Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato

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Manuscript received November 30, 1995
Accepted for publication May 13, 1996

ABSTRACT

Epistasis plays a role in determining the phenotype, yet quantitative trait loci (QTL) mapping has uncovered little evidence for it. To address this apparent contradiction, we analyzed interactions between individual *Lycopersicon pennellii* chromosome segments introgressed into an otherwise homogeneous genetic background of *L. esculentum* (cv. M82). Ten different homozygous introgression lines, each containing from 4 to 58 cM of introgressed DNA, were crossed in a half diallele scheme. The 45 derived double heterozygotes were evaluated in the field for four yield-associated traits, along with the 10 single heterozygotes and M82. Of 180 (45×4) tested interactions, 28% were epistatic ($P < 0.05$) on both linear and geometric scales. The detected epistasis was predominately less-than-additive, *i.e.*, the effect of the double heterozygotes was smaller than the sum of the effects of the corresponding single heterozygotes. Epistasis was also found for homozygous linked QTL affecting fruit mass and total soluble solids. Although the frequency of epistasis was high, additivity was the major component in the interaction of pairs of QTL. We propose that the diminishing additivity of QTL effects is amplified when more loci are involved; this mode of epistasis may be an important factor in phenotype canalization and in breeding.

THE phenotype of an organism results from the combined action of numerous Mendelian genes affecting qualitative and quantitative traits. Epistatic interactions of major genes have often been inferred from modified segregation ratios. For genes affecting quantitative traits, epistasis has been defined as the deviation from the sum of the independent effects of the individual genes (FALCONER 1989). Epistasis between quantitative trait loci (QTL) assayed in populations segregating for an entire genome has been found at a frequency close to that expected by chance alone (EDWARDS *et al.* 1987; DOEBLEY and STEC 1991; PATERSON *et al.* 1991; STUBER *et al.* 1992; DE-VICENTE and TANKSLEY 1993; LIN *et al.* 1995; XIAO *et al.* 1995). In contrast, recent studies focusing on nearly isogenic lines (NILs), in which most genetic variation is associated with the studied QTL, detected epistasis more frequently. Interaction between two QTL for number of spikelets in a maize-teosinte cross was not significant in the F_2 generation but was highly significant when a combination of NILs for these QTL were evaluated (DOEBLEY *et al.* 1995). The power of the nearly isogenic approach for detecting QTL epistasis was also demonstrated for genes affecting bristle number in Drosophila (SPICKETT and THODAY 1966; LONG *et al.* 1995). In F_2 generations, some of the genotypic classes are represented at a lower frequency than others, and therefore a very large population would have to be grown to detect QTL interactions (TANKSLEY 1993). In NIL experiments, the frequency of two-locus genotypes can be balanced and replicated measurements of identical genotypes can be assayed.

In this study we employed an introgression line (IL) population consisting of NILs carrying a single *Lycopersicon pennellii* chromosome segment in an otherwise homogeneous background of *L. esculentum* (cv. M82). The complete population is comprised of 50 NILs representing the entire wild species genome (ESHED and ZAMIR 1995). NILs carrying each of the introgressed segments in a homozygous and a heterozygous state were grown in the field and compared for yield-associated traits with M82. QTL affecting the measured traits were mapped to the introgressed segments where the effect of a QTL may be due to one or more linked genes.

Comparison of the phenotypic values of the parental species with those of the entire IL population showed that the cumulative effects of the individual QTL detected in the NILs was higher than the difference in the mean values of the parents. For example, the F_1 hybrid between *L. pennellii* and M82 had a total soluble solids content (Brix; B) in the fruit of 10.2, as compared to 4.3 for M82 (a difference of 5.9 B units). Summation of the estimated independent effects of the QTL detected in the heterozygous NILs yielded a B value of 12.5, twice as high as the difference between the interspecific hybrid and M82. This difference may reflect nonadditive QTL interactions. To assay for the prevalence of epistatic interactions in tomato, we selected 10 NILs with different *L. pennellii* introgressions, some of which possessed QTL that affect the measured traits in

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TABLE 1

Mean phenotypic values of M82 and the NIL hybrids heterozygous for single introgressions

Genotype	Introgressed region[a]	No. of replicates	Plant weight (kg)	Fruit mass (g)	Brix (°)	Yield (kg)	Brix × yield (g)
M82	None	79	1.82 ± 0.44	56.1 ± 4.9	4.54 ± 0.40	9.18 ± 1.54	417 ± 82
ILH1-1[b]	*1(CT233-TG71;* 58 cM)	26	3.56 ± 0.84*	48.6 ± 6.6*	5.23 ± 0.48*	11.15 ± 2.34*	580 ± 114*
ILH1-4	*1(TG245-TG259;* 35 cM)	23	2.10 ± 0.44	58.1 ± 5.4	5.16 ± 0.39*	9.84 ± 1.60	507 ± 83
ILH2-1	*2(R45S-TG276;* 16 cM)	26	1.27 ± 0.32*	52.4 ± 5.4*	4.01 ± 0.33*	7.19 ± 1.64*	289 ± 72*
ILH2-6-1[c]	*2(TG91-CT59;* 14 cM)	26	2.68 ± 0.46*	35.2 ± 4.5*	5.30 ± 0.44*	8.95 ± 1.81	474 ± 99
ILH5-4	*5(TG351-TG413;* 16 cM)	25	2.62 ± 0.59*	57.9 ± 7.1	5.07 ± 0.31*	10.85 ± 2.34*	551 ± 127*
ILH7-5	*7(TG61-TG131A;* 15 cM)	26	2.46 ± 0.46*	61.5 ± 6.3*	4.83 ± 0.33*	10.52 ± 1.45*	509 ± 87*
ILH9-2-5[c]	*9(CT283A-TG10;* 9 cM)	25	2.09 ± 0.47	51.7 ± 6.2*	5.52 ± 0.26*	9.58 ± 1.92	532 ± 122*
ILH10-1	*10(TG230-TG285;* 37 cM)	24	1.84 ± 0.33	46.5 ± 5.5*	5.11 ± 0.31*	8.38 ± 1.60	428 ± 81
ILH11-1	*11(TG497-TG523;* 27 cM)	26	2.06 ± 0.47	47.5 ± 3.6*	4.79 ± 0.41	8.50 ± 1.65	406 ± 73
ILH12-1-1[c]	*12(TG180-Aco-1;* 4 cM)	27	1.81 ± 0.32	63.3 ± 5.4*	4.70 ± 0.37	8.96 ± 1.21	422 ± 69

Mean phenotypic values ± SD of M82 and the ILHs that participated in the diallele crosses. All means were compared to M82 and the ones marked with * are significantly different (Dunnet's *t*-test, $P < 0.05$). Underlined mean values indicate a significant interaction with year (1993 *vs.* 1995; $0.01 < P < 0.05$).

[a] The introgressed regions in the ILHs are indicated by chromosome number, the markers flanking the introgression and its size in cM, according to TANKSLEY *et al.* (1992).

[b] ILH, hybrid of ILs crossed with M82.

[c] Interaction with year was based on unpublished results from a 1994 trial.

the heterozygous condition. The 10 homozygous NILs were crossed in a half diallele mode, and the phenotypic values of the 45 double heterozygotes were compared to their respective single heterozygotes and M82. The results indicate that QTL epistasis is prevalent and is generally less than additive.

MATERIALS AND METHODS

Plant material: *Intercrossed ILs:* The 10 ILs presented in this study (Table 1) were selected on the basis of the field performance of their hybrids with M82 and represent QTL affecting the means of the measured traits in both directions relative to the control, along with ILs having neutral effects (ESHED and ZAMIR 1995). We analyzed the ILs in a heterozygous condition because many of the homozygous ILs suffered from fertility problems associated with deleterious recessive alleles originating from the wild species. The phenotypic and marker data for all lines have been previously published (ESHED and ZAMIR 1995), except for IL2-6-1, IL9-2-5 and IL12-1-1, which were derived from the fine mapping of QTL of their parental ILs (IL2-6, IL9-2 and IL12-1; Y. ESHED and D. ZAMIR, unpublished results). Each selected homozygous IL was backcrossed to M82, and restriction fragment length polymorphism (RFLP) analysis was performed on 15–25 F_2 plants. Only plants homozygous for the selected introgression were used in the crosses. The 10 homozygous ILs were crossed to M82 and to each of the other lines to generate 10 introgression line hybrids (ILHs) heterozygous for single introgressions and 45 ILHs heterozygous for pairs of introgressions. The following genotypes were transplanted to a field in Akko in March 1995 in a completely randomized design: M82 (79 plants), hybrids of the ILs with M82, and the 45 double-heterozygous ILHs (23–27 plants each). Seedlings (35 days old) were transplanted to a drip-irrigated field with 50 cm between plants and 2 m between rows (1 m^2 per plant).

Linked QTL on chromosome 2: In a previous study, we demonstrated that a major QTL for fruit mass (FM) on the long arm of chromosome *2* is comprised of at least three linked genes (ESHED and ZAMIR 1995). To assay for interactions between these genes, we planted 12 homozygous ILs (15 plants each in a completely randomized design) with different introgression lengths (Figure 4). This analysis was conducted in 1994 and 1995, except for IL2-6-6 and IL2-5/2-6, which were planted only in 1995.

Phenotyping: Fruits of all lines were harvested when 95–100% of the tomatoes were red (105–115 days after transplanting in the field). The following measurements were taken for each of the plants: weight of the vegetative part (PW), mean fruit mass (FM) calculated from a random sample of 30 red fruits, total soluble solids concentration (Brix) of the fruit (B) assayed on a random sample of 20 red fruits per plant, and total fresh yield per plant (Y) (including both red and green fruits). The product of Y and B provided an estimate of the weight (g) of soluble solids produced per plant (BY) and represents horticultural yield in processing tomatoes (ESHED and ZAMIR 1994).

Statistical analysis: Statistical analyses were performed with the JMP V.3.1 software package for Macintosh (SAS INSTITUTE 1994). Mean values of the parameters measured for the single heterozygous ILHs were compared to those measured for M82 by multiple comparisons to a common control with an alpha level of 0.05 (DUNNET 1955). Results are presented as measured values in Table 1 and as percentage difference (Δ%) from the isogenic control (M82) in the margins of Figure 1; this difference is defined as the effect of the QTL that resides on the introgressed segment. Interaction of introgressed segment with year (1995 *vs.* 1993; ESHED and ZAMIR 1995) was determined for each of the ILHs using the model $Y = \mu + I + Y + I * Y + \epsilon$, where I is the presence or absence (as in M82) of the introgressed segment (fixed effect) and Y is the year (a random effect). The significance threshold for the detection of interactions with year was $P < 0.05$.

Assays for interactions between unlinked QTL were performed twice, before and after $\log_{10}$ transformation of the data. Transformation was included to test whether the QTL combine in a multiplicative manner. The significance of an interaction between two unlinked QTL was determined independently for each combination of introgression pairs by two-way analysis of variance. The participating lines in each test were M82, ILHa (IL(a) × M82), ILHb (IL(b) × M82) and ILHab (IL(a) × IL(b)). The model for the analysis was $Y = \mu + ILHa + ILHb + ILHa * ILHb + \epsilon$, where ILHa is the

TABLE 2

Correlations between the tested traits

	Plant weight	Fruit mass	Brix	Total fruit yield
Fruit mass	−0.13* (−0.21*)[a]			
Brix	0.38* (0.46*)	−0.26* (−0.29*)		
Total fruit yield	0.67* (0.24*)	0.22* (0.36*)	0.13* (−0.01)	
Brix × yield	0.72* (0.37*)	0.07 (0.24*)	0.54* (0.32*)	0.89* (0.94*)

[a] Numbers in parentheses are correlations following log transformation of the entire data set.
* Significant correlation at $P < 0.001$.

presence or absence (as in M82) of the introgressed segment a and ILHb is the presence or absence of the introgressed segment b. The interaction effect was estimated as ((M82 + ILHab) − (ILHa + ILHb)) and its significance was determined by the aforementioned F test.

Assuming complete additivity of the QTL, the effect of the double heterozygotes (ILHab − M82) should be equal to the sum of its components (expected value). This value was estimated as ((ILHa − M82) + (ILHb − M82)). The expected values were plotted against the observed values and the regression line was tested against a null hypothesis of complete additivity (expected = observed or Ho: $\beta = 1$ *vs.* H1: $\beta <> 1$).

To unify data representation, the deviation of each ILHab from its expected value (interaction effect) is presented in Δ% from M82. For example, ILH1-1 increased B by 15% as compared to M82; ILH9-2-5 increased B by 22% as compared to M82. The expected effect for the hybrid between the two homozygous ILs (IL1-1 and IL9-2-5) is a 37% increase in B relative to M82. The observed B for the hybrid heterozygous for the two introgressions was only 26% higher than M82, indicating a significant interaction ($P < 0.01$; Figure 1).

The NILs for the chromosome *2* trials were tested over two years (1994 and 1995). Since no interaction between line and year was detected (data not shown), the data from the two years were pooled. A multiple range test between the evaluated lines was performed as described previously (ESHED and ZAMIR 1995) with an alpha level of 0.05. After identification and mapping of the FM and B QTL, the data for lines with postulated identical genotype regarding the analyzed trait were pooled. The test for interaction between the different genotypic groups was performed as described for the unlinked QTL, except for the group with three FM QTL, which was tested against the two available combinations of single QTL and pairs of QTL.

RESULTS

Phenotype of the selected single ILHs: In the complete IL population composed of 50 ILHs that was analyzed for five yield-associated traits, 81 of the 250 ILH × trait combinations (32%) were significantly different from the isogenic controls ($P < 0.05$). For the subset of 10 ILHs selected for the present interaction study, 30 of the 50 combinations (60%) differed significantly from the control (ESHED and ZAMIR 1995). This indicates that the 10 ILHs were enriched for QTL affecting the measured traits.

In the present trial with the 10 ILHs (using the same experimentwise error), 28 of the 30 significant effects were consistent between the two experiments (Table 1; Y for ILH1-4 and BY for ILH2-6-1 were not significantly different from the control). The effects of ILH7-5 on PW, FM and B were found to be significant only in the present study, probably because of the larger number of replications tested here (26 as compared to six). Significant ILH by year interactions ($P < 0.05$) were detected for four of the 50 comparisons (Table 1); none of these four was significantly different from the control in either of the years. These results indicate a high overall reproducibility of the experimental system in different years, as has been demonstrated for other ILs from this population (ESHED *et al.* 1996).

Trait correlations: The traits PW, FM, B and Y are correlated yield-associated traits (Table 2). The highest correlation in the NIL population was found between PW and Y and was mainly contributed by ILH1-1, which had the highest Y and PW, and ILH2-1, which had the lowest Y and PW (Table 1). This correlation was lowered upon log transformation of the data, which reduced the effects of the extreme values. The other correlations between the four traits were weaker; the negative correlation between B and FM ($R^2 = 0.08$) is consistent with the observation of GOLDMAN *et al.* (1995), albeit of lower magnitude. The trait BY, which represents horticultural yield, is the product of B and Y and is therefore correlated to both its components. The higher correlation with Y is due to the larger variation for this trait relative to B (Table 1).

Most of the correlations between traits were not due to the introgressed *L. pennellii* chromosome segments. This is inferred from the correlation values obtained for the control genotype, M82 (data not presented), which were very similar to those calculated for all genotypes in the experiment (Table 2). Two differences were detected: for M82, no correlation was found between FM and B, and a positive correlation (0.22) was found between PW and FM for the nonscaled data.

Pleiotropic effects of IL2-1: Most of the ILHs had significant effects on some of the measured traits. However, the IL2-1 hybrid represented a unique genotype, showing transgressive segregation for reduced PW, B, Y and BY. These multiple effects can be attributed to either linkage of a number of QTL affecting these traits or pleiotropy. Because transgression for all these traits was restricted to the 16-cM introgression of IL2-1 (ESHED and ZAMIR 1995), it is unlikely that different QTL are clustered on the same segment by chance alone and therefore pleiotropy of a single gene is im-

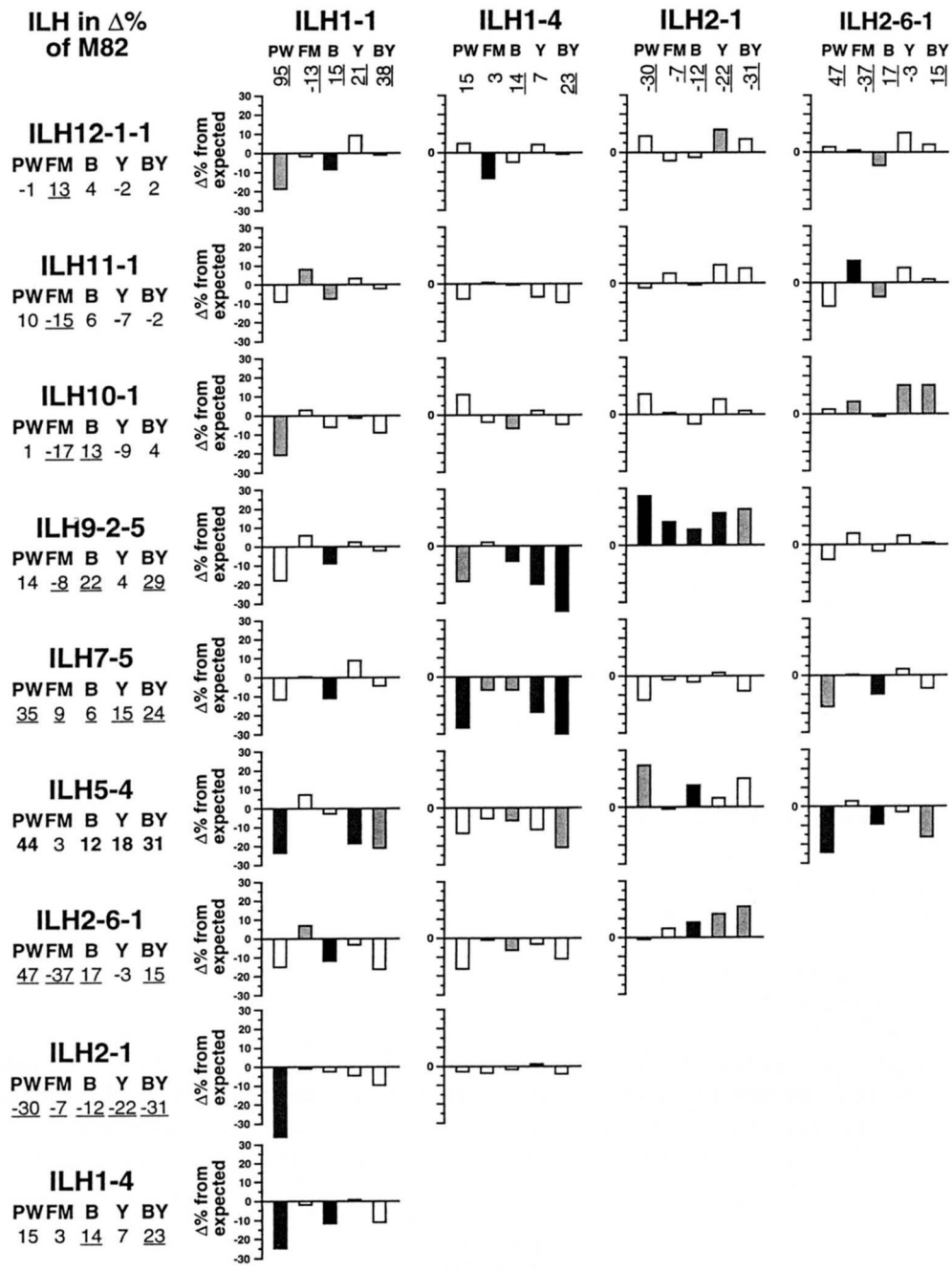

FIGURE 1.—Digenic interactions between unlinked QTL. Values on the left and top of the figure are the difference (in %) of each IL hybrid (ILH) from M82 according to Table 1. Underlined values are significant at $P < 0.05$ (Dunnet's *t*-test). Each histogram represents the interaction effect (in %) of the hybrid heterozygous for the two introgressions for all traits measured (PW, plant weight; FM, fruit mass; B, Brix; Y, total fruit yield; BY, the product of B and Y). Bars in white show no significant interaction and bars in light gray, gray and black indicate significant interactions of $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

plied. It is worth noting that this pleiotropic QTL resides on an introgression that carries the 45S ribosomal genes. A similar pattern of QTL clustering in the vicinity of the rDNA locus has been reported in a doubled haploid population of barley (POWELL *et al.* 1992).

Interactions between unlinked introgressions: The null hypothesis for the interaction analysis was complete additivity of the effects of the single ILHs. Significant deviation from complete additivity was considered evidence of epistasis (Figure 1). For each of the five traits measured, epistasis ($P < 0.05$) was detected at a frequency higher than would be expected by chance alone (ranging from 20 to 42%; Table 3). To estimate the overall number of interacting QTL, we excluded the BY trait from the analysis because of its high dependence on B and Y; the other traits used for the calculation exhibited lower correlations (Table 2). Of the 180 possible interactions (45 hybrids × four traits), 50, 25 and 10 were significant at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively (Figure 1). Since the traits are not completely independent, the presented number of significant interactions may be biased and result in

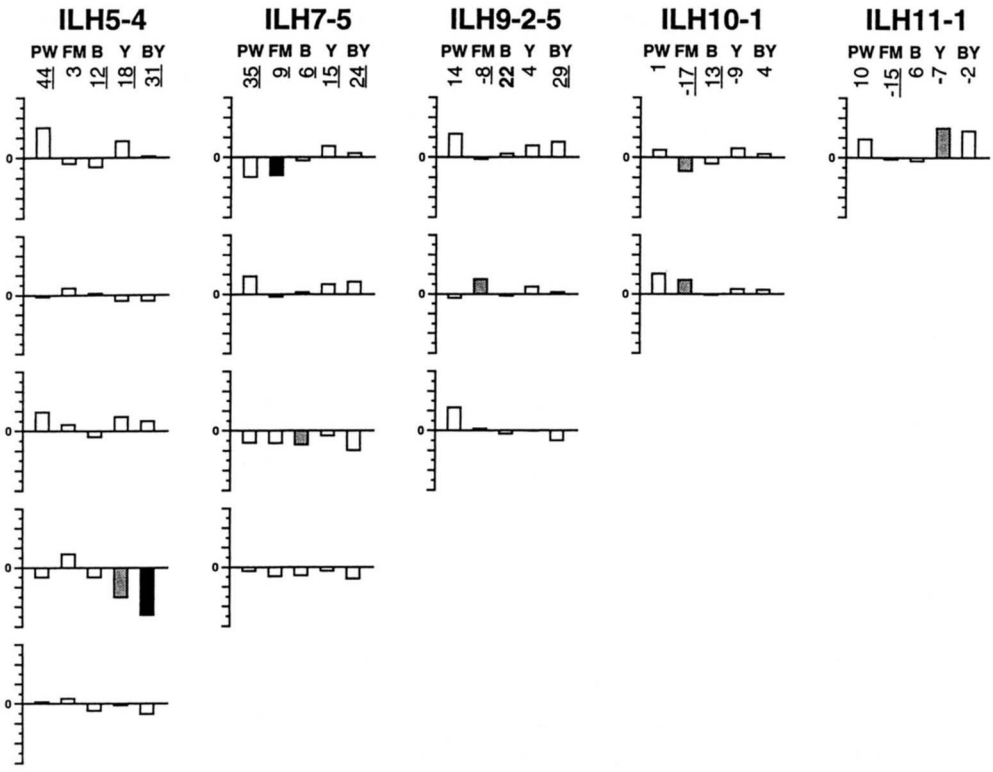

FIGURE 1.—*Continued*

clustering of epistatic effects in specific combinations of introgressions. To evaluate the effect of traits correlations on the prevalence of epistasis, we compared the observed and expected (under complete independence) distributions of the number of double introgression heterozygotes showing epistasis for zero, one, two, three or four traits (Figure 2). The calculations were based on the mean proportion of significant epistasis ($P < 0.05$) for a single trait (50/180; 28%). Overall, there is a good agreement between the observed and expected values except for two combinations of introgressions that affected all four traits (IL7-5 × IL1-4 and IL9-2-5 × IL2-1; Figure 1) as compared to an expected number of 0.3.

The detected epistasis could be merely a consequence of nonscaling of the measured values; therefore the entire data set was subjected to log transformation. Of the 180 possible interactions, 51, 24 and 14 were significant at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively. Of the 50 significant interactions ($P < 0.05$) of the nonscaled data, 43 were also detected upon log transformation of the data. The good correspondence between the tests of nonscaled and transformed data was common to all traits analyzed (Table 3). This comparison indicates that the high frequency of epistatic interactions is largely independent of the scale used.

To characterize the relationship between the effects

TABLE 3

Frequency of significant interactions ($P < 0.05$) between unlinked *L. pennellii* introgressions

Interacting QTL types[a]	Plant weight	Fruit mass	Brix	Total fruit yield	Brix × yield	Sum[d]
Sig.-Sig. (Same direc.)	3[b]/6[c]	8/16	12/21	1/3	5/15	24/46
Sig.-Sig. (Opposite direc.)	2/4	1/12	3/7	0/3	1/6	6/26
Sig.-Non Sig.	5/25	2/16	4/16	5/24	2/21	16/81
Non Sig.-Non Sig.	1/10	0/1	0/1	3/15	1/3	4/27
Sum	11/45	11/45	19/45	9/45	9/45	50/180
Sum after log transformation[e]	10 (8)	9 (7)	22 (19)	10 (9)	9 (7)	51 (43)

[a] QTL were classified according to the significance and direction of their effects relative to M82.
[b] Number of significant interactions.
[c] Number of tested combinations of two *L. pennellii* introgressions.
[d] The product of Brix × yield was excluded from the calculations of the general sum.
[e] The numbers in parentheses are the common significant interactions on linear and logarithmic scale.

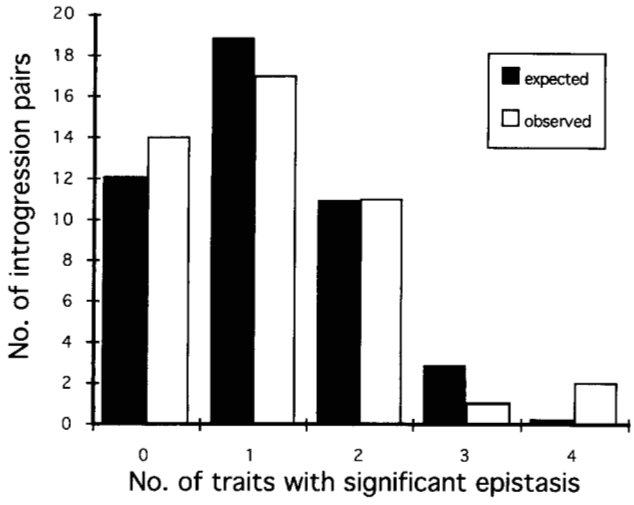

FIGURE 2.—Distribution of the observed and expected numbers of pairs of introgressions showing simultaneous significant epistasis ($P < 0.05$) for the traits PW, FM, B and Y. The expected values were calculated on the basis of complete independence between traits and a mean epistatic rate of 0.28 for each trait.

of the individual QTL and the prevalence of epistasis, the double-heterozygous combinations were divided into four groups based on the nonscaled performance of the single ILHs (Table 3). Of the 46 tested combinations between QTL affecting the four traits in the same direction, 24 (52%) were significant ($P < 0.05$). For the other three groups of combinations involving those *L. pennellii* QTL affecting the traits in opposite directions, those involving a single QTL and those with no QTL, the proportion of epistasis was markedly lower: 23, 20 and 15%, respectively. Epistasis in the latter two groups may result from the existence of minor QTL whose effects were below the significance threshold for detection or from genetic factors that affect the trait only when combined with a specific introgression.

To search for general trends in the interaction of QTL, the observed values of the 45 double-heterozygous hybrids were plotted against their expected values (Figure 3). Highly significant linear regressions were found for all five traits, indicating the overall additivity of the effects of the independent introgressions. Assuming complete additivity between the effects of the combined individual introgressions, one would expect a regression with a slope of 1. The slopes of the lines for the five traits were highly significantly lower than 1 (ranging from 0.71 to 0.79), indicating average combined effects that are less than additive. The slopes for the log-transformed data concur with the less-than-additive trend since all were highly significantly lower than 1 (for PW, B, Y and BY the slopes were reduced to 0.69, 0.75, 0.65 and 0.64, respectively, whereas for FM it increased to 0.85). The less-than-additive trend is also apparent in the group involving two QTL affecting the trait in the same direction; all 24 significant interactions (out of the 46 tested) were less than additive (Table 3). Only in this group is it possible to determine the proportion of cases of epistasis, where the phenotypic value of the double heterozygotes was of a smaller magnitude than the sum of the effects of the single heterozygotes.

Interactions between linked QTL (chromosome *2*): The previously discussed interactions were measured on lines heterozygous for the introgressed segments. Using a set of lines derived from ILs of the long arm of chromosome *2*, we tested for epistasis of QTL in a homozygous state. Twelve homozygous ILs with different introgression sizes in chromosome *2* were evaluated for FM and B; because 10 of the lines were tested in 1994 (ESHED and ZAMIR 1995) and no significant interactions between year and IL were detected (data not shown), the results from the two years were pooled. Based on the overlapping recombined chromosome segments and the phenotypic values of each of the ILs, two B QTL and three FM QTL, responsible for a similar reduction in FM, were identified and mapped (Figures 4 and 5). The mapping results were identical for the log-scaled data.

After determining the positions of these QTL, the lines were classified according to their postulated genotypes (Table 4). Epistasis for B and FM was tested by comparing the means of the pooled genotypic groups. The single interaction for B was significant, with both measured and scaled data, and the sum of the effects of the single QTL was higher than the mean value of the lines carrying both QTL. The four tests for FM QTL interactions were significant when the measured scale was used: two of them examined the combined action of a single QTL and two examined a single QTL and the remaining pair. The average diminishing effect for two QTL was 8.5% as compared to 14% for interactions involving the three QTL (Table 4). This result suggests that the effect of the less-than-additive epistasis is increased (*i.e.*, the effects are further diminished) when more QTL are involved. However, none of the epistatic effects for the linked FM QTL were significant upon log transformation of the data. This may indicate that the chromosome *2* QTL interact in a multiplicative manner, as has been shown previously for fruit size in certain tomato genotypes (POWERS 1941).

DISCUSSION

Unlike QTL mapping studies in conventional segregating populations that have generally uncovered little evidence for epistasis (see Introduction), we show here, using NILs, that QTL epistasis is a significant component in determining phenotypic values. The resolving power of the tomato NILs for a study of continuous traits was indicated previously when twice as many QTL affecting FM and B were identified as compared to those found using other conventional segregating interspecific populations (ESHED and ZAMIR 1995). In the present study we detected higher than expected frequency of epistatic combinations between introgression

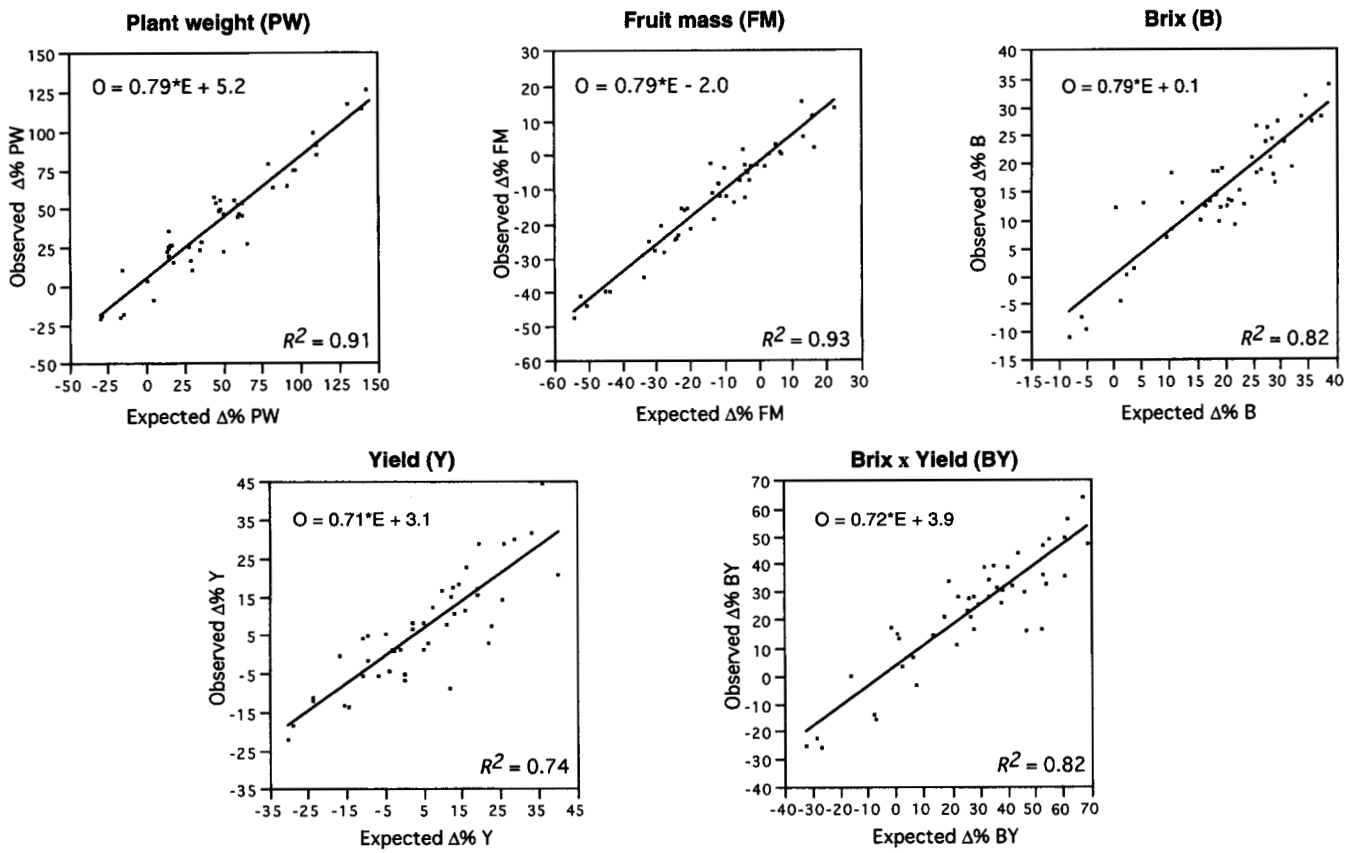

FIGURE 3.—Plots of expected *vs.* observed effects for the 45 tested combinations of pairs of heterozygous introgressions. Expected values were calculated on the basis of complete additivity of the effects of the individual ILHs. *, significant difference of the slope from 1, at $P < 0.001$.

that independently had no significant effect on the measured trait (Table 3). This observation indicates that the number of QTL is even higher than previously estimated.

The tomato NILs provide a number of advantages for the study of epistasis. (1) The lines contain single RFLP-defined introgressions some of which produce effects of relatively large magnitude in which all the quantitative genetic variation between the NILs is associated with the introgressed segment (ESHED *et al.* 1996). (2) The fixed genotypic constitution of the lines enables testing the introgression effects in different years. The results show high reproducibility of the effects of the QTL mapped to the different introgressed chromosome segments (Table 1; ESHED *et al.* 1996). (3) The genetic constitution of the lines can be easily manipulated to generate shorter introgressions that retain the phenotypic effects. For example, IL9-2 and IL12-1, with introgressed segments of 37 and 15 cM, respectively, were trimmed to generate IL9-2-5 and IL12-1-1 (9 and 4 cM, respectively). The quantitative effects associated with hybrids of these ILs were maintained (Table 1). This characteristic of the NILs enables the production of lines with a higher probability of containing a single QTL. (4) For the analysis of epistasis, a diallele crossing scheme is used to generate NILs containing a number of introgressions that are analyzed in a simple experiment with balanced representation of the different genotypes. The introgressions can be combined in different genetic constitutions enabling an analysis of all possible interactions between the components of the genetic variation. (5) The NIL population described in this study facilitated the utilization of wild germplasm in breeding processing tomatoes for increased horticultural yield (BY; ESHED *et al.* 1996). Since the *L. pennellii* introgressions were introduced into an elite genetic background, favorable QTL combinations can be used directly for variety development.

For the five yield-associated traits analyzed in this study, 20–42% of the 45 dichromosome segment combinations were epistatic ($P < 0.05$) and were generally independent of the scale used (Table 3). A higher-than-expected frequency of epistasis was also apparent when an experimentwise error correction was included for all tested traits, except Y (0.05/45, equivalent to $P < 0.001$ in Figure 1). Due to the common trend of less-than-additive epistasis (which will be discussed further on), we preferred the use of a less strict threshold ($P < 0.05$) for the characterization of the interaction prevalence. However, the use of an unadjusted Type I error does not allow us to draw conclusions on the interaction mode of a specific QTL combination; for this purpose we are presently replicating the trials with selected genotypes.

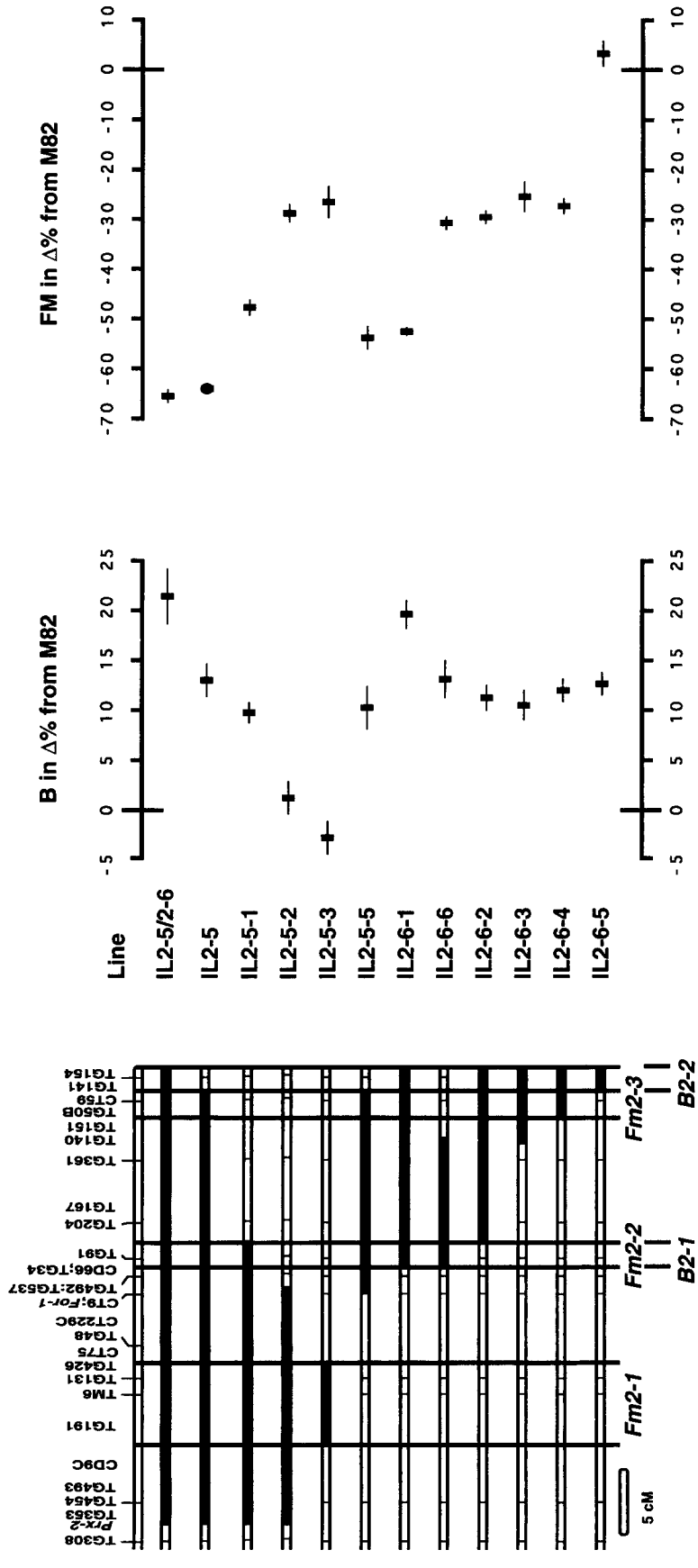

FIGURE 4.—Fine mapping of linked QTL for B and FM on the long arm of chromosome 2. The dark bars represent the *L. pennellii* chromosome segments introgressed into the presented lines. Each point represents the mean introgression effect and the bars give the standard errors of the means. The vertical lines represent the postulated position of the identified QTL.

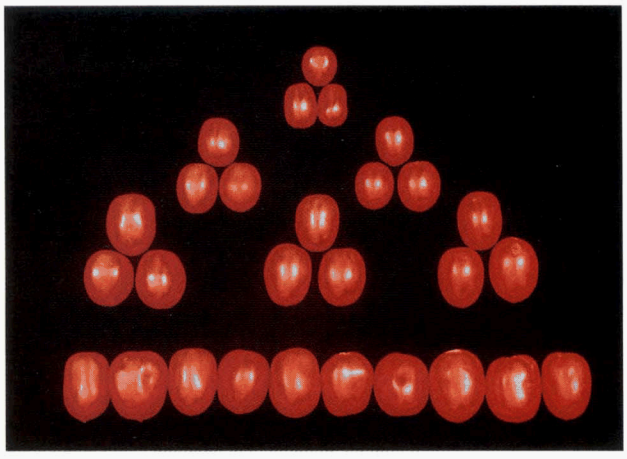

FIGURE 5.—Fruit size of lines used for the mapping analysis of the linked QTL on chromosome *2* (Figure 4; Table 4). (Top) IL2-5 containing *Fm2-1/2-2/2-3*. Second row, left: IL2-5-1 (*Fm2-1/2-2*); right, IL2-6-1 (*Fm2-2/2-3*). Third row, left: IL2-5-3 (*Fm2-1*); center, IL2-6-6 (*Fm2-2*); right, IL2-6-4 (*Fm2-3*). (Bottom) M82.

To assess the overall rate of epistasis in the experiment, we included the results for the traits PW, FM, B and Y, even though they were not completely independent (Table 2). Genetic correlations may result from pleiotropic effects of single QTL (as was indicated for ILH2-1) and from linkage of several genes controlling the traits (as was demonstrated for the FM and B QTL on the long arm of chromosome *2;* Figure 4). Irrespective of the cause for the correlations, our estimate of 28% epistatic combinations out of the 180 tested ($P < 0.05$) is hardly influenced by the correlation factor (Figure 2). This high frequency of epistasis is consistent with numerous classical studies on the nature of quantitative variation (HAYMAN 1958; MORENO-GONZALEZ and DUDLEY 1981) and with a Design III maize experiment using molecular markers (COCKERHAM and ZENG 1996).

A consistent trend of QTL interactions was detected, whereby the phenotypic effects of the double heterozygotes were of a lower magnitude than the sum of the effects of the single heterozygotes. The partially cumulative combined effect of the introgressions is indicated by the regression analyses of the observed *vs.* expected plots of the 45 combinations for each of the five yield-associated traits (Figure 3). The additive model explains a considerable portion of the variation in the observed values (74% for Y to 93% for FM); however, the slopes of the regression lines were significantly lower than 1. In natural and breeding populations where a number of QTL are segregating, there are numerous possible multi-way interactions that may account for greater diminishing effects than those detected for two-way interactions. This is supported by the observation that in the entire IL population, the cumulative effects of the individual QTL for B and FM were at least twice as high as the difference between the parental species, which differed in at least 23 QTL for B and 18 QTL for FM (ESHED and ZAMIR 1995). In addition, less-than-additive nonscaled effects on FM were more pronounced for three QTL than for pairs of QTL (Table 4). Assuming that the less-than-additive mode of epistasis detected in this study is common to other crosses, this interaction would be confounded with the effect of the individual QTL. As a consequence, the number of significant QTL would be underestimated and less-than-additive interactions of the magnitude described in this study would be

TABLE 4

Interactions of linked QTL for Brix (B) and fruit mass (FM)

Genotypic group[a]	Mean B (°Brix)	Mean B (Δ% from M82)	Interaction effect (Δ%)	*P* value of interaction
No QTL[b]	4.47	−0.2		
B2-1	5.00	11.7		
B2-2	4.98	11.6		
B2-1/2-2	5.37	19.9	−3.6	0.03 (0.02)[c]
Genotypic group[a]	**Mean FM (g)**	**Mean FM (Δ% from M82)**	**Interaction effect (Δ%)**	***P* value of interaction**
No QTL[b]	59.5	0.6		
Fm2-1	43.0	−27.3		
Fm2-2	41.0	−30.7		
Fm2-3	42.7	−27.8		
Fm2-1/2-2	30.7	−48.2	10.5	0.009 (0.5)
Fm2-2/2-3	28.0	−52.7	6.4	0.03 (0.15)
Fm2-1//2-2/2-3[d]	21.0	−64.6	16.0	<0.0001 (0.34)
Fm2-1/2-2//2-3[e]	21.0	−64.6	12.0	<0.0001 (0.19)

[a] Genotypic groups were pooled on the basis of the fine-mapping analysis presented in Figure 3.
[b] M82 was included in this group, which includes lines with no *L. pennellii* QTL affecting the trait.
[c] Numbers in brackets are the *P* values of interaction after log transformation.
[d] The tested interaction was *Fm2-1* × *Fm2-2/2-3*.
[e] The tested interaction was *Fm2-3* × *Fm2-1/2-2*.

undetectable. Recent studies that detected epistasis of selected QTL in Drosophila (LONG *et al.* 1995), soybean (LARK *et al.* 1995) and maize (DOEBLEY *et al.* 1995; COCKERHAM and ZENG 1996) did not show a less-than-additive trend. Only further evaluations of QTL interactions in additional experimental systems will reveal whether diminished additivity is a general phenomenon.

The observation that certain QTL that affect a continuous trait in the same direction interact in a less-than-additive manner, as compared to others that do not show such epistasis, provides a basis for the hypothesis that QTL can be grouped according to their mode of activity. In a review of the genetic interactions of flowering-time single-gene mutations in Arabidopsis, it was proposed that the mode of epistasis can be used to classify the genes according to their function. In general, when two mutations affecting the same developmental pathway are combined, they exhibit the phenotype of the more extreme mutation. When genes from different pathways are combined, their effects are additive (KOORNNEEF *et al.* 1991; COUPLAND 1995). The limitation for such an interpretation for QTL analysis in the IL population is that each introgression may carry a number of genes that affect the trait, as demonstrated here for the QTL reducing FM and the QTL increasing B on chromosome *2* (Figure 4). However, in the entire IL population, only two QTL of *L. pennellii* origin showed transgressive segregation for FM (ESHED and ZAMIR 1995). It is therefore unlikely that IL7-5 (introgression size, 15 cM) and IL12-1-1 (4 cM), which increase FM in a heterozygous state by 9 and 13%, respectively, harbor more than a single QTL for increased FM (the FM gene on chromosome *7* is designated *Fm7-1* and on chromosome *12, Fm12-1*). These genes thus interact in a less-than-additive manner, suggesting their potential classification into the same group. This suggestion is supported by the similar interaction patterns of these genes with the other *L. pennellii* introgressions: *Fm12-1* interacted only with IL1-4 and IL10-1, whereas *Fm7-1* interacted with IL1-4 and the significance level of its interaction with IL10-1 was $P = 0.08$. Further delimitation of QTL to smaller chromosome segments and studies of their interactions with genotypes and environments may provide the basis for their classification into groups. These aspects of QTL characterization may assist in the breeding of new varieties through cointrogression of QTL that show minimal diminishing effects.

Canalized characters are developmentally buffered such that the phenotype is kept within narrow boundaries, despite genetic and environmental disturbances (WADDINGTON 1942). For continuously distributed traits, a large number of QTL affect a particular phenotype. Less-than-additive interactions among QTL ensure that the "loss" of an allele affecting a fitness trait will have a minimal effect on the phenotype and that canalization will be maintained.

We thank G. GERA of Akko Experimental Station for assistance in conducting the field trials and T. PLEBAN, H. VAN OSS, A. EIZENBAND and O. EIZENBERG for technical assistance. We thank Drs. A. CAHANER, I. PARAN, S. D. TANKSLEY and J. I. WELLER for stimulating discussions. This research was supported in part by grant no. US-2427-94 from BARD, The United States-Israel Binational Agricultural Research and Development Fund, and was conducted under the auspices of the Israeli Plant Genome Center.

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Communicating editor: T. F. C. MACKAY