected by the genotype at the TG 43 locus, but *EE* and *EP* plants respond differently depending on the genotype at the TG 43 locus. (e, f) The TG 23 marker locus (not found associated with any yield parameter) interacts with two loci that affect FW:TG 48 and *Aco-1*, making some genotypic combinations at the three loci especially profitable.

Regression analysis of yield on the percentage of heterozygosity did not result in significant evidence of correlation, with very low r^2 values (3.9% for the regression of TW on H_1 and less than 1% for that of FN on H_2).

Discussion

Some of the markers studied exhibit significant deviations from expected Mendelian segregation ratios; abnormal gene flow has frequently been reported when working with interspecific crosses and its extent and direction varies from one cross to another (Vallejos and Tanksley 1983; Paterson et al. 1988, 1991). Six marker loci have shown association with QTLs involved in yield under salinity. The TG 123 marker locus is very relevant to the total fruit weight (TW), mainly due to its large effect on fruit number (FN). Thus, consideration of a quantitative trait, like total weight, which is the result of the combined effect of other metric characters, such as

Fig. 2a–f Between-loci interactions with significant effects on Fruit Number and Fruit Weight. The two interacting loci are specified on top of each figure. Capital letters indicate the genotype at the first marker locus, and lower case letters that at the second locus. Codes are like in Fig. 1 and text. In case **b**, the code "e_" indicates both genotypes homozygous and heterogygous for the "e" allele



fruit weight and fruit number, could lead to an underestimate of the number of QTLs involved in it, unless the basic traits are also included in the study. Such a situation often happens when dealing with yield-related parameters (Kahler and Wehrhahn 1986; Edwards et al. 1992).

Two of the three QTLs related to fruit weight (FW) identified here are placed in genomic areas where Paterson et al. (1991) also assigned a QTL, although they used *L. cheesmanii* Riley as the wild parent. The third, corresponding to a QTL associated with *Aco-1*, was not found in that survey, although both parents differed at this marker locus. A promising degree of agreement between QTLs identified when using different genetic backgrounds and different environmental conditions is not unusual. Thus, the studies of Kahler and Wehrhahn (1986), Beavis et al. (1991) and Edwards et al. (1992) all agree in several QTLs of maize. In tomatoes, fruit weight, soluble solids content and pH have been studied by several authors (Paterson et al. 1988, 1991; Tanksley and Hewitt 1988; Weller et al. 1988), using different wild relatives of the cultivated tomato, and some of the QTLs found are likely to be the same. The disagreement between our work and that of Paterson et al. (1991) concerning a QTL for FW linked to *Aco-1* could be a consequence of the non-saline conditions in which their study was carried out.

Since the strength of a detected association between a marker locus and a QTL depends on their degree of linkage and on the gene effects of the QTL alleles, the lack of a large number of markers dispersed throughout the genome has not allowed us to estimate the recombination fraction and the additive and dominance effects by the interval mapping methodology. Few studies have used a saturated linkage map in order to obtain precise estimates on the location and effects of QTLs (Paterson et al. 1988, 1991; Beavis et al. 1991). According to our results, shown in Table 2, in relation to FN there are dominant effects of the QTLs associated with markers TG 48, TG 24 and TG 123 (all of them in the same direction) but only additive effects of that QTL linked to TG 43. We have found previously (Asíns et al. 1983b) that only additivity was involved in the overall expression of this character under the 15 dS/m saline treatment. Thus, there must be more QTLs controlling FN (with gene effects compensating the observed dominance) than those identified here. Concerning FW, dominance and epistatic effects under saline conditions were reported in the aforementioned paper. The QTLs underlying FW reported here also exhibit dominance effects; thus, the number of QTLs must be considered as a minimum estimate.

In order to improve yield under saline conditions, selection should be directed at plants that have the Line 1 allele (*P*) at *Aco-1*, TG 24, TG 123 and TG 43 loci, and the Madrigal allele (*E*) at all other loci; two different lines with *EE* and *PP* genotypes at the TG 48 marker locus should be developed for increasing FW and FN respectively. Tanksley and Hewitt (1988) cautioned about the danger of using markers related to QTLs in breeding programs before evaluating their effects in different genetic backgrounds and their possible associated effects on other characters of agronomic interest. However, not much importance has beeen given to results of epistatic interactions, except in the work of Vallejos and Tanksley (1983) on cold tolerance in tomatoes. Epistatic interactions greatly affect the decision to be taken about which genotypes to select during a breeding program. For instance, given that the TG 63 marker locus was not associated with any QTL, if we disregard the epistatic interactions, the genotype at this locus at the end of the breeding program would be *EE*; on the other hand, if we want to increase FN under salinity we would select plants which are either *PP* or *EP* at the TG 123 locus. However, we now know (Fig. 2a) that plants with the genotype "TG 123 *PP*, TG 63 *EE*" are as low yielding as those homozygous for the *E* allele at both loci (like the salt-sensitive parental line). TG 23 is another case of a locus not found associated with any QTL but interacting epistatically with two QTLs for FW (see Fig. 2 e, f). Thus, regarding QTLs, we could select the genotypes TG 48 *EE* and *Aco-1 PP* or *EP* in order to improve the trait; but, when we pay attention to the interloci interactions, only the genotypes "*Aco-1 PP*, TG 48 *EE*, TG 23 *EE*" and "*Aco-1 EE/EP*, TG 48 *EP*, TG 23 *PP*" are expected to result in increasing values of the trait (the latter also increasing FN). It is also possible to detect epistatic effects on FN between two QTLs affecting this trait (case d). Although FN and FW can be viewed as traits which are very difficult to improve in the same breeding line, due to their negative correlation, there are two interacting QTLs that could allow their simultaneous increase: selection of the genotype "TG 43 *PP*, TG 134 *EE/EP*" would increase FN (see case b), while this same genotype at the TG 134 locus causes an increase in FW.

The TG 48 marker locus also affects both FW and FN; this could mean either that there is only one QTL linked to the TG 48 marker with a pleiotropic effect on both yield components, or else that more than one QTL is linked to this marker. If pleiotropy exists, one could not separate these opposite effects: selecting for a higher fruit weight would lead to a decrease in its number. But if the alternative applies, a precise location of QTLs by means of a detailed linkage map of this genomic area could allow for the detection of recombinant plants with the desired genotype. Multiple effects of some chromosomal regions have been previously reported; for example, there is a frequent association of factors at the same chromosomal location increasing the soluble solids content and decreasing the fruit weight of tomatoes; this accounts for the high degree of negative correlation existing between both traits (Paterson et al. 1988, 1991). Conversely, Edwards et al. (1992) reported that multiple effects previously associated with a single marker locus are, in fact, attributable to more than one underlying factor.

F_1 plants show heterosis for the total weight yielded at a salinity of 15 dS/m. There are several genetic explanations for heterosis; including accumulation of dominant alleles, overdominance, and epistasis. With regard to the former, the QTLs identified show dominant effects in the direction of the alleles which increase yield; significant epistatic interactions have also been demonstrated in the analysis, even including other genomic areas where no QTL was detected. However we have not found any correlation between yield and the percent heterozygosity; thus, the degree of heterozygosity does not seem to be an importaant factor for the observed heterotic effect.

Most of the QTLs affecting FN and FW act as expected from parental behavior: QTL alleles from Line 1 linked to the TG 48, TG 24, TG 123 and TG 43 loci lead to an increase in FN, which could be expected since Line 1 exhibits a much higher number of fruits than the other parent. On the other hand, the cultivated parent shows much bigger fruits than the wild parent, so it is not unexpected that Madrigal alleles at the QTLs linked to the TG 48 and TG 134 markers cause a rise in FW, although Madrigal is a salt-sensitive line. The only QTL not behaving according to expectation is that linked to *Aco-1*: plants carrying the Line 1 allele produce heavier fruits under salinity than plants homozygous for the Madrigal allele. De Vicente and Tanksley (1993) pointed out the importance in crop improvement of QTLs with effects opposite to those predicted by the parental means. They found a correlation between the presence of such QTLs and the occurrence of trangressive phenotypes in segregating populations. A combination of complementary positive QTL alleles from both parents might cause individuals to exceed the parental phenotypes. This is specially significant when dealing with interspecific crosses, since there should be useful QTL alleles masked in wild species that do not display the character of interest. Additionally, as Asíns et al. (1993b) pointed out, the process of fruit development under high osmotic pressure could be crucial for fruit yield under salinity; this would explain the need for a *P* allele from the salt-tolerant line at a QTL involved in FW under salinity to increase the expression of the target character. Aconitase is an enzyme involved in the energetic metabolism of plants. Responses of plants to salt involve many mechanisms, all of them implying high energetic costs. These include ion transport, exclusion or compartmentation, and carbon acquisition and allocation; the increases in carbohydrate accumulation with salinity is a well known phenomenon, whether for osmotic adjustment or for turgor maintenance (Cheesmann 1988). Thus, more efficient metabolic responses (for example, an allele of aconitase less affected by the saline stress) can account for better performances under salinity. Since this *Aco-1* allele shows a number of particularities, such as its unexpected behavior according to the parental means, the fact that it is not linked to any QTL for FW under non-saline conditions (Paterson et al. 1991), the fact that it is present in a number of salt-tolerant *L. pimpinellifolium* lines and in one line of *L. pennelliii* (Correll) D'Arcy but not in a salt-sensitive line of *L. pimpinellifolium* (Asíns et al. 1993a; Bretó et al. 1993), and the fact that Tanksley and Hewitt (1988) also reported a QTL for soluble solids content associated with *Aco-2*, suggests that this enzyme is playing an important role in determining the salinity tolerance in terms of yield of Line 1 plants. We therefore recommend a screen of germplasm resources for *Aco-1* when looking for other *Lycopersicon* entries to improve fruit weight under salinity.

To use a cross betwen species with a close genetic relationship is highly desirable in a breeding program since it reduces the time and effort required to recover the genome of the cultivated line. Its drawback is the smaller number of available polymorphic loci. Thus, we have found that only 41% of the loci assayed (14 out of 34 markers dispersed through the genome) were polymorphic between *L. esculentum* cv Madrigal and *L. pimpinellifolium* Line 1, its closest related species (Bretó et al. 1993). However, a high efficiency in detecting QTLs was achieved, since 43% of the marker loci (6 out of 14) proved to be linked to QTLs involved in yield under salinity. In a similar work, Weller et al. (1988) examined 18 quantitative traits and ten isozyme markers in a cross between *L. esculentum* × *L. pimpinellifolium*, and found that every marker locus was associated with FW. The proportion of QTLs identified in both cases is higher than those found in comparable studies when using crosses between less-related species: in *L. esculentum* × *L. hirsutum* Humb. and Bonpl. (Nienhius et al. 1987) and *L. esculentum* × *L. pennellii* (Martin et al. 1989) only 9 and 17%, respectively, of the polymorphic genomic regions carried QTLs for the trait under study. The greater efficiency in detecting QTLs in close crosses could indicate that, though polymorphic regions between the parental lines are scarce, it is more likely that they carry QTLs involved in traits for which the parental lines differ. Hence, the strategy employed in the present study is a highly useful alternative to locate QTLs for marker-assisted selection. However, care must be taken in the use of the statistical methodology. If linkage between a QTL and a marker locus exists, the variances within each genotypic class are different in the F_2 and their respective estimates of variance ought to be used for the test (Asíns and Carbonell 1988). Once QTLs are identified, a saturated map of the region of interest can be obtained by using the "bulked segregant analysis" methodology (Michelmore et al. 1991; Churchill et al. 1992); a high-resolution map of the genomic area would provide further knowledge of the location and the genetic effects of each QTL, and so expedite manipulation of the QTLs themselves.

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References

Asíns MJ, Carbonell EA (1988) Detection of linkage between restriction fragment length polymorphism markers and quantitative traits. Theor Appl Genet 76:623–626

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

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

Beavis WD, Grant D, Albertsen M, Fincher R (1991) Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor Appl Genet 83:141–145

Bretó MP, Asíns MJ, Carbonell EA (1993) Genetic variability in *Lycopersicon* species and their genetic relationships. Theor Appl Genet 86:113–120

Carbonell EA, Gerig TM, Balansard E, Asíns MJ (1992) Interval mapping in the analysis of nonadditive quantitative trait loci. Biometrics 48:305–315

Cheeseman JM (1988) Mechanisms of salinity tolerance in plants. Plant Physiol 87:547–550

Churchill GA, Giovanoni JJ, Tanksley SD (1992) Pooled-sampling makes high-resolution mapping practical with DNA markers. Proc Natl Acad Sci USA 90:16–20

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

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

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

Edwards MD, Helentjaris T, Wright S, Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative trait loci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. Theor Appl Genet 83:765–774

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

Gelderman H (1975) Investigation on inheritance of quantitative characters in animals by gene markers. 1. Methods. Theor Appl Genet 46:319–330

Kahler AL, Wehrhahn CF (1986) Association between quantitative traits and enzyme loci in the F_2 population of a maize hybrid. Theor Appl Genet 72:15–26

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

Maas EV, Hoffman GJ (1977) Crop salt tolerance – current assessment. J Irrig Drain Div Am Soc Civil Eng 103:115–134

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

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis. A rapid method to detect markers in a specific genomic region by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Nienhius J, Helentjaris T, Slocum M, Ruggero B, Schaefer A (1987) Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop Sci 27:797–803

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor Appl Genet 73:350–356

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits in to Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

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

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

Sacher RF, Staples RC, Robinson RW (1982) Saline tolerance in hybrids of *L. esculentum* × *S. pennellii* and selected breeding lines. In: San Pietro A (ed) Biosaline research: a look to the future. Plenum Press, New York, pp 325–336

Saranga Y, Cahaner A, Zamir D, Morani 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

Shannon MC (1984) Breeding, selection, and the genetics of salt tolerance. In: Staples RC, Toenniessen GH (eds) Salinity tolerance in plants. Wiley, New York, pp 231–254

Southern ED (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503

Tanksley SD, Hewitt J (1988) Use of molecular markers in breeding for soluble solids content in tomato – a re-examination. Theor Appl Genet 75:811–823

Vallejos CE, Tanksley SD (1983) Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato. Theor Appl Genet 66:241–247

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