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# Salt tolerance in *Lycopersicon* species. IV. Efficiency of marker-assisted selection for salt tolerance improvement

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**Abstract** The usefulness of marker-assisted selection (MAS) to develop salt-tolerant breeding lines from a $F_2$ derived from *L. esculentum* × *L. pimpinellifolium* has been studied. Interval mapping methodology of quantitative trait locus (QTL) analysis was used to locate more precisely previously detected salt tolerance QTLs. A new QTL for total fruit weight under salinity (*TW*) near TG24 was detected. Most of the detected QTLs [3 for *TW*, 5 for fruit number, (*FN*) and 4 for fruit weight (*FW*)] had low $R^2$ values, except the *FW* QTL in the TG180–TG48 interval, which explains 36.6% of the total variance. Dominant and overdominant effects were detected at the QTLs for *TW*, whereas gene effects at the QTLs for *FN* and *FW* ranged from additive to partial dominance. Phenotypic selection of $F_2$ familes and marker-assisted selection of $F_3$ families were carried out. Yield under salinity decreased in the $F_2$ generation. $F_3$ means were similar to those of the $F_1$ as a consequence of phentoypic selection. The most important selection response for every trait was obtained from the $F_3$ to $F_4$ where MAS was applied. While $F_3$ variation was mainly due to the within-family component, in the $F_4$ the *FN* and *FW* between-family component was larger than the within-family one, indicating an efficient compartmentalization and fixation of QTLs into the $F_4$ families. Comparison of the yield of these families under control versus saline conditions showed that fruit weight is a key trait to success in tomato salt-tolerance improvement using wild *Lycopersicon* germplasm. The QTLs we have detected under salinity seem to be also working under control conditions, although the interaction family × treatment was significant for *TW*, thereby explaining the fact that the selected families responded differently to salinity.

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## Introduction

For plant breeding to be able to contribute both to a more secure world food supply and to sustainable agricultural systems, plant breeders will have to develop varieties with wide adaptability as well as varieties for local, specific environments. Success in dealing with this demand in any crop requires access to a wide range of germplasm and a comprehensive and integrated breeding system that makes maximum use of classical breeding methods as well as of the new biotechnologies (Bosemark, 1994). Some crops have shown such a genetic variability for salt tolerance so as to be suitable to be used directly in a breeding program (Asharf and McNeilly 1990). Tomato (*Lycopersicon esculentum*) is considered to be moderately salt tolerant (Maas and Hoffman 1977). However, this crop cannot tolerate the high salinity levels (> 14 dS/m) found in many regions. This lack of tolerant accessions within the cultivated species has forced researchers to search among the wild relatives, such as *L. pimpinellifolium*, *L. pennelli*, *L. peruvianum* and *L. chesmanii*, which have been shown to be salt-tolerant (Cuartero et al. 1992; Saranga et al. 1991). Breeding programs involving wild species result in the introduction of many undesirable features. Hence, it is necessary to use powerful methodologies that provide a quick and efficient management of segregating generations to speed up and ensure the success of the breeding program. The development of DNA markers [restriction fragment length polymorphism (RFLPs) and random amplified polymorphic DNAs (RAPDs)] (Botstein et al. 1980; Welsh and McClelland 1990; Williams et al. 1990) and suitable statistical methods (Carbonell and Asins 1996) allows the breeder to locate quantitative trait loci (QTLs) and estimate their genetic effects,

thereby providing the possibility of improving quantitative characters by using wild species.

The number of QTLs controlling agronomically important traits that are being detected is continuously growing. One of the main purposes of such studies is their application in the construction of marker-assisted selection (MAS) schemes to speed up the breeding programs. Lande and Thompson (1990) have shown that MAS can be an useful tool improve quantitative traits; however, there is little information about its application in a breeding program, and what is available is not very positive. In fact, Stromberg et al. (1994) did not get a greater response to MAS than to conventional selection in a corn breeding program, and neither of the selected families performed significantly better than the unselected population. An important inconvenience of MAS might be the fact that when confidence intervals are calculated for the QTL positions, they may cover several intervals or even the whole linkage group if the heritability of the trait is low (Hyne et al. 1995); hence, it seems necessary to apply this methodology experimentally to confirm or deny the results found in theoretical studies.

The final aim of the present work is to study the effectiveness of selection based on genotypes at marker loci linked to QTLs involved in salt tolerance in order to develop salt-tolerant breeding lines. The association between molecular markers and yield traits under salinity conditions was investigated in a $F_2$ derived from an interspecific cross between *L. esculentum* cv 'Madrigal' and *L. pimpinellifolium* (Bretó et al. 1994). Taking into account these associations, we pursued the following three objectives:

– to verify or locate more precisely the salt tolerance QTLs already detected (Bretó et al. 1994) by screening new flanking RFLPs and using the interval mapping procedure;

– to study the response to marker-assisted selection at the $F_3$ level;

– to develop salt-tolerant recombinant breeding lines and comparatively study their behavior under control versus salinity conditions.

## Materials and methods

### Plant material

Plants were cultured in greenhouse with photoperiod (12 h) and temperature (25° ± 10 °C) control. Plants subject to the saline treatment were grown on sand and irrigated with one-half Hoagland solution plus 171.1 m*M* NaCl (conductivity of 15 dS/m). Control plants were grown in peat plus sand and irrigated with tap water ( ≈ 2 dS/m).

Three yield components were measured for each individual plant, total fruit weight (*TW*), fruit number (*FN*) and average fruit weight (*FW*), during the first 9 weeks of production.

### Molecular markers

Genomic DNA was extracted, digested and blotted to PVDF membranes (Millipore) following the procedure of Bretó et al. (1994) when the hybridization was carried with radioactive-labeled probes. The genomic DNA was also digested with *Xba*I, *Eco*RV and *Hae*III and transferred to Hybond N nylon membranes (Amersham) when hybridizations were carried with non-radioactive-labeled probes.

Twelve new tomato cDNA clones previously mapped around the most important markers ACO-1, TG24, TG123 (Bretó et al. 1994) were used to fully locate their associated QTLs. These clones were labeled with Dig-UTP (Boehringer Manheim) by means of the polymerase chain reaction (PCR) using pUC universal and reverse-derived primers with a Dig-UTP: ATP ratio of 5% in the reaction mixture. The probes were separated from unincorpored nucleotides with the Wizard PCR-preps Kit (Promega Corp). Southern blots were prehybridized at least for 2h in 5 × SSPE, 1% Blocking reagent (B.M.), 0.2% SDS, 0.01% *N*-Lauryl sarcosine. The hybridization solution was the same as the prehybridization one except for the addition of the probe at a concentration of 20 ng/ml. Washing of the filters and chemiluminescent detection were carried out following the instructions of the manufacturer (B.M.). RFLPs were scored only for those plants with trait values higher or lower than 1 SD from the trait mean. Linkage maps were made with the MAPMAKER 3.0 program (Lander et al. 1987) using a minimum LOD score of 3.0. Recombination frequencies were transformed into centimorgans (cM) using the Kosambi mapping function (Kosambi 1944).

### QTL analysis

Means of the quantitative trait at the marker genotype were compared by a *t*-test using non-pooled estimates of variance to define the associations between markers and putative QTLs (Asins and Carbonell 1988). Percentage of phenotypic variance explained by the markers ($R^2$) was calculated by regression analysis. Means of traits at the marker genotype were also used to estimate the dominant and additive effects of QTLs associated to single markers (Edwards et al. 1987).

QTLs were also analyzed by interval mapping using the MAPMAKER/QTL 1.1 (Paterson et al. 1988) and LINKQTL (Carbonell and Gerig 1991) computer programs. The LOD score threshold was 1.184 according to the number of intervals and a total level of significance of 5% (Lander and Botstein 1989).

### Development of salt-tolerant breeding lines

Six $F_2$ plants (named 2, 11, 17, 65, 83 and 184) were phenotypically selected according to their high yield components under salinity. Six $F_3$ families of 20 plants each were founded with the progenies derived by self-pollination from the selected $F_2$ plants. These families were labeled by the number of their parents and grown under salinity. Their genotypes at the marker loci associated to yield under the saline condition were determined, and yield traits were evaluated. Six $F_3$ plants having the most favorable genotype for the molecular markers [according to Bretó et al. (1994) and to the results of the interval mapping analysis] were selected. Plant $F_3$ 2–3 was selected due to its high level of expression of peptide 2', which is associated to salinity tolerance (Asins et al. 1993). The progeny by selfing $F_3$ selected plants were used to found $F_4$ families. Twenty-five plants of each family were grown under saline conditions, and comparable sets of 25 plants were grown under the control condition. $F_3$ 2–16 plant was heteorzygous at *Aco-1*; therefore its progeny was genotyped at the seedling stage for this locus, and homozygous seedlings for the "esculentum" allele were consequently discarded.

### Heritability, variances and response to selection

Data from yield components of the plants of each generation were used to estimate heritability by regression of the progeny on the parents. This estimator contains non-additive components depending on the generations for which it is calculated (Mather and Jinks 1971; Nyquist 1991), and it is not affected by the fact that selected parents are used, although it has reduced precision (Falconer 1989).

The estimators were:

$$h^2_{F_2:F_3} = b_{F_2F_3} = \frac{V_a + V_d/2}{V_a + V_d + V_e}$$

$$h^2_{F_3:F_4} = b_{F_3F_4}(1 + F(1 - b_{F_3F_4})) = \frac{V_a + V_d/8}{3V_a/2 + 3V_d/4 + V_e}$$

ignoring variances caused by interaction between genes $b_{F_2F_3}$ and $b_{F_3F_4}$ are the coefficients of the respective regressions; $F$, the inbreeding coefficient, (1.2 in our case) is used here to adjust the heritability for inbreeding (Nyquist 1991); $V_a$, $V_d$ and $V_e$ are the additive, dominance and environmental variances, respectively. Standard errors of regression were used as error of estimates (Nyquist 1991). The heritability so calculated is half-way between the narrow-sense and broad-sense In the absence of dominance, the above equations estimate heritability in the narrow-sense.

Between-families ($\sigma^2_b$) and within-families ($\sigma^2_w$) variances were estimated by one-way ANOVA for the corresponding family type. Selection differential ($S$) was calculated as the difference between the mean of the selected plants and the generation mean, and the response to selection ($R$) as the difference between parent and offspring generation means.

Comparison between treatments in $F_4$ families

Two-way ANOVA with family and treatments as factors was used to study the responses of the selected families to the saline treatment versus their behavior under the control condition

## Results

### Segregation and linkage of markers

Only 4 out of the 12 new clones were polymorphic between the parental lines. The distances and location of these RFLPs in the linkage map agreed with previously reported tomato genetic maps (Tanksley et al. 1992), except for the marker TG180, which is linked to TG48 (42.7 cM) in our population whereas for Tanksley et al. (1992) it is linked to *Aco-1* on chromosome 12. These linkage relationships were also confirmed in another $F_2$ population derived from a similar interspecific cross (data not shown). Linkage analysis resulted in four genomic regions suitable to QTL analysis by interval mapping.

### QTL analysis

#### *"A marker at a time" methodology*

A total of 14 marker-trait associations were significant (Table 1): 2 for *TW*, 6 for *FW* 6 for *FN*. The portion of phenotypic variance explained by individual marker loci ranged from 3.14 to 11.5. The ratio $d(1-2r)/a$ showed the genetic actions of the putative QTLs to be between strictly additive (0.04) to overdominant (4.24). QTLs linked to isozyme markers showed overdominant effects.

#### *"Interval mapping" methodology*

The probability plots obtained by MAPMAKER/QTL generally coincided with those obtained by LINKQTL and are found in Fig. 1. Table 2 shows the local maximum values of LOD scores, the position of this maximum in the interval relative to the leftmost marker, the

**Table 1** Marker loci associated with QTLs for yield under salinity. When two marker loci are connected by a vertical hyphen it means that they form an interval. The computed $t$-statistic for the difference between homozygous classes, the degree of dominance (estimated as $d(1-r)/a$) and the contribution of each QTL ($R^2$) are also shown. Presence of QTL genotypes with mean increasing alleles (+) mean decreasing alleles (−) or no effect ( ) is indicated at each QTL for the $F_4$ families. *, The TW QTL of TG24 was detected by interval mapping and also by comparison of means using only plants from both sides of the distribution (selective genotyping)

| Trait | Marker loci | Comparison | $t$-value | $d(1-2r)/a$ | $R^2$ | $F_4$ families | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 2–3 | 2–16 | 17–3 | 17–7 | 17–12 | 83–89 |
| Total | TG24–* | EP ≥ PP ≥ EE[a] | 3.14 | 4.05 | 7.24 | | − | + | + | + | |
| weight | ACO | EP ≥ PP ≥ EE | 2.44 | 3.05 | 3.24 | + | + | | | | |
| (TW) | TG123 | PP, EP > EE | 2.90 | 0.76 | 5.14 | − | + | + | + | + | + |
| Fruit | EST | EP ≥ PP ≥ EE | 2.25 | 4.24 | 3.14 | − | + | | | + | |
| number | TG123 | PP, EP > EE | 3.80 | 0.60 | 7 4 | − | + | + | + | + | + |
| (FN) | TG51 | PP > EE, EP | 3.35 | 0.04 | 11.15 | | | | | | |
| | \| | | | | | | − | + | + | + | |
| | TG24 | PP. EP > EE | 3.40 | 0.24 | 5.78 | | | | | | |
| | TG43 | PP ≥ EP ≥ EE | 2.5 | −0.10 | 4 01 | + | + | + | + | + | + |
| | TG48 | PP. EP > EE | 2.5 | 0.90 | 3.43 | + | − | + | + | + | + |
| Fruit | ACO | PP. EP > EE | 2.16 | 2.13 | 2.52 | + | + | + | + | + | + |
| weight | TG134 | E- > PP | 2.75 | | 4.04 | + | + | + | + | + | + |
| (FW) | TG180 | EE ≥ EP ≥ PP | 2.89 | −0.24 | 9.38 | | | | | | |
| | \| | | | | | − | + | − | − | − | − |
| | TG48 | EE > PP. EP | 2.99 | 0.789 | 5.41 | | | | | | |
| | TG51 | EE, EP > PP | 3.63 | −0 49 | 9.57 | | | | | | |
| | \| | | | | | | + | − | − | − | |
| | TG24 | EE, EP > PP | 2.65 | −0 61 | 4.70 | | | | | | |

[a] E, *L. esculentum* allele; P, *L. pimpinellifolium* allele

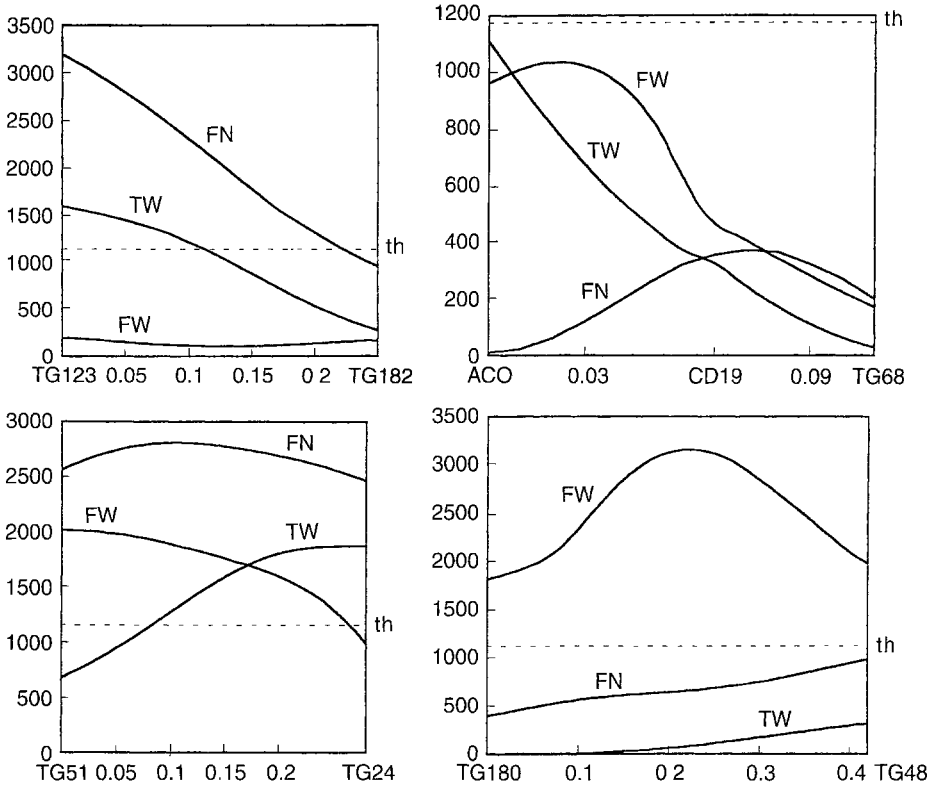



**Fig. 1** LOD score ($x \times 10^2$) plots for yield under salinity. *TW* Total fruit weight, *FN* fruit number, *FW* average fruit weight, *th* the threshold value for significance of the LOD score

**Table 2** QTL analysis by interval mapping. Local maximum values of LOD scores, the position of the maximum in the interval (cM), the estimated gene actions of the putative QTLs ($a, d, d/a$) and the percentage of phenotypic variance explained ($R^2$) are indicated

| Trait | Interval | LOD | cM | $a$ | $d$ | $d/a$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| Total weight | TG 51–TG 24 | 1.88 | 28 | 3 65 | 15.35 | 4 21 | 7.4 |
| | TG123–TG182 | 1.61 | 0 | 8 42 | 8.88 | 1 | 6.0 |
| | ACO-CD 19 | 1.10 | 0 | 4.68 | 10.43 | 2.2 | 4.2 |
| | All QTLs | | | | | | 15.5 |
| Fruit number | TG51–TG24 | 2.80 | 11 | 5 68 | 1.40 | 0.25 | 13 |
| | TG123–TG182 | 3.20 | 0 | 5 00 | 4.39 | 0.88 | 11.5 |
| | TG180–TG48 | 0 99 | 42 | 3.12 | 2.49 | 0.80 | 3.8 |
| | All QTLs | | | | | | 22.9 |
| Fruit weight | TG51–TG24 | 2 02 | 0 | −0.76 | 0.40 | −0.53 | 8.6 |
| | TG180–TG48 | 3.16 | 21 | −1.54 | −1.33 | 0.88 | 36.7 |
| | ACO-CD 19 | 1.03 | 2.0 | 0.33 | 0.82 | 2.34 | 5.3 |
| | All QTLs | | | | | | 47.1 |

estimated gene actions of the putative QTLs and the percentage of phenotypic variance explained ($R^2$).

According to the threshold previously determined, six significant LOD score maxima were obtained (Table 2): two for *TW* in the intervals TG51–TG24 and TG123–TG182, two for *FN* in the same intervals and two for *FW* in the intervals TG51–TG24 and TG180–TG48. The associations of TG48 with *FN* and *Aco-1* with *TW* and *FW* were not significant using the interval mapping approach, whereas a new QTL for *TW* was detected near TG24 only by using "interval mapping" methodology. The probability peaks of the putative QTLs for *FN* in interval TG51–TG24 and *FW* in TG180–TG48 interval were located inside the intervals. However, the other maxima of the LOD score curves were at one end of the interval, indicating that the actual position of the QTL might be at the other side of the marker locus. Two additional intervals that are almost significant are also included in Table 2.

Dominant and overdominant effects were detected at the QTLs for *TW*, whereas gene effects at the QTLs for *FN* and *FW* ranged from additive to partial dominant. The type of gene effects and the relative degree of dominance were consistent using both statistical methods.

Most detected QTLs had low $R^2$ values, except for the *FW* QTL in the TG180–TG48 interval, which explained 36.6% of the phenotypic variance.

Evolution of selected families

$F_3$ families (derived by self-pollination of the phenotypically selected $F_2$ plants) were genotyped for all the polymorphic molecular markers (16), and those plants with favorable QTL genotypes and the least heterozygosity were selected to found $F_4$ families. The QTL genotypes with mean increasing alleles (+) or mean decreasing alleles (−) are indicated in Table 1. The genotypic selection led to almost fully homozygous lines for the yield-associated markers. No recombination between linked markers was found, which practically ensured the inclusion of the QTLs in the intervals. With the establishment of these homozygous or near-homozygous lines, one of the objectives of our MAS program was achieved.

Yield means fell down in the $F_2$ generation due to the loss of heterotic effects found in the interspecific hybrid and the distortions in the segregation of markers, including some associated with yield (Asíns et al. 1993). In the $F_3$ generation, the yield parameters reached $F_1$ levels. An important increase in yield was obtained in the $F_4$, which was greater than the $F_1$ values. This evolution of yield parameters is graphically represented in Fig. 2.

Table 3 shows the evolution of yield parameters during the four generations. Estimates of heritability, within- and between-family variance components, selection differentials and responses to selection in the $F_3$ and $F_4$ generations are also shown. The most important selection response for every trait was obtained in the $F_4$ generation, in spite of the fact that the selection differentials were lower than those found in the previous generation. ANOVA showed an important change in the variance components from the $F_3$ to the $F_4$ generations. While in the $F_3$ most of the variation was located in the within-family component, in the $F_4$ the *FN* and *FW* between-family component was larger than the within-family one.

Variance components of $F_4$ families in the control conditions for *FW* and *FN* were similar to those under saline treatment. The variance of *TW* was greater under control conditions and, consequently, so were its variance components, especially the between-family component.

Most $F_4$ families underwent a decrease in *FW* and *TW* as an effect of saline treatment. Since *FN* remained constant, in general, (Fig. 3) the decrease in *TW* must be caused by the *FW* loss. No yield component of family 2–3 was significantly affected by the treatment. The family-by-treatment interaction was significant only for *TW* (Fig. 3).

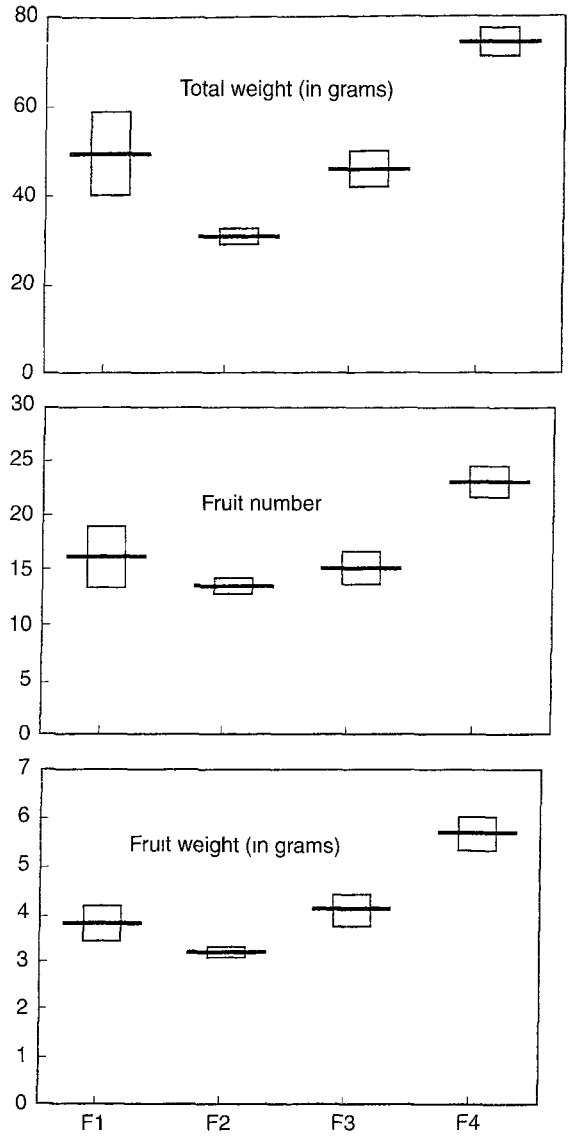


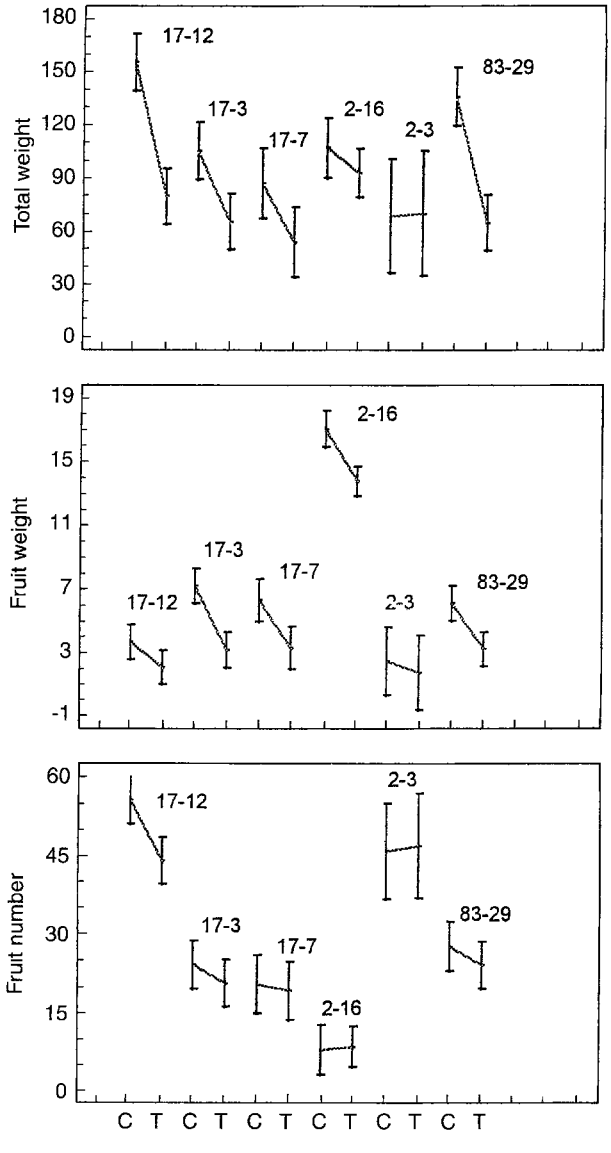

**Fig. 2** Evolution of total weight (*TW*) fruit number (*FN*) and fruit weight (*FW*) means of $F_1$, $F_2$, $F_3$ (phenotypically selected from $F_2$) and $F_4$ (MAS from $F_3$)

## Discussion

Nowadays, breeders are interested in the detection of QTLs by means of saturated genetic maps. Powerful statistical procedures to detect QTLs have been published (see Carbonell and Asíns 1996 for a review), and many papers report the application of these models in experimental populations (see Tanksley 1993 for a review). One of the main reasons for this effort is to apply this knowledge in a breeding program through marker-assisted selection. The most important drawback to MAS is its technical cost (Beckman and Soller 1983; Lande 1992). Theoretical studies in order to minimize the cost involved in the detection of QTLs (Asíns and Carbonell 1988; Darvasi and Soller 1994), evaluations of marker-assisted selection (Lande and Thompson 1990; Edwards and Page 1994; Zhang and Smith 1992) and the comparison of responses from MAS versus selection based on phenotypes through computer simulation have not been able to determine the economical benefits

**Table 3** Evolution of $F_1$, $F_2$ and selected families for *TW*, *FN* and *FW*. $\sigma_b^2$ and $\sigma_w^2$ are the between- and within-family variance components, $h^2$ is the estimated heritability, *S* is the selection differential, and *R* is response to selection

| Treatment | Family type | Statistic | Total weight (*TW*) | Fruit number (*FN*) | Fruit weight (*FW*) |
|---|---|---|---|---|---|
| Salinity | $F_1$ | Mean | 49.35 ± 22.7 | 16.33 ± 7 06 | 3.78 ± 0.7 |
| | $F_2$ | Mean | 30.64 ± 24.9 | 13.63 ± 10.2 | 3.17 ± 1.6 |
| | $F_3$ | Mean | 46.07 ± 40.5 | 15.37 ± 16.0 | 4.08 ± 3.1 |
| | | $\sigma_w^2$ | 0 | 45.09 | 5.46 |
| | | $\sigma_w^2$ | 1641 06 | 219.5 | 4.87 |
| | $F_2$–$F_3$ | $h^2$ | 0.24 ± 0.35 | 0.62 ± 0.17** | 0.86 ± 0.1* |
| | | *S* | 48.84 | 12.77 | 0.35 |
| | | *R* | 15.43 | 2.00 | 1 20 |
| | $F_4$ | Mean | 74.26 ± 35.7 | 23.8 ± 16.2 | 5.67 ± 5.6 |
| | | $\sigma_b^2$ | 160.0 | 187.4 | 27 83 |
| | | $\sigma_w^2$ | 1148.2 | 101.8 | 9.19 |
| | $F_3$–$F_4$ | $h^2$ | 0.05 ± 0.11 | 0.61 ± 0.08** | 1.17 ± 0.07* |
| | | *S* | 19 64 | 4.78 | 0.01 |
| | | *R* | 28.17 | 7.91 | 1.60 |
| Control | $F_4$ | Mean | 118.34 ± 52.0 | 28.65 ± 20.4 | 7.20 ± 5.1 |
| | | $\sigma_b^2$ | 708.7 | 311.7 | 25.19 |
| | | $\sigma_w^2$ | 2136 | 160 | 5.09 |




of MAS. Although computer simulation is a useful tool, our knowledge concerning the parameters involved in quantitative trait variation is still limited. Heterosis (Stuber et al. 1992), transgressive variation (de Vicente and Tanksley 1993), epistasis interactions between QTLs (Lark et al. 1994) or environment-by-QTL interactions (Asíns et al. 1994, Jensen et al. 1995) are not sufficiently understood to be satisfactory included in the theoretical models. Furthermore, the confidence intervals of the QTL positions often include two or more intervals (Hyne et al. 1995). These facts have led us to study the actual efficiency of MAS within the framework of a breeding program. Before applying MAS, we tried to locate the QTLs by interval mapping mainly to ensure their inclusion into the recombinant inbred lines, given that double crossing-over within an interval can be ignored from a practical point of view. Comparing both methods of detecting QTLs, we found some differences. When using the mean comparison procedure, three putative QTLs (for FN at TG48 and for *TW* and *FW* at *Aco-1*) were detected that had been missed by interval mapping, althouth it should be recognized that the probability plots of LOD score for these traits have local maxima, with values near the threshold at the correspondent marker loci (Fig. 1). These local maxima LOD scores have been commonly interpreted as an indication of additional QTLs with small effects (Paterson et al. 1988; Uzunova et al. 1995). It is difficult to calculate the proper threshold of a LOD score for QTL detection, and only approximate analyses are available (Lander and Botstein 1989; Rebai 1994; Carbonell et al. 1992), and this is crucial for QTLs that have very low

**Fig. 3** $F_4$ family by treatment interactions for total weight, fruit number and fruit weight. These interactions were significant only for total weight. *C* and *T* means control and saline treatment, respectively

contributions, as those mentioned before ($R^2$ from 2.52 to 3.43). In spite of that, we think these QTLs are real ones because Paterson et al. (1991), using a population derived from another interspecific cross (*L. esculentum* × *L. cheesmannii*), also detected a QTL for *FW* at chromosome 12 near *Aco-1*. By means of interval mapping, the detection of a new QTL for *TW* near TG24 (Fig. 2) has been possible. This QTL was not revealed by the comparison of means among the genotypes at this marker loci; however, when this comparison was performed only with the plants at both extremes of the distribution (selective genotyping), then it was clearly significant ($EP \geq PP \geq EE$, $t = 3.14$, $d(1 - 2r)a = 4.06$ and $R^2 = 7.24$). Though its contribution is not very small, variances for *TW* are large, making *t*-tests non-significant. Thus, if QTLs have a moderate contribution, interval mapping is more powerful for QTL detection.

By using interval mapping at TG180–TG48, we were able to distinguish between a pleitropic QTL or linked QTLs for *FW* and *FN*. However, for the interval TG51–TG24 the probability curve for *FN* was too flat for accurate positioning of the corresponding QTL and thus for allowing us to distinguish it from another QTL governing *FW* near TG51.

The relative abundance of QTLs with overdominant effects controlling *TW* under salinity should be pointed out. The practical non-existence of QTLs with additive genes might explain its low (non-significant) heritability, the presence of heterotic effects at the interspecific hybrid and the low response to phenotypic selection from the $F_2$ to the $F_3$, as well as its good response to MAS from the $F_3$ to the $F_4$.

We have found only 3 QTLs for *TW*, each with a small contribution. *TW* must be a very complex trait, with many genetic components (QTLs) showing small and, mostly, non-additive effects. These small individual contributions must be the cause of their difficult detection (Carbonell et al. 1993). Therefore, as stated by Jones (1986) phenotypic selection for this trait does not seem to be the best choice to obtain salt-tolerant lines. For this kind of character the study of its components may be more profitable than the study of the character itself.

Heritability estimates for *FW* and *FN* are high; 4 and 5 QTLs were detected for these traits, respectively. The fact that heritability of *FW* is larger than that for *FN* agrees with a larger contribution of QTLs governing FW than those controlling *FN* (see Table 2).

Marker-assisted selection in the $F_3$ has been very successful for obtaining salt-tolerant breeding lines. The response to this kind of selection has been higher than the response to phenotypic selection from the $F_2$ to $F_3$. This result contrasts with an expected theoretical reduction in the response in a second generation of selection (Falconer 1989). Due to the existence of pleitropic or linked QTLs with opposite effects, MAS had to be directed towards increasing *FN* or increasing *FW*. This kind of disruptive selection may explain the increase in the between-family component of the variance because the QTLs involved were efficiently compartmentalized into the $F_4$ families. Therefore, response to MAS has been larger than to phenotypic selection, especially for *TW* whose heritability was not significant, and has overcome the limitations of using only a sample of the actual QTLs involved and the fact that the estimate of the location was not very precise. Besides, it has enabled us to develop fully or almost fully homozygous lines for all markers with just one MAS generation. The trait means of the $F_4$ families are fully explained by their deduced QTL genotypes. Thus, the best family for *FW* is $F_4$ 2–16 (its genotype at the 4 *FW* QTLs increases this trait mean), and the best one for *FN* is $F_4$ 17–12, whose genotypes at the 5 *FN* QTLs increase this trait mean. Two salt-tolerant breeding lines derived by MAS from these two families (named "Salto" and "Tosal", respectively) have been registered, and their specific combining abilities are being evaluated. The existence of overdominance makes the heterozygous combination EP specially profitable at some QTLs.

When control and saline treatments are compared, it seems that salinity affects mainly fruit weight. Only family $F_4$ 2–3, selected because of its high expression of peptide 2' (Asins et al. 1993) did not change significantly at any of the yield components, including *FW*. Under saline conditions, all of the $F_4$ families except 2–16 had similar *FW*. This agrees with their genotypes at the FW QTLs, given that 2–16 is the only family that has all the "favorable" *FW* alleles Similarly, family 17–12, which shows all the *FN*-increasing alleles, shows the highest value for this trait (except for family 2–3, which was not selected because of its genotype at the QTLs but due to the presence of peptide 2'). These results and those concerning the partitioning of $\sigma_b^2$ and $\sigma_w^2$ for the crop yield traits of the $F_4$ families under control conditions seem to indicate that the QTLs we have analyzed are also working under the control condition. However, two-way ANOVA of treatments and families (genotypes) showed that the selected families responded differently to salinity. Thus, one of the highest yielding families under the control condition (83–29) showed one of the poorest yields under salinity, while one of the medium-yielding families (2–16) under control was our best one under salinity.

In conclusion, the results presented here show the importance of the following three points regarding crop yield improvement under high salinity levels:

1) Fruit weigth is a key trait for this process;

2) Marker-assisted selection has been proven to be very helpful in building up salt-tolerant recombinant inbred lines;

3) Selection under salinity should be more deeply investigated by means of QTL analysis given the presence of genotype × treatment interaction.

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