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Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: Deductions about natural variation and implications for germplasm utilization

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Abstract Quantitative Trait Locus (QTL) allelic variation was studied by analyzing near-isogenic lines (NILs) carrying homologous introgressions on chromosome *4* from three green-fruited wild tomato species. The NILs affect agronomic (yield, brix, fruit weight) and fruit (fruit shape, color, epidermal reticulation) traits in a similar manner. However, significant differences were detected in the magnitudes of the effects, the dominance deviations and epistatic interactions, indicating that those species carry different alleles for the QTL. As the QTL did not show any interaction across environments, genetic backgrounds or other QTLs, it can be used to introduce novel genetic variation into a broad range of cultivars. Analysis of new recombinant NILs showed that fruit traits are controlled by several linked genetic loci, whereas multiple genetic loci control the agronomic traits within the original introgression. The hypothesis that QTLs may be composed of multiple linked genes can not be rejected prior to implement projects for QTL isolation and cloning. Loci involved in color enhancement could not be related to any known gene involved in the carotenoid biosynthesis pathway, therefore it is hypothesized that the function of those loci must be related to the genetic regulation of the carotenoid biosynthetic pathway.

Keywords QTL · Fine-mapping · Epistasis · Pleiotropy · Breeding

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Introduction

The use of wild germplasm as a source of novel quantitative trait locus (QTL) allelic variation is one of the more important issues for crop improvement and breeders today. From a breeder's viewpoint, it would be most useful to identify new favorable QTL alleles that display low QTL × environment (QTL × E) and QTL × genotype (QTL × G) interactions. Also desirable would be the identification of combinations of QTLs' alleles at different loci that interact in an additive or complementary manner.

QTL effects and chromosomal position are usually estimated in early segregating populations, such as the F_2 or $BC_{1,}$ where QTLs can be detected using a relative small population size. However, QTL analysis in these populations is problematic for several reasons: (1) estimates of QTL effects and positions generally are biased (Van Ooijen 1992; Jiang and Zeng 1995; Melchinger et al. 1998); (2) QTL-by-environment interactions (QTL × E) are difficult to detect (Stuber et al. 1992; Ragot et al. 1995; Austin and Lee 1998; Melchinger et al. 1998); (3) the distinction between pleiotropy or close linkage when a chromosomal region shows an effect on several traits is not always possible (Lebreton et al. 1998). Additionally, epistatic and QTL-by-genetic background (QTL × G) interactions are difficult to estimate (Yu et al. 1997).

Advanced backcross QTL (AB-QTL) analysis has been proposed and tested as a method to mitigate these problems and facilitate utilization of the exotic germplasm (Tanksley and Nelson 1996; Tanksley et al. 1996; Fulton et al. 1997; Bernacchi et al. 1998a; Xiao et al. 1998). From an AB population, near-isogenic lines (NILs) carrying small introgressions from the donor parent, can be easily isolated by marker-assisted selection (MAS). NILs can then be used to obtain better estimates of the magnitudes of QTL × E, QTL × G and QTL × QTL interactions (Eshed and Zamir 1995, 1996), fine-map QTLs, eliminate undesirable affects caused by linkage drag and, eventually, perform positional cloning of the QTLs (Alpert and Tanksley 1996).

Bernacchi et al. (1998b) developed a set of NILs from a cross between *Lycopersicon esculentum* cv. E6203 (TA209) and *L. hirsutum* acc. LA1777. One of those, TA517 contains the distal 50-cM introgression of chromosome 4, a region previously shown to affect several agronomic important traits including brix, yield, brix*yield and fruit color (Bernacchi et al. 1998a, b). Other NILs carrying homologous introgressions on chromosome 4 from different wild species have been developed in parallel: IL4-4 from *L. pennellii* (LA716) on the processing tomato inbred variety M82 (Eshed and Zamir 1995) and TA1160 from *L. peruvianum* (LA1706) on TA209 background (Fulton and Tanksley, unpublished data). The goals of the study reported here were to use these recently developed NILs to address four issues: (1) genetic variability among QTLs on chromosome 4 across wild tomato species, (2) consistency of the QTL effect across testers and environments, (3) the potential for pyramiding QTLs from different wild species and (4) accurate estimation of the genetic effects of the QTLs and their location within the introgression by substitution mapping using a new set of recombinant subNILs from TA517 NIL. We herein present a comprehensive characterization of a single chromosomal region displaying multiple QTL effects that include verification of the effects, genetic variability, agronomic interest, fine mapping and implications on quantitative genetics.

Materials and methods

Near-isogenic lines

TA517 and TA1160 are NILs in the *Lycopersicon esculentum* cv. E6203 (TA209) background; each contains a small, single segment of DNA from the bottom of the chromosome 4 from a different wild tomato species (Fig. 1). These NILs were derived from advanced backcross populations aided by marker-assisted selection following the procedures described by Bernacchi et al. (1998b). TA517 was derived from *L. hirsutum* LA1777 (Bernacchi and Tanskley 1997; Bernacchi et al. 1998a) and TA1160 from *L. peruvianum* LA1706 (Fulton et al. 1997). IL4-4 (Fig. 1) contains the bottom of chromosome 4 from *L. pennellii* LA 716 in the M82 background (Eshed and Zamir 1994). TA523 contains a single introgression for the bottom of chromosome 1 from *L. hirsutum* LA 1777 covering the markers TG27 to TG161 (Bernacchi et al. 1998b; Monforte and Tanksley 2000), while TA1150 contains a slightly larger introgression on chromosome 1 from *L. chmielewskii* LA1063 extending from TG27 to TG607 (Tanskley unpublished results); both of them in the TA209 background. The double-NIL TA1451 was constructed by crossing TA1160 and TA1150.

SubNILs for the chromosome 4 introgression TA517 were isolated from 216 F_2 seedlings derived from the cross between TA517 × TA209 by screening the markers spanning the introgression after DNA digestion with the appropriate enzymes: TG155 (*Sca*I), CT133 (*Sca*I), CT199 (*Hind*III) and TG464 (*Hind*III) (Fig. 1). Fifteen independent recombinant plants were thus identified and allowed to self-pollinate. F_3 plants were scored with appropriate markers to fix the recombinant chromosomes. Additional markers were scored to determine the exact point of the recombination within each of the fixed subNILs (Fig. 1). TA1459 is a subNIL derived in a similar manner from TA1138 NIL by screening 360 F_2 seedlings from the cross TA1138 × TA209 with CT188 (*Sca*I), TG574 (*Sca*I) and CD39 (*Sca*I), which span the TA1138 introgression (Fig. 1).

All of the NILs and subNILs (except IL4-4) were crossed with TA209 to obtain the hybrid NILs for the phenotypic evaluations. Double-NILs containing two defined chromosomal segments in a heterozygous condition were obtained by crossing the selected NILs.

Field evaluations

NILs/subNILs (NS) trials

NILs and subNILs were evaluated in two locations: Akko (Israel) and Ithaca (N.Y., USA). The seedlings of the following genotypes were transplanted to 50 cm within rows and 2 m between rows in Akko in March 1998 in a completely randomized design: TA209 (50 plants), M82 (20 plants), TA517 (20 plants) and 10 plants each of IL4-4, TA1160, the subNILs from T517 (TA1232, TA1463, TA1464, TA1465, TA1466, TA1467, TA1468, TA1469, TA1470, TA1471, TA1473, TA1474, TA1475, TA1476, TA1477) and the corresponding hybrids with TA209. The following genotypes were transplanted in Ithaca, N.Y. (USA) in June 1998 in a completely randomized design: TA209 (50 plants), 10 plants each of TA517, TA1160 (both homozygous and heterozygous with TA209), the hybrids TA523 × TA209, TA523 × TA517, TA523 × TA1160 and TA517 × TA1160, the subNILs TA1232, TA1463, TA1464, TA1465, TA1466, TA1467, TA1468, TA1469, TA1470, TA1471, TA1473, TA1474, TA1475, TA1476, TA1477 (5 plants), and their respective hybrids with TA209 (10 plants). All plants were sown in flats in the greenhouse 40 days before being transplanted into the field allowing 1 m^2 per plant. The field in Israel was under drip irrigation, and the field in Ithaca was under natural rainfall.

Genetic background (GB) trials

To study the interaction of the genetic background on the effects of the QTL alleles included in the TA1160 introgression, we crossed TA1160 and TA209 with each other as well as with three different processing-type testers: TA502, a mid-season, firm-fruited inbred; TA446, a compact early inbred; TA1149, a mid-season inbred. Thus, TA1160 was evaluated on TA209 and hybrid TA209/tester genetic background.

TA1160, TA1150 and TA1451 were also crossed with TA209 to study the interactions among the QTLs included in the two NILs. The trials were conducted during the summer of 1998 at six locations worldwide: Akko (Israel), Badajoz (Spain), Davis (Calif., USA), Woodland (Calif., USA) and two locations in Stockton (Calif., USA). All trials comprised 15 plants per plot at commercial density with five plots per experimental line. Plots were randomized in five blocks with one plot per NIL in each block.

Phenotyping

Up to ten traits were measured for both the NS and the GB trials. The main difference between experiments was that in the NS trials the traits were measured on a single plant basis, whereas in the GB trials the traits were measured on a plot basis.

Fruit characteristics were scored before harvest from single plants (5 fruit) or plots (15 fruit randomly picked among the plants within the plot) in the NS and GB trials, respectively, except in Spain where fruit traits were not scored and in Ithaca where fruit traits were scored after harvest. Fruit characteristics were scored visually using a scale from 1 to 5: internal and external fruit color (ICOL and ECOL), 1 = pale to 5 = dark red; epidermal reticulation (ER), 1 = smooth to 5 = cracked, fruit shape (FS), 1 = round to 5 = very elongated. FS was also measured in Ithaca as the ratio longitude/diameter (L/D) for selected genotypes. ICOL and ER were scored on a similar scale by independent observers pre- and post-harvest in Israel.

The agronomic traits measured at harvest were: yield, measured as grams per plant (NS trial) or tons per hectare (GB trial) of

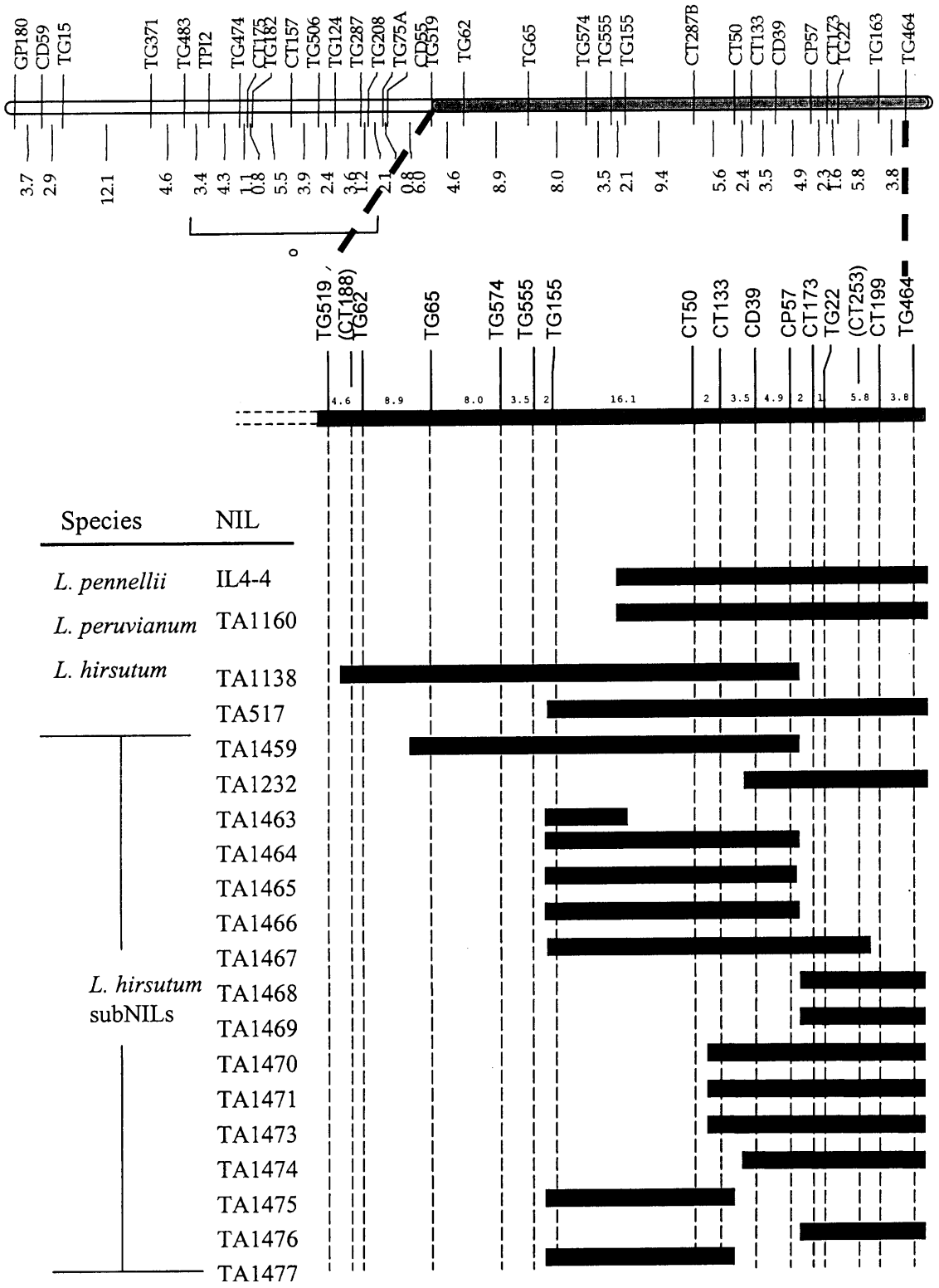

Fig. 1 Graphical genotypes for NILs and subNILs. The chromosome 4 linkage map depicted at *top* is adapted from Tanksley et al. (1992). The segment contained in the NILs and subNILs is shown *below. CT253* an *CT188* are bracketed because the distances from their respective flanking markers are not known. The *right column* refers to the wild species from which the NIL was developed. The extent of the introgressed segment for each NIL and subNIL is indicated by a solid *bar*

ripe fruit (YDR); in Ithaca a high percentage of the harvested fruit were not mature so yield was measured as the weight in grams of all green and mature fruit per plant (YDT); soluble solids content (SSC), measured as °Brix with a refractrometer from the juice from at least 5 mature fruit for each single plant in the NS trials and from serum from a raw, de-aerated cold-break puree derived from a sample of more than 40 randomly harvested fruit per plot in the GB trials; average fruit weight (FW), measured in grams from at least 5 mature fruit per plant in the NS trials and from at

least 40 ripe fruit per plot in the GB trials; Brix*Yield as the product of SSC and YDT (BTDY) or YDR (BYDR); and plant weight (PW), measured only in Israel in the NS trial on each single plant as the weight in kilograms of the vegetative portion of the harvest.

Data analysis

All statistical analysis presented here were performed using JMP 3.1 for Macintosh (SAS Institute 1994) and QGENE (Nelson 1997) software packages.

NIL/subNILs trials

Comparison of NILs carrying introgressions for chromosome 4 from different wild species. Means values of the traits studied in the NS trials for the NILs TA517, TA1160 and IL4-4 (Israel) and TA517 and TA1160 (Ithaca) were compared with the corresponding control genotypes using the Dunnet contrast (Dunnet 1955) with Type-I error $\alpha \leq 0.05$. TA209 was the common control for TA1160 and TA517. M82 was the control for IL4-4. TA1160 and TA517 mean values were also compared by a *t*-test with $\alpha \leq 0.05$.

Gene action estimates for QTLs on chromosome 4. The significance of the dominance deviation was evaluated from the Ithaca data by using the contrast (Wrike and Weber 1986):

$$2F_1–(P_1+P_2)$$

at $\alpha \leq 0.05$, where F_1 is the phenotypic mean for the heterozygote (e.g. TA517 × TA209, TA1160 × TA209 or TA1160 × TA517), and P_1 and P_2 are the phenotypic means of the respective parents (TA517, TA1160 or TA209). Gene action (d/[a], [a] being the absolute value of a) was calculated as:

$$\frac{F_1 - \frac{P_1 + P_2}{2}}{\left[\frac{P_1 - P_2}{2}\right]}.$$

Epistatic interactions estimates for chromosomes 1 and 4 introgressions. Interactions between the *L. hirsutum* and *L. peruvianum* introgressions on chromosome 4 with the *L. hirsutum* introgression from chromosome 1 were tested using data from heterozygous genotypes, TA523 × TA209, TA517 × TA209, TA1160 × TA209, TA517 × TA523, TA1160 × T523, as well as the control TA209 according to:

$$Y_{ijk} = \mu + C1_i + C4_j + C1*C4_{ij} + e_{ijk}$$

where, μ is the overall mean, $C1_i$ is the genotype in the region on chromosome 1 (scored as E for *L. esculentum* homozygous, H for *L. hirsutum* heterozygous), $C4_j$ is the genotype in the region on chromosome 4 (scored as E for *L. esculentum* homozygous, H for *L. hirsutum* heterozygous and P for *L. peruvianum* heterozygous), $C1*C4_{ij}$ is the interaction between the two chromosomal regions and e_{ijk} is the random error. The significance of the genotype effects and the interaction $C1*C4_{ij}$ was assessed by a *F*-test ($\alpha \leq 0.05$). The direction of the interaction effect specific for each species was confirmed and estimated by the respective contrast with the means:

(TA209 + TA517 × TA523)–(TA523 × TA209 + TA517 × TA209)

for the interaction among *L. hirsutum* introgressions

(TA209 + TA1160 × TA523)–(TA523 × TA209 + TA1160 × TA209);

for the interaction among *L. hirsutum* in chromosome 1 and *L. peruvianum* introgression in chromosome 4.

Fine mapping of QTLs within TA517 introgression. Both homozygous and hybrid subNILs means from the NS trials were compared with TA209 for each trait using Dunnett's contrast at $\alpha \leq 0.05$. Genotypes with less than four replications were excluded from the analysis. The subNILs also differed in marker genotype composition, so QTL analysis could also be made by marker genotype mean comparisons. This gave more replications for each chromosomal region and hence better estimates of the genetic effects. Comparisons of marker means by single-point analysis were performed using the software package QGENE. Due to linkage among markers, only seven different marker comparisons were performed. The Bonferroni correction that gives an overall $\alpha = 0.05$ has a threshold for each comparison $\alpha \leq 0.05/7 = 0.007$ or $-\log(p) \geq 2.14$. Both strategies (NILs comparison and single-point marker analysis) were combined to fine map and estimate the effects of a QTL within the TA517 subNIL when a subNIL showed significant effects compared with the TA209 control according to the Dunnett's contrast; a QTL was then considered to be within the genomic region covered by such subNIL. If several subNILs showed the effect, a QTL was deduced to be within the chromosomal region shared by the subNILs. QTL position was also estimated accordingly to marker mean comparison. When a QTL was located within a specific chromosomal region, the additive effects (a), measured as the percentage of deviation from TA209 control mean, and mode of gene action (d/[a]) of the QTLs were estimated according with marker means as a = 100*((HH–EE)/2EE), d/[a] = (EH–((HH+EE)/2))/[(HH–EE)/2], where HH is the phenotypic mean of individuals homozygous for the *L. hirsutum* alleles for the marker locus, EH is the phenotypic mean of individuals heterozygous for the same locus, EE is the TA209 mean and [(HH-EE)/2)] is the absolute value of the remainder. QTLs were named according previous experiments (Tanksley et al. 1996; Grandillo and Tanksley 1996; Fulton et al. 1997; Bernacchi et al. 1998b).

Genetic background (GB) trials

Genotype by genetic background interactions. The effect of the *L. peruvianum* introgression on chromosome 4 (TA1160) was tested in different genetic backgrounds (TA209 or hybrid TA209/tester) according to the model:

$$Y_{ijkl} = \mu + G_i + T_j + L_k + GT_{ij} + GL_{ik} + TL_{jl} + GTL_{ijk} + e_{ijkl}$$

where μ is the overall mean, G_i is the effect of the genotype in chromosome 4 (*L. peruvianum* (P) or *L. esculentum* (E), T_j is the effect of the testers (TA209, TA446, TA502, TA1149), L_k is the effect of the locations, followed by the respective interactions (GT_{ij}, GL_{ik}, TL_{jl}, GTL_{ijk}) and the random error (e_{ijkl}). The significance of each effect and their interactions was studied using an *F*-test.

Epistatic interactions between chromosome 1 and chromosome 4. Interactions among QTLs located on chromosomes 1 and 4 from different wild species (*L. chmiewleskii* and *L. peruvianum*, respectively) was studied according to the model:

$$Y_{ijkl} = \mu + P4_i + CH1_j + L_k + P4CH1_{ij} + P4L_{ik} + CH1L_{jl} + P4CH1L_{ijk} + e_{ijkl}$$

where μ is the overall mean, $P4_i$ is the effect of the *L. peruvianum* introgression on chromosome 4, $CH1_j$ is the effect of *L. chmiewleskii* introgression on chromosome 1, L_k is the effect of the locations, followed by the respective interactions ($P4CH1_{ij}$,

Fig. 2 Means and standard errors of the studied traits for controls (M82 and TA209), and homozygous chromosome 4 NILs (IL4-4, TA517 and TA1160) tested in both Ithaca, N.Y. and Akko (Israel). *Dark bars* indicate that there is a significant different between the NIL and its respective control; ★ indicates a significant difference between TA517 and TA1160 (all comparisons $\alpha < 0.05$) ▶

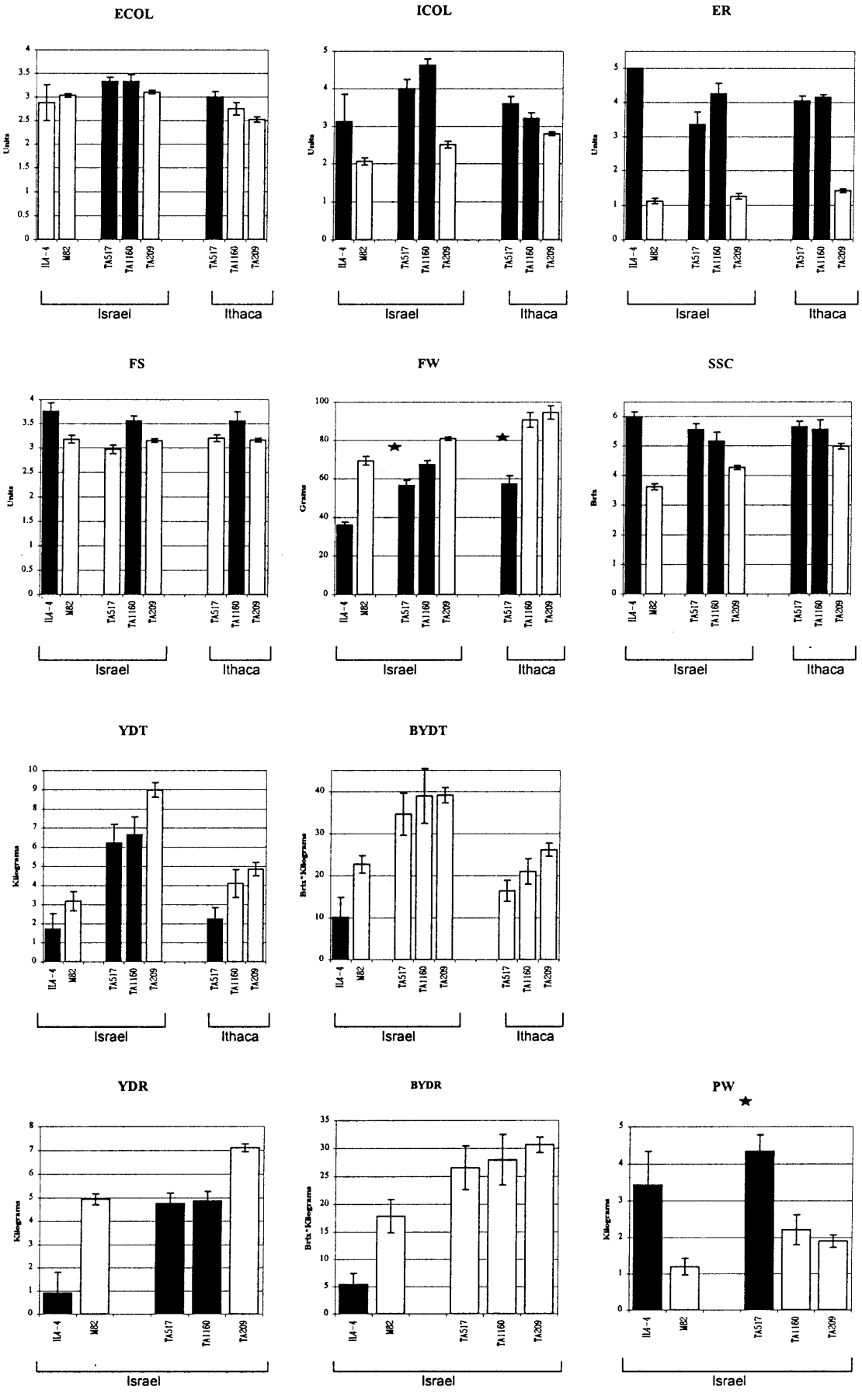

Fig. 2 Legend see page 575

$P4L_{ik}$, $CH1L_{jl}$, $P4CH1L_{ijk}$) and the random error (e_{ijkl}). The effect of the introgressions and their interactions were assessed using the *F*-test

Results

Comparing phenotypic effects and their magnitude for different species introgressions for the same region of chromosome 4

Comparison among homozygous NILs

NILs TA517 (*L. hirsutum* introgression), TA1160 (*L. peruvianum* introgression) and IL4-4 (*L. pennellii* introgression) carrying a homologous introgression on the bottom end of chromosome 4 (Fig. 1) from green-fruited species were significantly different from the control in at least one location with respect to most of the traits measured. The effects of the introgressed segment were similar in the three NILs: enhanced external (ECOL) and internal color (ICOL) and soluble solid concentration (SSC), decreased fruit weight (FW) and yield (YDT and YDR) and induced elongated fruit and epidermis reticulation (ER) (Fig. 2), although the magnitude of these effects was different for several traits. IL4-4 showed stronger undesirable effects than TA517 and TA1160, with a higher reduction of YRD, BYRD and FW and more severe ER. On the other hand, IL4-4 showed a higher increase of SSC than TA1160 and TA517, whereas the increase in ICOL, ECOL and plant weight (PW) was similar to that of TA517. Significant differences in the magnitude of the effects for PW and FW, FS and ECOL were also found between TA517 and TA1160 (Fig. 2).

Gene action for chromosome 4 NILs

The gene action (d/[a]) estimated with the hybrids TA517 × TA209 and TA1160 × TA209 was similar for several traits (Fig. 3): dominance for ER and additive for FW, YDT and BYDT. In contrast, TA517 × TA209 showed significant dominant gene action for ICOL, ECOL and SSC, whereas the gene action for these traits was not significant different from additivity for TA1160 × TA209. The mode of gene action for TA1160 × TA517 was additive for most the traits except for overdominance for SSC and L/D and recessive for ECOL.

Epistatic interactions between chromosome 1 (TA523) and chromosome 4 (TA517 and TA1160)

TA523, containing a 40-cM introgression to the bottom of chromosome 1 from *L. hirsutum*, has previously been shown to increase SSC, YDT, BYDT, ECOL and decrease FW and induce more rounded fruit (Bernacchi et al. 1998a, Monforte et al. 2000). Interactions among the QTLs on chromosome 1 and chromosome 4 were studied by crossing TA523 with TA517 and TA1160.

YDT, BYDT, FW, ER and L/D values of the double-NILs TA1160 × TA523 and TA517 × TA523 did not deviate from the expected value according to the additive model (Fig. 4). On the other hand, both double-NIL hybrids showed significant lower than expected values for ICOL , indicating negative epistatic interactions between the introgressions. Negative epistatic interactions were also detected for SSC and ECOL, but only in the TA517 × TA523 double-NIL.

Interactions among QTLs on chromosome 4 with genetic backgrounds and environments

TA1160 (*L. peruvianum* introgression) was crossed with four different inbred testers, TA209 (original genetic background), TA446, TA502 and TA1149, and evaluated in field trials in six locations around the world (Table 1). TA1160 showed significant effects on the agronomic traits (FW, B, YRD and BYRD) but not on the fruit traits (ECOL, ICOL, FS). Location (environment) had the most significant effect on all traits. In addition, the tester (genetic background) affected several agronomic traits (e.g. FW, SSC YDR and FS). However, despite the individual large effects observed for both environment and genetic background, significant interactions of the introgression with either environments or testers were not detected. QTL alleles on chromosome 4 from *L. peruvianum* maintain their effects across locations and testers. The only exception was for FS; TA1160 showed elongated fruits only in the TA209 and TA446 backgrounds but not in the TA502 and TA1149 backgrounds.

Combining introgressions from different wild species

Two introgressions were combined in a single double-NIL (TA1451) by crossing TA1150 (which carries an introgression on chromosome 1 from *L. chmiewleskii*, homologue to the introgression in TA523, and is known to increase SSC, YDR and BYDR; Paterson et al. 1990; Tanksley unpublished results) with TA1160 (Fig. 1). Hybrid NILs (TA1150 × TA209, TA1160 × TA209), heterozygous double-NIL (TA1451 × TA209) and control (TA209) were evaluated at six locations. Environmental effects were large and statistically significant for al-

Fig. 3 Gene action for chromosome 4 introgressions from *L. hirsutum* (TA517) and *L. peruvianum* (TA1160). Means for the traits of the NILs and crosses (TA1160, TA517, TA1160 × TA209 and TA517 × 1160) analyzed in Itahaca, N.Y. are expressed as the perentage of difference relative to the control (TA209). *Horizontal lines* indicate the expected value of the corresponding hybrid according to the additive model. *Dark vertical bars* indicate a significant deviation from additivity ($\alpha < 0.05$). The mode of gene action d/a) estimated from homozygous and hybrid NILs means is gen *below* each hybrid ▶

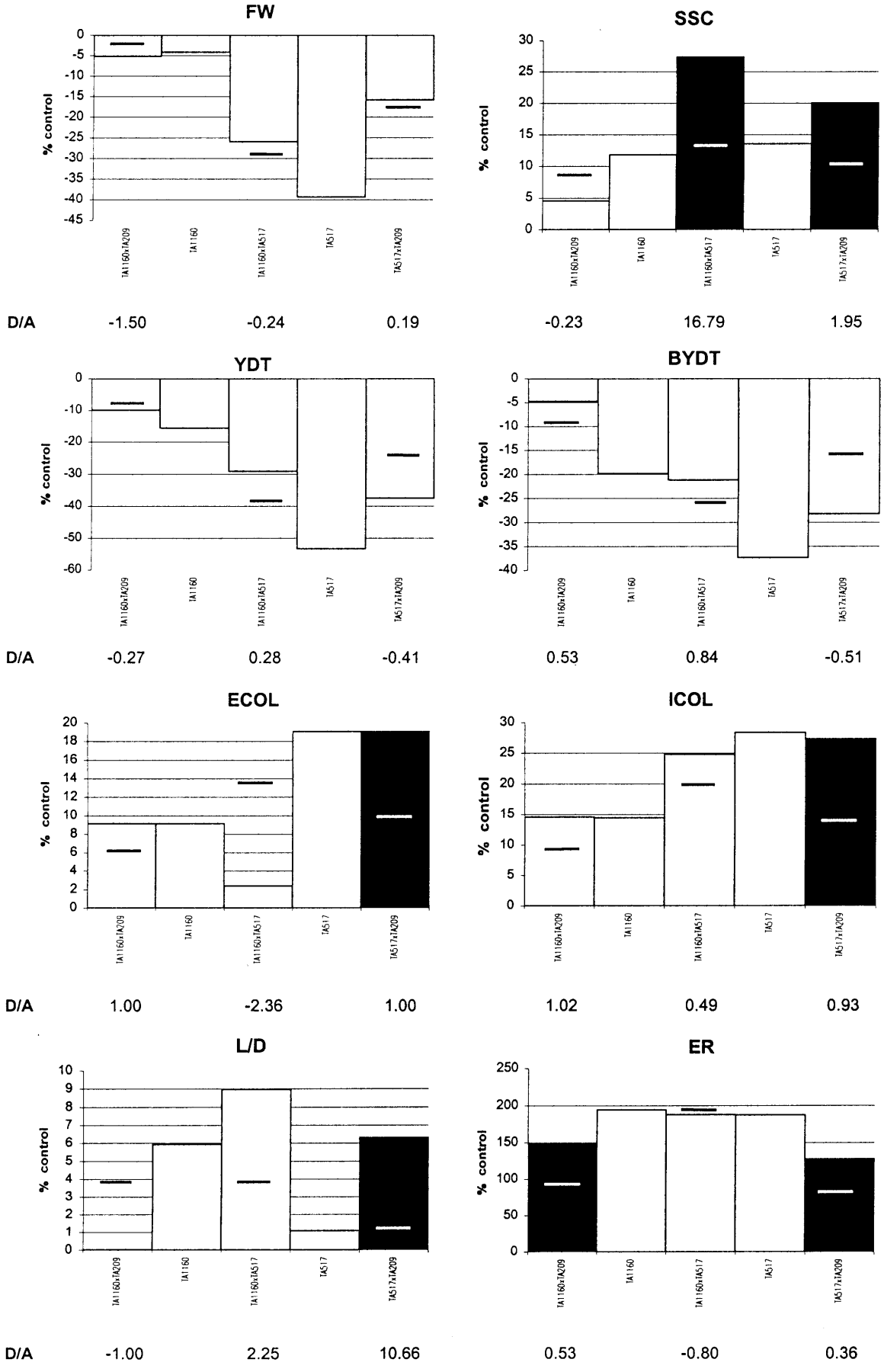

Fig. 3 Legend see page 577

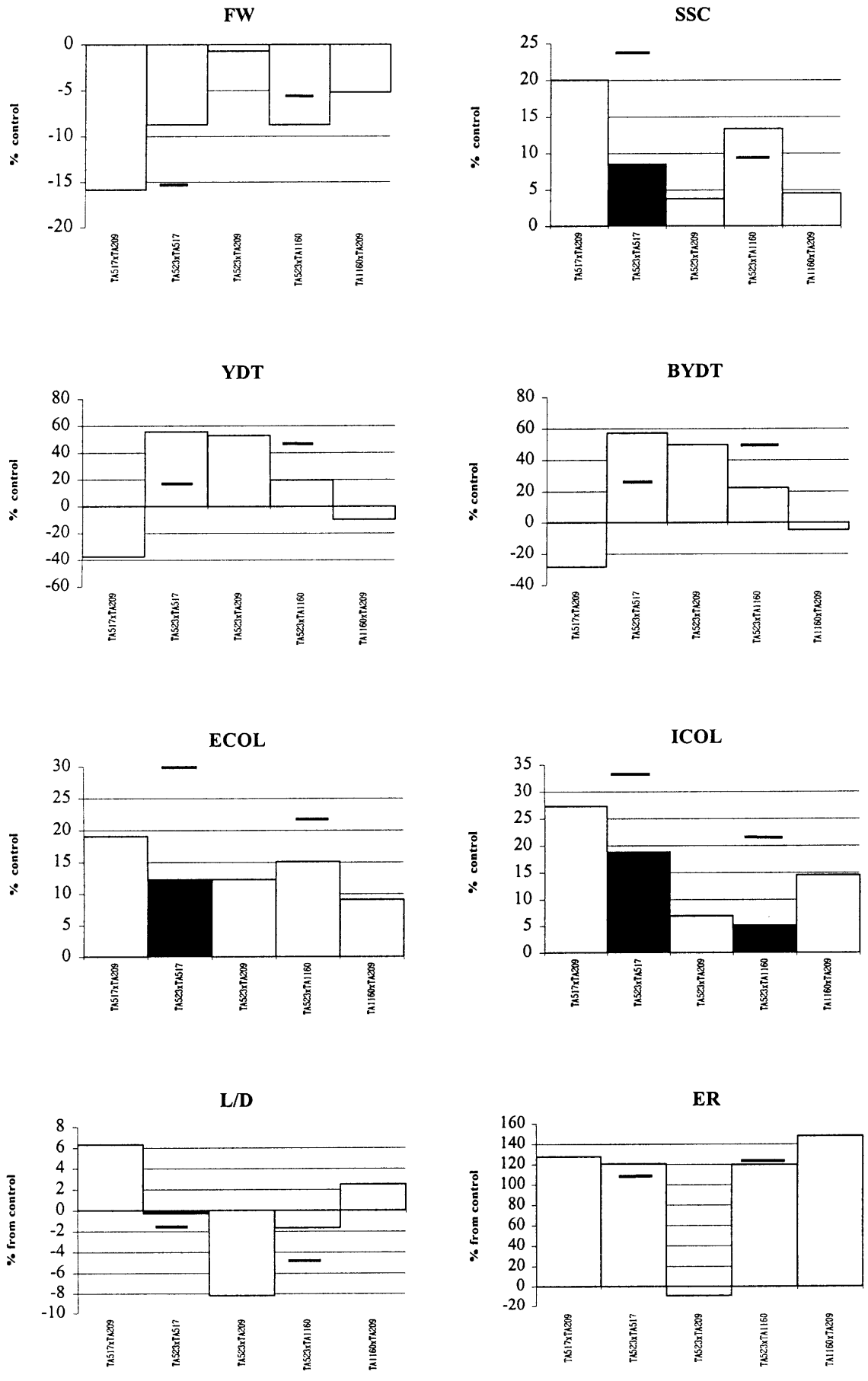

Fig. 4 Legend see page 580

Table 1 Significance (*P*) for the effects from ANOVA of TA1160 introgression on fruit traits (ECOL, ICOL and FS) and agronomic traits (FW, SSC, YRD ad BYRD) across testers and locations. The means across testers and locations are also shown

	ECOL	ICOL	FS	FW	SSC	YRD	BYRD
				P			
P4	0.242	0.171	0.251	<0.001	<0.001	0.008	0.452
Tester	0.254	0.342	<0.001	<0.001	<0.001	0.423	0.441
Location	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P4*Tester	0.354	0.222	0.003	0.591	0.591	0.594	0.573
P4*Location	0.842	0.081	0.821	0.732	0.732	0.152	0.393
Tester*location	0.974	0.264	0.242	0.641	0.641	0.392	0.322
P4*tester*location	0.857	0.112	0.304	0.202	0.202	0.883	0.954
Means across tester and locations							
TA209	2.89	2.72	3.18	74.2	4.9	87.31	419.42
TA1160 × TA209	2.86	2.80	3.22	68.04	5.19	80.37	409.14

Table 2 Significance (*P*) of the genetic and environmental effects from ANOVA for the interaction between the TA1160 and TA1150 introgressions. P4 indicates the effect of the *L. peruvianum* introgression on chromosomme 4 in TA1160. Chm1 indicates the effect of *L. chmielewskii* on the chromosomme 1 introgression in TA1150

	ECOL	ICOL	FS	FW	SSC	YRD	BYRD
				P			
P4	0.830	0.021	<0.001	<0.001	<0.001	0.152	0.698
Chm1	0.007	<0.001	<0.001	0.012	0.011	0.123	0.048
Location	<0.001	0.392	<0.001	<0.001	<0.001	<0.001	<0.001
P4*Chm1	0.500	0.552	0.590	0.246	0.832	0.415	0.612
P4*Location	0.240	0.244	0.783	0.618	0.866	0.851	0.897
Chm1*Location	0.480	0.466	0.008	0.330	0.136	0.029	0.090
P4*Ch1*Location	0.782	0.400	0.010	0.093	0.566	0.732	0.783

most all traits, but Genotype × Environment interactions were low and generally statistically non-significant. (Table 2). Both TA1160 (P4) and TA1150 (Chm1) introgressions showed significant effects for almost every trait (Table 2), however the direction of the effects was not always the same. Both introgressions increased SSC and significantly induced elongated fruits, whereas the direction of the effects was the opposite for FW and ICOL (Fig. 5). The TA1150 introgression decreased ECOL and ICOL, whereas the TA1160 introgression decreased FW and increased ICOL. Interactions between introgressions were not significant for any trait, indicating that the effects of both introgressions are additive (Table 2, Fig. 5).

Fine mapping of QTLs in the *L. hirsutum* chromosome 4 NIL

The introgressed segments for the chromosome 4 NILs (TA1160, TA517, IL4-4; Fig. 1) are sufficiently large to contain many genes. In an attempt to obtain a better characterization of the QTLs included in these region and to determine whether these multiple effects are due to pleiotropy or linkage, we studied a new set of recombinant lines (subNILs) with shorter introgressions than TA517 NIL (Fig. 1). SubNILs means were compared with the control TA209 to estimate QTL positions and effects, together with marker mean comparisons (Figs. 6, 7).

Plant weight (PW)

TA517 significantly increased PW by 125% (Fig. 6). TA1459, TA1463, TA1464, TA1465, TA1466, TA1467, TA1475 and TA1477 showed an average increment of 108% with respect to PW (Fig. 6). TA1463 was the shortest subNIL that still significantly increased PW. Together, these results are consistent with a single locus affecting plant weight near TG155, between TG555 and CT50 (18.2 cM). The estimated additive effect based on subNIL analysis for this locus (hereafter referred as *pw4.1*) was a = 54%, and the mode of gene action was partial recessive (d/a = –0.46, Fig. 8).

External color (ECOL)

TA517 increased significantly ECOL in both the homozygous and heterozygous conditions (Fig. 6). TA1475 was the only subNIL that showed a statistically significant improvement in ECOL at both locations, whereas TA1465, TA1466, TA1467, TA1473 and TA1477, carrying introgressions between CT50 and CP57 (Fig. 1), sig-

◀ **Fig. 4** Epistatic interactions between chromosome 1 (*TA523*) and chromosome 4 (*TA517* and *TA1160*) introgressions. Means for the traits of the NILs and corresponding hybrids (TA1160 × TA209, TA517 × 209, TA523 × TA209, TA1160 × TA523 and TA1160 × TA523) analyzed in Ithaca, N.Y. are expressed as a percentage relative to the control (*TA209*). *Horizontal lines* indicate the expected value of the double NIL according to the additive model. *Dark vertical bars* indicate significant deviation from additivity, i.e. significant epistasis ($\alpha < 0.05$)

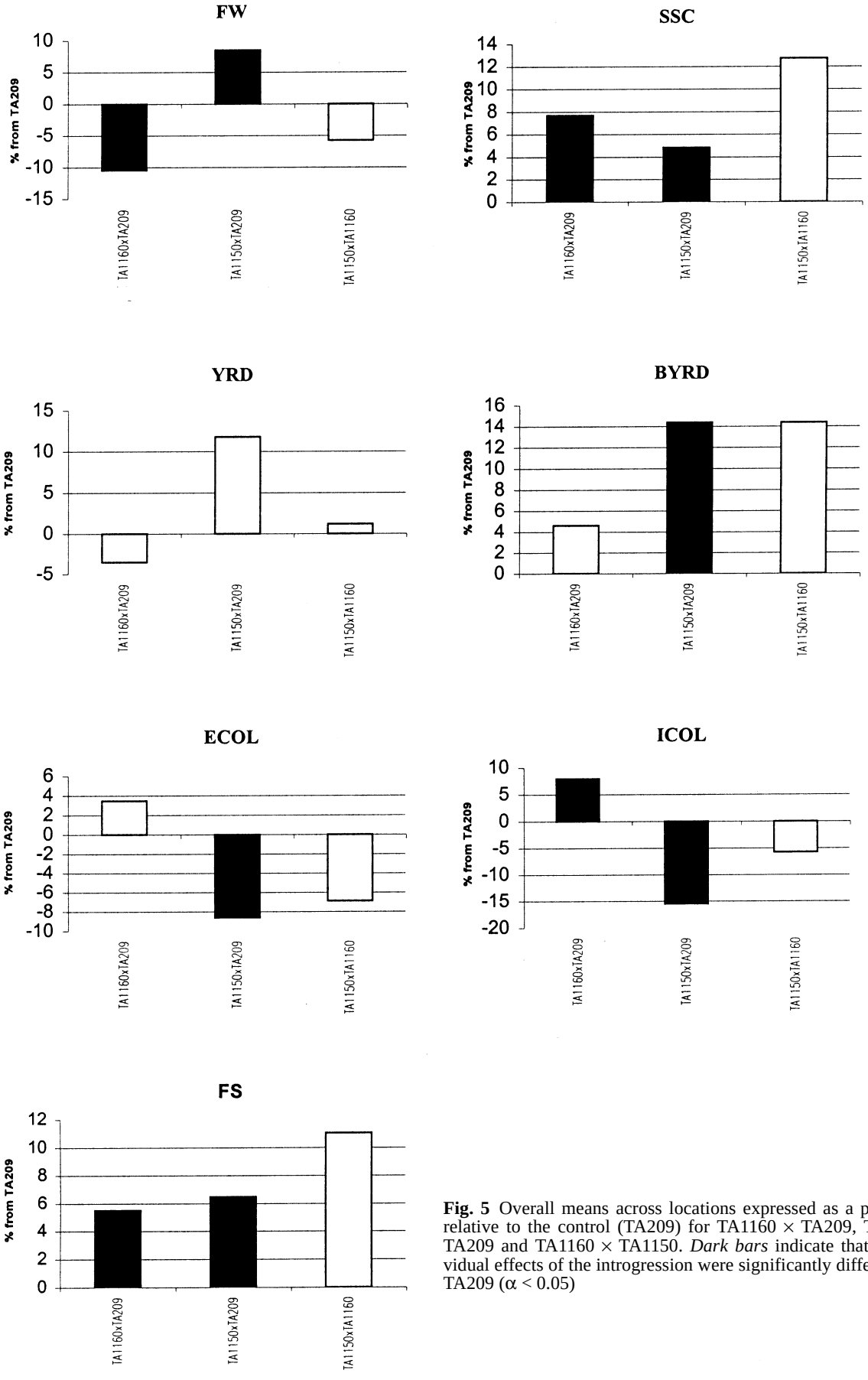

Fig. 5 Overall means across locations expressed as a percentage relative to the control (TA209) for TA1160 × TA209, TA1150 × TA209 and TA1160 × TA1150. *Dark bars* indicate that the individual effects of the introgression were significantly different from TA209 ($\alpha < 0.05$)

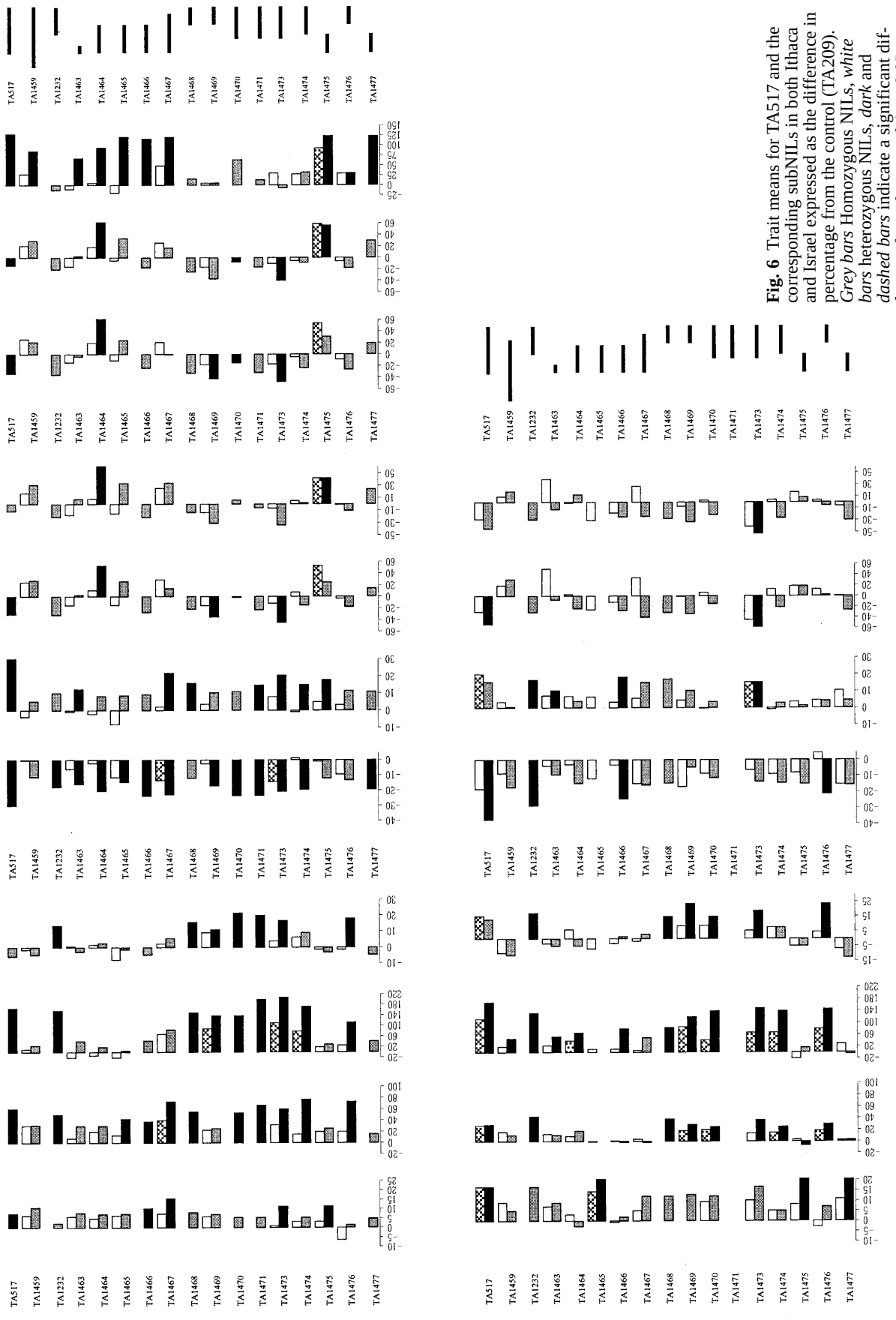

Fig. 6 Trait means for TA517 and the corresponding subNILs in both Ithaca and Israel expressed as the difference in percentage from the control (TA209). *Grey bars* Homozygous NILs, *white bars* heterozygous NILs, *dark* and *dashed bars* indicate a significant difference from the control ($\alpha < 0.05$, Dunnet contrast). Dark bars at the right indicate the extent of the introgression

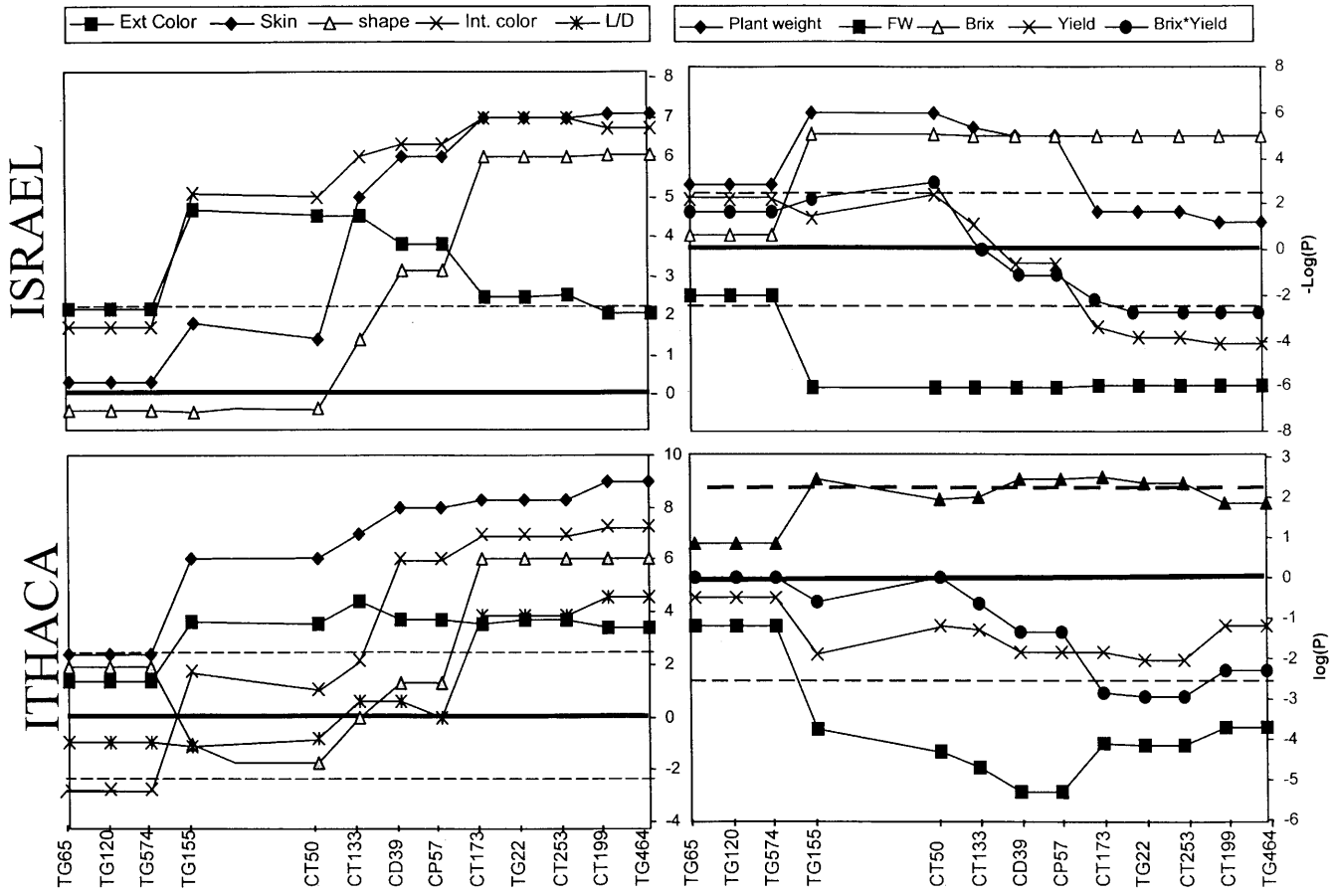

Fig. 7 Significance [Log(p)] of the marker means comparisons for various traits in the subNIL population in both Itahaca and Israel. Positive values of Log(p) indicate that *L. hirsutum* alleles increased the trait mean, whereas negatie values mean that *L. hirsutum* alleles decreased the trait mean

nificantly increased ECOL in at least one location (Fig. 6). The failure to detect significant effects in other NILs carrying similar introgressions effects among locations may be attributable to sampling errors in trial color evaluations and/or differences in the ripening stage of the fruits among NILs and locations at evaluation time. Furthermore, the high ECOL of TA1475 has been reconfirmed in subsequent trials (data not shown). The results are consistent with a single locus (hereafter *ecol4.1)* near CT133 between CT50 and CD39 (5.5 cM). The additive effect (Israel/Ithaca) was relatively modest (a = 4.7/7%), and the mode of gene action additive (d/a = 0.24/0.28) (Fig. 8).

Internal color (ICOL)

TA517 increased ICOL in both the homozygous and heterozygous conditions (Fig. 6). The TA1232, TA1468, TA1470, TA1473, TA1474, TA1476 subNILs sharing introgressions between CP57 and TG464 (13.5 cM, Fig. 1) increased significantly ICOL at both locations (Fig. 6). In addition, TA1467 (with an introgression from TG555 to CT199, Fig. 1), TA1465, TA1466 (both with an introgression from TG555 to CT173, Fig. 1), and TA1471 (introgression between CP57 and TG464, Fig. 1) significantly increased ICOL in Israel (Fig. 6). At the time of harvesting, ICOL was more difficult to evaluate in Ithaca than in Israel, which may explain some of the discrepancies between locations. The ripening stage of the plants was different at harvest between locations – in Israel the ratio of mature/green fruits was higher than in Ithaca – which could have influenced the color evaluation because the accumulation of color is achieved during the last stages of ripening. Also, the weather conditions generally are colder in Ithaca and the sunlight is less intense,both of which influence tomato ripening and color development (Grierson and Kader 1986). Furthermore, ICOL was evaluated in Israel pre- and postharvest by two independent observers with similar results (R^2 = 55%), making the Israel data more reliable. As final proof, TA1467 showed high ICOL in subsequent replicated experiments (data not shown), supporting the Israel data.

The results from Israel are consistent with a single locus controlling the ICOL (hereafter *icol4.2*) located between the CP57 and CT173 markers (2.3-cM interval). The additive effect was strong in Israel (a = 30%) and the gene action additive (d/a = 0) (Fig. 8).

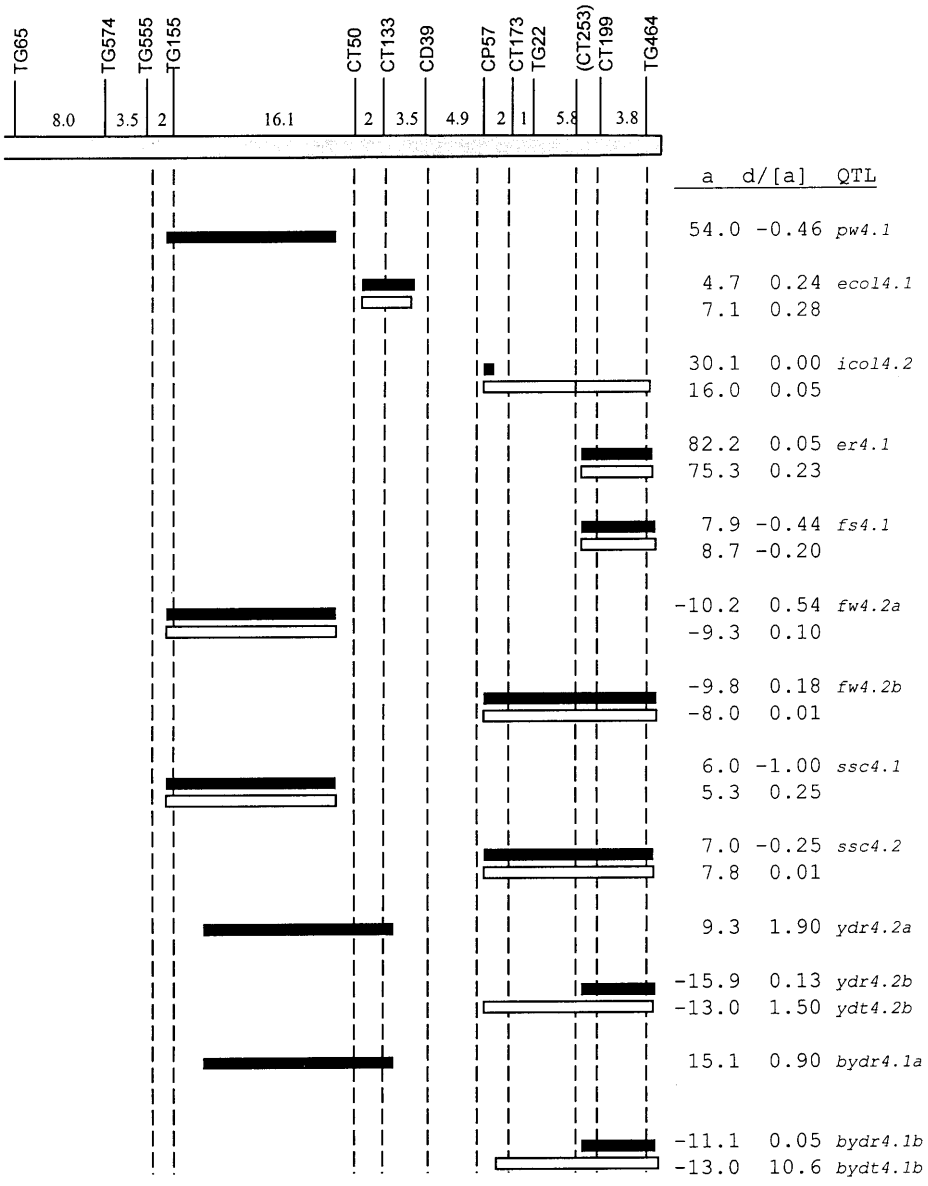

Fig. 8 Summary of the deduced map position of the QTL located within the TA517 introgression based on subNIL analysis. *Black bars* indicate the position according to Israel data, and *white bars* indicate the position according to Ithaca data. The estimate of additive effects (a) as a percentage from the control and ode of gene action (d/[a]) is also shown

Epidermal reticulation (ER)

TA517 showed ER in both the 73, TA1474, TA1476, with an introgression between CP57 and TG464 (Fig. 1), showed ER consistently in both locations (Fig. 6). These results suggest that there is a locus for ER (*er4.1*) between CT253 and TG464 (3.8 cM) with major effects (a = 82/75%) and that the mode of gene action is strictly additive (d/a = 0.05/0) (Fig. 8). TA1459, TA1463, TA1464 and TA1466 also showed ER in Ithaca, but much milder that the previous NILs. Thus, the possibility of another locus affecting ER with minor effects in another position within the introgression can not be rejected.

Fruit shape (FS)

TA517 only showed elongated fruits when heterozygous (Fig. 6), whereas TA1232, TA1468, TA1469, TA1470, TA1473 and TA1476 yielded elongated, oval fruit when homozygous in both locations (Fig. 6). The L/D ratio of TA517 fruit was L/D = 1.17, which was similar to that of TA209 fruit (L/D = 1.15), whereas TA517 × TA209, TA1232, TA1468, TA1469, TA1470, TA1471, TA1473, TA1474 and TA1476 fruit (average L/D = 1.24) were significantly more elongated than TA209 fruit. TA517 yielded very small fruit, smaller than that of the subNILs (see next paragraph); the low L/D ratio of TA517 fruit may due to the fact that the fruit were very small. The data is consistent with a single locus (*fs4.1*) located between CT253 and TG464 (3.8 cM), the same interval where *er4.1* was located. The additive effect was a = 7.9/8.7%, and the mode of gene action was partially recessive (d/a = –0.2/–0.44).

Fruit weight (FW)

TA517 produced fruit 40% lighter than TA209 (Fig. 6). In Israel most subNILs also produced fruit significantly smaller than TA209 (Fig. 6), yet larger than TA517. All markers in the introgression showed significant effects on FW in both locations (Fig. 7). These results, and the fact that subNILs with non-overlapping introgressions (e.g. TA1463 and TA1469 in Israel) showed significantly lower FW than TA209, suggest that there are at least two FW loci located on the TA517 introgressed segment: *fw4.2a* located between TG555 and CT50 (18.2 cM), with additive effect a = –10/–9% and a partial dominant mode of gene action (d/a = 0.54/0.1), and *fw4.2b* between CP57 and TG464 (13.5 cM), with a = –9.8/–8% and the additive mode of gene action (d/a = 0.18/0.01, Fig. 8). The fact that the FW of the subNILs was in general lower than that of TA209 but higher than that of TA517 suggests that the effects of the *fw4.2a* and *fw4.2b* are additive.

Soluble solids concentration (SSC)

TA517 significantly increased SSC in both the homozygous and heterozygous conditions (Fig. 6). Similarly to FW, most of the homozygous subNILs had a significant higher SSC than the TA209 control (Fig. 6) but lower than TA517. This fact and the observation that subNILs carrying non-overlapping introgressions increased SSC (as TA1463 and TA1468 in Israel) also suggest that there are at least two SSC loci within the TA517 introgressed segment: *ssc4.1* between TG555 and CT50 (18.2 cM), with additive effects a = 6/5.3% and a mode of gene action variable between locations (d/a = –1/0.25), and *ssc4.2* between CP57 and TG464 (13.5 cM), with additive effects a = 7/4.8% and an additive mode of gene action (d/a = –0.28/–0.01, Fig. 8).

Total yield (YDT) and red yield (YDR)

TA517 significantly decreased YDT and YDR (Fig. 6). TA1464 and the TA1475 × TA209 hybrid significantly increased YDR, whereas TA1469 and TA1473 significantly decreased YDR in Israel (Fig. 6). Other subNILs did not show significant effects on these traits, which may be explained by the high variability of the traits. Comparisons of the marker means indicate that markers on the distal part of the chromosome are associated with reduced yield, whereas markers towards the centromere are associated with increased yield (Fig. 7). No significant effects on YDT were detected among the subNILs in Ithaca, which may be attributed to the smaller sample size of the families as suggested by subthershold effects detected by marker mean comparison (Fig. 7). Data from Israel suggested that there are at least two yield QTLs located in the opposite extremes of the introgression (Fig. 8): *ydr4.2a* (between TG155 and CD39, 21.5 cM), with positive additive effects (a = 9.3%) and a dominant made of gene action (d/a = 1.9), and *ydr4.2b* (between CT253 and TG464, 3.8 cM) with negative additive effects (a = –15.85%) and an additive mode of gene action (d/a = 0.13). Other NILs sharing the same introgression as TA1475 and TA1464 (e.g. TA1465, TA1466) were not associated with increased yield, rendering the assumptions about *ydr4.2a* inconclusive. Further experiments must be carried out to verify the presence of that QTL in the TA517 introgressions.

*Brix*Yield (BYDT and BYDR)*

TA1464 and TA1475 and the TA1475 × TA209 hybrid significantly increased BYDT and BYDR in Israel (Fig. 6). These data together with the comparison of the marker means (Fig. 7) suggest that there is a BYDR locus (*bydr4.1a*) between TG155 and CD39 (21.5 cM) with positive additive effects (a = 15.07 %) and a dominant mode of gene action (d/a = 0.9). *bydr4.1a* may be the combined effect of the *ydr4.1a* and *ssc4.1a* loci (Figs. 7 and 8). TA1473 significantly decreased BYDR and BYDT in Israel, suggesting the presence of a second locus (*bydr4.1b* or *bydt4.1b*) between CT253 and TG464 (3.5 cM) with negative additive effects (a = –11/–13 %) and an additive mode of gene action, d/a = 0.05/ 0.96, probably induced by the negative effects of *ydt4.1b/ ydr4.1a*. The increased BYDR in TA1475 and TA1464, as with YDR, was not observed in similar subNILs; again, the effects of this QTL need to be verified by further experimentation.

Discussion

Common allelic effects from green-fruited species

QTL alleles with favorable effects from an agronomical perspective have been reported in several independent studies involving different wild tomato species including *L. pennellii* (Eshed and Zamir 1995), *L. pimpinellifolium* (Tanksley et al. 1996), *L. hirsutum* (Bernacchi et al. 1998b) and *L. peruvianum* (Fulton et al. 1997). However, due to confounding factors, such as the use of different sets of markers, different environments and sampling errors in the estimate of the genetic effects, it has not been possible to directly compare allelic effects among those different wild species. To remedy this situation, a set of NILs has been constructed in which direct comparisons can be made in common experiments. NILs with introgressions in the bottom end of chromosome 4 from the green-fruited species *L. pennellii*, *L. peruvianum* and *L. hirsutum* showed significant effects for most of the same traits (Fig. 2), including a number of important agronomic traits such as SSC, FW, YDT and YDR. The three NILs also showed effects on fruit traits that were opposite to those predicted by the donor wild parent phenotype. *L. pennellii*, *L. peruvianum* and *L. hirsutum* pro-

duce green, round and smooth-skinned fruit, however the NILs derived from these species (IL4-4, TA1160 and TA517) increased ECOL, ICOL, had rough skin (epidermal reticulation, ER) and elongated oval fruit. These findings reinforce the usefulness of introgressing chromosomal segment from wild species to reveal novel and unexpected phenotypic variation.

Although the direction of the effects was similar among NILs, some facts suggest that there is genetic variability for the traits among the NILs: (1) the magnitude of the effects in TA1160 and TA517 were different for FW, FS, ECOL and PW (Fig. 2); (2) the dominance deviation of the respective hybrids and/or the magnitude of the epistatic interactions with the introgression on chromosome 1 included in TA523 were clearly different for traits such as SSC and ECOL (Figs. 3, 4). This genetic variability can be used to design different breeding programs with these NILs and provide the basis for the biological characterization of the QTLs included within them.

QTL × environment interaction

Genotype × environment (G × E) interaction is a common phenomenon encountered in quantitative genetics and plant breeding (Falconer 1989; Hallauer and Miranda 1988). Ironically, QTL × E interactions are not encountered nearly as frequently in QTL studies (Stuber et al. 1992; Ragot et al. 1995; Melchinger et al. 1998; Lübberstedt et al. 1998; Austin and Lee 1998). This discrepancy may result from the statistic tests used to detect QTL × E interactions as these are less powerful that those for G × E interactions (Melchinger et al. 1998). Consequently, the lack of detection of QTL × E interactions in segregating populations is not a good indicator of the actual QTL × E interactions.

Estimating G × E interactions using NILs provides a more robust estimate of QTL × E interactions because the latter can then be studied with the same power as G × E interactions. While the magnitude of the QTL effects estimated in the GB trials differed among environments, the QTL × E interactions were minimal and significant only for FS and YDR. The QTLs on chromosome 1 and 4 had been identified previously in advanced backcross populations in replicated trials among several locations and were selected because of their potential stable effects across environments; hence the QTLs studied here are not a random sample, which may explain the low level of QTL × E interactions observed.

QTL × genetic background interaction

Studies in maize and barley have shown that QTL × genetic background interactions can be important (Doebley et al. 1995; Melchinger 1998; Toojinda et al. 1998). The magnitude of QTL × G interactions is a critical issue if QTL alleles discovered in one genetic background are to be transferred via marker-assisted selection into another genetic background. Specifically, low QTL × G interactions are desirable because: (1) the QTL allele could be transferred to a broad range of cultivars; (2) the tester may change over time in breeding programs, so the introgressed QTL allele could be useful over a longer time period. In the current experiment, the testers showed significant effects for traits as FS, FW and SSC, but the NIL × tester interactions were not significant, except for FS (Table 1). These results demonstrate that the QTLs alleles from *L. peruvianum* included within the TA1160 introgressions may be used in a broad range of breeding programs involving different elite tomato cultivars and that TA1160 likely could be used in future breeding programs for developing new inbred and hybrids.

QTL allele interactions among species

The exploitation of wild germplasm genetic resources requires the identification and introgression of alleles with favorable effects on specific traits as well as the exploitation of the new genetic interactions arising after the introgression into the elite modern cultivars. Thus, once a wild QTL allele is successfully incorporated into elite germplasm, the next step is to combine it with other wild QTL alleles located in the same or other genomic region. In these cases, specific QTL × QTL allele interactions (inter- and intra-locus) must be either positive or at least not negative.

Intra-loci interactions were studied in the hybrid TA1160 (*L. peruvianum*) × TA517 (*L. hirsutum*). The most remarkable result was that the hybrid TA1160 × TA517 showed heterosis for SSC (d/a = 16.79) compared with the additive (d/a = –0.23) and dominant (d/a = 1.95) gene action of their respective hybrids with TA209.

The study of inter-loci (epistasis) interactions showed that *L. hirsutum* alleles on chromosomes 1 (TA523) and 4 (TA517) affecting SSC and ECOL interacted negatively, whereas *L. peruvianum* alleles on chromosome 4 (TA1160) did not interact with any of the *L. hirsutum* (TA523) and *L. chmiewleskii* (TA1150) alleles on chromosome 1 for any of the studied traits (Fig. 4, Table 2). These results suggest that the genetic interactions could be different depending on the wild accession alleles combined. Less-than-additive epistatic interaction can occur when two introgressions are combined (e.g. Eshed and Zamir 1996), but they could be avoided or minimized by combining the appropriate wild alleles. Furthermore, the combination of two introgressions can also be useful to minimize the undesirable effects and increase the breeding potential. For example, pyramiding QTLs from TA1160 and TA1150 yielded two major benefits (Fig. 5):

1) SSC improvement. The average increment of SSC for each individual NIL TA1160 and TA1150 compared with TA209 control was 6% and 5%, respectively. The increment in the double-NIL TA1160+TA1150

was 18%. The double NIL also showed a combined increase of 14% on BYRD.

2) Compensation of deleterious undesirable effects. TA1160 reduced FW and YDR, whereas TA1150 reduced ICOL and ECOL. These undesirable effects were, at least in part, compensated for in the double-NIL as TA1160 + TA1150 did not show significant statistic differences in YDR, FW, ECOL and ICOL when compared with the TA209 control, having a better agronomic performance than any of the original NILs.

The results presented in the current study indicate that the exploitation of the genetic interactions (intra- and inter-locus) justifies the effort to develop NILs carrying the same introgression from different wild species.

Fine mapping of QTLs from *L. hirsutum* on chromosome 4

The distal region of chromosome 4 is a very complex genetic region affecting several important agronomic and fruit traits (Eshed and Zamir 1995; Tanksley et al. 1996; Bernacchi et al. 1998a,b; Fulton et al. 1997; Figs. 3 and 4, this report). By substitution mapping it was possible to distinguish between linkage and pleiotropy for several loci (Fig. 8). Thus, *icol4.2*, *ecol4.1*, *er4.1, fs4.1* and *pw4.1* can be clearly separated as single independent loci in the introgressed region.

In addition, the fine mapping results show that the agronomic traits (FW, SSC, YDR, YDT, BYDR) are controlled by multiple loci located within the 50-cM introgression of TA517. Two genetic loci are postulated for most agronomic traits (e.g. *fw4.2a*, *fw4.2b*, *ssc4.1*, *ssc4.2*, *ydr4.2a*, *ydr4.2b*, *byr4.1a*, *byr4.1b*), but we can not reject the possibility that there actually are more loci within this region affecting some of those traits. The loci affecting these traits are grouped in two clusters, *fw4.2a-ssc4.1-ydr4.2a-byr4.1a* and *fw4.2b-ssc4.2-ydr4.2b-byr4.1b*, which are located in opposite regions of the introgression (Fig. 8), but no recombination within any of those cluster was observed. In most of the previous investigations FW and SSC have been found to be usually negatively correlated, and FW and SSC QTLs often map on similar genomic regions (Goldman et al. 1995; Tanksley et al. 1996; Fulton et al. 1997). Similarly, YDR and SSC usually are negatively correlated (Stevens and Rudich 1978). A reduction in FW could lead to an increase in the sugar concentration in the fruit and also a reduction of yield. Therefore, these effects may be the consequence of pleiotropy rather than several linked genes, although a more thorough study is necessary to discern between these hypotheses.

Both pairs of loci, *ssc4.1-ssc4.2* and *fw4.2a-fw4.2b*, seem to act additively, as suggested by the fact that subNILs carrying only one of them showed larger fruit and lower SSC than TA517 but smaller fruits and higher SSC than TA209. The genetic dissection of YDR loci revealed more complexity. TA517 homozygous subNIL decreased YDR by 32 % compared with the TA209 control in Israel. However, an increase of YDR (above TA209) was found to be associated with the interval between TG155 and CT50 (*ydr4.2a*), whereas the negative effects were associated with the most distal region of the chromosome between CT253 and TG464 (*ydr4.2b*). The interaction between the two loci seems to be very complex, *yrt4.2b* (or any other locus linked to it) seems to repress the positive effects of *ydr4.2a* when both loci are together in the TA517 original NIL.

New loci for fruit color

Among the traits we studied, fruit color is the one for which our knowledge of the biochemical pathways and genetic control is the most complete. The red color of ripe tomatoes is due to the accumulation of the carotenoid lycopene (Laval-Martin et al. 1975; Cunningham and Gantt 1998)). SubNILs for chromosome 4 showing an increase in color also accumulate more lycopene (Monforte and van der Hoeven, unpublished results), therefore, genes coding for enzymes involved in the carotenoid pathway could be candidate genes for the QTLs affecting color. For example, Ronen et al. (1999) found that the orange fruit color of the tomato mutant *Delta* is caused by a mutation in the lycopene δ-cyclase gene, whose enzymatic product converts lycopene into δ-carotene, thereby giving the orange color. In the mutant *Delta*, lycopene δ-cyclase is not down-regulated as in the normal tomato, thus preventing the accumulation of lycopene and resulting in the accumulation of δ-carotene. Neither the lycopene cyclases, other genes known to be involved in the carotenoid pathway, such as phytoene synthase, phytone desaturase and zeta-carotene desaturase nor other ripening-related genes map within the introgression in chromosome 4 (Tanksley et al. 1992; Bartley and Scolnick 1993; Ronen et al. 1999; Giovanonni et al. 1999; D. Zamir, personal communication; Monforte and van der Hoeven, unpublished results), consequently ruling out the possibility that the high color observed in the chromosome 4 NILs is due to allelic variation of a gene involved in the carotenoid pathway. Two independent loci affecting fruit color were detected within the introgression, *ecol4.1* and *icol4.2*, indicating that color accumulation in the external and internal organs of the fruit is, at least in part, under different genetic control. Fulton et al. (1997) also detected loci affecting ECOL but not ICOL in an AB population from a cross between *L. esculentum* and *L. peruvianum*. *ecol4.1* and *icol4.2* could be newly discovered regulatory genes of the carotenoid pathway genes predominantly affecting quantitative aspects of the pathway and possibly up-regulating enzymes in the upstream steps of the biosynthetic pathway.

Implications for QTL analysis

The number of QTLs detected using the common segregation populations varies from 1 to 18, but is usually less

than 8 (Tanksley 1993; Kearsey and Farquhar 1998). This might suggest that quantitative traits are usually controlled by a few QTLs, however it might also reflect the limitations of QTL mapping in such populations with the current experiment designs. The analysis of NILs carrying small introgressions of a donor genome supports the second argument. For example, Eshed and Zamir (1995) detected 23 QTL for SSC using an introgression line population from *L. pennellii*, whereas only 4–13 were detected using segregating populations (Paterson et al. 1988, 1991; Goldman et al. 1995; Fulton et al. 1998; Grandillo and Tanksley 1996; Tanksley et al. 1996; Bernacchi et al. 1998a). Similarly, Bernacchi et al. (1998b) found that NILs showed significant effects for traits not detected in the previous BC_3 population from which those NIL were developed (Bernacchi et al. 1998a). The results from the current study support these last observations; for example, no fruit shape QTL had been reported previously in the bottom end of chromosome 4 in any tomato interspecific cross (Grandillo et al. 1999), whereas IL4-4, TA517 and TA1160 have a FS QTL within their respective introgressions on chromosome 4, which makes *fs4.1* a newly discovered QTL for fruit shape.

Moreover, a distinction between pleiotropy and closely linked QTLs using segregating population is normally not possible due to the low resolution of the QTL mapping (Maguin et al. 1998; Lebreton et al. 1998), also resulting in an underestimate of the number of QTLs affecting a trait. Recently, fine mapping experiments have shown that it is not uncommon that a genomic region displaying QTL activity contains several tightly linked QTLs (Eshed and Zamir 1995; Tunistra et al. 1998; Graham et al. 1998). In agreement with these studies, we report a minimum of two loci for SSC and FW and YRD and BYRD included in the 50-cM TA517 introgression. Although this is not always necessarily the case (e.g. *fs8.1* and *fw2.2* are most likely single loci affecting fruit shape and size in tomato, respectively; Grandillo et al. 1996; Alpert and Tanksley 1996), the fine-mapping studies suggest that the hypothesis of linkage of QTLs can not be rejected *a priori*.

Recently, there is growing concern about the reliability and accuracy of the QTL effects estimated in the common segregating populations. It is well-documented that QTLs effects are generally overestimated (Lande and Thompson 1990; Melchinger et al. 1998). As Kearsey and Farquhar (1998) remark "Actual data must be viewed with due awareness of these biases and limitations on reliability". In the present report, QTL effects detected in AB populations (Fulton et al. 1997; Bernacchi et al. 1998a) have been verified in several tomato wild species, thereby validating the data obtained in the previous populations. These results reinforce the idea that segregating populations are a good starting point to detect QTLs. With regards to the accuracy of the estimates of the effects, our results support the observations of Lande and Thompson (1990) and Melchinger et al. (1998). One added problem to the estimation of the QTL effects in the bottom region of the chromosome 4 is that, as revealed by the fine mapping experiment, this region contains multiple QTLs affecting several agronomic and fruit traits. The estimate of the genetic effects of a region with multiple QTLs would depend on the genetic interactions among them so that estimates based on the effect of the region covering all of them could not be valid for each single QTL. For example, the gene action of the SSC QTLs included in the TA517 introgression was expected to be dominant based on previous experiments (Bernacchi et al. 1998a,b) and also the current study (Fig. 2). However, fine mapping suggested that the actual mode of gene action is additive, or even recessive (Fig. 8). This incongruity was not found in every trait; for example, the mode of gene action of *fw4.2a* and *fw4.2*b was additive, as expected.

The low accuracy of the estimation of the QTL effects in a segregating population is, at least in part, due to the great genetic complexity underlying some quantitative traits. Early generations are efficient material in which to detect genomic regions displaying QTL effects. However, the estimation of genetic effects are not so accurate in such populations and must be further evaluated in more advanced generations.

Wild germplasm and breeding

With regards to the current study, the introduction of QTL alleles from wild germplasm into a modern cultivar has been achieved after following a series of steps:

1) Genomic regions carrying putative favorable QTL alleles were detected using molecular markers and QTL analysis from tomato wild species *L. peruvianum* (Fulton et al. 1997) and *L. hirsutum* (Bernacchi et al. 1998a).
2) The genomic region carrying the desirable effect was isolated by means of the development of near-isogenic lines (NILs) carrying known introgressions from the wild donor.
3) Selected NILs were tested across several environments and elite genetic backgrounds. The effects were consistent for most traits across locations and elite genetic backgrounds, demonstrating the suitability of exotic germplasm as a source of useful and stable alleles.
4) QTL alleles from two different wild species were combined and their effects showed additivity. Furthermore, the negative effects showed for one of the introgressions were partially compensated by the other.
5) Undesirable linkage drag has been broken by developing new recombinant lines (subNILs). Thus, TA1467 showed high SSC, increased in ICOL and ECOL, reduced ER, did not have a significant reduction in YRD and showed only minimal effects on FW.

The favorable effects of wild QTL alleles and the possibility of combining QTL alleles from different wild species open new perspectives in plant breeding. It is now

feasible to exploit more efficiently hidden genetic variation in wild germplasm and to combine derived alleles in elite cultivars. Even though the development of NILs requires a large amount of work and resources, the new possibilities in breeding make this effort worthwhile as the pay-off can be multiplicative.

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