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Identification of quantitative trait loci associated with acylsugar accumulation using intraspecific populations of the wild tomato, *Lycopersicon pennellii*

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Abstract *Lycopersicon pennellii* LA716, a wild relative of tomato, is resistant to a number of insect pests due to the accumulation of acylsugars exuded from type IV trichomes. These acylsugars are a class of compounds including both acylglucoses and acylsucroses. Intraspecific populations between *L. pennellii* LA716 and *L. pennellii* LA1912, the latter an accession that assorts for low-level acylsugar accumulation, were created to study the inheritance of type IV trichome density, acylsugar accumulation levels, percentage of acylsugars that are acylglucoses, and leaf area. The F_2 population was subsequently used to determine genomic regions associated with these traits. The relative proportion of acylglucoses and acylsucroses was found to be largely controlled by a single locus near TG549 on chromosome 3. One locus on chromosome 10 showed significant associations with acylsugar levels. In addition, 1 locus on chromosome 4 showed significant associations with leaf area. Ten additional loci showed modest associations with one or more of the traits examined, 5 of which have been previously reported.

Key words Pest resistance · Insect resistance · QTL analysis · *Lycopersicon esculentum* · Sugar ester

Introduction

Due to increasing health and environmental concerns and the evolution of pesticide-resistant insects, there is an increasing need for alternatives to the use of pesticides for the control of insect pests. The acylsugars of *Lycopersicon pennellii* (Corr.) D'Arcy accession LA716, a wild relative of the cultivated tomato *L. esculentum* Mill., confer resistance to several insect pests of tomato including potato aphid (*Macrosiphum euphorbiae*), leafminer (*Liriomyza trifolii*), tomato fruitworm (*Helicoverpa zea*), beet armyworm (*Spodoptera exigua*), silverleaf whitefly (*Bemisia argentifolii*), and green peach aphid (*Myzus persicae*) (Goffreda et al. 1989; Hawthorne et al. 1992; Juvik et al. 1993; Liedl et al. 1995; Rodriguez et al. 1993). These acylsugars comprise approximately 90% of type IV trichome exudate of *L. pennellii* LA716 (Burke et al. 1987). As cultivated tomato does not accumulate detectable levels of acylsugars, the transfer of acylsugar accumulation to cultivated tomato promises significant pest resistance. A better understanding of the genetic control of acylsugar accumulation is needed in order to utilize acylsugar mediated resistance.

The acylsugars of *L. pennellii* LA716 are a complex chiefly comprised of 2,3,4-tri-*O*-acylated glucose esters containing C4 to C12 fatty acids (Fobes et al. 1985). Accessions of *L. pennellii* vary considerably in the level ane composition of acylsugars accumulated (Shapiro et al. 1994). The compositions of acylsugars vary in their sugar substituents (glucose or sucrose) as well as in the length and branching structure of their fatty acid substituents.

Trichome density is correlated with level of insect resistance in several solanaceous species (Fery and Kennedy 1987; Tingey and Sinden 1982; Weston et al. 1989). Type IV trichome density was associated with mite resistance in studies of *L. hirsutum* (Carter and Snyder 1985) and with acylsugar levels in studies of *L. pennelli* (Goffreda et al. 1990). One quantitative trait locus (QTL) was found to be associated with both acylsugar accumulation and type IV trichome density in *Solanum berthaultii*, suggesting a genetic basis for this correlation (Bonierbale et al. 1994).

An interspecific F_2 population created from a cross between *L. esculentum* and *L. pennellii* LA716 was used

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to identify 5 QTLs associated with acylsugar accumulation (Mutschler et al. 1996). These results were subsequently used in a marker-assisted breeding program to incorporate acylsugar accumulation into *L. esculentum* (Lawson et al. 1997). Plants identified as containing all 5 QTLs in the heterozygous condition produce acylsugars at levels a fraction of that of the interspecific F_1 control. One explanation for this would be the requirement of 1 or more additional, but as of yet unidentified, QTLs for moderate levels of acylsugar accumulation.

Analysis of intraspecific populations created with two accessions of *L. pennellii* should be useful to further elucidate the genetic control of acylsugar accumulation. The use of intraspecific populations will avoid any errors introduced by aberrancies in plant growth, development, and gene segregation ratios commonly observed in interspecific populations (Zamir and Tadmor 1986; Liu 1994). The identification of accessions of *L. pennellii* that are incapable of acylsugar accumulation (Shapiro et al. 1994) provides an opportunity to create intraspecific populations segregating for genes affecting acylsugar level and type. *L. pennellii* LA1912 is a heterogeneous accession that assorts for acylsugar accumulation, with values ranging from undetectable amounts to approximately 15% of the amount typically accumulated by *L. pennellii* LA716. The close evolutionary relationship of these two parents suggests that intraspecific populations may segregate for fewer of the genes involved in acylsugar accumulation. Since many individuals within *L. pennellii* LA1912 are capable of accumulating acylsugars, it is likely that individuals that do not accumulate detectable levels of acylsugars lack at least 1 gene required for acylsugar accumulation, but possess a number of the other genes. This may yield a less complex genetic model for this intraspecific population which may increase the power of QTL detection. Therefore, the study presented here uses intraspecific populations between *L. pennellii* LA716 and *L. pennellii* LA1912 to determine the inheritance of acylsugar accumulation and related traits and to identify QTLs affecting these traits.

Materials and methods

Plant materials

An F_1 was created by crossing *L. pennellii* LA716 (PI246502) to an *L. pennellii* LA1912 individual that did not accumulate detectable amounts of acylsugars (non-accumulating). This F_1 was self pollinated to create an F_2 population, cross pollinated to *L. pennellii* LA716 to create backcross population BC716, cross pollinated to non-accumulating *L. pennellii* LA1912 individuals to create backcross population BC1912NA, and cross pollinated to *L. pennellii* LA1912 individuals that accumulate low levels of acylsugars to create backcross population BC1912A, respectively. Two hundred and thirty-one F_2 plants, 63 BC716 plants, 63 BC1912NA plants, and 58 BC1912A plants were grown under normal greenhouse conditions along with *L. pennellii* LA716, LA1912 and their F_1 as controls.

Phenotypic screening

Luckwill (1943) described two trichomes with a similar size and structure in *Lycopersicon*, the type IV and type V trichomes. The key feature distinguishing these trichomes is the presence or absence, respectively, of an exuded droplet at the trichome's tip. The type IV, but not the type V, trichome is present on *L. pennellii* LA716 and acylsugar-accumulating individuals within LA1912. The non-accumulating LA1912 plants have similar densities of a trichome that could be either a non-functional type IV or a type V trichome. However, since all non-accumulating segregants have non-droplet-bearing trichomes, and the intraspecific F_1 and acylsugar-accumulating segregants in the intraspecific F_2 and backcross populations possess type IV but not type V trichomes, the non-droplet-bearing trichome is probably a non-functional type IV trichome. Since both trichomes are considered to be type IV trichomes in this study, the estimates of type IV trichome densities include trichomes that bear and do not bear droplets.

Abaxial type IV trichome densities were estimated on the control, backcross, and F_2 populations at 12–13 weeks of age. Leaflets adjacent to the terminal leaflet at the fourth node were sampled. Since the mid-vein area has higher trichome densities than the rest of the leaf blade, this area was avoided to ensure comparable estimates between samples. Only abaxial surfaces were sampled, since trichome densities on the adaxial and abaxial surfaces are strongly correlated (Lemke et al. 1984). Determinations of the trichome density were made in a 2.14 mm^2 area using a dissecting microscope at 60×. The values of three replicates were averaged to give a mean value for each plant. Leaf samples were retained, and their area measured with a LI-COR model LI 3000 area meter. Leaf area was measured to test whether associations with leaf expansion rates, as reported by Snyder and Carter (1984), were controlled for by consistently sampling at the same node.

Acylsugars on the leaf surfaces of control, backcross, and F_2 populations were assayed at 15 weeks of age. Three samples per plant, each containing three to five leaflets from the fourth node, were collected and processed as described in Mutschler et al. (1996). These samples were then assayed for acylsugar content as described by Goffreda et al. (1990). The samples were assayed with and without invertase in order to estimate both acylglucose and acylsucrose content. Leaflet samples were retained and their area measured as described above to estimate the accumulation of acylsugars in units of nmol/cm^2. The values of the three replicates were averaged for each plant.

Marker genotyping

Genomic DNAs were extracted from the F_2 plants and controls as described by Doyle and Dickson (1987). Each polymerase chain reaction (PCR) with total volume of 25 µl contained 20 ng of DNA, 1 × *Taq* polymerase buffer (Boehringer Mannheim), 0.8 µ*M* random amplified polymorphic DNA (RAPD) primer, 0.2 m*M* each dNTP (Boehringer Mannheim), 1 m*M* $MgCl_2$, and 0.5 U *Taq* polymerase (Boehringer Mannheim). Once overlaid with 25 µl of mineral oil, reactions were run using an Ericomp Twinblock thermal cycler model TCX20A (San Diego, Calif.) for 40 cycles of 1 min at 94°C, 1.5 min at 35°C, 2 min at 72°C, followed by 7 min at 72°C. Amplified products were separated on 1.5% agarose gels (Maniatis et al. 1982). Ten-mer RAPD primers were synthesized at the New York State Center for Advanced Technology in Biotechnology, Cornell University, using sequences obtained from Dr. S.D. Tanksley. From an initial survey using 155 RAPD primers, 120 RAPD markers were found to be polymorphic and subsequently included in the intraspecific map. The acylglucose transferase gene (Li and Steffens, in preparation) was amplified using 20-mer primers (XL) supplied by Dr. J. C. Steffens. A similar protocol to that above was followed for amplification of this locus, where each primer was used at a concentration of 0.8 µ*M*.

Restriction fragment length polymorphism (RFLP) marker genotyping was conducted as described in Mutschler et al. (1996).

Clones supplied by Dr. S.D. Tanksley were chosen based upon their position on the interspecific map (Tanksley et al. 1992). Out of 164 clones surveyed, 52 clones were found to be polymorphic within the intraspecific population and were included in the intraspecific map.

A cleaved amplified polymorphic sequence (CAPS) marker of known location on the interspecific map (Alpert and Tanksley 1996) was included in this study. For this marker the PCR with a total volume of 25 µl contained 20 ng of DNA, 1 × *Taq* polymerase buffer (Boehringer Mannheim), 0.2 µ*M* each of forward and reverse CAP TG167 primer supplied by Dr. S.D. Tanksley, 0.8 m*M* dNTPs (Boehringer Mannheim) and 1.0 U *Taq* polymerase (Boehringer Mannheim). Once overlaid with 25 µl of mineral oil, reactions were run using the following program: 35 cycles of 1 min at 94°C, 1 min at 50°C, 2 min at 72°C, followed by 5 min at 72°C. Amplified products were digested with *Asc*I and separated on 1.5% agarose gels as indicated above.

Map construction and QTL analysis

The linkage map was constructed using MAPMAKER/EXP v3.0 (Lander et al. 1987). Markers were grouped with a minimum LOD threshold of 3.0 and maximum distance of 30 cM. Six markers were included that do not fit this criteria. Four of these markers are RFLP markers whose position on this map agrees with the position on the interspecific map (Tanksley et al. 1992); the remaining 2 are RAPD markers. Five of the markers, including both RAPD markers, no longer fit criteria due to removal of markers from the data set that skewed distances when placed on the linkage map. The LOD scores for grouping all 6 markers to their corresponding linkage groups range from 5 to 12, and the distances from the nearest markers range from 31 cM to 39 cM. All distances were expressed in Haldane centiMorgans (Haldane 1919). Order was determined using a minimum LOD of 3.0, except where noted. Triple marker error detection was used to find data indicating rare recombination events; these data were checked against the original film and corrected as needed.

Identification of QTLs associated with acylsugar accumulation and related traits was conducted using regression-based analysis as implemented in the program QGENE (Nelson 1994). This analysis was applied to each marker separately, and thus was not affected by any possible errors in the linkage map. Experiment-wise 95% and comparison-wise 99% confidence threshold values were calculated based upon 1,000 permutations of the original data (Churchill and Doerge 1994) using QGENE software.

Residual analysis was conducted as decribed by Doerge and Churchill (1996) to control for the effects of a single QTL on a given trait and search for additional QTLs. Percentage of variance of a trait explained by a QTL and potential interactions between QTLs were examined using regressional analysis.

Results

Trait distribution

L. pennellii LA716 and LA1912 have high and moderate densities of type IV trichomes, respectively (Table 1), while the F_1 has trichome densities intermediate to that of the two parents. Trichome densities of the F_2 population show a unimodal distribution with a mean between that of the F_1 and LA1912. Similar distributions are shown using data from the BC716, BC1912A and BC1912NA populations.

Acylsugar accumulation levels are high in *L. pennellii* LA716 (Table 1), but range from undetectable to low levels in the accession LA1912. Levels of acylsugars in the F_2 population show a unimodal distribution with a mean between that of the F_1 and LA1912. Similar distributions are shown using data from the BC716, BC1912A and BC1912NA populations.

A high percentage of acylsugars produced by *L. pennellii* LA716 are acylglucoses as opposed to acylsucroses (Table 1). The F_1 produce a high percentage acylglucoses indistinguishable from that of the *L. pennellii*

Table 1 Means and standard errors (in brackets) for traits measured for F_2, backcross, and control populations. Traits measured are type IV trichome density, acylsugar level (nmol/cm^2 acylglucoses + nmol/cm^2 acylsucroses), percentage acylglucoses (nmol/cm^2 acylglucoses/nmol/cm^2 acylsugar level), and leaf area measurements. Controls from F_2 and backcross populations were combined for all traits except the leaf area measurements at 12–13 weeks. For this trait, control values are based upon F_2 control populations only. Means within a column that are followed by different letters are significantly different from each other ($\alpha = 0.05$) (*NT* data not tested)

Population	Trichome density (mm^{-1})	Acylsugar level (nmole/cm^2)	Percentage acylglucoses	Leaf area (cm^2)	
				12–13 weeks	15 weeks
L. pennellii	92.5 a	612.2 a	86.5 a	3.4 a	2.0 abc
LA716	(1.1)	(44.4)	(1.0)	(0.1)	(0.1)
L. pennellii	41.5 d	27.1 f	55.6 b	2.7 b	2.4 abc
LA1912	(1.2)	(4.3)	(7.0)	(0.1)	(0.1)
F_1	72.3 b	239.2 c	84.1 a	2.9 b	2.2 abc
	(1.8)	(21.4)	(2.3)	(0.1)	(0.2)
F_2	62.0 c	189.9 c	71.4 b	2.8 b	2.0 b
	(1.0)	(5.3)	(1.13)	(0.1)	(0.0)
BC716	95.9 a	345.7 b	86.5 a	NT	2.6 c
	(1.8)	(12.0)	(0.8)		(0.1)
BC1912NA	56.8 c	49.6 e	67.8 b	NT	2.5 c
	(1.6)	(4.5)	(4.2)		(0.1)
BC1912A	56.4 c	104.5 d	72.6 b	NT	1.6 a
	(1.6)	(9.6)	(2.2)		(0.1)

LA716 parent. Percentage acylglucoses for *L. pennellii* LA1912 was difficult to accurately measure since many individuals produced extremely low or undetectable amounts of acylsugars. Therefore, in all populations, plants that accumulated less than 50 nmol/cm^2 of acylsugars were removed from the data set for analysis of percentage acylglucoses. The low-accumulating plants comprised 4.3% of the F_2, 0% of the BC716, 58.7% of the BC1912NA, and 17% of the BC1912A populations. If one considers only those individuals that accumulated 50 nmol/cm^2 or more, *L. pennellii* LA1912 produced a moderate percentage acylglucoses (Table 1).

The bimodal distribution in the F_2 population for percentage acylglucoses (Fig. 1), in which the number of plants in the two modes are in an approximate 3 : 1 ratio, indicates that this trait is largely governed by the action of a single locus in which the LA716 allele conferring high percentage acylglucose is dominant. Since percentage acylglucose is largely determined by a single locus, this trait can be converted into a qualitative trait by comparing F_2 percentages to those of the parents. Chi-square tests using the qualitative data were consistent with the above model for the F_2 ($0.25 < P < 0.50$), BC716 ($P > 0.90$), and BC1912NA ($0.20 < P < 0.25$) populations. The data from the BC1912A population were consistent with the single-gene model if one assumes the LA1912 parent is heterozygous for this trait ($0.50 < P < 0.75$). The LA1912 parent produces an unusually high percentage acylglucoses for this accession (65%), suggesting that this plant may have one or more dominant alleles at this locus. Since the accession LA1912 is self-incompatible, it is plausible that the LA1912 parent is heterozygous at this locus.

Using percentage acylglucose data converted to qualitative data and determining linkage distances using MAPMAKER/EXP, we estimated the position of this locus to be 6 cM below TG549. Due to possible misclassifications when converting percentage acylglucose data into qualitative data, 6 cM is possibly an overestimate of this distance (Lincoln and Lander 1992). Using the gene-effect model presented in the appendix, we estimated that the LA716 allele increased the proportion of acylglucoses by 35.6% of the acylsugar level and that the second LA716 allele in homozygotes increased the proportion of acylglucoses by an additionl 4.9% of the acylsugar level.

Correlations among traits

No significant correlations were observed between type IV trichome density and leaf area in the F_2 population, indicating a lack of bias in estimates from differences in leaf expansion rates. A moderate positive correlation ($R^2 = 0.13$; $P < 0.001$) was observed in the F_2 population

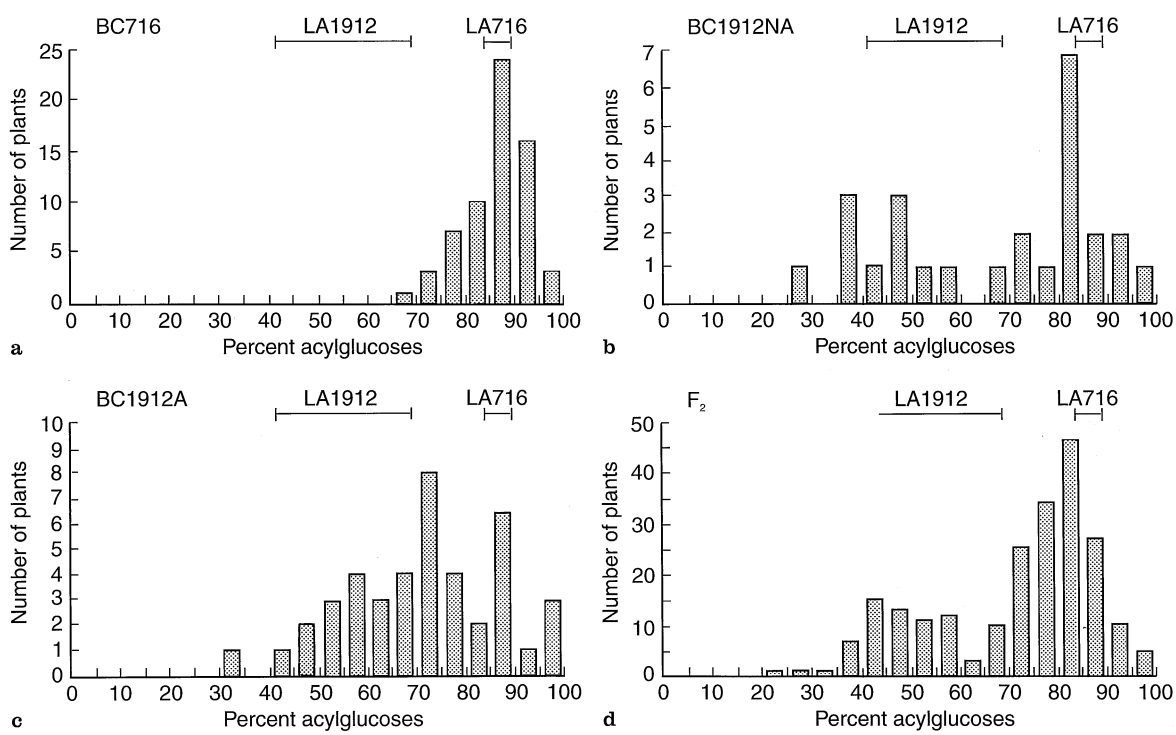

Fig. 1a–d Histograms of percentage acylglucoses for **a** BC716, **b** BC1912NA, **c** BC1912A, and **d** F_2 populations. Plants producing less than 50 nmol/cm^2 are not included. The 95% confidence intervals for parental controls *L. pennellii* LA716 (*LA716*) and *L. pennellii* LA1912 (*LA1912*) are shown *above* histograms

between type IV trichome density and acylsugar level. This correlation indicates that acylsugar level might be increased as type IV trichome density increases, or that some other common factor affects both acylsugar level and trichome density.

A moderate positive correlation was observed in the F_2 population between percentage acylglucoses and acylsugar levels ($R^2 = 0.05$; $P < 0.001$). If one considers plants with high and low percentage acylglucoses separately, it is apparent that the correlation detected is due to correlations with plants with a high percentage acylglucoses ($R^2 = 0.16$; $P < 0.001$) and not a low percentage acylglucoses ($R^2 = 0.01$; $P > 0.5$). This suggests an epistatic interaction between genes controlling percentage acylglucoses over levels of acylsugar accumulation. All other correlations were found to be either statistically or biologically insignificant.

Map construction

A linkage map was constructed using the F_2 population and includes 120 RAPD markers, 55 RFLP markers, one PCR-based marker corresponding to acylglucose transferase and one CAPS marker (Fig. 2). This map covers 1449.1 cM with intervals between markers ranging from 0.0 to 43.4 cM. The clustering of RAPD markers strongly parallels the regions at which clustering of RFLP markers has been observed (Pillen et al. 1996). No differences in the order of RFLP markers from single-copy clones were detected from that of Tanksley et al. (1992). However, 3 multiple-copy clones produced RFLP markers which mapped to areas of the genome other than those found on the interspecific map. These RFLPs, generated using TG260, CT234 and CT92 as probes, mapped to chromosome 1, 7, and 11, respectively.

QTL mapping analysis

Seven QTLs were associated with type IV trichome density (QTLs 2A, 4A, 5A, 6A, 7B, 10A, and 11A; see usage in Table 2). QTLs 2A, 4A, 6A and 11A each show linkage to only 1 marker. *L. pennellii* LA716 alleles decrease trichome density at QTL 7B and increase trichome density at the other 6 QTLs. When a linear model with all 7 QTLs as predictors is used, the effects of QTLs 2A, 4A, and 11A become insignificant. Together, QTLs 5A, 6A, 7B and 10A explain 19.1% of the variance of type IV trichome density.

Six QTLs were associated with acylsugar accumulation levels (QTLs 2B, 3A, 4A, 5A, 7B, and 10A). QTLs 2B, 4A, and 7B each show linkage to only 1 marker. The F-value ($F = 16.39$) corresponding to QTL 10A falls just below the 95% experiment-wise cutoff ($F = 16.45$). *L. pennellii* LA716 alleles at all 6 QTLs increase acylsugar levels. When a linear model with all 7 QTLs as predictors is used, the effect of QTL 2B becomes insignificant. Together, the remaining QTLs explain 38.3% of the variance of acylsugar levels.

Four QTLs were associated with percentage acylglucoses (QTLs 3B, 7A, 9A and 10A). The F-value ($F = 140.26$) corresponding to QTL 3B greatly exceeds the 95% experiment-wise cutoff ($F = 17.23$); this QTL corresponds to the major locus detected near TG549 discussed earlier. QTLs 7A, 9A, and 10A each show linkage to only 1 marker. *L. pennellii* LA716 alleles at QTLs 3B, 9A, and 10A result in an increased percentage acylglucoses while those at QTL 7A decrease percentage acylglucoses. When a linear model with all 4 QTLs as predictors is used, the effects of QTLs 7A and 9A become insignificant. Together, QTLs 3B and 10A explain 51.1% of the variance of percentage acylglucoses.

QTL analysis was conducted using leaf area data (Eggleston 1997). One QTL on chromosome 4 (QTL 4B) was found to have a F-value ($F = 17.71$) that exceeds the 95% experiment-wise cutoff ($F = 16.78$). All the other QTLs detected were not investigated further since these QTLs have fairly low F-values, their combined QTL explain only up to 12.5% of the variance of leaf area, and leaf area has no direct effect on acylsugar level.

Analysis using MAPMAKER/EXP v1.1 (Paterson et al. 1988) with a LOD threshold of 2.4 support the identification of QTLs using regression analysis, except for QTL 2A originally found to be associated with trichome density (data not shown). Additional QTLs were found using this program, but these QTLs were not pursued due to their low LOD scores. Residual analysis for all traits based on each QTL detected failed to reveal additional loci. There was no evidence for epistatic interactions between QTLs for each trait.

Discussion

Twelve putative QTLs were found to be associated with type IV trichome density, acylsugar level, and percentage acylglucose. Of these 12 loci, 1 exceeds and 1 falls just below the 95% experiment-wise cutoff. These 2 QTLs, on chromosomes 3 and 10, respectively, are primarily associated with percentage acylglucoses and acylsugar levels, respectively. In addition, 1 QTL on chromosome 4 was found to be associated with leaf area, with an F-value that exceeds the 95% experiment-wise cutoff. The detection of 1 or more of the remaining 10 putative QTLs is potentially due to spurious associations. Therefore, it would be prudent to verify these QTLs through comparisons to previous studies.

The results of this study can be compared to those from three previous studies. The first examined acylsugar accumulation using an interspecific population between *L. esculentum* and *L. pennellii* LA716

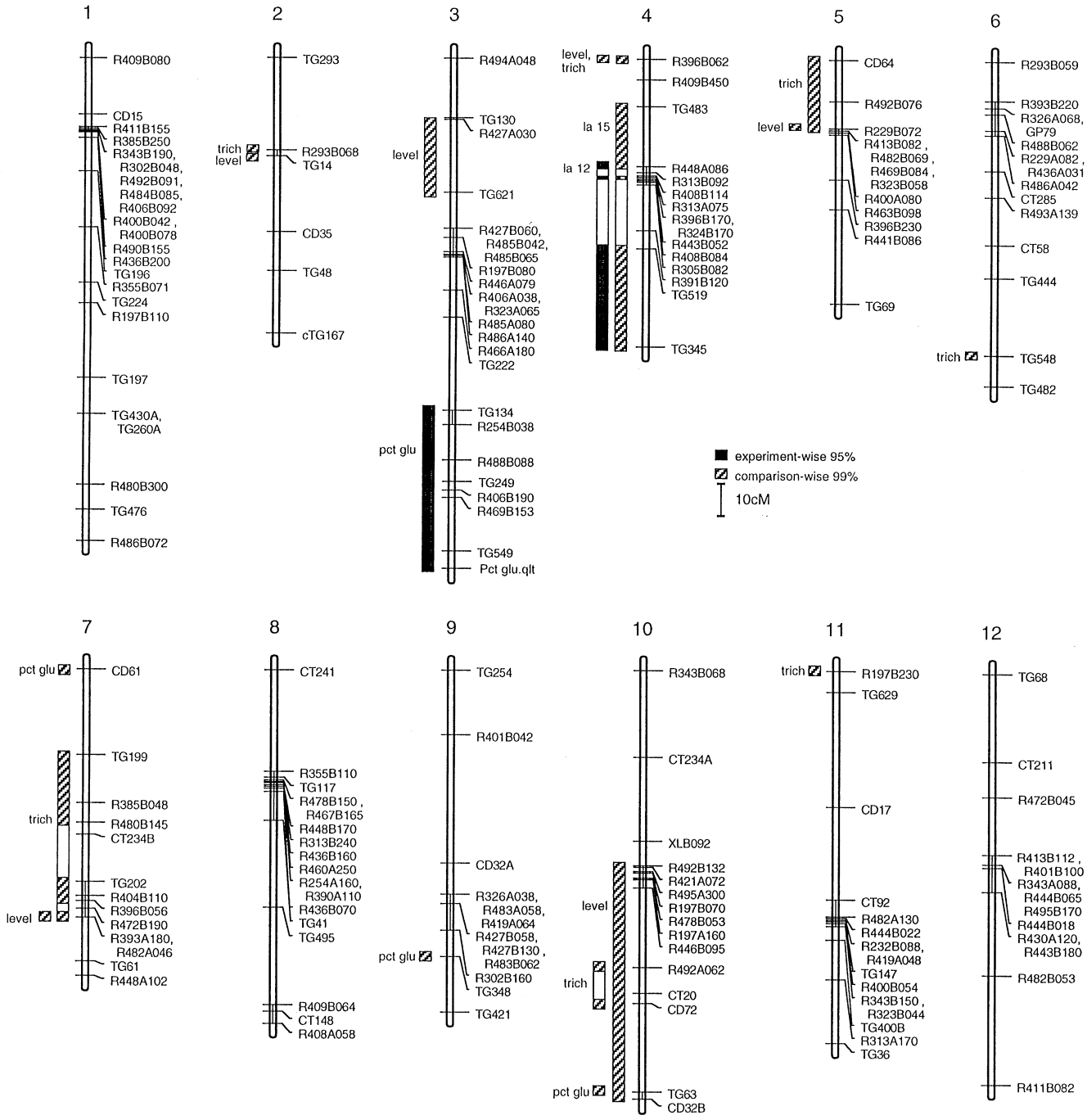

Fig. 2 Linkage of intraspecific F_2 population. RFLP markers are labeled as in Tanksley et al. (1992); RAPD markers are labelled with *R* followed by the primer *number*, a *letter* denoting specificity to LA716 (*A*) or LA1912 (*B*) and size of the band scored in 10 bp. A marker corresponding to acylglucose transferase is labeled *XL* followed by specificity and size. Pct glu.qlt is generated by converting percentage acylglucose data to qualitative data. Order was determined using minimum LOD 3.0; *vertical bars* indicate regions where marker order does not fit this criterium. *Bars* to the *left* of chromosomes indicate QTL associated with type IV trichome density (*trich*), acylsugar level (*level*), and percentage acylglucoses (*pct glu*). One QTL associated with leaf area measured at 12–13 weeks (*la12*) and 15 weeks (*la15*) is included. Pattern of *bars* denotes level of significance at corresponding markers as follows: exceeds 95% experiment-wise cutoff (*solid*), or 99% comparison-wise cutoff (*diagonal lines*), or falls below 99% comparison-wise cutoff (*white*)

(Mutschler et al. 1996). Acylsugar accumulation levels and percentage acylglucoses were both measured in this population. Since type IV trichome density did not segregate in other *L. esculentum* × *L. pennellii* LA716 F_2 populations studied by Mutschler et al. (unpublished data), this trait was not measured. The second study examined type IV trichome density and greenhouse whitefly resistance using a population derived from crosses between *L. esculentum* and *L. hirsutum* f. *glabratum* (Maliepaard et al. 1995). Since 2-tridecanone in type VI trichome glands of *L. hirsutum* has insecticide activity (Williams et al. 1980), we cannot assume that QTLs associated with resistance will include QTLs conferring acylsugar accumulation. The third study examined type IV trichome density and acylsugar accumulation levels in a population derived from crosses between *Solanum tuberosum* and *S. berthaultii* (Bonierbale et al. 1994). Percentage acylglucoses was not measured in this population, since *S. berthaultii* accumulates acylsucroses exclusively.

Five QTLs identified in the current study were also reported in one or more of the prior studies (Table 2). QTL 2B associated with acylsugar accumulation levels was also detected by Mutschler et al. (1996) and Bonierbale et al. (1994). Mutschler et al. also found this QTL to be associated with percentage acylglucoses. QTL 3A associated with acylsugar accumulation levels was detected by Mutschler et al. (1996) and was also found to be associated with percentage acylglucoses. QTL 4A associated with acylsugar accumulation levels was also detected by Mutschler et al. (1996) and Bonierbale et al. (1994). In addition, this QTL was found to be linked to type IV trichome density in this study. Mutschler et al. (1996) also found this QTL to be associated with percentage acylglucoses. QTL 5A affecting type IV trichome density and acylsugar accumulation levels was found to be associated with both traits by Bonierbale et al. (1994), and associated with type IV trichome density by Maliepaard et al. (1995). QTL 11A affecting type IV trichome density was also detected by Bonierbale et al. (1994). The remaining 5 QTLs that were not identified in previous studies all exceed the 99% comparison-wise cutoffs, but not the 95% experiment-wise cutoffs. Therefore, it is possible that the identification of 1 or more of the QTLs may be spurious.

Several QTLs detected in the above-mentioned studies were not detected in this study. It is likely that QTLs were detected in this population that have not been found in other populations, and vice versa, due to the great differences in genetic background of the different populations used. For example, since the interspecific population studied by Mutschler et al. (1996) did not segregate for type IV trichome density, any QTLs that affect acylsugar levels through their effects on trichome density cannot be detected.

The results of our study furthers our understanding of acylsugar accumulation and aids in the transfer of acylsugar-mediated resistance to cultivated tomato.

Table 2 QTL detected for type IV trichome density (*trich*), acylsugar level (*level*) and percentage acylglucoses (*pct glu*). One QTL associated with leaf area at 12–13 weeks (la 12), and leaf area at 15 weeks (la 15) is included. Marker range of the QTL corresponds to that of the trait with the highest *F*-value. Non-contiguous ranges are marked with an asterisk. Highest *F*-value for each QTL and trait exceeds 99% comparison-wise cutoff. Those *F*-values that exceed 95% experiment-wise cutoff are marked with an asterisk. Similar findings for QTL of acylsugar and affiliated traits are noted from *MM* Mutschler et al. (1996), *CM* Maliepaard et al. (1995), and *MB* Bonierbale et al. (1994)

Chromosome	QTL	Marker at peak (marker range)	*F* value	R^2	Traits	Similar findings
Ch2	2A	R293B068	7	2.6	Trich	
	2B	TG14	7	0.0	Level	Level, pct glu (MM) Level (MB)
Ch3	3A	R427A030 (R427A030-TG621)	9	2.3	Level	Level, pct glu (MM)
	3B	TG549 (TG134-TG549)*	140*	50	Pct glu	
Ch4	4A	R396B062	11	7.7	Level	Level, pct glu (MM)
			7	5.2	Trich	Level (MB)
	4B	R313A075	− 18*	4.9	La 12	
		(TG483-TG345)*	− 13	1.7	La 15	
Ch5	5A	R400A080	11	5.1	Trich	Level, trich (MB)
		(CD64-R400A080)*	10	2.5	Level	Trich (CM)
Ch6	6A	TG548	6	4.7	Trich	
Ch7	7A	CD61	− 11	1.3	Pct glu	
	7B	R396B056	− 12	3.0	Trich	
		(TG199-R482A046)*	7	2.8	Level	
Ch9	9A	TG348	5	5.1	Pct glu	
Ch10	10A	R492A062	16	13	Level	
		(R492B132-CD32B)	7	4.6	Trich	
			6	4.7	Pct glu	
Ch11	11A	R197B230	11	8.1	Trich	Trich (MB)

QTL 10A, which we found to be primarily associated with acylsugar level, has not been reported previously. This QTL may be useful in increasing acylsugar accumulation levels in breeding populations, if the LA716 allele proves to be the active allele and if this allele is not present in *L. esculentum*. This study also confirms previously identified QTLs and identifies several putative QTLs for further study.

A positive correlation between acylsugar accumulation levels and type IV trichome density was previously observed by Bonierbale et al. (1994) in *Solanum*. The current study also shows a significant correlation between the two traits, and 3 QTLs were detected in which *L. pennellii* LA716 alleles increase both type IV trichome density and acylsugar accumulation levels. This suggests the possibility that acylsugar levels can be raised by increasing the density of trichomes on the leaf surface. However, a fourth locus was found in this study for which *L. pennellii* LA716 alleles increase acylsugar levels but decrease trichome density. Also, 3 loci were found to be associated with trichome density without any effect on acylsugar levels. These results imply that increasing trichome density may not be an effective strategy for raising acylsugar level in breeding populations.

Percentage acylglucoses was found to be primarily controlled by a single locus on chromosome 3. In contrast, in the interspecific F_2 population between *L. esculentum* and *L. pennellii* LA716 Mutschler et al. (1996) observed that this trait is largely determined by a single QTL on chromosome 11. In the intraspecific population, the *L. pennellii* LA716 allele is dominant and confers high percentage acylglucose; in the interspecific population, the *L. esculentum* allele is dominant and confers low percentage acylglucoses. Thus, these 2 QTLs correspond to separate loci affecting percentage acylglucoses. The ability to convert the percentage acylglucose data into qualitative data opens the possibility for isolating genes involved in the accumulation of acylsugars by positional cloning.

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Appendix

A model was developed to estimate the effect of an allele (δ) given the distance in recombination frequency (θ) from the nearest marker (M) and the mean values of a given trait for each marker class (Y_M). The mean trait value for each marker genotypic class can be expressed as the mean trait value for trait genotypic class Q_2Q_2 with the additional effect on the phenotype due to the presence of Q_1 alleles which is unique for each marker genotype (Δ_M).

$$Y_M = Y_{Q_2Q_2} + \Delta_M \quad (1)$$

The expectation of the effect on the phenotype due to the presence of Q_1 alleles given the marker genotype can be calculated using a simple recombination model (Table 3). By summing across all

Table 3 Development of gene effect model using Mendelian genetics

Configuration	M[a]	Q[b]	P(Q|M)[c]	Effect|Q[d]	Effect|M[e]
M_1Q_1/M_1Q_1	M_1M_1	Q_1Q_1	$(1-\theta)^{2}$[f]	δ	$\delta(1-\theta)^2$
M_1Q_1/M_1Q_2	M_1M_1	Q_1Q_2	$\theta(1-\theta)$	xδ	x$\delta\theta(1-\theta)$
M_1Q_2/M_1Q_1	M_1M_1	Q_1Q_2	$\theta(1-\theta)$	xδ	x$\delta\theta(1-\theta)$
M_1Q_2/M_1Q_2	M_1M_1	Q_2Q_2	θ^2	0	0
M_1Q_1/M_2Q_1	M_1M_2	Q_1Q_1	$\theta(1-\theta)/2$	δ	$\delta\theta(1-\theta)/2$
M_1Q_1/M_2Q_2	M_1M_2	Q_1Q_2	$(1-\theta)^2/2$	xδ	x$\delta(1-\theta)^2/2$
M_1Q_2/M_2Q_1	M_1M_2	Q_1Q_2	$\theta^2/2$	xδ	x$\delta\theta^2/2$
M_1Q_2/M_2Q_2	M_1M_2	Q_2Q_2	$\theta(1-\theta)/2$	0	0
M_2Q_1/M_1Q_1	M_1M_2	Q_1Q_1	$\theta(1-\theta)/2$	δ	$\delta\theta(1-\theta)/2$
M_2Q_1/M_1Q_2	M_1M_2	Q_1Q_2	$\theta^2/2$	xδ	x$\delta\theta^2/2$
M_2Q_2/M_1Q_1	M_1M_2	Q_1Q_2	$(1-\theta)^2/2$	xδ	x$\delta(1-\theta)^2/2$
M_2Q_2/M_1Q_2	M_1M_2	Q_2Q_2	$\theta(1-\theta)/2$	0	0
M_2Q_1/M_2Q_1	M_2M_2	Q_1Q_1	θ^2	δ	$\delta\theta^2$
M_2Q_1/M_2Q_2	M_2M_2	Q_1Q_2	$\theta(1-\theta)$	xδ	x$\delta\theta(1-\theta)$
M_2Q_2/M_2Q_1	M_2M_2	Q_1Q_2	$\theta(1-\theta)$	xδ	x$\delta\theta(1-\theta)$
M_2Q_2/M_2Q_2	M_2M_2	Q_2Q_2	$(1-\theta)^2$	0	0

[a] Marker genotye (alleles M_1 or M_2)
[b] QTL genotype (alleles Q_1 or Q_2)
[c] Probability of the QTL genotype given the marker genotype based upon the recombination frequency between the marker and the QTL
[d] Change in trait phenotype from Q_2Q_2 genotype due to the presence of one or more Q_1 allele
[e] Expectation of effect on phenotype due to QTL genotype given marker genotype
[f] Variebles are defined as follows: θ, recombination frequency; x, dominance of the Q_1 allele where 0 = fully recessive and 1 = fully dominant; δ, the effect of a single Q_1 allele

QTL genotypes (Q) for each marker genotype (M), assuming that the original F_1 parent had the configuration M_1Q_1/M_2Q_2, the expectation of effect given marker genotype is determined.

$$\Delta_{M_1M_1} = \delta(1-\theta)^2 + 2x\delta\theta(1-\theta) \quad (2a)$$

$$\Delta_{M_1M_2} = 2\delta\theta(1-\theta)/2 + 2x\delta(1-\theta)^2/2 + 2x\delta\theta^2/2 \quad (2b)$$

$$\Delta_{M_2M_2} = \delta\theta^2 + 2x\delta\theta(1-\theta) \quad (2c)$$

For each pair of marker genotypes, the difference between trait means can be calculated as

$$Y_{M_i} - Y_{M_j} = \Delta_{M_i} - \Delta_{M_j} \quad (3)$$

where i and j are two different genotypic classes for marker M. This can be expanded using Eqs. 2a–c:

$$Y_{M_1M_1} - Y_{M_1M_2} = \delta(1-\theta)^2 + 2x\delta\theta(1-\theta) - 2\delta\theta(1-\theta)/2 - 2x\delta(1-\theta)^2/2 - 2x\delta\theta^2/2 \quad (4a)$$

$$Y_{M_1M_1} - Y_{M_2M_2} = \delta(1-\theta)^2 + 2x\delta\theta(1-\theta) - \delta\theta^2 - 2x\delta\theta(1-\theta) \quad (4b)$$

$$Y_{M_1M_2} - Y_{M_2M_2} = 2\delta\theta(1-\theta)/2 + 2x\delta(1-\theta)^2/2 + 2x\delta\theta^2/2 - \delta\theta^2 - 2x\delta\theta(1-\theta), \quad (4c)$$

At this point, estimates for recombination frequency (θ), and trait means for each marker genotype (Y_M) can be substituted in Eqs. 4a–c estimates can be found for x and δ.

This model can be adapted for dominant markers or other segregating populations by adjusting $P(Q \mid M)$ in Table 3 and making similar corrections in Eqs. 1–4. In the case of dominant markers, both x and δ cannot be calculated since there are only two marker genotypic classes, resulting in one equation corresponding to Eq. 4 above. In this case, 0 and 1 can be entered for x, and a range of values for δ can be calculated.

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