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Identification of QTLs controlling acylsugar fatty acid composition in an intraspecific population of *Lycopersicon pennellii* (Corr.) D'Arcy

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Abstract Acylsugars exuded by type IV glandular trichomes are responsible for insect resistances found in many *Lycopersicon pennellii* accessions. Acylsugars are complex mixtures composed of polyacylated sugars (glucose or sucrose) esterified to branched and straight-chain 4:0 to 12:0 fatty acids. The biogeneses of these unusual fatty acid constituents have their origins in branched-chain amino acid pathways. However, the mechanism of fatty acid elongation in these systems and the genetic control of carbon flux from amino acid to fatty acid pathways remain unclear. In this study, we used an intraspecific F_2 population derived from the cross between *L. pennellii* LA716 and *L. pennellii* LA1912 to examine the genetic basis of acylsugar fatty acid composition. Six QTLs were detected which, combined, explain 23–60% of the variance observed for each of the nine segregating fatty acid constituents. Both correlation data and QTL analysis data indicate that branched medium-chain fatty acids are synthesized through elongation of short-chain precursors in two-carbon increments. The proportion of *iso*-branched acylsugar fatty acids that have an even-carbon chain length was found to be primarily determined by a single locus that maps to a location 5.5 cM above TG117 on chromosome 8. QTL function in several cases can be inferred from discrete patterns of fatty acid composition; in other cases, control of acylsugar fatty acid composition appears to be complex.

Key words Sugar ester · Quantitative trait loci · QTL mapping · Transglucosylase · Transacylase

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Abbreviations *i*4:0 2-Methylpropanoate · *i*10:0 8-Methylnonanoate · *i*5:0 3-Methylbutanoate · *i*9:0 7-Methyloctanoate · *i*11:0 9-Methyldecanoate · *ai*5:0 2-Methylbutanoate · *ai*6:0 3-Methylpentanoate · *n*6:0 *n*-Hexanoate · *n*10:0 *n*-Decanoate · *n*12:0 *n*-Dodecanoate · *iso*-branched *i*4:0, *i*10:0, *i*5:0, *i*9:0, *i*11:0 · straight-chain (normal) *n*10:0, *n*12:0 · *anteiso*-branched *ai*5:0, *ai*6:0 · *iso*-evens *i*4:0, *i*10:0 · *iso*-odds *i*5:0, *i*9:0, *i*11:0

Introduction

Type IV glandular trichome exudates of certain accessions of *Lycopersicon pennellii* (Corr.) D'Arcy, a wild relative of the cultivated tomato *L. esculentum* Mill., are primarily composed of acylsugars (Burke et al. 1987). These acylsugars 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). Accessions of *L. pennellii* vary considerably in their acylsugar accumulation level and composition (Shapiro et al. 1994). Structurally, acylsugars vary in their sugar moieties (glucose or sucrose), the number and regiochemistry of acyl groups esterified, the length of their fatty acid constituents (even or odd length, 4–12 carbons) and fatty acid branching structure (*iso*-, *anteiso*- or straight).

The acylsugars of *L. pennellii* LA716 are chiefly comprised of 2,3,4-tri-*O*-acyl glucose esters possessing short-chain, *iso*- and *anteiso*-branched fatty acids (4:0 and 5:0) and medium-chain length, straight and *iso*-branched fatty acids (10:0, 11:0 and 12:0) (Burke et al. 1987). The fatty acid constituents of *L. pennellii* LA716 include 2-methylpropanoate (*i*4:0), 2-methylbutanoate

(*ai*5 : 0), 3-methylbutanoate (*i*5 : 0), 8-methylnonanoate (*i*10 : 0), *n*-decanoate (*n*10 : 0), 9-methyldecanoate (*i*11 : 0), 10-methyl-undecanoate (*i*12 : 0) and *n*-dodecanoate (*n*12 : 0) (Burke et al. 1987).

The short-chain fatty acid constituents of *L. pennellii* LA716 and *Nicotiana tabacum* acylsugars are synthesized from branched amino acids through a process of transamination and oxidative decarboxylation of the resulting branched 2-oxo acid (Walters and Steffens 1990; Kandra et al. 1990). For example, Val is the precursor for *i*4 : 0, Leu is the precursor for *i*5 : 0, and Ile is the precursor for *ai*5 : 0. Walters and Steffens (1990), using stable isotope labeling and GC-MS, showed that *i*4 : 0 and *i*5 : 0 act as primers for the biosynthesis of *iso*-branched 9 : 0 to 12 : 0 fatty acids in two-carbon increments. Similarly, straight-chain fatty acids were shown to be elongated in a manner consistent with the two-carbon extension reactions characteristic of de novo fatty acid biosynthesis. In contrast, Kroumova et al. (1994) proposed a novel mechanism for fatty acid elongation which involves extension of straight- and branched-chain 2-oxo acids in one-carbon increments. This mechanism is hypothesized to utilize the enzymology of the Leu biosynthetic pathway, i.e. α-isopropyl malate synthase, α-isopropyl malate dehydratase and isopropyl malate dehydrogenase, to accomplish not only the biosynthesis of Leu but also that of even and odd chain length, *iso*- and *anteiso*-branched and straight chain fatty acids ranging from 3 to 12 carbon atoms in length.

Esterification of fatty acids to glucose in *L. pennellii* follows a multi-step process (Ghangas and Steffens 1993, 1995). Fatty acids are first activated to 1-*O*-acyl-*β*-glucose derivatives by UDPGlc : fatty acid transglucosylases (Kuai et al. 1997). The activated fatty acid is then transferred to non-anomeric positions of other glucose molecules or partially acylated glucose molecules by *β*-1-*O*-acylglucose : glucose acyltransferase. Although UDPGlc : fatty acid transglucosylases are chain-length specific, they exhibit a broad range of activities (Kuai et al. 1997), whereas the acyltransferase exhibits relatively little specificity for chain length of the 1-*O*-acyl-*β*-glucose acyl donor (Ghangas and Steffens unpublished). These observations suggest that neither of these enzymes exerts a significant effect on acylglucose fatty acid composition (Kuai et al. 1997). In fact, substrate feeding studies indicate that the fatty acid composition of *L. pennellii* acylsugars is controlled at the level of substrate availability (amino acids or 2-oxo acids) (Walters and Steffens 1990). Since acylsugars are secreted extracellularly, they do not undergo turnover after synthesis. Therefore, the profile of fatty acids in acylsugars can be taken as an indication of the underlying biosynthetic activities leading to availability of fatty acids to the activation/acylation/secretion machinery.

L. pennellii exhibits a high degree of acylsugar structural diversity and variation in acylsugar production levels (Shapiro et al. 1994; Blauth et al. 1998). Alleles affecting acylsugar fatty acid profiles are likely to exert either substrate-level control of fatty acids derived from branched amino acid biosynthesis, control of de novo fatty acid biosynthesis or control at the level of elongation of branched-chain fatty acid primers. Therefore, an examination of the genetic control of acylsugar fatty acid composition may shed light not only on the mode of fatty acid elongation (Walters and Steffens 1990; Kroumova et al. 1994) and the interactions of these pathways but may also provide information useful for the utilization of acylsugar-mediated resistance. In the investigation reported here, we used an intraspecific F_2 population derived from the cross between *L. pennellii* LA716 and *L. pennellii* LA1912 to examine the genetic control of amino acid and fatty acid pathways that lead to the biosynthesis of fatty acid constituents of acylsugars.

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. This F_1 was self-pollinated to create an F_2 population. Two hundred and thirty-one F_2 plants were grown under normal greenhouse conditions, along with *L. pennellii* LA716, LA1912 and their F_1 as controls.

Phenotypic screening

Leaf rinsates were collected as described in Blauth et al. (1998) and processed as described in Mutschler et al. (1996). These samples were then assayed for acylsugar content as described by Goffreda et al. (1990). Using these acylsugar level estimates and assuming an average of three fatty acids per acylsugar molecule we calculated the fraction of total volume containing 300 μg of fatty acid constituents. Leaf rinsates were resuspended in methanol, and the calculated fraction of total volume was removed, placed in a disposable glass test tube and left to evaporate. This sample of approximately 300 μg of fatty acids was then transesterified with sodium ethoxide (Walters and Steffens 1990). Following transesterification, ethyl ester samples were analyzed using gas chromatography (GC) as described by Walters and Steffens (1990). All samples were analyzed within 12 h of transesterification. Data for each fatty acid were calculated as a percentage of the total area of fatty acids detected through gas chromatography.

Identification of fatty acid constituents was accomplished by comparing retention times to those of fatty acid ethyl ester standards and to those of ethyl esters from*L. pennellii* LA716. One peak with a retention time (11.9) between those of *i*5 : 0 and control *n*-hexanaote (*n*6 : 0) has not been found in *L. pennellii* LA716. The fatty acid corresponding to this retention time was identified as 3-methylpentanoate (*ai*6 : 0) through GC-MS as described by Walters and Steffens (1990).

Data analysis

Marker genotyping and map construction were conducted as described in Blauth et al. (1998). All map distances were calculated

using MAPMAKER/EXP (Lander et al. 1987) and are reported in Haldane centiMorgans (Haldane 1919) unless otherwise specified. Estimation of quantitative trait locus (QTL) effects was conducted using the gene effects model described by Blauth et al. (1998). Identification of QTLs associated with fatty acid composition 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% 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 described 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. In addition to univariate trait analysis, a number of combination traits were analyzed including major principal components and other linear combinations motivated by proposed biochemical pathways of acylsugar biosynthesis.

Results and discussion

Fatty acid profiles for *L. pennellii* LA716, LA1912, F_1 and F_2 plants are shown in Table 1. The profile for *L. pennellii* LA716 is very similar to that reported in previous studies (Burke et al. 1987; Walters and Steffens 1990; Shapiro et al. 1994). Means and standard deviations of the fatty acid constituents for the F_2 population and controls indicate that the F_2 population segregates for the relative proportions of nine acylsugar fatty acids: *i*4:0, *i*10:0, *i*5:0, *i*9:0, *i*11:0, *ai*5:0, *ai*6:0, *n*10:0 and *n*12:0 (Table 1). Although the *L. pennellii* LA1912 control population assorts for acylsugar accumulation levels, including individuals whose levels are undetectable by the acylsugar assay reported by Goffreda et al. (1990), the sensitivity of gas chromatography allowed detection of acylsugar fatty acids in all LA1912 individuals.

It is interesting to note that for several of the fatty acids, i.e. *i*4:0, *i*10:0, *i*5:0 and *n*10:0, the means for the parental controls are not significantly different from each other, while the means of the F_1 or F_2 population are significantly different from one or both of the parental controls. This may be an example of transgressive segregation but may also be due to the self-incompatibility of *L. pennellii* LA1912, resulting in heterogeneous populations in which the LA1912 and F_1 means may not accurately depict the values for the actual parent of the F_2 population.

Correlations between traits

No significant correlations were found between relative fatty acid levels and the traits acylsugar levels, trichome densities or glucose:sucrose ratio previously discussed by Blauth et al. (1998). However, a regular pattern of high correlations was observed among the different fatty acid constituents (Table 2). The levels of fatty acids are positively correlated within the same category of branching pattern and chain length (even or odd carbon length). However, fatty acid classes between these categories are negatively correlated. These positive fatty acid correlations are likely to represent

Table 1 Mean and standard deviations (in parentheses) for traits measured for F_2 and control populations. Values for the nine fatty acids with segregating levels are expressed as a percentage of all fatty acid constituents detected. Other fatty acids detected but not included in this table failed to segregate in the F_2 population and comprise approximately 2% or less of the total amount detected (*n* population size)

Population (*n*)	*L. pennellii* LA716 (6)	*L. pennellii* LA1912 (23)	F_1 (16)	F_2 (231)
*i*4:0 (2-methylpropanoate)	34.1[a] a, b (8.8)	26.1 a (9.5)	33.7 b (8.0)	29.0 a (7.6)
*i*10:0 (8-methylnonanoate)	11.8 a, b (7.6)	11.9 a (6.2)	12.7 b (4.0)	12.8 b (4.6)
*i*5:0 (3-methylbutanoate)	9.8 a (2.7)	14.8 a, b, c (9.8)	13.3 b (2.4)	15.4 c (6.1)
*i*9:0 (7-methyloctanoate)	0.1 a (0.2)	3.4 d (3.4)	1.3 b (0.8)	1.9 c (1.5)
*i*11:0 (9-methyldecanoate)	0.2 a (0.5)	5.9 b, c (5.8)	3.8 b (1.2)	4.7 c (3.0)
*ai*5:0 (2-methylbutanoate)	33.0 a (14.9)	15.9 b (4.6)	15.1 b (3.2)	15.4 b (6.1)
*ai*6:0 (3-methylpentanoate)	0.2 a (0.2)	2.0 b (1.7)	3.7 c (1.6)	4.7 c (3.0)
*n*10:0 (*n*-decanoate)	4.1 a (2.7)	5.5 a (2.7)	6.3 a (2.4)	7.7 b (3.0)
*n*12:0 (*n*-dodecanoate)	0.9 a (1.2)	14.3 c (15.8)	7.0 b, c (5.6)	5.7 b (3.0)

[a] Means within a row that are followed by the same letter are not significantly different from each other ($\alpha = 0.5$) by Fisher's protected LSD (Ott 1993)

Table 2 Correlation table for nine segregating fatty acids. Table includes correlation coefficients followed by *P*-values. Bold numerals denote significant correlation ($P < 0.001$)

	*i*4:0	*i*10:0	*i*5:0	*i*9:0	*i*11:0	*ai*5:0	*ai*6:0	*n*10:0	*n*12:0
*i*4:0	1.00								
*i*10:0	**+0.66 (0.000)**	1.00							
*i*5:0	**−0.62 (0.000)**	**−0.84 (0.000)**	1.00						
*i*9:0	−0.15 (0.026)	**−0.25 (0.000)**	**+0.44 (0.000)**	1.00					
*i*11:0	**−0.40 (0.000)**	**−0.30 (0.000)**	**+0.47 (0.000)**	**+0.81 (0.000)**	1.00				
*ai*5:0	**+0.27 (0.000)**	0.13 (0.049)	**−0.36 (0.000)**	**−0.37 (0.000)**	**−0.53 (0.000)**	1.00			
*ai*6:0	**−0.66 (0.000)**	**+0.79 (0.000)**	**+0.86 (0.000)**	**+0.36 (0.000)**	**+0.44 (0.000)**	**−0.40 (0.000)**	1.00		
*n*10:0	**−0.38 (0.000)**	0.03 (0.659)	−0.11 (0.081)	**−0.43 (0.000)**	−0.15 (0.027)	**−0.29 (0.000)**	0.01 (0.858)	1.00	
*n*12:0	**−0.41 (0.000)**	0.04 (0.586)	−0.15 (0.023)	**−0.47 (0.000)**	−0.09 (0.185)	**−0.23 (0.000)**	−0.07 (0.284)	**+0.76 (0.000)**	1.00

precursor : product relationships between short-chain and elongated fatty acids within the same category and, in fact, are in accordance with the biosynthetic patterns of primer utilization and extension reactions established by Walters and Steffens (1990). For example, *i*4 : 0 was shown by Walters and Steffens to be derived from the Val pathway and to serve as a primer for two-carbon extension reactions. Hence, in these populations the level of *i*4 : 0 are highly, positively correlated with those of *i*10 : 0, its extension product. Similarly, *i*5 : 0 is known as a biosynthetic primer for *i*9 : 0 and *i*11 : 0, and this precursor : product relationship results in a similar correlation pattern. The negative correlations observed may be due to competition between the synthesis of short-chain fatty acids of different categories. Alternatively, the negative correlations may be an artifact of data construction, since normalization of peak area to a percentage of total area detected may produce negative correlations between fatty acids derived from independent pathways.

*N*10 : 0 and *n*12 : 0 show a strong positive correlation as expected since *n*12 : 0 is an extension product of *n*10 : 0. Both *n*10 : 0 and *n*12 : 0 show significant negative correlations with *i*4 : 0, *ai*5 : 0 and *i*9 : 0 which may reflect competition between the de novo fatty acid biosynthetic pathway responsible for straight-chain acids and the extension pathways that utilize branched short chain primers.

Nevertheless, our knowledge of the biosynthesis of these fatty acids does not explain some strong correlations. For example *ai*5 : 0, derived from the Ile biosynthetic pathway, shows significant positive correlations with *i*4 : 0, a product of the Val biosynthetic pathway, and significant negative correlations with all other fatty acids except *i*10 : 0. *Ai*6 : 0, which has been proposed to be derived from a two-carbon extension of *ai*5 : 0 followed by C1 oxidation (VanderHooven and Steffens, in preparation), shows significant positive correlations with *i*5 : 0 and its extension products (*i*9 : 0 and *i*11 : 0), and negative correlations with *i*4 : 0 and its extension product, *i*10 : 0. The biosynthesis of *iso*-branched and *anteiso*-branched fatty acids both share pyruvate and acetolactate synthase; the basis of these correlations may reside in these biosynthetic components.

Combining fatty acid data

Since the biosyntheses of many fatty acid constituents are related to each other through primer : product relationships, it is likely that any one QTL detected in this population will contain gene(s) affecting the level of more than one fatty acid. Thus, our power to detect QTLs may be increased by combining information from more than one fatty acid constituent. This can be approached in two ways. One approach is to combine fatty acid data to create new trait data based upon precursor : product relationships between fatty acids. A second, automated approach is to use principle component analysis and determine whether the components found have any clear biological interpretation.

The first approach was used to generate the trait percentage of *iso*-branched fatty acids that have even carbon chain length (percent *iso*-even). These data were calculated based upon the observation that *i*4 : 0 is a precursor for the extension reactions leading to *i*10 : 0

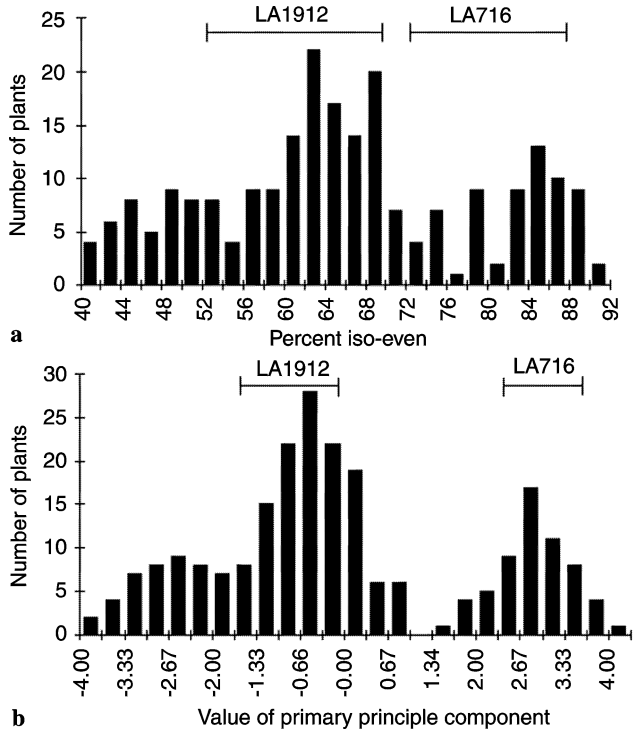

Fig. 1a, b Histograms of combined fatty acid data for percent *iso*-even (**a**) and primary principle component (**b**). The 95% confidence intervals for the parental controls *L. pennellii* LA716 (*LA716*) and *L. pennellii* LA1912 (*LA1912*) are shown *above* the histograms

and that *i*5 : 0 is a precursor for *i*9 : 0 and *i*11 : 0 (Walters and Steffens 1990). The trimodal distribution of percent *iso*-even (Fig. 1a), in which the number of individuals in each mode follows a 1 : 2 : 1 ratio, suggests that this trait is largely governed by a single locus for which the LA716 allele conferring high percent *iso*-even is additive. The *L. pennellii* LA716 values are consistent with this model. However, under this model, the *L. pennellii* LA1912 controls appear to be heterozygous for this locus. This is likely since LA1912 is largely self-incompatible and heterogeneous.

Since percent *iso*-even appears to be largely determined by a single locus, this trait can be converted into a qualitative trait by comparing F_2 percentages to those of the parental controls. Chi-square tests using the qualitative data are consistent with the above genetic model, using cutoff values of 56% and 76% *iso*-even ($0.50 < P < 0.75$). Using qualitative data and MAPMAKER/EXP software, we found the location of the single locus to be 5.5 cM above TG117 on chromosome 8. Due to possible misclassifications when converting percent *iso*-even data into qualitative data, 5.5 cM is possibly an overestimate of this distance (Lincoln and Lander 1992). With the gene-effect model, the LA716 allele increases the level of *iso*-even fatty acids incorporated into acylsugars by 16.5% of the *iso*-branched fatty acid constituents. The second LA716 allele in homozygotes increases the level of *iso*-even fatty acids incorporated into acylsugars by an additional 20.2% of the *iso*-branched fatty acid constituents. These values are consistent with the additive model proposed from the distribution of this trait in the F_2 population. Several other linear combinations of the data were investigated including percent *iso*-branched, percent straight-chain, percent *anteiso*-branched, percent *iso*-evens that are extension products, percent *iso*-odds that are extension products and percent of all fatty acid constituents that are extension products. None of these combinations have bi- or tri-modal distributions in the F_2 population.

Table 3 Coefficients for primary and secondary principle components

Fatty acid constituent	Coefficients	
	Primary	Secondary
*i*4 : 0	0.364	0.334
*i*10 : 0	0.403	0.065
*i*5 : 0	−0.456	0.021
*i*9 : 0	−0.312	0.380
*i*11 : 0	−0.360	0.147
*ai*5 : 0	0.264	0.146
*ai*6 : 0	−0.446	−0.056
*n*10 : 0	0.019	−0.589
*n*12 : 0	0.033	−0.589

Principle component analysis was used to combine data from all nine segregating fatty acids. Coefficients for the primary principle component are shown in Table 3. The trimodal distribution of the primary principle component (Fig. 1b), in which the number of plants in each mode follows a 1 : 2 : 1 ratio, indicates that this trait is largely determined by a single locus. In this model, the range of LA1912 values and the nature of this population (as discussed above) suggest that these LA1912 individuals are heterozygous for this QTL. The quantitative data for this trait can be converted into qualitative data by comparing values to those of the parental controls. Chi-square tests using the qualitative data are consistent with the above model using cutoff values of −1.33 and 1 ($0.75 < P < 0.90$). Using qualitative data and MAPMAKER/EXP software, we found the location of this locus to be 3.9 cM above TG117 on chromosome 8. It is likely this is the same locus found for percent *iso*-even, since it maps to the same location, and when looking at the equation for the primary component, the coefficients are strongly positive for *iso*-even fatty acids and strongly negative for *iso*-odd fatty acids. Since it is easier to interpret analysis conducted with percent *iso*-evens than with the primary principle component, additional QTL analysis was conducted using percent *iso*-even data only. All other principle components do not have a strong bi- or tri-modal distribution.

QTL mapping

Using the 95% experiment-wise cutoffs, QTL analysis identifies four regions associated with one or more of the nine segregating fatty acid constituents (Fig. 2, Table 4). The QTL on chromosome 2 shows significant associations with only *n*10:0. LA716 alleles at this locus increase the proportion of *n*10:0 fatty acids incorporated into acylsugars. The QTL on chromosome 5 shows significant associations with *i*9:0 and *i*11:0. LA716 alleles at this locus decreases the proportion of *i*9:0 and *i*11:0 fatty acids that are incorporated into acylsugars. The QTL on chromosome 7 shows significant associations with *i*4:0, *i*9:0, *n*10:0, and *n*12:0. LA716 alleles at this locus decrease the proportion of *n*10:0 and *n*12:0 fatty acids and increases the proportion of *i*4:0 and *i*9:0 fatty acids that are incorporated into acylsugars. The QTL on chromosome 8 shows significant associations with *i*4:0, *ai*5:0, *i*5:0, *ai*6:0, *i*9:0 and *i*11:0. LA716 alleles at this locus increase the proportion of *ai*5:0 and *iso*-even fatty acids and decrease the proportion of *ai*6:0 and *iso*-odd fatty acids that are incorporated into acylsugars. After the appropriate QTLs for each of the nine fatty acids are controlled for residual analysis identifies one QTL on chromosome 12. This QTL shows significant associations with *n*10:0 when residuals are calculated based on the QTL on chromosome 7. LA716 alleles at this locus increase the proportion of *n*10:0 fatty acids that are incorporated into acylsugars.

For each fatty acid constituent, the appropriate QTLs from the five identified above are used in a multiple regression equation to determine the percentage of variance observed in the trait that can be explained by the identified QTLs (Table 5). I5:0 is the trait for which the largest proportion of the variance can be explained by the identified QTLs. For this trait, the effect of all three QTLs are significant when a linear equation is constructed using the QTLs on chromosomes 5, 6 and 8. Together, these three QTLs explain 60.3% of the variance of the proportion of *i*5:0 fatty acids incorporated into acylsugars. When the appropriate combination of QTLs is used in a multiple regression equation for each trait, they explain 23–60% of the variance observed for the nine individual fatty acids.

Combined data were also used for QTL analysis (Table 4). Percent *iso*-evens is significantly associated with the QTL on chromosome 8. Using residual data based upon this QTL, we detected a second QTL on chromosome 6. LA716 alleles at both of these loci result in an increased proportion of *iso*-even fatty acids incorporated into acylsugars. The coefficients for the secondary principle component are shown in Table 3. The secondary principle component shows associations with the QTL previously detected on chromosome 7. Biochemical interpretation of this component

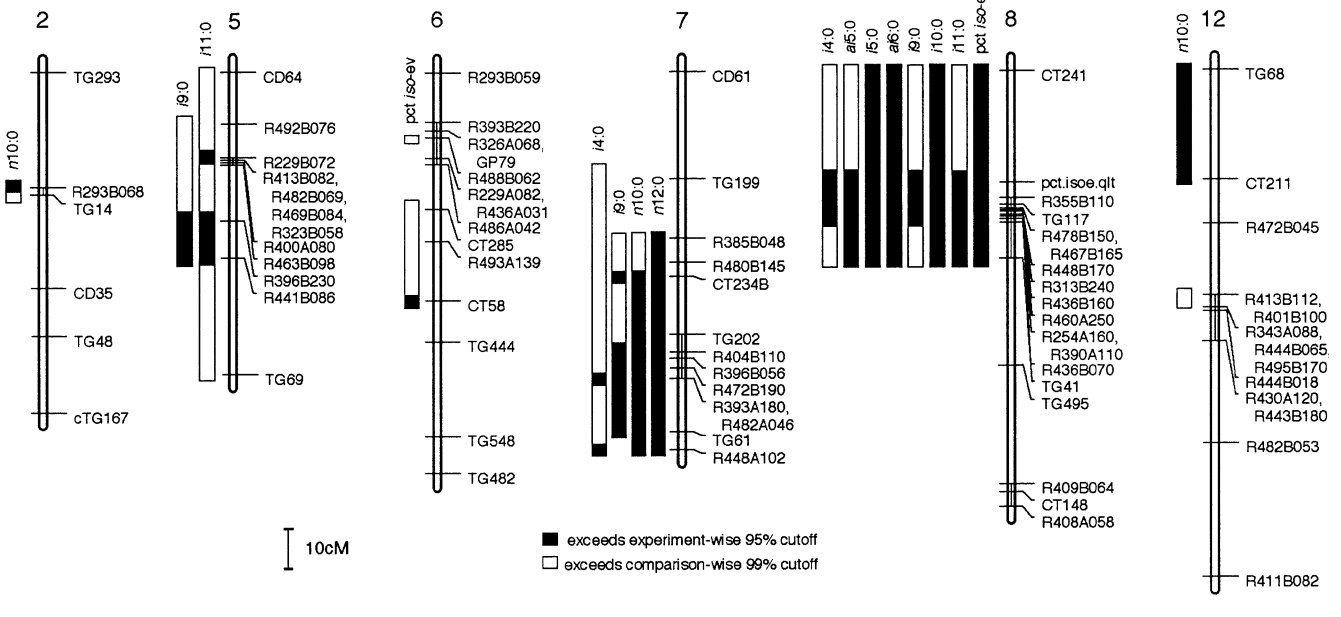

Fig. 2 Linkage map of the intraspecific F_2 population. RFLP markers are labeled as in Tanksley et al. (1992). RAPD markers are labeled with *R* followed by the primer *number*, a letter denoting specificity to LA716 (*A*) or LA1912 (*B*) and the size of the band scored in 10 bp. Pct.isoe.qlt is generated by converting percent *iso*-even 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 markers with significant associations with fatty acid constituents. *Pattern* of *bars* denotes level of significance at corresponding markers as follows: exceeds 95% experiment-wise cutoff using original data (*black*), or exceeds 99% comparison-wise cutoff (*white*). Bars do not represent confidence intervals

Table 4 QTLs with significant associations with proportion of the nine fatty acid constituents segregating in the intraspecific population. Traits include the nine segregating fatty acids (e.g. *i*4:0) and percent *iso*-evens (pct.isoe). All *F*-values exceed 95% experiment-wise cutoffs

Chromosome	QTL	Traits	*F* value	Marker at peak (Marker range[a])
Ch2	2	*n*10:0	17	R293B068 (R293B068-TG14)
Ch5	5	*i*11:0	−48	R396B230 (CD64-TG69)
		*i*9.0	−25	
Ch6	6	pct.isoe	19[b]	CT58 (R488B062-CT58)[nc]
Ch7	7	*n*12:0	−204	R482A046 (R385B048-R448A102)
		*n*10:0	−61	
		*i*9.0	52	
		*i*4:0	47	
Ch8	8	*ai*6:0	−281	R313B240 (CT241-TG495)
		*i*5:0	−221	
		pct.isoe	209	
		*i*10:0	159	
		*i*11:0	−63	
		*ai*5:0	62	
		*i*4:0	56	
		*i*9.0	−46	
Ch12	12	*n*10:0	24[b]	CT211 (TG68-R401B100)[nc]

[a] Marker range of the QTL corresponds to that of the trait with the highest *F*-value
[nc] Non-contiguous ranges
[b] *F*-values resulting from residual analysis are marked with an asterisk

Table 5 R^2 values for QTLs associated with fatty acid constituents of acylsugars. Markers used in regression equations have the highest R^2 value for all markers in QTL range

Trait	QTL	Marker	R^2[a] (%)	Multiple regression	
				Sig. coef.[b]	R^2 (%)[c]
*i*4:0	8	R460A/R313B	27.2	*	
	7	R482A	16.7	*	
	6	CT58	3.8	*	
	2	R293B	1.9	*	
	12	CT211	1.7	*	46.4
*i*10:0	8	R460A/R313B	41.5	*	
	7	R482A	1.7		
	6	CT58	1.2	*	43.0
*i*5:0	8	R406A/R313B	57.9	*	
	6	CT58	0.7	*	
	5	R396B	0.0	*	60.3
*i*9:0	8	R460/R313B	22.8	*	
	7	R482A/R472B	16.1	*	
	5	R396B	10.7	*	
	2	R293B	0.0		48.1
*i*11:0	8	R460AR313B	32.2	*	
	5	R396B	19.1	*	
	7	R482A/R472B	0.6		
	6	CT58	0.1		50.0
*ai*5:0	8	R460A/R313B	23.3	*	
	12	CT211	0.3		
	5	R396B	0.0		23.3
*ai*6:0	8	R460A/R313B	57.2	*	57.2
*n*10:0	7	R482A/R472B	27.2	*	
	12	CT211	7.0	*	
	2	R293B	6.6	*	
	5	R396B	1.3		45.4
*n*12:0	7	R482A/R472B	54.4	*	
	12	CT211	1.3	*	
	6	CT58	1.2		
	2	R293B	0.0	*	59.6

[a] R^2: Values when a single QTL is used in the regression equation
[b] Multiple regression sig. coef.: QTL with asterisks have coefficients that significantly deviate from 0 ($\alpha = 0.05$) and were included in the multiple regression equation
[c] Multiple regression R^2: values for multiple regression with multiple QTL

is unclear. However, the coefficients are consistent with the univariate associations previously detected on chromosome 7 (Tables 3, 4). The tertiary principle component fails to show significant associations with any of the markers. Thus, this component and all subsequent components were not further analyzed.

Analysis with MAPMAKER/QTL (Paterson et al. 1988) verified the QTLs detected with the experiment-wise cutoffs. All other QTLs detected using MAPMAKER/QTL were not pursued due to low LOD scores and R^2 values.

Conclusions

Correlations of acylsugar fatty acid constituents in an intraspecific population of *L. pennellii* reveal patterns which clearly support the biosynthetic origin of acylsugar fatty acids proposed by Walters and Steffens (1990): short-chain branched primers are extended in two-carbon increments to generate branched medium-chain length fatty acids. A one-carbon elongation mechanism, such as that hypothesized by Kroumova et al. (1993), would predict an entirely different pattern of correlations and, thus, is inconsistent with the genetic evidence presented here.

The function of segregating alleles at QTLs strongly associated with fatty acid profiles may be inferred from a knowledge of fatty acid biosynthetic origin. The following hypothesized functions are based upon the assumption that each QTL contains one gene with pleiotropic effects, as opposed to multiple genes. Since the QTLs on chromosomes 2 and 12 are only associated with *n*10:0, it is possible their biosynthetic function could be associated with specific termination of de novo elongation at *n*10:0. The lack of associations between these QTLs and other fatty acids is likely to be due to the low effect of gene(s) at these loci compared to those at other QTLs.

Segregating alleles at the QTL on chromosome 5 are likely to affect the extension of *i*5:0 to *i*9:0 and *i*11:0. This hypothesis is supported by the observation that LA716 alleles at this QTL similarly affect the levels of *i*9:0 and *i*11:0.

The associations of *i*4:0, *i*9:0, *n*10:0 and *n*12:0 with the QTL on chromosome 7 are consistent with the significant negative correlations observed between the straight-chain and *iso*-branched fatty acids. Although it appears this QTL could be involved in an early biosynthetic commitment to straight-chain or *iso*-branched fatty acids, it is not clear why it does not appear to affect levels of *iso*-branched fatty acids such as *i*10:0.

The QTL on chromosome 8 exerts a complex effect. Since LA716 alleles at this QTL increase the relative proportion of *iso*-evens and decrease the proportion of *iso*-odds, it is likely that one component of this QTL's effect is exerted at the branch point between *i*4:0 and *i*5:0. This occurs at the level of α-ketoisopentanoic acid, which may either undergo decarboxylation to form *i*4:0 or be incorporated into the Leu biosynthetic pathway by α-isopropylmalate synthase for formation of α-ketoisohexanoic acid, which undergoes decarboxylation to produce *i*5:0. In addition, the strong associations between this QTL and the levels of *ai*5:0 and *ai*6:0 indicate that the segregating alleles at this locus are likely to regulate the conversion of *ai*5:0 to *ai*6:0. The pleiotropic effects of this locus may be due to tightly linked genes, where one gene affects the proportion of *i*4:0 and *i*5:0, and the other affects the proportion of *ai*5:0 and *ai*6:0. Alternatively, the pleiotropic effects of this locus may be due to one gene causing both functions, either by differential availability of pyruvate to the Val or IIe pathways or by affecting the activity or association of acetolactate synthase within these pathways (Walters and Steffens 1990).

Six QTLs were detected in this study, which together explain 23–60% of the variance of the nine segregating acylsugar fatty acid constituents in the intraspecific *L. pennellii* population. None of these QTLs have been previously reported. The QTL on chromosome 8 controlling percent *iso*-even has the strongest associations with any of the traits discussed here in terms of *F*-values, affects the relative proportion of seven out of nine fatty acids, has the highest R^2 values of the six QTLs identified and is the primary principle component of the fatty acid profile. Since percent *iso*-even can be converted into qualitative data, this QTL can potentially be isolated via positional cloning without the time-consuming and costly development of introgression lines between *L. pennellii* LA716 and LA1912. Understanding the functional significance of this QTL and other QTLs controlling the diversity of plant secondary chemistry will provide novel insight into the complexity of control mechanisms in metabolic pathways.

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