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D. Bernacchi · T. Beck-Bunn · Y. Eshed · J. Lopez
V. Petiard · J. Uhlig · D. Zamir · S. Tanksley

Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*

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Abstract Advanced backcross QTL (AB-QTL) analysis is a new strategy for studying the effect of unadapted alleles on the agronomic performance of elite cultivated lines. In this paper we report results from the application of the AB-QTL strategy to cultivated tomato using the wild species *Lycopersicon hirsutum* LA1777 as the donor parent. RFLP genomic fingerprints were determined for 315 BC_2 plants and phenotypic data were collected for 19 agronomic traits from approximately 200 derived BC_3 lines which were grown in replicated field trials in three locations worldwide. Between 1 and 12 significant QTLs were identified for each of the 19 traits evaluated, with a total of 121 QTLs identified for all traits. For 25 of the QTLs (20%) corresponding to 12 traits (60%), the *L. hirsutum* allele was associated with an improvement of the trait from a horticultural perspective, despite the fact that *L. hirsutum* is overall phenotypically inferior to the elite parent. For example, *L. hirsutum* has fruit that remains green when ripe (lack of red pigment) yet alleles were found in this species that significantly increase red color when transferred into cultivated tomatoes. Wild alleles were also associated with increases in total yield and soluble solids (up to 15%) and brix × red yield (up to 41%). These results support the idea that one cannot predict the genetic potential of exotic germplasm based on phenotype alone and that marker-based methods, such as the AB-QTL strategy, should be applied to fully exploit exotic germplasm.

Key words Molecular breeding · Germplasm utilization · Quantitative traits

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D. Bernacchi · S. Tanskley (✉)
Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca, NY 14853-1902, USA,
Fax: +1 607-255-6683
E-mail: sdt4@cornell.edu

T. Beck-Bunn · J. Uhlig
Seminis Vegetable Seeds, 37437 State Highway 16, Woodland, CA 95695, USA

Y. Eshed · D. Zamir
The Faculty of Agriculture, Department of Field Crops, Hebrew University of Jerusalem, Kennedy Lee Building, Rm 222, Box 12, Rehovot 76-100, Israel

J. Lopez
Nestlé Research and Development Center, Apartado 435, E-06080, Badajoz, Spain

V. Petiard
Centre Recherche Nestlé, 101 Avenue Gustave Eiffel, 37390 Notre Dame D'OE, Tours, France

Introduction

DNA marker technology has greatly enhanced our ability to study the genetic factors underlying continuous phenotypic variation. Over the past decade there have been numerous reports on the use of DNA markers for the identification of quantitative trait loci (QTLs) (Tanksley 1993; Paterson 1996). These studies have shown that the majority of quantitative traits tend to be controlled by a few QTLs with major effects plus additional minor QTLs of lesser effect (Tanksley 1993). Some examples of QTL studies include growth in *Populus sp.* (Bradshaw and Stettler 1995), weight gain in mice (Horvat and Medrano 1995), yield components in rice (Xiao et al. 1995, 1997), and many traits in tomato (Paterson et al. 1988, 1990; de Vicente and Tanksley 1993; Grandillo and Tanksley 1996). This discovery has important implications for plant breeders working the quantitative traits because QTLs of major effect should be most amenable to manipulation as discrete units of simple Mendelian inheritance via marker-assisted selection.

QTL analysis can be used to dissect genetic variance in populations derived from crosses both within species as well as between elite and exotic or wild germplasm. Since the majority of our seed banks are comprised of

exotic germplasm, one can ask what role QTL mapping may play in the utilization of these genetic resources. Historically, in tomato and in most other crops, wild genetic resources have not been used in the improvement of quantitative traits of agronomic importance because of the difficulties associated with regaining adaptation and quality. Currently, a reduction of linkage drag and the recovery of the recurrent parent genotype can be facilitated by marker-assisted selection (MAS) (Tanksley et al. 1989). Also wild germplasm, in general, has not been viewed as harboring useful allelic variation on account of its interior phenotype. Instead, breeders have relied on repeated intercrossing of adapted elite materials to improve quantitative traits; this has led to the narrow genetic base characteristic of many crops (Ladizinsky 1985; Miller and Tanksley 1990; Wang et al. 1992).

QTL studies in plants have shown that the phenotype of a plant is not always a good predictor of its genetic potential. This is especially true in crosses between wild and cultivated species (de Vicente and Tanksley 1993; Eshed and Zamir 1995; Xiao et al. 1997). For example, de Vicente and Tanksley (1993) in an F_2 population of a cross between the wild tomato species *L. pennellii* and the cultivated tomato *L. esculentum* observed that, depending on the trait, 11–57% of the *L. pennellii* alleles had effects opposite to those predicted by phenotype. These findings create a new perception of the potential value of wild germplasm in which the mere phenotypic characterization of a wild accession is insufficient to fully reveal its potential as a donor of agronomically useful alleles.

While QTL mapping can potentially facilitate the breeding process, most QTL studies have been uncoupled from breeding activities or else have been performed in a context that did not allow for the direct creation of improved lines for breeding programs. A recently proposed strategy for molecular breeding, referred to as advanced backcross QTL (AB-QTL) analysis, attempts to reverse this trend by integrating QTL analysis and variety improvement efforts (Tanksley and Nelson 1996). The AB-QTL strategy is also designed for the exploitation of exotic germplasm. Using this method it is predicted that beneficial alleles can be identified in unadapted germplasm and simultaneously transferred into elite cultivars, thus exploiting the hidden value of exotic germplasm. The general strategy of AB-QTL analysis is comprised of the following experimental phases: (1) generation of an elite × unadapted donor hybrid, (2) backcross to the elite parent to produce BC_1 and BC_2 populations which are subjected to marker and/or phenotypic selection against undesirable donor alleles (e.g., for indeterminate growth habit, photoperiod response or small fruit size), (3) molecular-marker characterization of the BC_2 or BC_3 population, (4) generation of BC_3 or BC_4 families which are evaluated for agronomic performance and analyzed for QTLs, (5) selection of target genomic regions containing useful donor alleles for the production of near-isogenic lines (NILs) in the elite genetic background using marker-assisted selection and (6) evaluation of the agronomic performance of the NILs and elite parent controls in replicated environments.

The current study is part of an AB-QTL analysis project in tomato using the wild species *Lycopersicon hirsutum* LA1777 as the unadapted donor parent. This paper reports the results of QTL analysis in BC_3 families that had been evaluated for agronomic performance in three locations worldwide. An accompanying paper (Bernacchi et al. 1998) details the production and evaluation of near-isogenic lines (NILs) derived from this project, as well as from a parallel AB-QTL project using the wild species *L. pimpinellifolium* as a donor parent (Tanksley et al. 1996). Jointly, these reports represent the first full cycle of AB-QTL analysis from the production of the initial interspecific hybrid to the development of breeding lines improved for several important agronomic traits.

Materials and methods

Population development and linkage map

The BC_1 population (*L. esculentum* cv E6203 × *L. hirsutum* LA1777) × E6203 and the RFLP map used in this study are the same as that described in Bernacchi and Tanksley (1997). E6203 is hereafter referred to as E, and LA1777 as H. The initial BC_1 population of 395 plants was subjected to two rounds of genomic selection. First, marker-assisted selection (MAS) was used to remove plants containing the H allele at the *Sp*(*self-pruning*) locus on chromosome 6 (between markers CT109 and TG279), which is dominant for indeterminate growth habit and renders the plant type unacceptable for processing tomatoes (Paterson et al. 1990; Grandillo and Tanksley 1996). A total of 149 determinate individuals (*sp*/*sp*) was selected. These plants were allowed to set self fruit and were also fertilized with E pollen in order to produce BC_2 seed. These same plants were then subjected to phenotypic selection for the production of larger fruit, higher fertility, acceptable fruit color and fruit firmness. Twenty one BC_1 plants were selected using these traits. A final selection criterion applied to the group of BC_1 individuals that were to produce the BC_2 generation was that they should provide a representation of H alleles at all genomic regions not fixed by either the initial MAS selection or the phenotypic selection. The effects of the phenotypic selection in the BC_1 marker segregation were evaluated by comparing the allelic frequencies (excluding the *Sp* locus region) of the selected BC_1 individuals with the unselected BC_1 population using an adjusted χ^2 test of independence (1 *df* and $\alpha = 0.05$).

Fifteen BC_2 plants were derived from each of the selected BC_1 plants, resulting in a BC_2 population of 315 plants. The same 122 informative RFLP markers used to construct the E × HBC_1 linkage map (Bernacchi and Tanksley 1997) were probed onto DNA from each of the 315 BC_2 plants to generate the fingerprints required for QTL analysis. Each BC_2 plant was crossed as the pistillate parent to E to produce BC_3 seed for replicated field evaluation.

Field evaluation of BC_3 families

BC_3 families were evaluated for agronomic performance in three field locations worldwide: Akko, Israel (IS), Badajoz, Spain (SP) and

Woodland, CA (CA) (Table 1). In each location, between 180 and 246 BC_3 plots of 40 plants each were randomized along with nine plots of the E parental control. Nineteen traits of agronomic importance were evaluated at each location and are briefly described below.

Green yield and mature red yield (YDR) were recorded separately as described in Tanksley et al. (1996). Total yield (YDT) is the sum of red yield and green yield. Percent green yield (PGY) was calculated as (green yield/total yield) × 100. Also measured as described in Tanksley et al. (1996) were: soluble-solids content (SSC), brix × red yield (BYR), fruit firmness (FIR), average fruit weight (AFW), cover (COV), pH (pH), viscosity (BOS) and fruit puffiness (PUF).

The intensity of red color in mature fruit (FC) was estimated in several different ways. In IS the internal color of 10–40 randomly selected fruits per plot was rated visually on a scale from 1 to 5 by two independent observers, one pre-harvest (IS.a) and one post harvest (IS.b). In CA redness was measured with an Agtron (LA/B) on raw de-aerated puree. Lower Agtron readings indicate a more intense red color. Two color measurements were made in SP: a visual estimation of color intensity of canned paste was made using a scale from 1 to 20 (SP.a) and a quantification of redness using a Gardner Colorgard 2000/05 optical sensor (A/B) calibrated with Black Tile and Red Tile B.C.R. 801 (SP.b). A higher A/B reading indicates more intense red color. For correlation analysis, the evaluations of fruit color are separated into visual ratings (FC.1) and analytical measurements (FC.2).

The percentage of mature fruit with flower peduncles still attached after harvest (referred to as stem retention, STR) was recorded from the same sample used to calculate average fruit weight. Stem retention is an undesirable trait in processing tomato because stems puncture other fruit during harvest and transport.

Fruit shape (FS) was evaluated visually from the same samples used to determine average fruit weight. In SP the rating system scaled from 1 (round) to 3 (elongated). In CA a similar scale was used but with values ranging from 1 to 4, and in IS the scale went from 1 to 7. In all cases, higher numbers indicate more elongated fruit.

The maturity (MAT) of the different lines was estimated in only two of the locations, IS and SP. Prior to harvest, a subjective visual rating of maturity was made on the basis of foliage and fruit characteristics using a scale from 1 (early maturing) to 5 (late maturing). The percent of total yield consisting of unripe green fruit was recorded in all locations as an additional index of maturation.

The horticultural acceptability of the lines was estimated in two different ways. In IS, fruit set (SET) was evaluated prior to harvest using a scale from 1 (low set) to 5 (heavy set). At the same time the lines were also characterized with a similar scale for their general performance or commercial suitability as reflected primarily by general fruit set (GRL; 1 = poor and 5 = high). In CA fertility was characterized by the total number of fruit (NF) and by a rating of vine uniformity (VU) describing overall horticultural acceptability on a scale from 1 (low) to 3 (high). In parts of this report, these four traits (SET, GRL, FN and VU) are grouped under the designation of horticultural acceptability (HA).

Trait correlation

Pearson correlation coefficients were calculated for each trait combination within locations using the QGENE software package (Nelson 1997).

QTL analysis

The association between phenotype and marker genotype was investigated by regression analysis using the software package QGENE (Nelson 1997). Each trait and location was treated separately. A QTL was declared to be associated with a marker locus if the results met one or more of the following criteria: (1) one location showed association at a significance of $P \leq 0.001$, (2) two locations showed association with the same allelic effect, both at a significance of $P \leq 0.01$, (3) three locations, or three or more independent ratings, showed association with the same allelic effect, each at a significance of $P \leq 0.1$.

The percent phenotypic change (Δ%) associated with the presence of the *L. hirsutum* (H) allele at a given marker locus was estimated as $2 \times 100 \times [(EH\text{-}EE)/EE]$, where EH is the phenotypic mean of individuals heterozygous (E/H) for the marker locus and EE is the phenotypic mean of individuals homozygous (E/E) for the same locus. Because the BC_3 families had a probability of 0.5 of being heterozygous for any one fragment that was heterozygous in the BC_2 generation, a factor of 2 was included to obtain the final estimation of the percent phenotypic change or the additive effect of the H alleles.

Results from this QTL study were compared with those obtained in a similar AB-QTL study utilizing the wild species *L. pimpinellifolium* (PM) (Tanksley et al. 1996). The linkage maps of H and PM are based on a different subset of RFLP markers from the high-density tomato map (Pillen et al. 1996), but both cover the entire length of every chromosome. QTLs from the two wild species were determined to be potentially orthologous if they mapped to the same 20-cM region on the same chromosome.

Results and discussion

Segregation

The BC_1 population E × (E × H) was subjected first to MAS against indeterminate growth habit (*Sp*) and then to phenotypic selection in favor of increased fruit set and better fruit quality. The effects of marker-assisted selection against H alleles at the *Sp* locus on chromosome 6 are described in Bernacchi and Tanksley (1997). The 21 selected BC_1 individuals represented H alleles for over 95% of the genome based on the E × H linkage-map fingerprints (Bernacchi and Tanksley 1997). Chi-square analysis was used to evaluate the effects of the phenotypic selection for higher fertility, larger fruit, and better color and firmness in the BC_1. Significant deviations ($P \leq 0.05$) in segregation between the selected subset and the entire population were detected for six regions on four chromosomes (Table 2, and see Fig. 2).

Because *L. hirsutum* is self-incompatible and displays unilateral incongruity, using the BC_1 plants as females to generate the BC_2 population resulted in selection against LA1777 alleles at the *S* locus region on chromosome 1 as well as in other regions affecting fertility (Bernacchi and Tanksley 1997). The *S* locus on chromosome 1 (near marker CT62), as expected, showed strong skewing against H alleles, which cause self-sterility. This skewing was detected from maker TG301 to marker CT231. The five remaining regions were also skewed in favor of the homozygous E/E class: two regions on chromosome 3 (TG585 and CT170), two regions on chromosome 11 (CT182 and TG393) and one region on chromosome 12 ((TG380) (see Fig. 2).

Table 1 Field information for BC_3 plots

Location (symbol)	Soil	Irrig.[a]	Plants/plot	Spacing bed (cm)	Spacing plant (cm)	Number BC_3 lines
California, Woodland (CA)	Sandy loam	F/S	30	150	30	180
Israel, Akko (IS)	Clay loam	D	40	196[b]	25	246
Spain, Badajoz (SP)	Sandy	F	30	150	25	221

[a] Irrig. = irrigation method: F = furrow irrigation; D = drip irrigation; S = sprinkler
[b] Double-rowed beds (35 cm between rows within a bed)

Table 2 Significant ($P \leq 0.05$) differences in marker segregation comparing the entire BC_1 population with a subset selected based on fruit set, fruit size, fruit color and firmness. *P* values based on χ^2 test of heterogeneity

Marker	Chr.	BC_1		Selected BC_1		*P* value
		E/E	E/H	E/E	E/H	
CT62 (*S* locus)	1	103	65	21	0	< 0.01
TG585	3	93	74	17	4	0.02
CT170	3	64	102	13	7	0.01
CT182	11	123	44	20	1	0.04
TG393	11	89	72	17	4	0.02
TG380	12	98	70	19	2	< 0.01

Trait correlations

The four different measures of horticultural acceptability (SET, GRL, NF and VU) were all positively correlated among themselves and were also strongly correlated with YDR and YBR (Fig. 1). Likewise, total yield (YDT), red yield (YDR) and brix × red yield (BYR) were positively correlated (Fig. 1). Also, as expected, brix was inversely correlated with total and red yield. The association between brix and brix × red yield was positive in IS, not significant in CA, and strongly negative in SP. These results suggest that increases in brix in CA, and particularly in SP, were compensated for by reductions in red yield. On the other hand, in IS red yield appears to be less affected by increases in brix. Percent green yield (PGY) and later maturity were also positively correlated. Yield traits and horticultural acceptability traits were negatively correlated with percent green fruit.

Average fruit weight (AFW) was positively correlated with yield traits (all locations for YDT, two locations for YDR, and two locations for BYR) and negatively correlated with soluble solids in all locations. These correlations are consistent with those reported by Tanksley et al. (1996). Both AB-QTL studies also revealed negative correlations between soluble solids and total and red yield (two locations for YDT, $r = -0.55$; all locations for YDR, $r = -0.3$). In both locations where it was evaluated, elongated fruit shape was correlated with increased total yield ($r = 0.25$) and with increased fruit firmness ($r = 0.32$). Similar associations were also identified by Tanksley et al. (1996).

The Bostwick index of viscosity, measured in only one location (SP), was negatively correlated with soluble solids ($r = -0.48$) and with both ratings of fruit color (SP.a $r = -0.32$ and SP.b $r = 0.28$), indicating that more viscous paste is associated with higher solids and more intense red color. Working with another interspecific cross, Tanksley et al. (1996) reported an opposite correlation between these two variables. Positive correlations were detected between Bostwick index and yield, average fruit weight and pH, indicating that higher yield and larger fruit size correlate with less viscous and more basic paste.

Identification of QTLs and comparison across species

QTLs were identified from the BC_3 data for all traits (ranging from 1 to 15 QTLs/trait). Putative QTLs for each trait are listed in Table 3 and shown in Fig. 2.

Total yield

Twelve QTLs were identified for total yield. At 11 of these loci (92%) the H allele caused significant reductions in yield (Table 3, Fig. 2). However, for *ydt4.1* on chromosome 4, the H allele caused an increase in yield. Lines carrying the H allele at QTL *ydt4.1* produced a 15% greater yield on average than lines homozygous E/E for the same genomic region.

Several QTLs associated with total yield in the current study may be conserved with the QTLs identified by Tanksley et al. (1996) in the advanced backcross study of *L. pimpinellifolium* (PM) and by Eshed and Zamir (1995) in their study of *L. pennellii* (P) introgression lines. All three studies detected QTLs for which the wild alleles reduced total yield at the *ydt2.1* region on chromosome 2, the *ydt3.2* region on chromosome 3, and the *ydt7.1* region on chromosome 7. Interestingly, an H allele in the *ydt3.1* region caused a reduction in

		YDT																
YDR	IS	0.98																
	SP	0.93																
	CA	0.19	YDR															
SSC	IS	-0.12	-0.14															
	SP	-0.64	-0.60															
	CA	-0.43	-0.27	SSC														
BYR	IS	0.86	0.82	0.40														
	SP	0.87	0.96	-0.39														
	CA	0.06	0.95	0.03	BYR													
FC.1	IS.a	0.16	0.18	0.10	0.20													
	SP.a	-0.16	-0.02	0.26	0.07													
	CA	-0.09	-0.11	0.05	-0.09	FC.1												
FC.2	IS.b	0.13	0.14	0.00	0.11	0.08												
	SP.b	-0.13	-0.02	0.20	0.05	0.78	FC.2											
FIR	IS	0.07	0.08	0.01	0.08	0.20	-0.03											
	SP	0.18	0.18	-0.23	0.16	0.06	0.14											
	CA	0.06	0.11	-0.02	0.11	-0.10		FIR										
AFW	IS	0.38	0.41	-0.19	0.24	0.10	0.02	0.10										
	SP	0.55	0.54	-0.54	0.48	-0.03	-0.21	0.17										
	CA	0.26	0.01	-0.38	-0.09	0.08		0.06	AFW									
pH	IS	-0.02	0.03	-0.19	-0.11	-0.02	-0.18	-0.08	0.12									
	SP	-0.02	-0.01	-0.05	-0.02	0.04	-0.08	-0.02	0.08									
	CA	-0.04	-0.01	0.26	0.07	-0.09		0.01	-0.31	pH								
STR	IS	0.09	0.08	-0.01	0.09	-0.03	-0.19	-0.11	-0.04	-0.07								
	SP	-0.24	-0.25	0.08	-0.27	-0.12	-0.15	0.10	-0.20	-0.01								
	CA	-0.21	-0.11	0.17	-0.06	0.18		0.06	-0.22	-0.05	STR							
COV	IS	-0.04	-0.10	0.23	0.08	0.14	-0.01	-0.03	-0.24	-0.26	-0.06							
	SP	0.25	0.22	-0.21	0.19	-0.01	0.00	-0.01	0.16	0.03	-0.17							
	CA	-0.09	-0.31	0.06	-0.29	0.05		0.10	0.13	-0.06	0.06	COV						
PUF	IS	-0.13	-0.12	0.10	-0.08	0.02	0.20	0.00	-0.17	-0.10	-0.11	-0.02	PUF					
BOS	SP	0.36	0.34	-0.48	0.22	-0.32	-0.28	-0.06	0.24	0.20	-0.01	0.09		BOS				
FS	IS	0.30	0.30	-0.31	0.27	0.13	0.12	0.42	0.38	0.00	-0.11	0.19		0.06				
	SP	0.16	0.00	-0.08	-0.02	-0.24		0.16	0.07	0.06	-0.14	0.12			FS			
PGY	IS	-0.69	-0.82	0.15	-0.55	-0.18	-0.15	-0.10	-0.41	-0.15	-0.02	0.25	0.08					
	SP	-0.22	-0.43	0.24	-0.42	-0.02	0.03	0.00	-0.26	-0.32	0.11	-0.07		-0.21	-0.07			
	CA	-0.07	-0.45	-0.08	-0.48	0.17		0.04	0.60	-0.20	-0.03	0.44			-0.06	PGY		
MAT	IS	-0.52	-0.58	0.04	-0.46	-0.14	-0.10	-0.09	-0.33	-0.14	0.01	0.18	0.15			0.62		
	SP	0.13	0.12	-0.03	0.12	-0.08	-0.01	0.12	0.00	-0.08	0.03	-0.21		-0.06	0.03	-0.02	MAT	
SET	IS	0.23	0.24	-0.10	0.16	0.01	0.13	0.01	0.27	0.05	-0.05	-0.10	-0.14			-0.23	-0.26	SET
GRL	IS	0.46	0.50	0.01	0.43	0.33	0.11	0.33	0.33	0.02	-0.10	0.04	-0.18			-0.51	-0.46	0.16
NF	CA	-0.01	0.87	-0.04	0.89	-0.10		0.09	-0.38	0.11	0.04	-0.29			-0.08	-0.50		
VU	CA	0.15	0.45	-0.31	0.34	-0.16		-0.04	-0.16	0.00	-0.12	-0.53			0.02	-0.54		

Fig. 1 Correlation between traits scores from BC_3 families within locations. *YDT* = total yield; *YDR* = red yield; *SSC* = soluble solids; *BYR* = brix × red yield; *FC* = internal fruit color; *FC.1* = visual ratings; *FC.2* = analytical measures (*IS.a* = IS observer "a", *IS.b* = IS observer "b", *SP.a* = SP visual rating, *SP.b* = SP A/B index); *FIR* = firmness; *AFW* = average fruit weight; *pH* = pH; *STR* = stem retention; *COV* = cover; *PUF* = puffiness; *BOS* = viscosity Bostwick; *FS* = fruit shape; *PGY* = percent green yield; *MAT* = maturity; *SET* = fruit set; *GRL* = general score; *NF* = total number of fruits; *VU* = vine uniformity. *CA* = California, *IS* = Israel and *SP* = Spain. *Shadowed cells* indicate significant correlations ($P \leq 0.01$)

total yield, whereas the PM study identified *ydt3.1* as the only QTL for yield for which the PM allele was associated with an increased total yield.

Red yield

Variation in red fruit yield was associated with 11 chromosomal regions (Table 3, Fig. 2). For all loci, H alleles were associated with reduced red yield. Seven red yield QTLs (60%) map to identical markers as total yield QTLs (on chromosomes 1, 3, 7, 8, and 12). For all the other regions (chromosomes 2, 5, 10 and 11), total and red yield QTLs were associated with adjacent markers or else the association failed to reach QTL significance (for one of the two traits). The PM AB-QTL study also identified similar QTLs in the *ydr2.1* and *ydr7.1* regions (Tanksley et al. 1996).

Table 3 List of significant QTL detected in BC_3 evaluations

Trait	QTL	Locus	Chr.	Loc.	R^2	*p*-value	E/E	N	E/H	N	Add%
Total yield	*ydt1.1*	CT81	1	IS	0.06	0.0002	102.0 ± 1.3	207	89.3 ± 2.6	32	− 25
	ydt1.2	CT191A	1	IS	0.1	< 0.0001	103.8 ± 1.3	164	85.5 ± 2.9	21	− 35
	ydt2.1	TG204	2	CA	0.12	0.0001	70.3 ± 0.8	99	63.1 ± 1.7	24	− 21
	ydt3.1	TG251	3	IS	0.17	< 0.0001	104.8 ± 1.2	179	87.0 ± 2.2	59	− 34
	ydt3.2	CT243	3	IS	0.07	< 0.0001	103.0 ± 1.3	183	91.1 ± 2.5	52	− 23
	ydt4.1	CD59	4	IS	0.02	0.02	99.4 ± 1.3	202	107.3 ± 3.5	30	16
	ydt5.1	TG69	5	IS	0.06	0.0001	101.8 ± 1.2	217	86.3 ± 3.5	22	− 30
	ydt6.1	TG356B	6	IS	0.05	0.0006	101.7 ± 1.2	216	86.8 ± 3.7	19	− 29
	ydt7.1	TG331	7	IS	0.08	< 0.0001	102.6 ± 1.2	194	87.0 ± 3.3	32	− 30
	ydt8.1	TG553	8	IS	0.06	0.0001	103.0 ± 1.4	168	93.3 ± 2.2	68	− 19
	ydt12.1	CT79B	12	IS	0.06	0.0003	102.4 ± 1.3	194	90.3 ± 2.5	35	− 24
	ydt12.2	TG296	12	IS	0.06	0.0001	101.2 ± 1.2	222	83.2 ± 3.6	17	− 36
Red yield	*ydr1.1*	CT81	1	IS	0.06	0.0001	91.7 ± 1.4	207	75.9 ± 3.1	32	− 35
	ydr1.2	CT191A	1	IS	0.12	< 0.0001	94.0 ± 1.5	164	70.8 ± 3.5	21	− 49
	ydr2.1	CT9	2	CA	0.06	0.0006	23.3 ± 0.5	148	18.8 ± 1.5	30	− 39
	ydr3.1	TG251	3	IS	0.18	< 0.0001	95.0 ± 1.4	179	74.0 ± 2.5	59	− 44
	ydr5.1	CT138	5	IS	0.05	0.0006	91.7 ± 1.5	200	78.1 ± 3.4	32	− 30
	ydr7.1	TG331	7	IS	0.12	< 0.0001	92.8 ± 1.4	194	71.6 ± 3.7	32	− 46
	ydr8.1	TG553	8	IS	0.08	< 0.0001	93.5 ± 1.5	168	80.1 ± 2.5	68	− 29
	ydr10.1	CT95	10	SP	0.03	0.2	48.8 ± 1.25	157	43.9 ± 2	61	− 20
	ydr11.1	TG400B	11	CA	0.07	0.0005	23.1 ± 0.5	162	17.0 ± 1.5	16	− 53
	ydr12.1	CT79B	12	IS	0.05	0.001	91.8 ± 1.5	194	79.1 ± 2.9	35	− 28
	ydr12.1	TG296	12	IS	0.08	< 0.0001	91.5 ± 1.4	222	68.0 ± 4.6	17	− 51
Soluble solids	*ssc3.1*	TG247	3	IS	0.03	0.01	4.2 ± 0.03	177	4.3 ± 0.07	59	8
	ssc3.2	CT243	3	SP	0.05	0.0006	6.0 ± 0.05	171	6.4 ± 0.09	42	12
	ssc5.1	TG69	5	IS	0.01	0.05	4.2 ± 0.03	217	4.5 ± 0.1	22	12
	ssc6.1	CT216	6	IS	0.04	0.001	4.3 ± 0.03	179	4.1 ± 0.05	58	− 10
	ssc9.1	CT112A	9	CA	0.07	0.0004	4.8 ± 0.04	146	5.1 ± 0.1	32	14
Brix × red yield	*byr1.1*	TG29	1	IS	0.06	0.0003	437.1 ± 6.6	168	381.9 ± 13.1	39	− 25
	byr1.2	CT191A	1	IS	0.09	< 0.0001	435.6 ± 6.3	164	356.2 ± 15.8	21	− 36
	byr2.1	CT9	2	CA	0.08	0.0002	113.0 ± 2.4	148	89.6 ± 7	30	− 41
	byr3.1	TG251	3	IS	0.12	< 0.0001	442.0 ± 5.8	179	372.0 ± 10.8	59	− 31
	byr4.1	TG163	4	CA	0.07	0.0004	104.9 ± 2.7	134	126.4 ± 5.4	35	41
	byr6.1	TG356B	6	IS	0.05	0.0009	430.6 ± 5.7	216	364.3 ± 17	19	− 31
	byr8.1	TG553	8	IS	0.06	0.0001	437.5 ± 6.3	168	391.0 ± 9.5	68	− 21
	byr11.1	TG400B	11	CA	0.07	0.0003	112.0 ± 2.4	162	82.3 ± 7.3	16	− 53
	byr12.1	TG296	12	IS	0.06	0.0001	431.6 ± 5.6	222	350.5 ± 15.4	17	− 37
Fruit color	*fc1.1*	TG460	1	CA	0.06	0.0007	25.3 ± 0.12	143	26.3 ± 0.24	28	8
	fc1.2	CT267	1	CA	0.09	0.0001	25.3 ± 0.1	125	26.3 ± 0.3	41	8
	fc1.3	CT190	1	IS.a	0.07	< 0.0001	3.4 ± 0.07	166	2.9 ± 0.12	62	− 33
	fc2.1	TG276	2	SP.b	0.05	0.004	9.8 ± 0.18	107	10.6 ± 0.23	55	17
	fc2.2	TG620B	2	IS.b	0.06	0.0001	2.9 ± 0.08	185	3.7 ± 0.18	40	53
	fc4.1	TG182	4	SP.a	0.02	0.06	2.0 ± 0.01	191	1.9 ± 0.02	20	− 5
	fc4.2	TG163	4	IS.b	0.05	0.0006	3.2 ± 0.07	176	3.6 ± 0.11	54	30
	fc5.1	TG441	5	IS.b	0.03	0.005	2.9 ± 0.08	194	3.5 ± 0.22	33	42
	fc7.1	TG61	7	IS.a	0.05	0.0007	3.4 ± 0.06	207	2.8 ± 0.16	34	− 33
	fc8.1	CT88	8	SP.b	0.2	< 0.0001	10.6 ± 0.15	118	8.7 ± 0.26	49	− 35
	fc9.1	TG390	9	SP.a	0.1	< 0.0001	2.0 ± 0.01	154	1.9 ± 0.02	43	− 9
	fc9.2	TG421	9	CA	0.12	< 0.0001	25.5 ± 0.09	169	28.7 ± 2.5	4	25
	fc10.1	TG241	10	SP.a	0.06	0.0005	2.0 ± 0.01	148	1.9 ± 0.01	68	− 5
	fc11.1	TG36	11	SP.a	0.05	0.001	2.0 ± 0.01	211	1.8 ± 0.05	9	− 13
	fc11.2	TG393	11	IS.a	0.06	0.0002	3.2 ± 0.06	213	3.9 ± 0.14	22	46
Firmness	*fir2.1*	TG492	2	SP	0.06	0.0002	4.2 ± 0.05	187	3.7 ± 0.2	29	− 27
	fir5.1	CT138	5	SP	0.08	< 0.0001	4.2 ± 0.05	184	3.5 ± 0.2	26	− 32
	fir11.1	CT107	11	SP	0.05	0.001	4.2 ± 0.05	203	3.6 ± 0.2	18	− 29
Fruit weight	*fw2.2*	TG204	2	IS	0.11	< 0.0001	56.8 ± 0.7	145	48.3 ± 1.7	26	− 30
	fw3.1	CT170	3	IS	0.16	< 0.0001	58.5 ± 0.7	177	50.2 ± 0.9	61	− 29
	fw4.1	TG182	4	IS	0.03	0.008	57.1 ± 0.7	199	53.2 ± 1.4	39	− 15
pH	*pH1.1*	CT231	1	SP	0.08	< 0.0001	4.6 ± 0.01	156	4.5 ± 0.02	38	− 3
	pH2.1	TG140	2	SP	0.1	< 0.0001	4.5 ± 0.01	200	4.4 ± 0.02	40	− 5
	pH3.1	TG247	3	IS	0.06	0.0002	4.5 ± 0.01	177	4.4 ± 0.02	59	− 3

Table 3 Continued

Trait	QTL	Locus	Chr.	Loc.	R^2	*p*-value	E/E	N	E/H	N	Add%
pH	*pH3.2*	CT170	3	IS	0.06	0.0002	4.5 ± 0.01	177	4.4 ± 0.02	61	− 3
	pH4.1	TG182	4	IS	0.05	0.0003	4.4 ± 0.01	206	4.5 ± 0.03	27	4
	pH6.1	TG356B	6	IS	0.06	0.0001	4.4 ± 0.01	216	4.6 ± 0.03	19	5
	pH8.1	CT27	8	SP	0.11	< 0.0001	4.5 ± 0.01	172	4.6 ± 0.01	46	4
	pH9.1	TG390	9	SP	0.06	< 0.0001	4.6 ± 0.01	154	4.5 ± 0.02	43	− 3
	pH10.1	CT234	10	SP	0.06	0.0004	4.6 ± 0.01	166	4.6 ± 0.01	43	2
	pH12.1	TG350	12	IS	0.06	0.0002	4.4 ± 0.01	158	4.5 ± 0.01	78	3
% Stem retention	*str2.1*	CT9	2	SP	0.07	0.0001	28.0 ± 0.8	184	36.1 ± 2	34	58
	str2.2	TG140	2	SP	0.06	0.0002	27.7 ± 0.84	177	35.2 ± 1.72	41	54
	str8.1	CD40	8	SP	0.03	0.009	28.2 ± 0.8	166	31.0 ± 1.8	49	35
	str9.1	TG390	9	SP	0.07	0.0002	27.8 ± 0.8	154	35.3 ± 2.2	43	54
	str10.1	CT234	10	IS	0.06	0.0003	53.8 ± 0.9	176	47.0 ± 1.7	55	− 25
	str11.1	CT107	11	SP	0.08	< 0.0001	28.3 ± 0.7	203	39.8 ± 3.7	18	81
Cover	*cov2.1*	CT9	2	CA	0.07	0.0005	3.4 ± 0.06	148	4.0 ± 0.18	30	34
	cov3.1	TG251	3	CA	0.14	< 0.0001	3.3 ± 0.06	127	4.0 ± 0.13	48	42
	cov6.1	CT216	6	IS	0.06	0.0001	4.7 ± 0.05	179	4.3 ± 0.12	58	− 18
	cov7.1	TG331	7	IS	0.03	0.007	4.5 ± 0.06	194	4.9 ± 0.06	32	17
	cov8.1	CD40	8	SP	0.03	0.007	4.4 ± 0.06	188	4.1 ± 0.1	49	− 14
	cov8.2	CT265	8	SP	0.05	0.001	4.5 ± 0.06	189	4.1 ± 0.11	49	− 18
Puffiness viscocity	*puf4.1*	TG163	4	IS	0.06	0.0001	2.7 ± 0.1	176	3.5 ± 0.17	54	59
	vis2.1	TG308	2	SP	0.05	0.0012	231.5 ± 2.8	162	250.7 ± 5.2	49	17
	vis10.1	CT20	10	SP	0.06	0.0004	231.0 ± 3	155	251.3 ± 4.8	56	18
Fruit shape	*fs2.1*	TG204	2	IS	0.06	0.0002	3.8 ± 0.09	202	2.9 ± 0.19	33	− 45
	fs3.1	TG585	3	SP	0.05	0.0014	2.8 ± 0.03	197	2.4 ± 0.12	19	− 24
	fs7.1	TG639	7	IS	0.05	0.0008	3.6 ± 0.09	188	4.3 ± 0.2	43	39
	fs8.1	CD40	8	CA	0.06	0.0009	3.8 ± 0.05	134	3.3 ± 0.15	41	− 22
	fs9.1	CT112A	9	CA	0.04	0.008	3.7 ± 0.05	146	3.3 ± 0.17	32	− 20
	fs10.1	TG241	10	IS	0.05	0.0005	3.9 ± 0.1	170	3.2 ± 0.14	69	− 33
	fs11.1	CT107	11	SP	0.07	0.0001	2.8 ± 0.03	203	2.3 ± 0.16	18	− 30
	fs11.2	TG393	11	CA	0.12	< 0.0001	3.7 ± 0.05	157	2.9 ± 0.25	17	− 43
	fs12.1	TG296	12	SP	0.09	< 0.0001	2.8 ± 0.03	203	2.2 ± 0.13	14	− 37
% Green yield	*pgy1.1*	CT191A	1	IS	0.11	< 0.0001	9.0 ± 1	164	15.0 ± 1.5	21	111
	pgy2.1	TG492	2	CA	0.09	< 0.0001	9.0 ± 1	150	17.0 ± 2	26	150
	pgy2.2	TG140	2	CA	0.09	< 0.0001	9.0 ± 1	140	16.0 ± 2	38	132
	pgy3.1	CT263	3	IS	0.15	< 0.0001	9.0 ± 0.5	187	14.0 ± 1	46	112
	pgy3.2	CT170	3	IS	0.17	< 0.0001	9.0 ± 0.5	177	14.0 ± 1	61	115
	pgy7.1	CT195	7	IS	0.05	0.0005	10.0 + 0.6	226	15.0 ± 2	15	83
	pgy7.2	TG331	7	IS	0.17	< 0.0001	9.0 ± 0.3	194	15.0 ± 1	32	133
	pgy8.1	TG553	8	IS	0.11	< 0.0001	9.0 ± 0.2	168	13.0 ± 1	68	68
	pgy9.1	CT112A	9	IS	0.04	0.0009	9.0 ± 0.3	201	12.0 ± 1	40	64
	pgy11.1	TG400B	11	CA	0.04	0.007	10.0 ± 1	162	16.0 ± 2	16	121
	pgy12.1	TG380	12	IS	0.11	< 0.0001	10.0 ± 0.5	231	19.0 ± 2	10	188
Maturity	*mat3.1*	CT170	3	IS	0.14	< 0.0001	1.5 ± 0.06	177	2.3 ± 0.14	61	107
	mat5.1	TG69	5	IS	0.05	0.0007	1.7 ± 0.06	217	2.4 ± 0.24	22	86
	mat7.1	TG331	7	IS	0.07	< 0.0001	1.6 ± 0.06	194	2.4 ± 0.2	32	89
	mat8.1	TG553	8	IS	0.06	0.0001	1.6 ± 0.07	168	2.1 ± 0.14	68	66
	mat9.1	CT112A	9	IS	0.06	0.0001	1.6 ± 0.07	201	2.3 ± 0.16	40	75
	mat12.1	TG296	12	SP	0.05	0.001	3.5 ± 0.24	203	4.1 ± 0.04	14	27
Hort. accept. (Fertility, general, number of fruits, vine uniformity)	*set1.1*	CT81	1	IS	0.04	0.0015	2.7 ± 0.05	207	2.2 ± 0.13	32	− 35
	vu1.1	CT190	1	CA	0.04	0.01	2.1 ± 0.08	129	1.7 ± 0.15	41	− 37
	set2.1	TG140	2	IS	0.04	0.0015	2.7 ± 0.05	200	2.6 ± 0.13	26	− 31
	nf3.1	TG251	3	CA	0.17	< 0.0001	254.0 ± 6	127	192.6 ± 8.5	48	− 49
	nf4.1	TG574	4	CA	0.06	0.001	230.3 ± 5.5	153	282.2 ± 12	20	45
	vu5.1	TG69	5	CA	0.07	0.0004	2.1 ± 0.07	162	1.2 ± 0.18	15	− 80
	grl7.1	TG331	7	IS	0.07	< 0.0001	3.5 ± 0.08	194	2.6 ± 0.18	32	− 51
	grl8.1	CT88	8	IS	0.07	< 0.0001	3.5 ± 0.08	180	2.8 ± 0.17	54	− 32
	nf9.1	TG223	9	CA	0.04	0.006	2.1 ± 0.08	144	1.6 ± 0.16	28	− 48
	vu10.1	TG241	10	CA	0.03	0.01	1.2 ± 0.08	114	1.8 ± 0.11	62	− 32
	nf11.1	TG400B	11	CA	0.06	0.001	241.5 ± 5.4	162	183.1 ± 13.8	16	− 48
	set12.1	TG350	12	IS	0.03	0.01	2.5 ± 0.06	158	2.8 ± 0.09	78	20

Locus = marker showing strongest association with trait. Chr = chromosome. Loc. = location. Data is shown only for the location showing the highest significance. Add% = percent additive variance attributable to the H allele. Trait units are described in Materials and methods

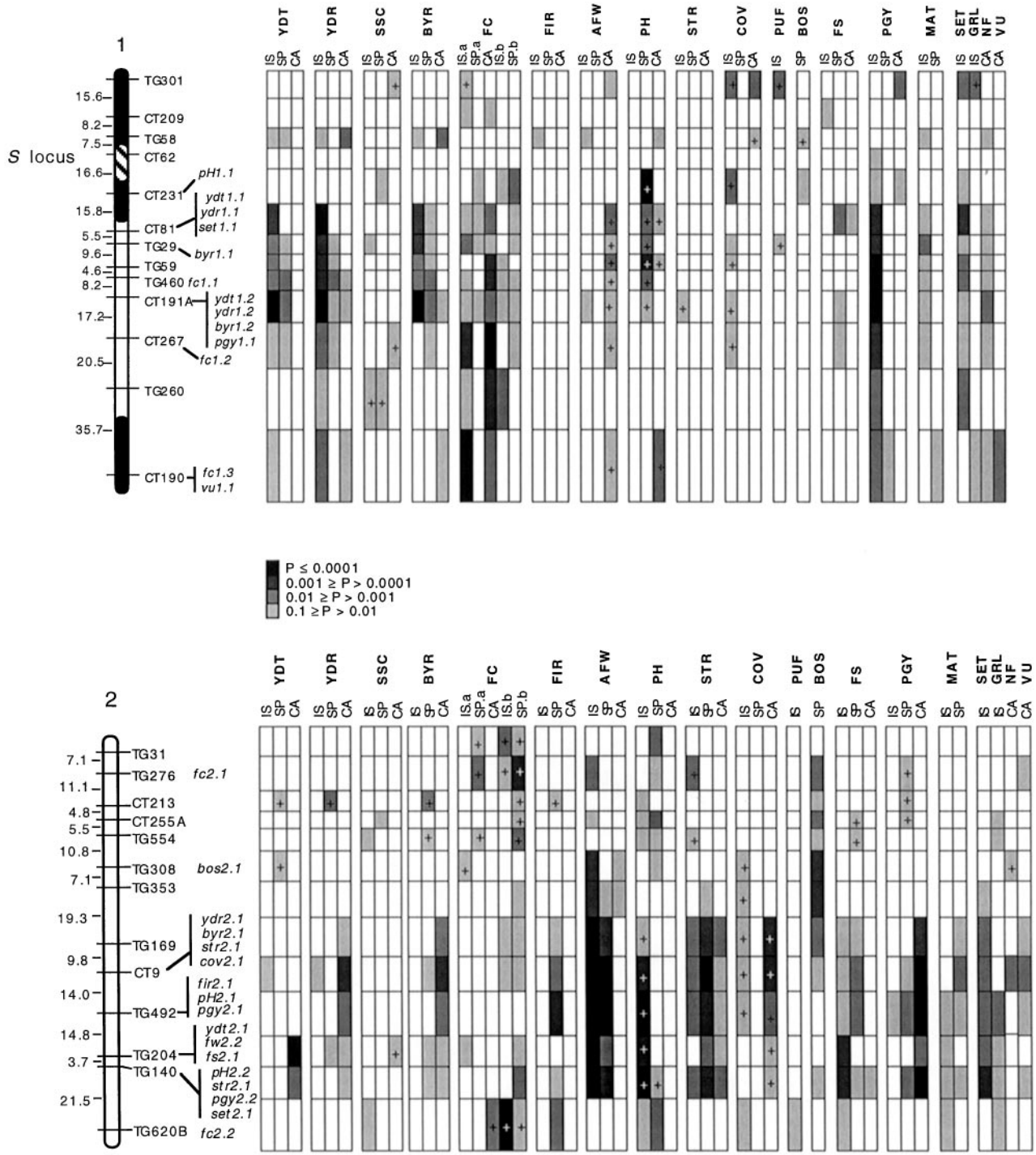

Fig. 2 QTL results classified by trait and location with their position on the *L. esculentum* × *L. hirsutum* BC_1 linkage map (Bernacchi and Tanksley 1997). Map distances are given in centimorgans calculated using the Kosambi mapping function. Chromosomal regions shaded in *black* indicate areas of the genome with segregation significantly ($P \leq 0.05$) affected by BC_1 phenotypic selection. *Striped* chromosomal regions indicate areas of the genome fixed for E alleles. The *self-incompatibility* (*S*) locus and *self-pruning* (*Sp*) locus are indicated to the left of the chromosomes. The *shaded bars* to the right of the chromosomes show the individual analyses for the following traits and locations: *YDT* = total yield; *YDR* = red yield; *SSC* = soluble solids; *BYR* = brix × red yield; *FC* = fruit color (*IS.a* = IS observer a, *SP.a* = visual rating, *SP.b* = SP A/B index); *FIR* = firmness; *AFW* = average fruit weight; *STR* = stem retention, *COV* = cover; *PUF* = puffiness; *BOS* = viscosity Bostwick; *FS* = fruit shape; *PGY* = percent green yield; *MAT* = maturity; *SET* = fruit set; *GRL* = general score; *NF* = total number of fruit; *VU* = vine uniformity. *CA* = California, *IS* = Israel and *SP* = Spain. Levels of significance for $0.1 \geq P > 0.01$; $0.01 \geq P > 0.001$; $0.001 \geq P > 0.0001$ and $P \leq 0.0001$ are indicated by the intensity of the shading (see key on figure). *Positive signs* indicate that the H allele positively affects the trait from a horticultural point of view. Putative QTLs are indicated by *symbols* to the right of each chromosome

Soluble solids

Five QTLs were identified for soluble-solids content (*ssc3.1*, *ssc3.2*, *ssc5.1*, *ssc6.1*, and *ssc9.1*). The only QTL for which H alleles decreased solids was *ssc6.1*. The H allele produced an increase in soluble-solids content in the other four QTLs identified but it was also associated with reduced yield. The increase in soluble-solids content may therefore be a direct consequence of a yield reduction and a concentration of photosynthates (Stevens and Rick 1987). Equivalent soluble-solids QTLs have been identified at all these chromosomal positions by Tanksley et al. (1996) in PM (except that of chromosome 9) and by Eshed and Zamir (1995) in P. Both the PM and the P studies observed correlations between increased solids and reduced yield QTLs in all regions except that on chromosome 6. An earlier study of *L. cheesmanii* (CM) by Paterson et al. (1991) identified a similar QTL for brix for the *ssc3.1* and *ssc6.1* regions, though in this case yield was not measured.

Brix × red yield

Nine QTLs were identified for brix × red yield (*byr1.1*, *byr1.2*, *byr2.1*, *byr3.1*, *byr4.1*, *byr6.1*, *byr8.1*, *byr11.1*, *byr12.1*). For only one QTL, *byr4.1*, was the H allele associated with an increase in brix × red yield (41%

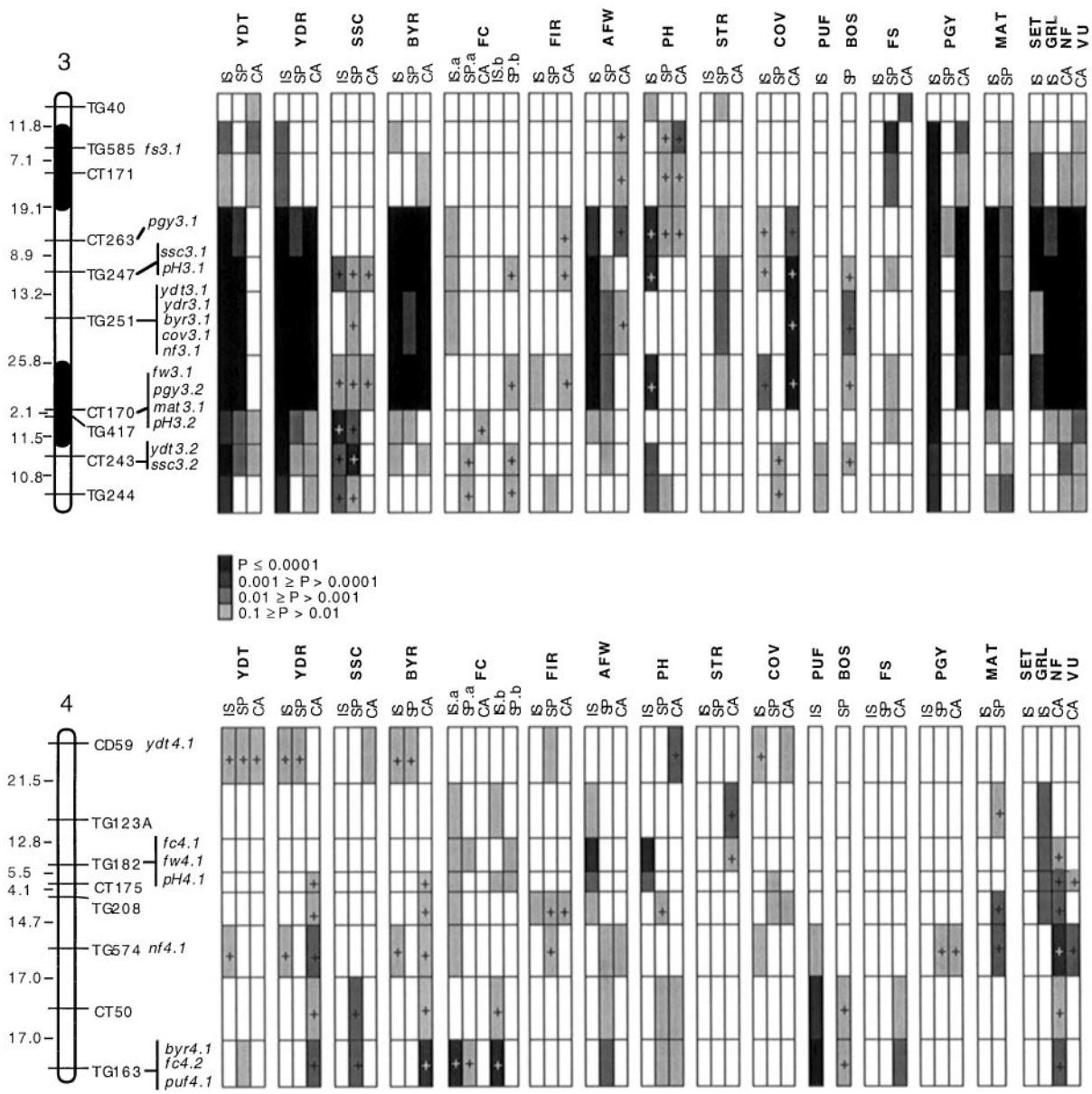

Fig. 2 See page 388 for legend

increase). Tanksley et al. (1996) reported a brix × red-yield QTL on a similar region of chromosome 3 as *byr3.1*, though in these two studies the wild alleles had opposite effects. Eshed and Zamir (1995) also reported QTLs from P in the same region as *byr3.1* and *byr4.1*; however, in this case, the wild allele had the same effect on the trait as H in both regions. Eshed and Zamir (1995) also found brix × yield QTLs in similar regions of chromosome 6, chromosome 11 and chromosome 12 of *L. pennellii*. However, for these QTLs, the wild allele was associated with increased brix × yield while the opposite occurred for the H alleles. These differences may be due in part to the fact that the P study plants were widely spaced and phenotyped individually, while the present evaluation was based on plots. Eshed and Zamir (1995) have shown that there is very little correspondence between the performance of single plants versus plots with respect to brix × yield.

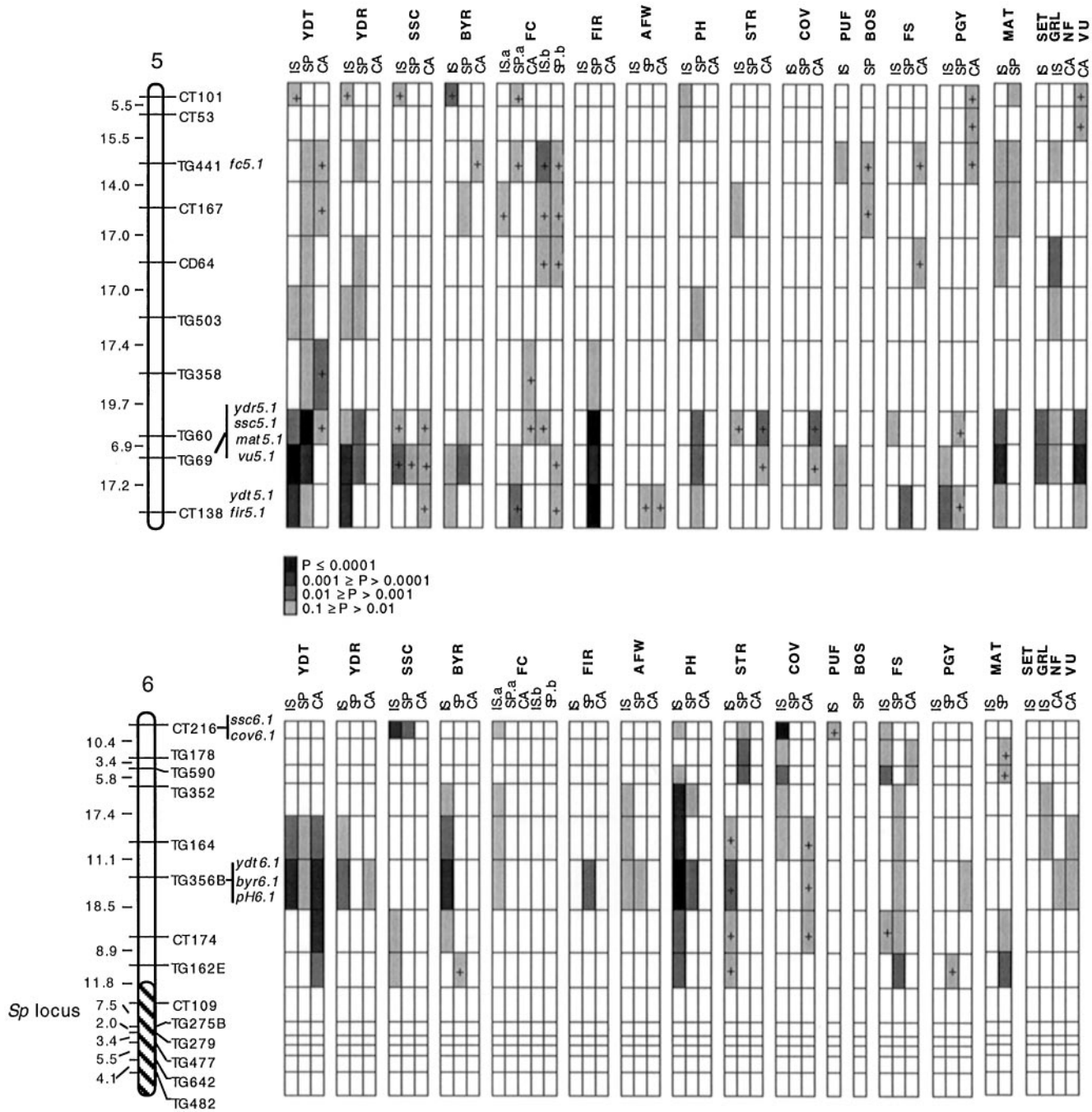

Fig. 2 See page 388 for legend

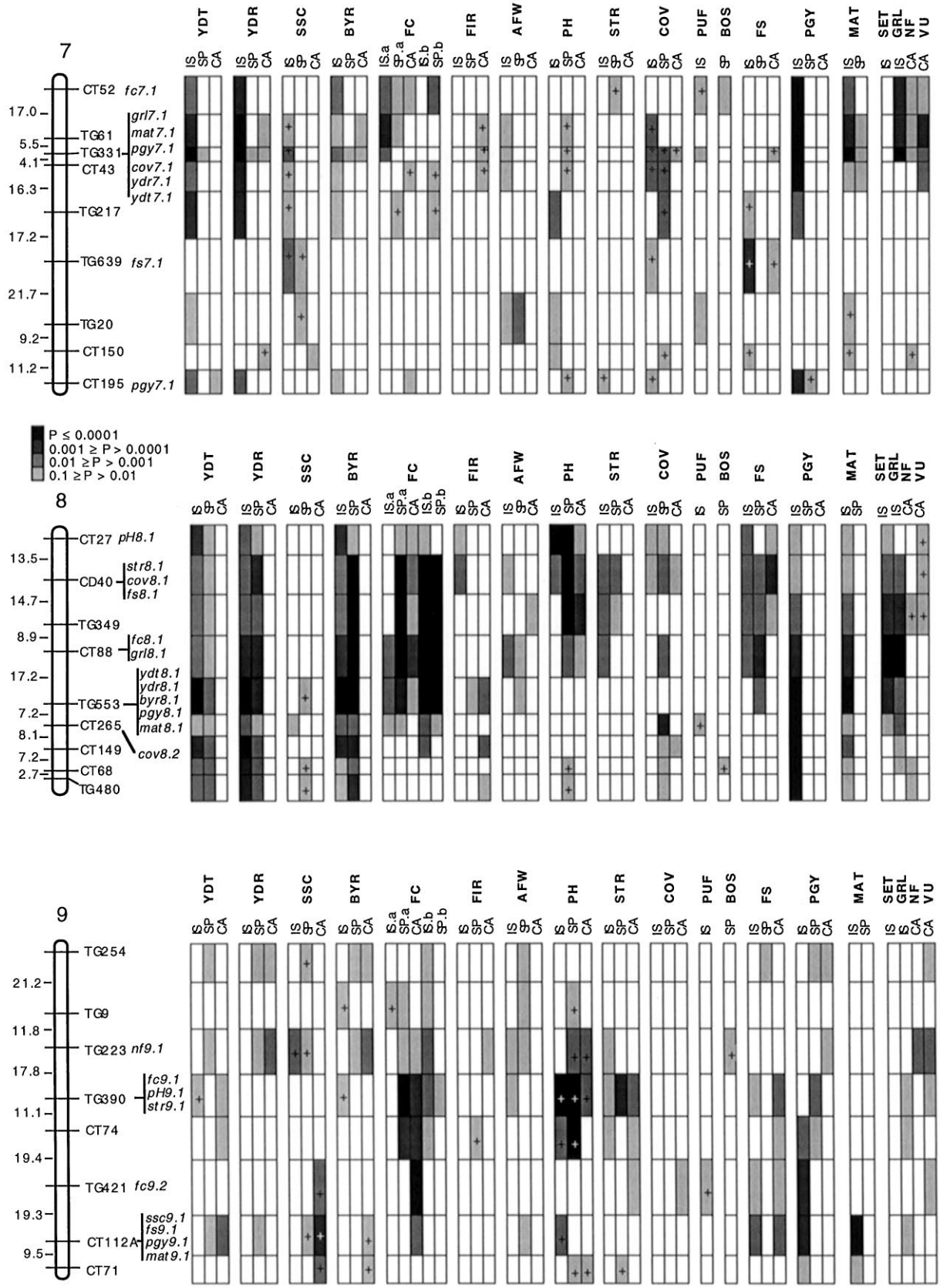

Fig. 2 See page 388 for legend

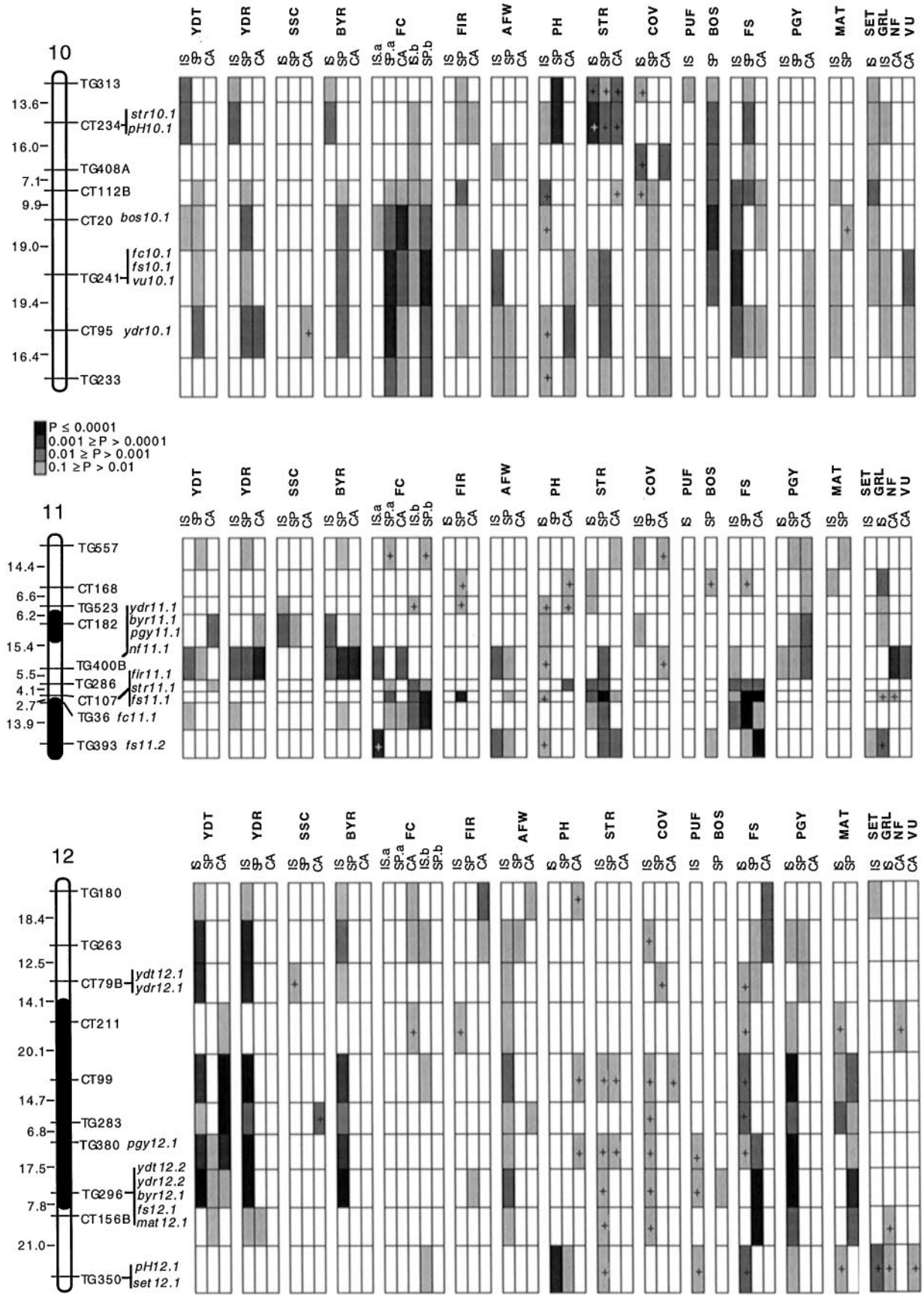

Fig. 2 See page 388 for legend

Fruit color

Fifteen QTLs were identified for fruit color (Table 3, Fig. 2). For five QTLs (30%), the H allele was associated with an increased redness of the fruits. This is despite the fact that *L. hirsutum* does not develop red pigments upon ripening. The percent phenotypic change for these QTLs ranged from 17% (*fc2.1*) to 52% (*fc2.2*). There was strong agreement for the color QTLs detected at different locations and by different observers or procedures. The *fc1.1*, *fc1.2*, *fc7.1*, *fc8.1*, *fc9.1*, *fc10.1* and *fc11.1* regions were detected in all three locations regardless of whether visual or photometric regions were used. *fc2.1*, *fc2.2*, *fc4.2* and *fc5.1* were all QTLs for which the H allele increased color in three out of the five independent color evaluations. The AB-QTL study of donor parent PM (Tanksley et al. 1996) identified five fruit color QTLs, three of which mapped to equivalent chromosomal regions (*fc4.1*, *fc7.1* and *fc8.1*). However, for *fc7.1* the wild PM allele was associated with increased color while the H allele at the same position was associated with reduced fruit color.

Fruit firmness

Three QTLs were associated with fruit firmness, *fir2.1*, *fir5.1* and *fir11.1*, all detected only in SP. For all three QTLs, the H allele induced a significant reduction in fruit firmness. No common QTLs for firmness were detected between this and the PM study (Tanksley et al. 1996).

Average fruit weight

The average weight of ripe fruit was associated with three QTLs *fw2.2*, *fw3.1* and *fw4.1*. Plants carrying the H allele at any of these loci showed significant reductions in average fruit weight. *fw2.2* has been detected in several other tomato interspecific crosses involving both red- and green-fruited species (Alpert and Tanksley 1996; Alpert et al. 1995). Similar, *fw3.1* on chromosome 3 appears to be conserved between H and PM (Tanksley et al. 1996). CM alleles (Paterson et al. 1991), and P alleles (Eshed and Zamir 1995). Paterson et al. (1991) mapped a fruit-mass QTL to the same region of chromosome 4 as *fw4.1*.

pH

pH was associated with ten QTLs (Table 3, Fig. 2). For *pH1.1*, *pH2.1*, *pH3.1*, *pH3.2* and *pH9.1* the H allele gave rise to a reduced pH. Paterson et al. (1988, 1991) mapped QTLs at the same position as *pH3.1*, for which the wild alleles from *L. chmielewskii* (CL) and CM also reduced pH. At the same region of *pH9.1*, these authors also detected a pH-QTL with a LOD score of 2.3, marginally below QTL threshold, associating the presence of the CL allele with a reduced pH (Paterson et al. 1988). Other examples of similar effects of H and CM alleles on pH occur for *pH1.1*, *pH6.1*, *pH8.1* and *pH12.1* (Paterson et al. 1991). For the *pH4.1* region on chromosome 4, the H and PM studies show QTLs with opposite effects.

Stem retention

Stem retention was associated with six QTLs (*str2.1*, *str2.2*, *str8.1*, *str9.1*, *str10.1*, and *str11.1*). The H allele caused a reduction in stem retention at only one of the six (*str10.1*). In this case a 25% reduction in stem retention was attributed to the H allele. QTLs potentially orthologous to *str2.1*, *str2.2* and *str10.1* in PM regions were reported by Tanksley et al. (1996) for PM alleles.

Cover

Cover in the BC_3 plots was associated with six QTLs, *cov2.1*, *cov3.1*, *cov6.1*, *cov7.1*, *cov8.1* and *cov8.2*. Amongst these, *cov2.1*, *cov3.1* and *cov7.1* controlled increases in cover if the H allele was present. The AB-QTL study of PM alleles identified six QTLs scattered over five chromosomes (Tanksley et al. 1996). The only H QTL that appears to have a counterpart in PM is that on chromosome 7 (*cov7.1*).

Fruit puffiness

A single QTL, *puf4.1*, was associated with fruit puffiness. In this case, the H allele increased puffiness. While the PM AB-QTL study (Tanksley et al. 1996) identified five QTLs associated with fruit puffiness, none of these regions corresponded to the *puf4.1* region.

Viscosity

Viscosity, evaluated only in SP, was associated with two QTLs, *bos2.1* and *bos10.1*. In both cases, the presence of the H allele reduced the viscosity of the paste. None of the H viscosity QTLs were identified by the PM study (Tanksley et al. 1996).

Fruit shape

Nine QTLs on six different chromosomes were associated with fruit shape (Table 3). The H alleles were associated with elongated fruit in only one case, *fs7.1*. For all other QTLs, the effect of the H allele was to produce rounder fruit. This is consistent with the fact

that *L. hirsutum* has round fruit whereas the cultivated tomato fruit is more elongated. QTLs potentially orthologous to *fs8.1* and *fs2.1* have been reported for PM (Grandillo and Tanksley 1996; Tanksley et al. 1996).

Percent green yield and maturity

Eleven and nine QTLs were identified for percent green fruit and maturity respectively. In most instances, the map position of QTLs for these traits coincided. The H allele was always associated with an increased percent of green fruit at harvest time (delayed maturity). The central region of chromosome 3 displayed strong associations with maturity, as indicated by the presence of *ma+3.1*, *pgy3.1* and *pgy3.2*. In a similar fashion, QTLs for the two traits coincide with, or else map to, adjacent markers on the bottom of chromosome 7 (*mat7.1* and *pgy7.2*), chromosome 8 (*mat8.1* and *pgy8.1*), chromosome 9 (*mat9.1*, and *pgy9.1*) and chromosome 12 (*mat12.1* and *pgy12.1*). In the regions of *pgy1.1*, *pgy2.1* and *pgy2.2* there are parallel associations with maturity, though they just failed to reach the QTL significance threshold. An additional QTL was detected for percent green yield alone on chromosome 11, *pgy11.1*. In the PM study (Tanksley et al. 1996), the plant life cycle was also evaluated by measuring the number of days from transplant to first ripe fruit (fruit ripening) and by using a score similar to the one used in this study (maturity). Seven of eight regions where putative QTLs were identified for fruit ripening or for maturity in Tanksley et al. (1996) were among those detected in the present study (all except chromosome 4). Interestingly, while the H allele on chromosomes 2, 8 and 9 was responsible for lengthening the plant life cycle, the PM alleles in the same regions shortened it.

Horticultural acceptability

Several traits were evaluated that reflect the horticultural acceptability of the BC_3 lines. These traits were not evaluated in all locations. Fruit set (SET) and general performance (GRL) were measured in IS, and the total number of fruits (NF) and the vine uniformity (VU) were measured in CA. Seven QTLs were identified for these traits. Two regions on chromosome 1 were associated with fruit set. For both *set1.1* and *vu1.1* the H allele was associated with a reduced fruit set. Other QTLs identified for which the wild allele reduced fertility were *nf3.1* on chromosome 3, *vu5.1* on chromosome 4, *grl7.1* on chromosome 7, *grl8.1* on chromosome 8, *vu10.1* on chromosome 10, and *nf11.1* on chromosome 11. For all these factors the wild allele reduced either fertility or fruit set. Only two QTLs out of a total of 12 were identified for which the wild H allele increased either fertility or the acceptability of the line. Heterozygosity for QTL *nf4.1* on chromosome 4 was associated with a 45% greater fruit set as compared to plants homozygous E/E at the same locus. Similarly, at the QTL *set12.1* on chromosome 12, the H allele was associated with a 20% increase in fruit set. Fertility was also evaluated by Tanksley et al. (1996) resulting in the identification of only two QTLs, possibly orthologous to *vu5.1* and *grl7.1*, at which the wild allele led to reduced fertility.

Wild alleles with favorable effects and the direction of allelic effects

A clear distinction should be made between whether or not the effect of a donor allele is agronomically

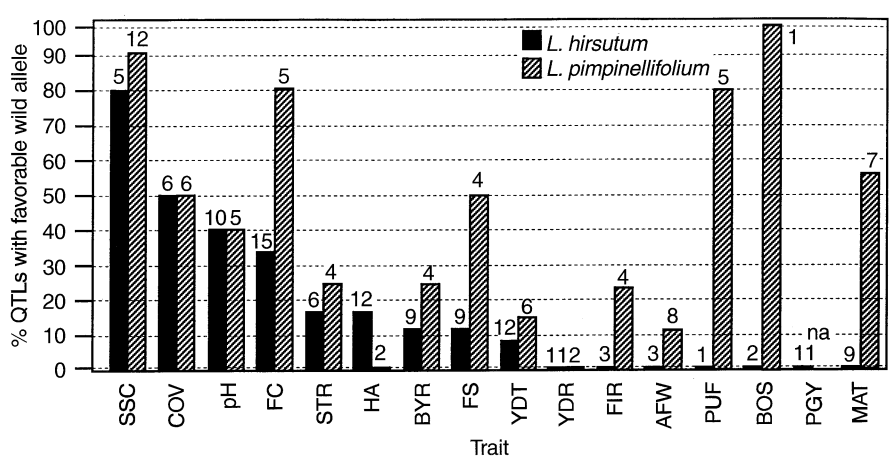

Fig. 3 Percentage of QTLs with favorable alleles (from an horticultural perspective) detected for *L. hirsutum* (*dark bars*) and for *L. pimpinellifolium* (*striped bars*) (Tanksley et al. 1996). The total number of QTLs detected for the trait are indicated above the bars. *SSC* = soluble solids; *COV* = cover; *FC* = fruit color; *STR* = stem retention; *HA* = horticultural acceptability traits (*SET* = fruit set; *GRL* = genereal score; *NF* = total number of fruits and *VU* = vine uniformity); *BYR* = brix × red yield; *FS* = fruit shape; *YDT* = total yield; *YDR* = red yield; *FIR* = firmness; *AFW* = average fruit weight; *PUF* = puffiness; *BOS* = viscosity Bostwick; *PGY* = percent green yield; *MAT* = maturity. *na* = not available

favorable (from an horticultural perspective) versus whether or not the effect of a donor allele is in accordance with the donor phenotype. This is an important issue because, unless there is a strong association between the parental phenotype and the direction of allelic effects, it is unlikely that phenotypic evaluations of wild parents will be the best criteria for selecting potential donor parents for breeding.

In this study, superior wild alleles were found both for traits for which the H phenotype is superior from an agronomic perspective (SSC, COV), as well as for traits for which the H phenotype is agronomically inferior (STR, FC, HA, BYR, FS, YDT) (Fig. 3). For 60% of the traits evaluated, at least one QTL was detected where the H allele was agronomically superior to the cultivated allele (Fig. 3). No QTLs with superior H alleles were found for YDR, FIR, AFW, PUF, BOS, PGY and MAT (Fig. 3). On the other hand, SSC had a maximum of 80% QTLs for which the H allele was superior to the E allele (Fig. 3). For the other traits the values were 50% (COV), 40% (pH), 33% (FC), 18% (STR), 18% (HA), 12% (BYR), 12% (FS), and 9% (YDT) (Fig. 3). Similar variation in this parameter was reported by Tanksley et al. (1996) in their study of PM (Fig. 3). In that study SSC was also the trait for which the wild allele was favourable for the greatest percentage of QTLs (91%). As seen in Fig. 3, the percentage of favorable QTLs per trait, from either *L. hirsutum* or *L. pimpinellifolium*, was very similar for SSC, COV, pH, STR, BYR and YDT, despite the fact that sometimes the total numbers of QTLs identified for the traits varies between the studies. Comparing only traits for which more than three QTLs were identified in both the H and the PM studies, those that had a very different percent of favorable QTLs were FC, FS, FIR, and MAT (Fig. 3).

The percentage QTLs with an allelic effect opposite to that expected based on the parental phenotype was 20% for SSC, 50% for COV, 34% for FC, 17% for STR, 17% for HA, 11% for BYR, 11% for FS and 9% for YDT. Tanksley et al. (1996) in their QTL study of *L. pimpinellifolium* also found a similar percent of QTLs with an unpredicted effect for many of these same traits. For SSC, 10% of the QTLs had allelic effects opposite to those predicted by the parental phenotype, 50% for COV, 25% for STR, 25% for BYR, and 16% for YDT. For FC, the percent QTLs with allelic effects opposite to that expected differed considerably for *L. pimpinellifolium* (80%) and *L. hirsutum* (30%). A similar situation was seen for FS, for which the percent QTLs with opposite allelic effects were 33% and 12%, respectively.

Overall, for most traits the total number of favorable QTLs was similar between this study and that of PM (Tanksley et al. 1996). However, there are some exceptions. For example, despite having a similar percentage of favorable QTLs, 4 favorable QTLs were detected for SSC in the H study whereas 11 favorable QTLs were detected for the same trait in the PM study. FC analysis showed an opposite pattern in that the two wild donors (H and PM) had similar total numbers, but very different percentages of favorable QTLs. Interestingly, for FC, *L. hirsutum*, a green tomato, appears to have more favorable alleles than the red-fruited *L. pimpinellifolium*. Results such as these further exemplify the limitations of selecting donor parents exclusively on the basis of their phenotypic characterization.

For traits without significant favorable QTLs (YDR, FIR, FW, PUF, BOS, PGY and MAT), marker-phenotype analysis identified regions for which the H allele had a favorable effect on the trait but which marginally failed to reach the QTL threshold (Fig. 3). For example, H alleles at the TG260-CT260 region of chromosome 1 showed a marginal association with an increased SSC in all three locations, and H alleles at the middle and bottom of chromosome 4 (TG574 to TG163) showed marginal associations with improved YDT, YDR, SSC, FIR, BOS, PGY and MAT. As shown in the NIL analysis (Bernacchi et al. 1997) some of the NILs displaying the best genetic gains over the elite controls contained H introgressions at regions that failed to reach QTL significance (in the QTL mapping study).

The results from the present study and that of *L. pimpinellifolium* (Tanksley et al. 1996) show that for most traits, most QTLs have an allelic effect in the direction predicted by the parental phenotypes. However, it is important to note that a significant portion of the QTLs (10% to 30%) had allelic effects opposite to those predicted by the parents. It is these agronomically useful and novel alleles, that would be overlooked in phenotypic evaluations of exotic germplasm, which can be detected and transferred with a marker-based breeding approach.

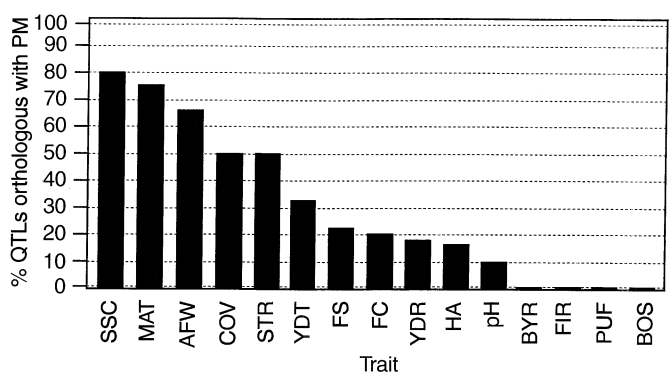

Fig. 4 Percentage of QTLs potentially orthologous between *L. hirsutum* (*H*) and *L. pimpinellifolium* (*PM*). *SSC* = soluble solids; *MAT* = maturity; *AFW* = average fruit weight; *COV* = cover; *STR* = stem retention; *YDT* = total yield; *FS* = fruit shape; *FC* = fruit color; *YDR* = red yield; *HA* = horticultural acceptability traits (*SET* = fruit set; *GRL* = general score; *NF* = total number of fruits and *VU* = vine uniformity); *BYR* = brix × red yield; *FIR* = firmness; *PUF* = puffiness; *BOS* = viscosity Bostwick. *Note*: QTLs for HA traits were compared to those reported for fertility in *L. pimipinellifolium*. Data for *L. pimpinellifolium* are from Tanksley et al. (1996)

Conservation of QTLs across species

Overall, an estimated 30% of the QTLs identified in the current study have potentially orthologous counterparts in PM according to map position; however, this percentage varied greatly among traits (Fig. 4) (Tanksley et al. 1996). For SSC, AFW, MAT, COV and STR, 50% or more of the QTLs are possibly orthologous with loci detected in the PM study. For YDT, FS, YDR, horticultural acceptability traits, FC and pH, the percentage of common QTLs ranged from 10% to 25%. For BYR, FIR, PUF and BOS, no common QTLs were identified.

The traits that have the most QTLs in common with PM are traits for which the wild parents have a strong detrimental effect (Fig. 4). For example, for all common QTLs for YDT, YDR, AFW and MAT, the wild alleles were associated with inferior performance (Fig. 4). In fewer cases, potentially orthologous QTLs were identified between H and PM for QTLs at which both exotic alleles had a positive effect on the trait, such as QTLs for SSC on chromosomes 3 and 5, a QTL for STR on chromosome 10, and a QTL for COV on chromosome 7. In other cases, apparently orthologous QTL-alleles for H and PM had opposite effects. For example, for *ydt3.1*, *fc7.1*, *ph4.1*, *pgy2.2*, *mat8.1*, *mat9.1* the H allele had an adverse effect on the respective traits, while the PM allele at the same regions had positive effects on the same traits (Tanksley et al. 1996).

The fact that there is low conservation between H and PM for the positive effect QTLs, or that sometimes the direction of the allelic effect of species (relative to the E parent) varies for a given QTL, is encouraging from the perspective of the utilization of wild alleles for breeding. This suggests that different exotic accessions are likely to contain different favorable alleles for a given trait, encouraging further marker-based exploration of additional exotic accessions.

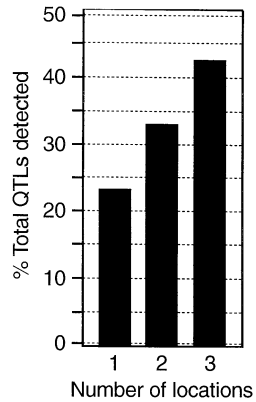

Fig. 5 Percentage of QTLs detected in 1, 2 and 3 locations. Only QTLs for traits evaluated in two or more locations were considered

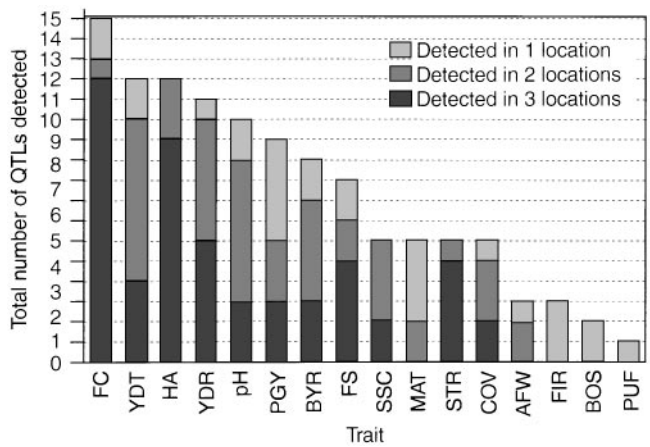

Fig. 6 Number of QTLs detected per trait, classified according to the number of locations in which the effect was detected. YDT = total yield; YDR = red yield; SSC = soluble solids; BYR = brix × red yield; FC = fruit color; FIR = firmness; AFW = average fruit weight; STR = stem retention; COV = cover; PUF = puffiness; BOS = viscosity; FS = fruit shape; PGY = percent green yield; MAT = maturity; HA = horticultural acceptability traits (SET = fruit set; GRL = general score; NF = total number of fruits and VU = vine uniformity)

Conservation of QTLs across environments

Of the 119 QTLs reported for traits evaluated in two or more locations, 23% were detected in only one location, 33% were detected in two locations, and 43% were detected in all three locations, at the same RFLP loci or flanking loci in the individual locations (Fig. 5). These results suggest that a significant fraction of the QTLs are conserved across locations. This is consistent with findings of other tomato QTL projects (Paterson et al. 1991; Tanksley et al. 1996) and QTL studies in maize (Ragot et al. 1995). However, there appears to be little relationship between the magnitude of the QTL effect and the number of locations at which a QTL was detected. The number of locations at which a QTL was detected and the percentage of phenotypic variance associated with that QTL were only mildly correlated ($r = 0.17$, $P = 0.05$). This suggests a weak tendency for major-effect QTLs to be detected more consistently across multiple locations.

The degree to which QTLs were constant across locations varied with the trait (Fig. 6). For example, fruit color showed consistent QTL effects across locations despite the different rating procedures employed. Thus, *fc1.1*, *fc2.2*, *fc7.1*, *fc8.1* and *fc9.1* had significant effects in all three locations. Stem retention also showed a high consistency across locations with five out of the six QTLs showing some degree of association in all three locations. Traits for horticultural acceptability too were consistently detected in two or more locations or by two or more independent evaluations of HA. Most QTLs for yield traits were also detected in at least two locations. For all traits for which positive-effect H QTLs were detected, (YDT, BYR, FC, STR, COV, FS, SSC and horticultural acceptability traits),

the majority of QTLs were independently identified in two or more locations (Fig. 6).

Implications for the use of exotic germplasm in breeding

Results from this study demonstrate that, despite its inferior horticultural characteristics, *L. hirsutum* contains alleles capable of improving many traits of economic importance in processing tomatoes. While most H alleles are deleterious from an agronomic perspective, specific H alleles were associated with a 44% increase in brix × red yield, a 7–11% increase in soluble solids, a 16% increase in total yield, a 17–52% increase in fruit color, and a 25% reduction in stem retention. Importantly, these favorable wild alleles often showed their effect across experimental locations. Also, this study shows that this wild species contains novel and useful alleles which can be readily detected through QTL mapping and that the true genetic value of exotic germplasm in many instances may lay hidden until exposed by the AB-QTL, or other similar, procedures.

Ultimately, the value of such wild alleles, and thus the feasibility of breeding on the basis of QTL mapping data, can only be assessed by developing and testing near-isogenic lines for the selected wild QTL-alleles in replicated environments. In a companion paper, we report the generation of NILs containing specific selected introgressed QTL-alleles from *L. hirsutum* and *L. pimpinellifolium* and the evaluation of these NILs in replicated trials worldwide (Bernacchi et al. 1997).

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