Theor Appl Genet (1996) 93. 877–886 

Y. Eshed · G. Gera · D. Zamir

# A genome-wide search for wild-species alleles that increase horticultural yield of processing tomatoes

Received: 21 February 1996 / Accepted: 1 March 1996

**Abstract** To identify QTLs associated with horticultural yield it is necessary to conduct replicated plot trials of the tested genotypes. The first step in the utilization of an introgression-line (IL) population of *Lycopersicon pennellii* in a processing-tomato variety (M82) for mapping such QTLs was to screen 51 ILs in a non-replicated plot trial. The results of this survey were compared to those obtained in a replicated trial of the same genotypes grown as single plants at wide spacing. Fruit characteristics were similar between the two stands, but yield was generally different. Eight lines that outperformed the control in the plot survey were subjected to detailed analysis in the following year. The effects of these introgressions, measured on single plants, were reproducible relative to the previous year's results. In a replicated plot trial of these ILs and their hybrids involving two genetic backgrounds, the product of yield and total soluble solids (horticultural yield) in seven of the eight hybrids was 7–13% higher than that of their nearly isogenic controls. The results revealed a consistent trend in the interaction between introgression effects and genetic background. Combining the two introgressions with the largest contribution to horticultural yield in plots resulted in a 20% increase relative to the control in the third year. This research highlights the potential of wild germ plasm for yield improvement and the ability of nearly isogenic populations to achieve this goal.

**Key words** Breeding · QTL · Wild germ plasm · Introgression lines · *Lycopersicon pennellii*

Communicated by F. Salamini

Y. Eshed · D. Zamir (✉)
Department of Field and Vegetable Crops
and The Otto Warburg Center for Biotechnology,
Faculty of Agriculture,
The Hebrew University of Jerusalem,
P.O. Box 12, Rehovot 76100, Israel

G. Gera
Akko Experiment Station, Western Galilee 25212, Israel

## Introduction

Genetic factors underlying traits showing continuous variation have been successfully mapped for a wide range of species, traits, environments and populations (Tanksley 1993). Due to limited resolution power, the QTL identified in a classical segregating population ($F_2$, BC) is actually a chromosome segment carrying one or more genes which have a significant effect on the measured trait (Darvasi et al. 1993). To reduce genetic variation in such studies, it is preferable to perform both genetic and phenotypic analyses on the same plants. This is feasible for characteristics measured in single plants, but complex traits such as horticultural yield have to be measured in plots containing many plants. Two general approaches have been used to circumvent this problem: (1) progenies of plants subjected to genotypic analysis are analyzed (Stuber et al. 1992; Tanksley et al. 1996) or (2) segregating populations of fixed genotypes are used such as doubled haploids (Powell et al. 1992) or recombinant inbreds (Lark et al. 1995; Xiao et al. 1995). However both these strategies still require large-scale trials to enable a meaningful statistical analysis.

In a previous study, we used an introgression-line (IL) population of *Lycopersicon pennellii* in the genetic background of the processing-tomato variety M82 to identify yield-associated QTLs as measured in widely spaced single plants (Eshed and Zamir 1995). The major advantage of this nearly isogenic approach lay in the elimination of genetic variation which was not associated with the specific introgressions. The present study was aimed at identifying the *L. pennellii* chromosome segments contributing to elevated horticultural yield as measured in a replicated plot trial mimicking the common growing practices of the crop. Bearing in mind the practical space limitations for plot trials and those of harvest schedules, we looked for ways to increase the efficiency of the QTL identification process. One possible approach was to measure the same yield-associated traits on single widely spaced plants where each plant constitutes a replicate.

The processing-tomato industry requires varieties that are adapted to a single machine harvest and produce high fruit yield per unit area combined with high levels of total soluble solids (Brix: mainly sugars and organic acids) in the fruit. The use of such varieties renders the manufacturing of concentrates more cost-effective via a reduction in expenses associated with transportation and water evaporation at the processing plant. In tomato, yield (Y) and Brix (B) are inversely related (Stevens and Rick 1986). Horticultural yield in this study was therefore calculated as the product of Y and B (BY), which provides an estimate of the weight of soluble solids produced per unit area. BY is therefore directly related to the amount of processed product (e.g. tomato paste) that can be produced by each genotype.

The underlying genetic assumption in this study was that any difference between an IL and its nearly isogenic control is due to a QTL that resides on the chromosome segment introgressed from the wild species. The following questions were raised during the search for the yield-associated QTLs: (1) Are QTLs identified in single widely spaced plants consistent with those obtained for plants grown in densely spaced plots? (2) Does the difference between the IL and its nearly isogenic control lie solely in the introgressed segment? (3) What is the value of a non-replicated whole-genome plot trial in the identification of outperforming genotypes? (4) Are QTLs for yield-associated traits consistent in different genetic backgrounds and years?, and (5) What is the mode of interaction between two QTLs? Stepwise trials performed over 3 years in two types of field stands and two genetic backgrounds are presented. The results demonstrate that wild germ plasm can be used to modify the inverse relationship between yield and quality, and might serve as a resource for improving cultivated crops with respect to a wide range of traits. The resolving power of the nearly isogenic approach for the identification of agronomically important QTLs should encourage the development of similar resources for other crop plants.

## Materials and methods

### Plant material and field trials

The genetic constitutions of the 51 ILs in the genetic background of the processing-tomato var. M82 are presented in Fig. 1. M82 is the most common inbred processing variety in Israel, producing a small plant with medium-size blocky fruit and relatively low B. The sizes and identities of the introgressed *L. pennellii* (LA 716) segments were determined by RFLP analysis of 375 markers chosen to cover the entire tomato genetic map at minimal intervals. The lines contained on average, introgressions of 33 cM from a total genome size of 1200 cM (Eshed and Zamir 1994a).

The effect of the introgressed segments on the measured traits was determined by comparison of each IL to the nearly isogenic M82. The effect of one dose of *L. pennellii* introgression was determined by comparing the hybrid IL×M82 to M82. To evaluate the ILs in genetic background other than M82 while maintaining their nearly isogenic nature, the homozygous ILs were crossed with another processing tester, A8 (an inbred processing line with larger fruit and higher B than M82). M82×A8 was used as the nearly isogenic control for these IL hybrids.

The different trials conducted in the 3 different years are summarized in Table 1. All experiments were performed in fields at the Western Galilee Experimental Station in Akko, Israel. Cultural practices were according to the recommendations for this region. The plants were grown in a greenhouse for 35–40 days and transplanted in the field at the end of March. For the single-plant trials, lines were planted at a wide spacing (0.5 m between plants and 2 m between rows). For the plot trials, the lines were planted in 10-$m^2$ plots with 35 plants per plot. IL8-1 was not tested as an inbred: it could not be fixed in a homozygous condition due to elimination of the *L. pennellii* male gametes, and its hybrids were selected by the isozyme *Aps-2* prior to planting.

Eight hybrid ILs which outperformed the control with regard to BY (trial B) were chosen for detailed evaluation (trials C and D). Seed production of these eight ILs and their hybrids was manipulated to minimize the effects of possible undetected introgressions in the lines. $F_2$ populations from crosses of each of the eight ILs with M82 were subjected to RFLP analysis with markers flanking the introgression. An average of six segregants that were homozygous for the *L. pennellii* introgression (namely $IL^P$) were selected to represent each of the tested introgressions. Pollen was harvested in bulk from the $IL^P$s and used in crosses with M82 and A8. $F_2$ plants homozygous for the *L. esculentum* alleles (namely $IL^e$) represent true isogenic lines of the different $IL^P$s.

Hybrids of $IL^P1$–4 and $IL^P7$–5 with M82 showed the largest BY increase over the control in the 1994 replicated plot trial (trial D). Therefore, an additional trial using these $IL^P$s was executed in the following year (trial E). The combined effect of these introgressions was determined upon evaluation of the hybrid between the two $IL^P$s; in such a cross, both introgressions are in a heterozygous state. The single-introgression hybrids, along with M82, therefore enabled the measurement of intergenic interaction.

### Measurements

In all experiments, fruits were harvested when 90–100% of the tomatoes were red (red and green fruits were weighed separately to estimate earliness). Total fresh yield per plot or per plant (Y) included both the red and green fruit since a few days' delay in the picking of processing tomatoes allows most of the green fruit to turn red. Experiments were harvested according to the stage of maturity, except in trial A where tomatoes were picked according to the order of planting. Since in the wide-spacing experiments each plant occupied an area of 1 $m^2$, we compared the yields of the two stands on the basis of unit area (1 plant per $m^2$ vs 3.5 plants per $m^2$ in the plots). Mean fruit mass (FM) was determined on a random sampling of 100 fruit per plot, and from all red fruits in the single-plant experiment. Concentrations of total soluble solids (B, measured in degrees Brix) were measured from two random samplings of 40 fruits per plot or from a single sampling of 20 fruits per single plant. Fruits were blended at room temperature and B was measured using an RFM-80 BS digital refractometer. The product of Y and mean B (BY) provided an estimate of the weight of soluble solids produced per 1 $m^2$. The same measurements were taken from plots for 3 consecutive years and from the single plants for 2 years.

### Statistical analysis

Statistical analyses were performed with the JMP V.3.1 software package for Macintosh (SAS institute 1994). Results from the non-replicate survey trials were correlated with the mean values obtained from the replicated trials of widely spaced plants of the same genotypes (Eshed and Zamir 1995). Pairwise comparisons of the mean introgression effect in the two field stands was executed using the "Fit y by x" function and "Paired *t*-test" (Table 2). For the replicated trials, an experiment-wise error of 0.05 was taken as a standard for significant effect. Comparison of the mean values of multiple genotypes to a common control (M82 was the common control for the ILs and their hybrids with M82; M82×A8 was the common control for the IL×A8 hybrids) was performed using the "Fit y by x" function and "Compare with control" with an alpha level of 0.05

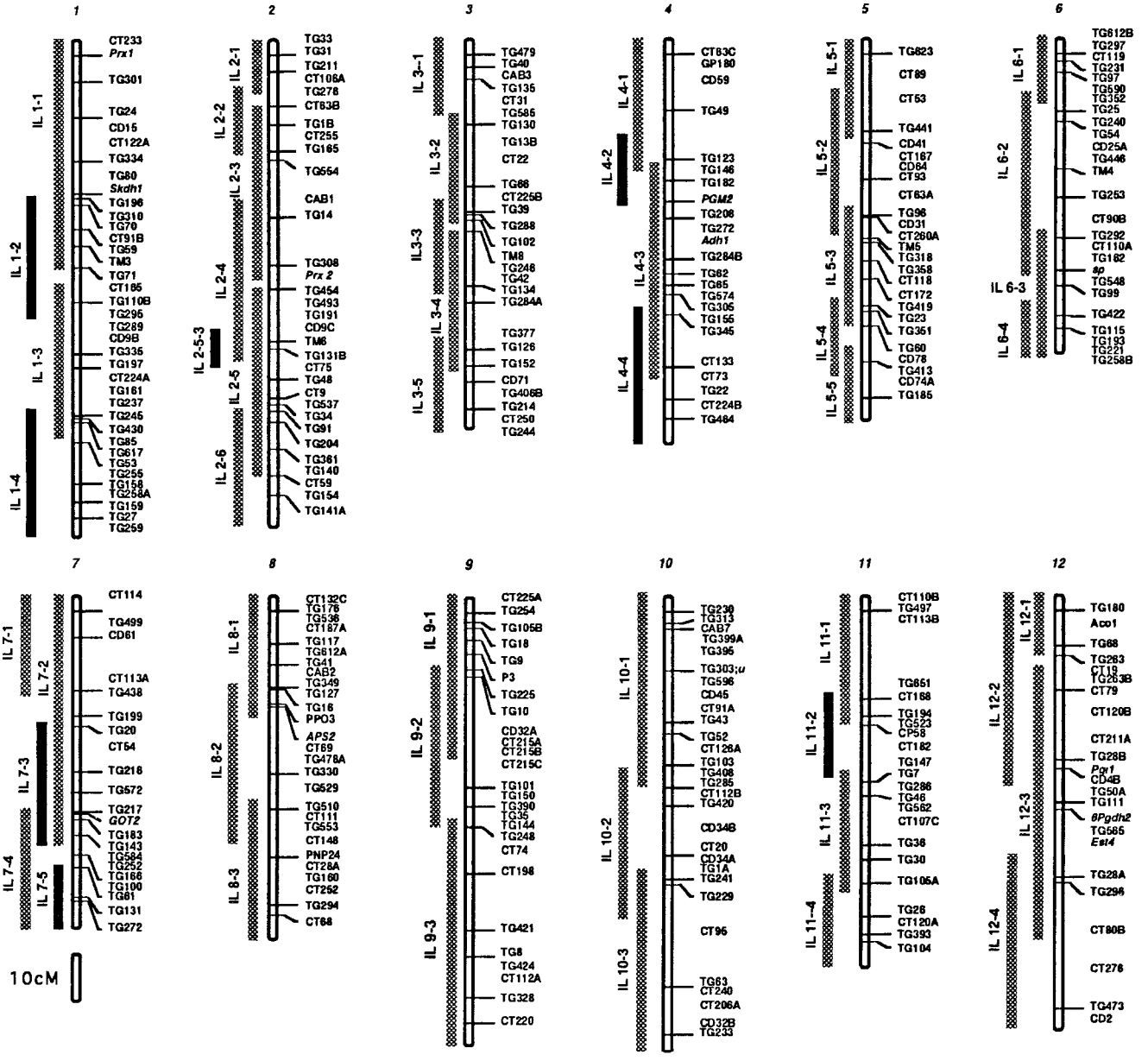



**Fig 1** Chromosomal locations, sizes and identities of the 51 *L. pennellii* ILs. The genetic map was constructed on the basis of 119 $BC_1$ plants as described by Eshed et al. (1992). Mapped markers are connected to the chromosome by a *line* and markers not assayed on the $BC_1$ map are placed at their approximate positions according to Tanksley et al. (1992). Each line was probed with all the markers, and the ones showing wild-species alleles are marked by *bars* to the left of the chromosome. The ILs with *black bars* were tested in the replicated trials C and D

(Dunnet 1955). For multiple tests in the same experiment (e.g interaction with year, stand or genetic background), the significance threshold was $0.05/n$ (where $n$ is the number of independent tests performed in the same experiment). The additive effect (a) of each introgression was half of the difference between the inbred IL and M82, and its significance level was determined by comparison between the IL and M82. The dominance deviation (d) and its significance were calculated by contrasting IL×M82 (+1) with M82 (−0.5) and the appropriate IL (−0.5). The threshold level for significant d values was 0.006, yielding an experiment-wise error of 0.05 for the eight compared genotypes. When multiple interaction tests were executed, the analysis was made by a two-way ANOVA with one factor being the introgression (presence or absence) and the other factor being year, stand or genetic background. For the 1994 single-plant experiments, all IL's were compared to M82 (see Fig. 3).

For the 1995 replicated-plot trial, a multiple range test was conducted using the "Fit y by x" function and "Compare all pairs" with an alpha level of 0.05. Interaction with year was tested against the results of the 1994 plot trial. A test for intergenic interaction was performed for the two independent introgressions using a two-way ANOVA (Fig. 4).

To unify data representation of the different experiments, all results are presented as percent difference ($\Delta\%$) from the nearly isogenic control (M82 or M82×A8).

## Results

### Comparison of the effects of the introgressed segments in individual plants and plots

The entire population, composed of 51 ILs and their hybrids, had been previously evaluated for yield-associated

**Table 1** Technical specifications of introgression lines (ILs) field trials conducted in 3 years, two stands and two genetic backgrounds

| Trial code | Year tested | Genotypes tested | Trial mode | Replicates per genotype | Replicates per control | Experimental design |
|---|---|---|---|---|---|---|
| A[a] | 1993 | 50 ILs self | Plants | 6 | 30 | CRD[b] |
| A | 1993 | 51 ILs×M82 | Plants | 6 | 30 | CRD |
| A | 1993 | 51 ILs×A8 | Plants | 6 | 20 | CRD |
| B | 1993 | 50 ILs self | Plots | 1 | 6 | CRD |
| B | 1993 | 51 ILs×M82 | Plots | 1 | 6 | CRD |
| B | 1993 | 51 ILs×A8 | Plots | 1 | 6 | CRD |
| C | 1994 | 8 IL$^e$s self | Plants | 18 | 18 | CRD |
| C | 1994 | 8 IL$^p$s self | Plants | 9 | 18 | CRD |
| C | 1994 | 8 IL$^p$s×M82 | Plants | 9 | 18 | CRD |
| D | 1994 | 8 IL$^p$s self | Plots | 6 | 9 | RBD[c] |
| D | 1994 | 8 IL$^p$s×M82 | Plots | 8 | 9 | RBD |
| D | 1994 | 8 IL$^p$s×A8 | Plots | 11 | 12 | RBD |
| E | 1995 | 2 IL$^p$s×M82 | Plots | 16 | 20 | RBD |

[a] The results of trial A were described in detail by Eshed and Zamir (1995)
[b] CRD – completely randomized design
[c] RBD – randomized block design

**Table 2** The effect of field stand on the mean introgression effects. Mean values and standard deviations for M82 and for "all genotypes" are given in the measured scale, while other values are given in percent difference from M82, except for ILs×A8 which are presented in percent difference from M82×A8 Pairwise comparisons were made between the same genotypes in the two field stands, and underlined values represent significant differences at $P<0.05$. Correlation coefficients were calculated for the same genotypes in the two field stands. *** denote a significant $r^2$ at $P<0.001$

| Trial code | Genotypes tested | Replicated unit | Fruit mass (FM) g | | Total soluble solids (B) °Brix | | Yield (Y) Kg/m$^2$ | | TSS×yield (BY) g/m$^2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | $r^2$ | Mean | $r^2$ | Mean | $r^2$ | Mean | $r^2$ |
| A | M82 | Plant | 58.2±7.1 | | 4.3±0.2 | | 8.2±1 7 | | 361±82 | |
| B | M82 | Plot | 55.9±4.5 | | 4.2±0.3 | | 13.1±0 7 | | 549±23 | |
| A | M82×A8 | Plant | 11±15 | | 9±7 | | 5±18 | | 10±18 | |
| B | M82×A8 | Plot | 7±10 | | 6±7 | | −8±6 | | −4±8 | |
| A | 50 ILs self | Plant | −13±19 | 0.64*** | 14±11 | 0.67*** | −13±31 | 0.56*** | −2±35 | 0.52*** |
| B | 50 ILs self | Plot | −18±17 | | 6±14 | | −25±25 | | −21±26 | |
| A | 51 ILs×M82 | Plant | −4±11 | 0.31*** | 11±9 | 0 63*** | 8±17 | 0.01 | 18±22 | 0 |
| B | 51 ILs×M82 | Plot | −10±14 | | 4±10 | | −4±13 | | −1±12 | |
| A | 51 ILs×A8 | Plant | −6±11 | 0.39*** | 6±8 | 0 50*** | 8±18 | 0.03 | 17±25 | 0 |
| B | 51 ILs×A8 | Plot | −11±11 | | 3±8 | | 1±10 | | 5±10 | |
| A | All genotypes | Plant | 55.9±9.0 | 0.45*** | 4.9±0.4 | 0.61*** | 8.4±2.1 | 0.29*** | 413±111 | 0.29*** |
| B | All genotypes | Plot | 50.0±8.5 | | 4.5±0.5 | | 11.6±2.5 | | 510±107 | |

traits in a field trial where individual plants were the replicated unit (trial A). To conduct a similar experiment using plots as the replicated unit was unrealistic. We therefore determined the relationship between results obtained for the same genotypes on the basis of replicated individual plants and a single plot.

A single 10-m$^2$ plot was evaluated for each IL and its hybrids (trial B). The results of the non-replicated surveys are presented as the frequency distribution of the performance of the ILs and their hybrids relative to their nearly isogenic controls (Fig. 2). The measured values of the IL hybrids in the two genetic backgrounds (IL×M82 and IL×A8) were pooled, since relatively high correlations were obtained between the two (Table 3). The ILs and their hybrids generally exhibited smaller fruit with higher B values relative to the control. Fifteen inbred ILs showed a 30–90% reduction in fresh yield and only three exhibited yield equal to, or slightly higher than that of the control. In contrast, only two hybrid ILs were substantially inferior and 26 were equal to, or higher than the control. These results demonstrate that most of the unfavorable alleles for yield originating from *L. pennellii* are recessive.

To evaluate the effect of the two types of planting stands, we compared the mean values for the measured traits in plants and plots by summing up all the genotypes tested in the two experiments (see Table 2). For all traits, the effect of the replicated unit was highly significant: in plots, the fruits were smaller than in individual plants, with lower B, and higher Y and BY. To compare the two stands we also correlated the pooled results of all genotypes from the wide and dense spacings; highly significant correlation coefficients were observed for all measured traits. For FM and

**Table 3** The effect of genetic background on the mean introgression effects. Mean values and standard deviations for M82 and for M82×A8 are given in the measured scale, while other values are given in percent difference from the nearly isogenic control. Pairwise comparisons were made between the same genotypes in the two genetic backgrounds, and underlined values represent significant differences at $P<0.05$. Correlation coefficients were calculated for the same ILs in the two genetic backgrounds. *, **, *** represent a significant $r^2$ at $P<0.05$, $P<0.01$ and $P<0.01$ respectively

| Trial code | Genotypes tested | Replicated unit | Fruit mass (FM) g | | Total soluble solids (B) °Brix | | Yield (Y) kg/m$^2$ | | TSS×yield (BY) g/m$^2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | $r^2$ | Mean | $r^2$ | Mean | $r^2$ | Mean | $r^2$ |
| A | M82 | Plant | 58.2±7.1 | | 4.3±0.2 | | 8.2±1.7 | | 361±82 | |
| A | M82×A8 | Plant | 64.7±8.1 | | 4.7±0.3 | | 8.6±1.5 | | 396±66 | |
| A | 51 ILs×M82 | Plant | −4±11 | 0.75*** | 11±9 | 0.78*** | 8±17 | 0.62*** | 18±22 | 0.75*** |
| A | 51 ILs×A8 | Plant | −6±11 | | 6±8 | | 8±18 | | 17±25 | |
| B | M82 | Plot | 55.9±4.5 | | 4.2±0.3 | | 13.1±0.7 | | 549±23 | |
| B | M82×A8 | Plot | 59.6±5.6 | | 4.4±0.3 | | 12.1±0.8 | | 529±44 | |
| B | 51 ILs×M82 | Plot | −10±14 | 0.27*** | 4±10 | 0.42*** | −4±13 | 0.28*** | −1±12 | 0.18** |
| B | 51 ILs×A8 | Plot | −11±11 | | 3±8 | | 1±10 | | 5±10 | |
| D | M82 | Plot | 55.2±4.5 | | 4.4±0.2 | | 13.8±0.6 | | 603±32 | |
| D | M82×A8 | Plot | 62.7±6.5 | | 4.8±0.2 | | 12.1±0.9 | | 574±59 | |
| D | 8 IL$^P$s×M82 | Plot | −8±8 | 0.55* | 7±6 | 0.52* | 1±8 | 0.46 | 8±6 | 0.30 |
| D | 8 IL$^P$s×A8 | Plot | −9±6 | | 4±4 | | 6±4 | | 11±2 | |

B the correlation coefficients were highly significant both for the inbred ILs and their hybrids. However, for Y and BY, high correlations were found only for the inbred ILs and not for their hybrids. The results for the inbred ILs can be attributed to 15 lines which were almost sterile in both stands. When these lines were eliminated from the analysis, the correlations were close to zero.

## Verification of the effects of the introgressed segments

On the basis of the non-replicated survey in plots (Fig. 2), eight ILs whose hybrids outperformed the controls for BY in both genetic backgrounds were selected for detailed analysis. To verify the effect of the selected chromosome segments on the measured traits, we produced a set of true isogenic lines for these ILs. If the difference between any IL or its hybrid from their nearly isogenic control lies solely in the introgressed *L. pennellii* region, then a line derived from an $F_2$ of the IL crossed to M82 and selected against the introgressed region should be identical to M82. We therefore planted the $F_2$ progeny of the eight selected ILs which contained the *L. esculentum* segment (trial C). These genotypes were evaluated as individual plants; none of the true isogenic *L. esculentum* lines (IL$^e$s) was significantly different from M82 for any of the measured parameters, whereas all *L. pennellii* ILs (IL$^P$s) were significantly different from the control with respect to at least one trait (Fig. 3).

These eight IL$^P$s had been tested previously for the same traits in the same stand (trial A). As a result, genotype-by-year interactions could be estimated. For the four traits, 18 QTLs were identified in these eight lines in 1993. All these QTLs were re-identified in the present trial with the addition of QTLs for FM and Y in IL4-4. The magnitude of the effects were not always the same between the 2 years, and of the 60 interaction tests for genotype-by-year eight were significant (five interactions involved the inbred or hybrid of IL4-4). This experiment confirmed that the observed effects, which were generally consistent over the years, were associated with the *L. pennellii* introgressed segments.

## Field performance in replicated plots of the selected ILs and their hybrids

The effects of the eight introgressions on the measured traits in plots were determined for the homozygous IL$^P$s and their hybrids with M82 (trial D). A significant QTL was identified by comparing the IL$^P$s, or their hybrids with M82, to the nearly isogenic M82. Five QTLs with a significant additive effect (a) for reduced FM were identified (Fig. 3). For B, four IL$^P$s had a significant increasing additive effect, and a significant dominance deviation was found only for IL$^P$7–3 where the *L. pennellii* allele for higher B was dominant. Four inbred IL$^P$s exhibited reduced yield and only IL$^P$7–5×M82 yielded significantly more than the control. The dominance deviation for Y was significant for five of the IL$^P$s, where in all cases the hybrid was higher than the mid-value of its parents. These results are in agreement with the general trend observed in the survey experiment of the entire IL population; namely, lower yield for the inbred ILs than for the hybrids (Fig. 2). For BY, three inbred lines were inferior to the control and one, IL$^P$7–5, was higher. Here too, dominance deviation was significant for five of the ILs. The advantage over the control detected for the eight selected hybrid IL$^P$s in the non-replicated survey trial ranged from 14 to 22% for BY values. When evaluated in the replicated trial in the following year, seven of the eight hybrid

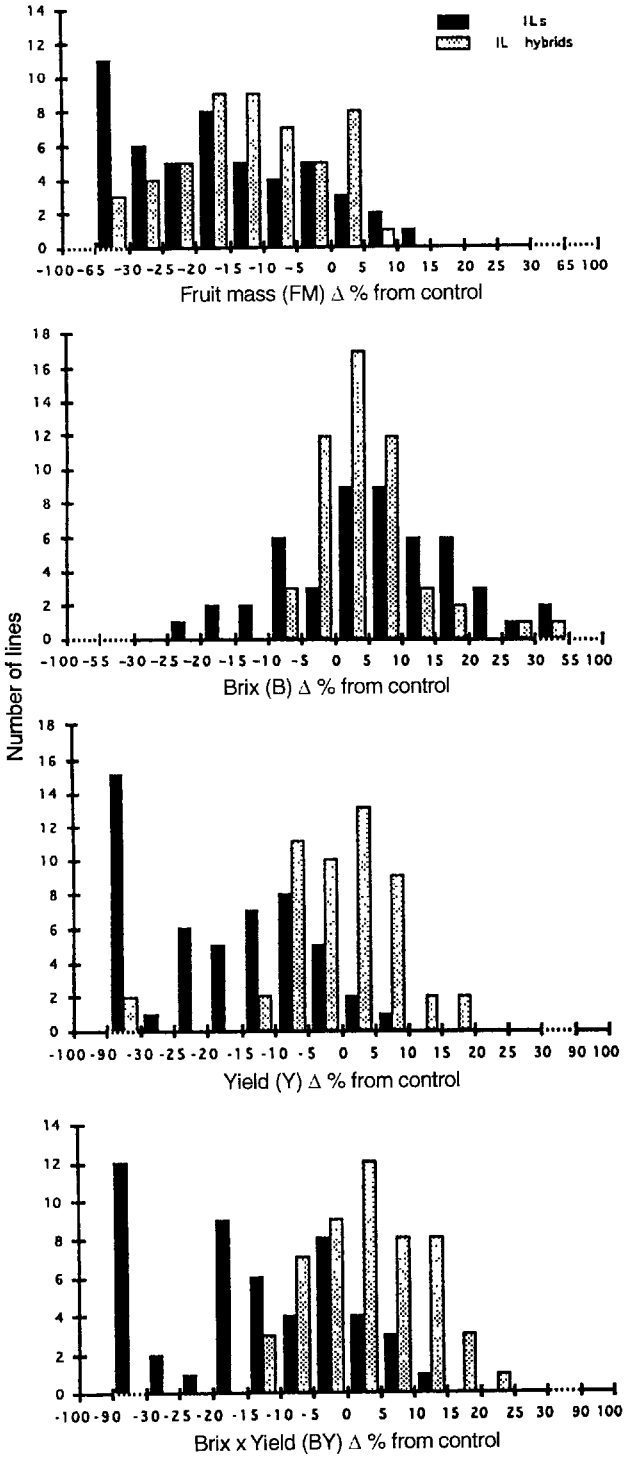



**Fig. 2** Frequency distribution of the relative performance of ILs and their hybrids as measured on non-replicated 10-$m^2$ plots [trial B; expressed in percent difference ($\Delta$%) of control]. The means and standard deviations of the IL population and the controls are presented in Table 2

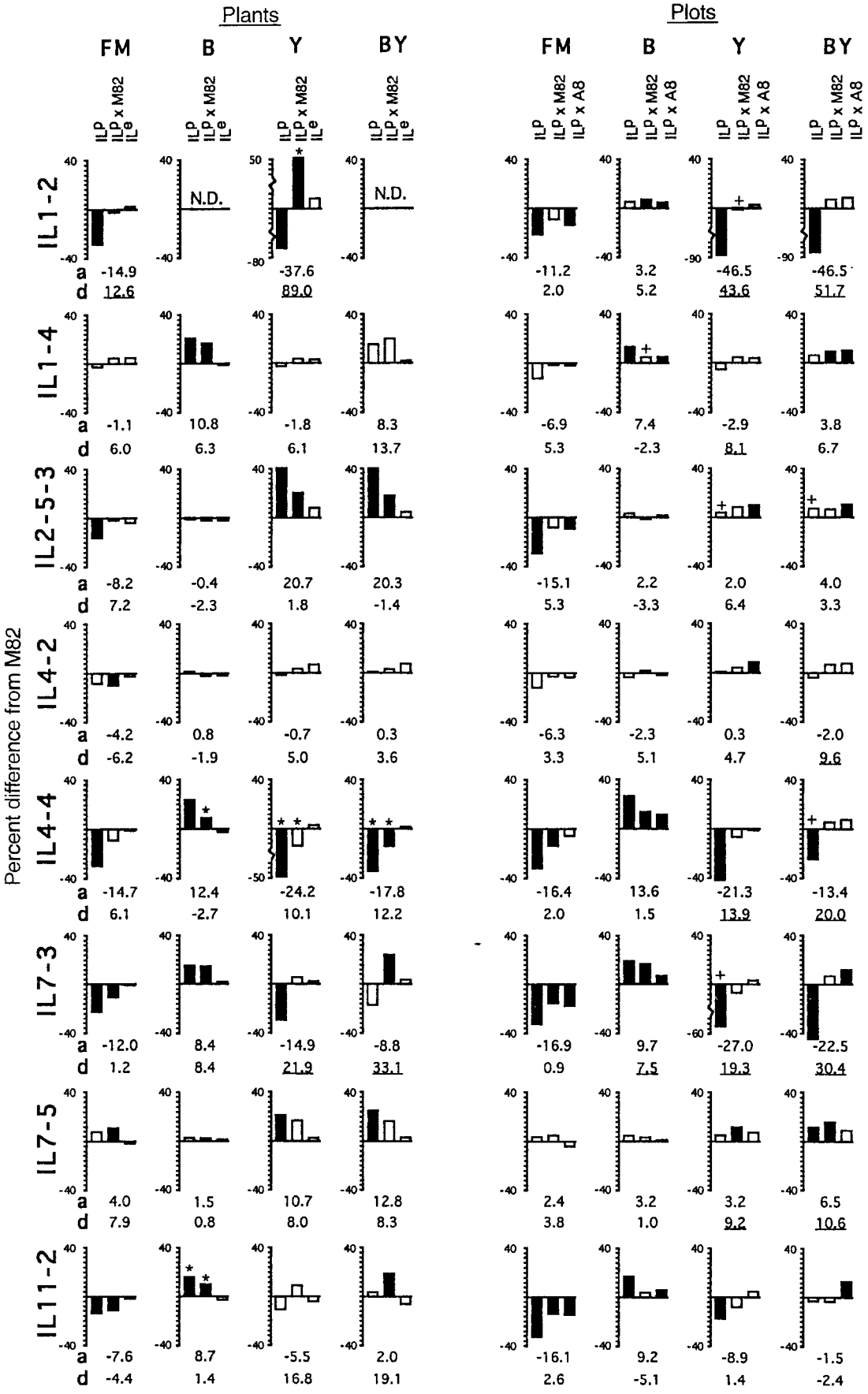
IL$^P$s outperformed the control for BY by 7–17%. Only the hybrids of IL$^P$7–5 and IL$^P$1–4 were significantly higher than the control (using an experiment-wise error of 0.05), but all the other hybrids except for IL$^P$11–2×M82 were close to being significantly different.

The five IL$^P$s exhibiting a significant effect on FM in plots were also effective in the wide-spacing trial (Fig. 3). Only IL$^P$7–5 had significantly larger fruits in the wide spacing and a non-significant effect in plots. For B, the same inbred IL$^P$s were significant in both trials, and the hybrids of IL$^P$1–4 and IL$^P$11–2 with M82 were significant with respect to wide spacing only. One significant interaction between the introgression effect and the planting stand was found for B (IL$^P$1–4×M82), and none was found for FM. Unlike fruit characteristics, Y displayed greater differences between the two stands and three significant interactions were detected. The inbred IL$^P$s which exhibited a poor Y in plots showed the same in wide spacing, but a high Y in wide spacing was not reflected in plots. Significant elevation in Y in the wide spacing ranged from 21 to 50%, whereas the same genotypes had no effect in plots. The consistency between the stands for BY was intermediate between its components. Genotypes with low BY values performed similarly but only some of the lines with high BY in single plants were high in plots, and vice versa. Significant interactions for BY between genotype and stand were detected for two IL$^P$s.

## Introgression-effect consistency between genetic backgrounds

To evaluate the effects of the selected introgressed segments in a different genetic background, we crossed the IL$^P$s to the inbred tomato line, A8. The nearly isogenic control for

**Fig. 3** The effects of selected *L. pennellii* introgressions on FM (fruit mass), *B* (total soluble solids measured in °Brix), *Y* (yield) and *BY* (the product of B and Y – horticultural yield) as measured in individual plants (trial C) and plots (trial D). The ILs homozygous for the *L. pennellii* introgression (*IL$^P$*) and their hybrids with M82 were measured at the two planting densities, whereas the true isogenic lines homozygous for the *L. esculentum* chromosome segments (*IL$^e$s*) were assayed only at the wide spacing. The hybrids of IL$^P$s with the tester A8 were assayed only in plots. For each trait, the *left bar* represents the relative performance of the IL$^P$s and the *central bar* shows the effects of the IL$^P$ M82 hybrids. *IL$^P$* represents a bulk of $F_2$ progenies of these ILs selected for the homozygous *L. pennellii* chromosome segment. The *bar on the right* represents the relative performance of the IL$^e$s in the wide-spacing trial and the IL$^P$s×A8 hybrids in the plot trial. *IL$^e$* represents a bulk of the same $F_2$ that was selected against the *L. pennellii* chromosome segment. All genotypes were compared with M82 except for the IL$^P$s×A8, which were compared with the M82×A8 hybrid. The values are presented as percent difference from the nearly isogenic control; *black bars* indicate a significant difference at $P<0.05$ and *empty bars* indicate non-significant differences. The following components of genetic variability for each IL×trait are presented as a percent of the control (M82): the additive effect (*a*) is half of the difference between IL$^P$ and M82, and its significance was calculated on the basis of the comparison between them. The dominance deviation (*d*) is the difference between IL$^P$×M82 and the mid-value of its parents. Significant d values at $P<0.05$ are *underlined*. The mean values and standard deviation for the controls in plots are presented in Table 3 and the values for the control M82 in the single-plant experiment were as follows. FM 66.8±5.8, B 4.16±0.2, Y 10.50±1.15 and BY 437±56. * denotes a significant interaction with year (as compared to the 1993 trial: Eshed and Zamir 1995). + denotes a significant interaction with the field stand


Plants
Plots
FM
B
Y
BY
ILP
ILP x M82
ILP x A8
ILe
Percent difference from M82
IL1-2
IL1-4
IL2-5-3
IL4-2
IL4-4
IL7-3
IL7-5
IL11-2
N.D.
a -14.9 -37.6 -11.2 3.2 -46.5 -46.5
d 12.6 89.0 2.0 5.2 43.6 51.7
a -1.1 10.8 -1.8 8.3 -6.9 7.4 -2.9 3.8
d 6.0 6.3 6.1 13.7 5.3 -2.3 8.1 6.7
a -8.2 -0.4 20.7 20.3 -15.1 2.2 2.0 4.0
d 7.2 -2.3 1.8 -1.4 5.3 -3.3 6.4 3.3
a -4.2 0.8 -0.7 0.3 -6.3 -2.3 0.3 -2.0
d -6.2 -1.9 5.0 3.6 3.3 5.1 4.7 9.6
a -14.7 12.4 -24.2 -17.8 -16.4 13.6 -21.3 -13.4
d 6.1 -2.7 10.1 12.2 2.0 1.5 13.9 20.0
a -12.0 8.4 -14.9 -8.8 -16.9 9.7 -27.0 -22.5
d 1.2 8.4 21.9 33.1 0.9 7.5 19.3 30.4
a 4.0 1.5 10.7 12.8 2.4 3.2 3.2 6.5
d 7.9 0.8 8.0 8.3 3.8 1.0 9.2 10.6
a -7.6 8.7 -5.5 2.0 -16.1 9.2 -8.9 -1.5
d -4.4 1.4 16.8 19.1 2.6 -5.1 1.4 -2.4

this comparison was M82 crossed to A8. The control hybrid was significantly different from M82 with a larger FM, a higher B and a lower Y (Table 3, trial D). Among the eight $IL^P$ hybrids tested, four had a significant reducing effect on FM, six caused an elevated B, two had a higher Y and four had higher BY values (Fig. 3). No significant interaction between an introgressed segment and genetic background (M82 and M82×A8) was detected for any of the measured traits. After pooling the data from both genetic backgrounds, all the tested hybrid $IL^P$s except $IL^P11–2$ had significantly higher BY values than the control (7–13% increase). The lack of significant interaction between the wild-species chromosome segments and genetic background had been previously reported for the entire IL population tested under wide spacing (Eshed and Zamir 1995). These results demonstrate the broad potential of novel genetic variation introduced from exotic germ plasm.

## Magnitude of the mean introgression effects in the different genetic backgrounds

In the previous section we describe no significant interaction for individual introgressions with genetic background. In this section we approach the question of genetic background effect in a different manner, by comparing the general means in the two genetic backgrounds over all introgressions for each of the evaluated traits. Such an analysis is meaningful since the heterozygous *L. pennellii* introgressions generally had a common effect of reducing FM, increasing B and increasing BY. Using a pairwise comparison, we ran the analysis for the experiment conducted on replicated plants. In this experiment (Table 3, trial A), M82×A8 had significantly larger FM and higher B than M82, with no difference in Y and BY. Whereas the correlation coefficients between the performance of the introgressions in the two genetic backgrounds were very high, significant differences in the general means of FM and B were detected. The mean FM of the IL hybrids with M82 was reduced by 4% (as compared to M82) and the mean FM of the hybrids with A8 was reduced by 6% (as compared to M82×A8). This difference in the average effects of the introgressions corresponded to the difference between the mean values of the control genotypes. The larger fruit of M82×A8 was reduced more than that of M82. A similar pattern was found for B where the introgressions had a larger effect in the M82 background (low B) than in that of M82×A8 (high B). For Y and BY, the background effects were observed in the plots (trials B and D) where the higher-yielding background of M82 showed less BY increase than the lower-yielding background of M82×A8. These results indicate that the magnitude of the effects of novel genetic variation originating from the wild species is influenced by genetic background. The lack of significant interactions described in the previous section can be attributed to the small number of replications analyzed for each individual introgression, to the threshold level determined for multiple tests, and to the relatively small difference in the mean values of the two control genotypes.

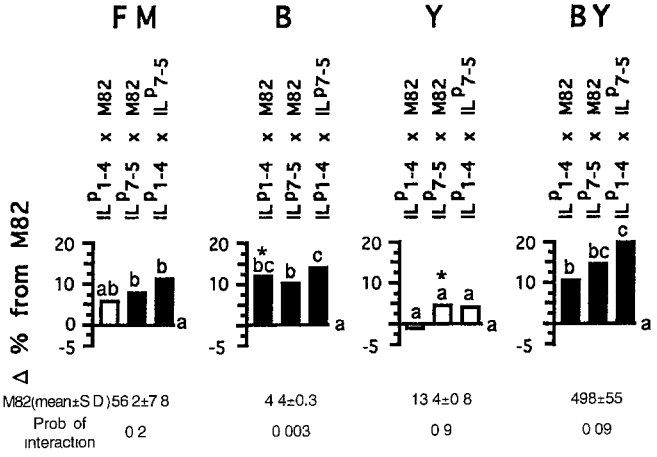



**Fig. 4** The single and combined effects of heterozygous *L. pennellii* introgressions of $IL^P1–4$ and $IL^P7–5$ (trial E). All genotypes were compared to each other and different *letters above the bars* denote significant differences at $P<0.05$. The values are presented as a percent difference ($\Delta\%$) from M82 (ranked *a* in the multiple comparison tests); *black bars* indicate a significant difference from M82 and *empty bars* indicate non-significant differences. * denotes a significant interaction with year (as compared to trial D). A test for genetic interaction between the two introgressions was evaluated as described in M and M and *P* values for this interaction are presented

## The combined effect of two introgressions on yield in plots

Hybrids of M82 with $IL^P1–4$ and $IL^P7–5$ were the only ones showing significantly increased values of BY in plots (Fig. 3); hybrids of IL1-4 have been previously shown to increase horticultural yield in plots in three genetic backgrounds, in two different years (Eshed and Zamir 1994b). To test whether the effects of the two introgressions are additive, we re-evaluated the $IL^P1–4$×M82 and $IL^P7–5$× M82 hybrids and compared the results to the hybrid of $IL^P7–5$×$IL^P1–4$ (trial E). For FM, no interaction was detected for the individual introgressions between the 2 years, although a significant increasing effect of $IL^P7–5$ was detected in 1995 and not in 1994 (Fig. 4). For B, both introgressions had significant increasing effects relative to M82, and for $IL^P1–4$×M82 a significant interaction with year was detected. For Y, neither hybrid showed a significant effect, and $IL^P7–5$×M82 showed a significant interaction with year. For BY, both hybrids had a significant increasing effect, in a manner similar to that of the 1994 experiment. Since Y and B have a negative relationship in tomato, the combined parameter (BY) compensated for the environmental fluctuations in its components. In the 1995 experiment, the effects of the introgressions on Y were of a smaller magnitude as compared to 1994 but their effects on B were higher. The effects on BY were therefore almost identical for the 2 years.

The mean values obtained for the double heterozygous hybrid were not significantly different from the single introgression with the largest effect on the measured traits; however, its values were highest for FM, B and BY (Fig. 4). A significant interaction between the individual introgres-

sions was detected for B, in which the double heterozygotes exhibited significantly lower values than those stemming from the additive effect of the individual introgressions. For BY, the double heterozygous hybrid had 20% higher values than the commercial variety M82.

## Discussion

The preferred approach for the identification and fine mapping of QTLs consists of developing nearly isogenic lines which differ in the traits of interest (Tanksley 1993). We developed an IL population of *L. pennellii* in *L. esculentum* var. M82 in which each line carries a single RFLP-defined wild-species chromosome segment while between lines the exotic genome is completely represented. The problem addressed in this paper is how to screen this population efficiently for QTLs associated with increased horticultural yield, which in processing tomatoes is represented by the total soluble solids output per unit area (BY).

The first stage of this study dealt with the consistency of results obtained for the same genotypes from analyses of individual widely spaced plants and plots. A comparable identification of QTLs for FM and B was achieved in the two field stands (Table 2, Fig. 3), validating the more efficient use of individual plants for an analysis of fruit characteristics. However, for Y and BY the results of single plants and plots differed, indicating the necessity of evaluating the material under conditions of commercial crop production, i.e. in plots. These findings are in agreement with numerous other studies in different crops, where growth practices and especially planting densities were associated with variations in yield. These differences are most commonly related to a variation in competition between plants (Jensen 1988).

In the development of nearly isogenic lines for QTLs, additional undetected chromosome segments may differ between the lines and the measured effects may be associated with these segments. To confirm the effects of eight introgressions, we analyzed plants of isogenic lines derived from an $F_2$ generation and selected using RFLPs for and against the *L. pennellii* introgression. The results obtained for the eight *L. esculentum* homozygous lines showed their high similarity to the recurrent parent M82 (Fig. 3), confirming the nearly isogenic nature of the ILs. The effects of the *L. pennellii* introgressions of these lines were consistent with the results obtained in an earlier experiment indicating little genotype-by-year interaction. Verification of the effect of a QTL through the analysis of truly nearly isogenic lines is crucial for any long-term study of that QTL (Paterson et al. 1990; Doebley et al. 1995).

On the basis of the non-replicated plot survey, we selected eight IL hybrids with the highest BY values. Although the Y of these hybrids was not supported by the replicated single-plant experiment, their higher B values were generally consistent with those of the single-plant experiment. When evaluated in the replicated trial, seven of the eight hybrid ILs (in the two genetic backgrounds combined) outperformed their nearly isogenic control for BY by 7–13%. Unlike fruit characteristics, the inheritance of which was generally additive, dominance deviation for Y and BY was the predominant mode of gene action in both wide and dense spacings (Fig. 3). Dominance deviation is the primary reason for heterotic effects. In the entire IL population grown at wide spacing, five ILs showed overdominance for BY over the highest parent (Eshed and Zamir 1995). In the present study, no significant heterosis over the highest parent was detected for the most productive ILs in plots. These results are in agreement with previous studies in which heterosis was profoundly affected by spacing (Severson and Rasmusson 1968) and cultural practices (Griffing 1990).

A major question concerning a QTL is whether it is effective in different genetic backgrounds. Upon analysis of the IL hybrids with a different inbred (A8), we did not identify an interaction with genetic background. The statistical procedure for the test relied on a null hypothesis of no interaction. Since the analyzed traits generally exhibit large environmental variation, only large interaction effects (having a magnitude in the order of the main effect) would have been detected as significant. This limitation may explain the lack of identification of specific interactions between QTLs and genetic background. The same statistical rationale might explain the lack of QTL-by-environment interaction in some other recent studies (Stuber et al. 1992).

An alternative approach was used to assay for interaction with genetic background by comparing the mean effect of all the introgressions in the two genetic backgrounds. This analysis suggests a trend for QTL-by-genotype interaction where the mean introgression effect depends on the phenotypic value of the genetic background. The effects of the *L. pennellii* alleles on reducing FM are more pronounced in the larger-fruited genetic background, while their effects of increasing B are of higher magnitude in the low B background. The dependence of the introgression effects on the genetic background is also exemplified by the interaction of the two introgressions for increased B (Fig. 4). The hybrids of IL1-4 and IL7-5 in the M82 background increased B by 12% and 10%, respectively. The hybrid containing both introgressions increased B by only 14%, which is significantly less than expected based on the assumption of additivity of QTL effects. Such a pattern of less-than-additive epistatic interactions of QTLs has been recently shown for a number of IL combinations in wide-spacing trials (Eshed and Zamir 1996). These results suggest that the increasing effect of a QTL will be diminished in a genetic background which has a higher mean value.

The results of the first screening of the inbred ILs clearly demonstrate that selection for one component of horticultural yield – fruit B – may be associated with a marked reduction in the other component, i.e. fruit Y. The inverse relationship between B and Y can also be inferred from a recent study in which the production of tomatoes with high B was achieved through molecular modification of the level of the plant-growth regulator, cytokinin (Martineau et al. 1995). In all cases, Y of the transgenic plants expressing the hormone was lower than that of the control. The

B increase in the transgenic plants did not compensate for the reduction in fruit yield, resulting in a higher BY in the control than in the transformed plants. The use of the combined parameter (BY) in our study allowed us to select for ILs that were not extreme for either parameter but were significantly higher than the control in terms of horticultural yield.

The use of wild germ plasm to increase B in tomato was elegantly demonstrated by Rick (1974). The genome-wide survey presented in the present study facilitated mapping of the QTL that "break" the inverse relationship between Y and B. This information can be used in marker-assisted breeding programs aimed at transferring the identified chromosome segments to various genetic backgrounds. The availability of nearly isogenic lines which differ with respect to a single quantitative trait allows us to further recombine the introgressed segments and to achieve finer mapping of the QTLs (Eshed and Zamir 1995). In recombined lines, linkages between favored and undesirable alleles can be broken, thus possibly allowing the introgressions to be used in the development of non-hybrid varieties. Furthermore, by combining a number of QTLs on different chromosome segments, the performance of the novel germ plasm could be further enhanced (Fig. 4).

In addition to Y and B, which were the main focus of this study, we identified *L. pennellii* alleles that contribute to firmer fruit, better internal color, a higher level of carotenoids and vitamins, earlier setting and less stem retention. In all of these cases, the *L. pennellii* alleles acted in a manner contrary to that expected from the performance of the parents. This mode of transgressive variation has been reported for a wide range of morphological seedling characteristics in tomato (De Vicente and Tanksley 1993) and is extended here to traits of agronomic importance.

In this study we describe a nearly isogenic population structure which facilitates the utilization of wild germ plasm by providing the means for genome-wide screening of QTLs associated with agricultural yield. An important advantage of the IL population is that the genetic analysis is performed on an elite genetic background that can be used directly for variety development.

**Acknowledgements** We thank T. Pleban, H. van Oss, T. Bloch, M. Emanuel, A. Eizenband and A. Nator for their technical assistance and E. Fridman for valuable comments. This research was supported by Grant no. US-2427-94 from BARD, The United States-Israel Binational Agricultural Research and Development Fund.

## References

Darvasi A, Weinreb A, Minke V, Weller JI, Soller M (1993) Detecting marker – QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134:943–951

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

Doebley J, Stec A, Gustus C (1995) *Teosinte branched 1* and the origin of maize: evidence for epistasis and the evolution of dominance. Genetics 141:333–346

Dunnet CW (1955) A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 50:1096–1121

Eshed Y, Zamir D (1994a) Genomic library of *Lycopersicon pennellii* in *L. esculentum*: a tool for fine mapping of genes. Euphytica 79:175–179

Eshed Y, Zamir D (1994b) Introgressions from *Lycopersicon pennellii* can improve the soluble solids yield of tomato hybrids. Theor Appl Genet 88:891–897

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTLs. Genetics 141:1147–1162

Eshed Y, Zamir D (1996) Less than additive epistatic interactions of quantitative trait loci in tomato. Genetics (in press)

Eshed Y, Abu-Abied M, Saranga Y, Zamir D (1992) *Lycopersicon esculentum* lines containing small overlapping introgressions from *L. pennellii*. Theor Appl Genet 83:1027–1034

Griffing B (1990) Use of a controlled nutrient experiment to test the heterosis hypothesis. Genetics 126:753–767

Jensen NF (1988) Methods shaped by competitive forces. In: Plant breeding methodology. A Wiley Interscience Publication, New York, pp 63–104

Lark GK, Chase K, Adler F, Mansur LM, Orf JH (1995) Interaction between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proc Natl Acad Sci USA 92:4656–4660

Martineau B, Summerfelt KR, Adams DF, DeVerna JW (1995) Production of high-solids tomatoes through molecular modification of levels of the plant growth regulator cytokinin. Bio/Technology 13:250–254

Paterson AH, DeVerna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecific cross of tomato. Genetics 124:735–742

Powell W, Thomas WTB, Thompson DM, Swanston JS, Waugh R (1992) Association between rDNA alleles and quantitative traits in doubled-haploid populations of barley. Genetics 130.187–194

Rick CM (1974) High soluble-solids content in large-fruited tomato lines derived from a wild green-fruited species Hilgardia 42.493–510

Sas Institute (1994) JMP Statistics and graphics guide: version 3. SAS Institute Inc., Cary, North Carolina

Severson DA, Rasmusson DC (1968) Performance of barley hybrids at four seeding rates. Crop Sci 8:339–341

Stevens MA, Rick CM (1986) Genetics and breeding. In: The tomato crop, a scientific basis for improvement. Atherton JG, Rudich J (eds), Chapman and Hall, New York, pp35-109

Stuber CW, Lincoln SE, Wolff DE, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Tanksley SD (1993) Mapping polygenes Annu Rev Genet 27:205–233

Tanksley SD, Ganal MW, Prince JC, de Vicente MC, Bonierabale MW, Broun P, Fulton TM, Giovanonni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes: biological inferences and practical applications. Genetics 132:1141–1160

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

Xiao J, Li J, Yuan L, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140:745–754