Quantitative Trait Loci Controlling Amounts and Types of Epicuticular Waxes in Onion

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ABSTRACT. Natural variation exists in onion (*Allium cepa* L.) for amounts and types of epicuticular waxes on leaves. Wild-type waxy onion possesses copious amounts of these waxes, whereas the foliage of semiglossy and glossy phenotypes accumulates significantly less wax. Reduced amounts of epicuticular waxes have been associated with resistance to onion thrips (*Thrips tabaci* Lindeman), an important insect pest of onion. A segregating family from the cross of waxy and semiglossy onions was used to map single nucleotide polymorphisms (SNPs) and identify chromosome regions affecting amounts and types of epicuticular waxes as measured by gas chromatography–mass spectrometry. The amount of the primary epicuticular wax on onion leaves, hentriacontanone-16, was controlled by one region on chromosome 5. One region on chromosome 2 affected concentrations of several primary fatty alcohols. Results indicate that the region on chromosome 2 may be associated with the acyl reduction pathway, and the region on chromosome 5 may affect the decarbonylation pathway of epicuticular wax biosynthesis. Because lower amounts of epicuticular waxes are recessively inherited, SNPs tagging regions on chromosomes 2 and 5 will be useful for marker-assisted breeding to vary amounts and types of epicuticular waxes on onion foliage with the goal to develop cultivars resistant to onion thrips.

Onion thrips is an important insect pest of onion causing damage to foliage and reducing bulb and seed yields (Diaz-Montano et al., 2010; Elmore, 1949). Thrips also vector serious diseases such as iris yellow spot virus (Gent et al., 2004; Shock et al., 2008). Onion thrips populations can increase rapidly during warmer conditions requiring frequent pesticide applications, and consequently the insect has developed resistance to pyrethroid and organophosphate insecticides (Allen et al., 2005; Herron et al., 2008; Shelton et al., 2006). Because it is desirable to reduce pesticide use and increase integrated pest management strategies in vegetable crops (Eigenbrode and Trumble, 1994), genetic resistance to onion thrips would be beneficial for growers and, combined with fewer pesticide applications, offers a potentially affordable and sustainable management strategy for this important pest.

Reduced amounts of leaf epicuticular waxes (often referred to as glossy or bloomless phenotypes) have been associated with insect resistance in several crops. Bloomless sorghum [*Sorghum bicolor* (L.) Moench.] showed resistance to greenbug [*Schizaphis graminum* Rondani (Starks and Weibel, 1981)], glossy wheat [*Triticum aestivum* L. emend. Thell.] to grain aphid [*Sitobion avenae* F. (Lowe et al., 1985)], and glossy *Brassica oleracea* L. to cabbage worm (*Artogeia rapae* L.) and cabbage aphid (*Brevicoryne brassicae* L.) (Stoner, 1990). An explanation for these observations is that insects respond to

cues from the chemistry of the waxes, and the broad diversity of compounds found in epicuticular waxes (long-chain fatty acids, esters, ketones, alkanes, and alcohols) may help insects to identify their host plants (Blaney and Chapman, 1970; Eigenbrode and Espelie, 1995; Städler, 1986; Thibout et al., 1982).

The biosynthetic pathway for epicuticular waxes has been studied in several plants (Kunst and Samuels, 2003). De novo fatty acid synthesis occurs in the plastid (Ohlrogge and Browse, 1995) and a substantial portion of fatty acids in epidermal cells is allocated toward wax production (Liu and Post-Beittenmiller, 1995). C18 fatty acids destined for wax synthesis are catalyzed into long chain molecules by fatty acid elongases associated with the endoplasmic reticulum (Fehling and Mukherjee, 1991). In leek (*Allium ampeloprasum* L.), peak activities of these enzymes were detected in basal regions of leaves immediately before the accumulation of epicuticular waxes (Rhee et al., 1998). Two main pathways have been suggested for production of epicuticular waxes (Millar et al., 1999). Long-chain fatty acids are converted into primary alcohols and esters through the acyl reduction pathway or into secondary alcohols and ketones through the decarbonylation pathway (Millar et al., 1999). Aldehydes are an intermediate step in both pathways and alkanes are either a product or intermediate step in the decarbonylation pathway. Enzymes that catalyze many of the later steps of these pathways are not well understood (Kunst and Samuels, 2003).

Natural variation exists in onion for amounts and types of epicuticular waxes (Damon et al., 2014; Molenaar, 1984). The most prevalent epicuticular waxes on the foliage of wild-type (waxy) onion are ketones (hentriacontanone-16), alkanes (2-methyl octacosane, 1-ethenyl octadecane, and heptacosane), and fatty alcohols (heptadecanol-1, hexacosanol-1, octacosanol-1, and triacontanol-1) (Damon et al., 2014). Amounts of these waxes are significantly less on leaves of the glossy (lowest

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amounts of waxes) and semiglossy (intermediate amounts of waxes) phenotypes relative to waxy plants (Damon et al., 2014). Although glossy onions show significant resistance to onion thrips (Alimousavi et al., 2007; Damon et al., 2014; Jones et al., 1934; Molenaar, 1984), this phenotype is not commercially useful because of susceptibility to leaf pathogens, excessive transpiration, and spray injury (Baker, 1982; Mohan and Molenaar 2005; Wirth et al., 1991). Semiglossy onions possess epicuticular wax amounts intermediate between the waxy and glossy phenotypes (Damon et al., 2014) as well as show significant resistance to onion thrips (Damon et al., 2014; Diaz-Montano et al., 2010, 2012). Therefore, this phenotype may be useful for integrated management of this important pest.

Genetic analyses of amounts and types of epicuticular waxes may reveal genes controlling the different branches of the wax–biosynthetic pathway and lead to a better understanding of epicuticular wax accumulation in plants. Quantitative trait loci (QTL) controlling amounts of epicuticular waxes have been mapped in several crops, including canola [*Brassica napus* L. (Pu et al., 2013)], rice [*Oryza sativa* L. (Srinivasan et al., 2008)], maize [*Zea mays* L. (Liu et al., 2012)], and sorghum (Burow et al., 2009). Mapping of QTL controlling amounts and types of waxes in onion would provide markers associated with onion thrips resistance and contribute to our understanding of the inheritance of different foliar wax phenotypes. In this study, we developed a waxy by semiglossy mapping population to identify QTL controlling amounts and types of epicuticular waxes in onion.

Materials and Methods

GENETIC MATERIALS. ‘DehyA’ is an S-cytoplasmic male-sterile inbred line with white bulbs and wild-type waxy foliage (gift of Olam International, Hanford, CA). ‘B5351C’ is an inbred line of yellow onion selected from the open-pollinated population ‘Colorado #6’, restores male fertility in S cytoplasm, and has semiglossy foliage (Damon et al., 2014). A single bulb from the cross of ‘DehyA’ by B5351C was self-pollinated to produce the segregating family. F₂ seeds were planted in Jan. 2012 in a greenhouse on the campus of the University of Wisconsin (UW)–Madison. Initial photoperiod was set at 12 h and was increased on the first of each subsequent month after the spring equinox to the natural daylength in Madison, WI. Temperatures were 25 °C days and 20 °C nights.

DNA ISOLATION. In Feb. 2012, 120 F₂ plants were transplanted into 20-cm pots and individually labeled. In March, fresh tissue was harvested from each plant into polystyrene tubes and frozen in liquid nitrogen. Samples were stored at –80 °C for ≈48 h before lyophilization and then subsequently stored at –20 °C. Samples were individually ground using a mortar and pestle with ≈20 mL of liquid nitrogen and 0.1 g of silicon dioxide (Sigma-Aldrich, St. Louis, MO). Ground samples were placed in 50-mL polypropylene centrifuge tubes and DNA was isolated using the DNeasy Plant Maxi DNA isolation kit (Qiagen, Hilden, Germany). DNA concentrations were spectrophotometrically determined and 1 µg of DNA from each plant was electrophorized through a 0.8% agarose gel to assess intactness and quantity.

GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS AND DATA COLLECTION. In Apr. 2012 at approximately the eight-leaf stage, three samples were collected from a single leaf from each of the 120 F₂ plants for a total of 360 samples. In May 2012

(approximately the 14-leaf stage), the same procedure was followed to yield another 360 samples. In June 2012 (≈20-leaf stage), two samples were collected from an older leaf and two from a younger leaf from each of the 120 plants for a total of 480 samples. Visual phenotyping of F₂ plants as either waxy or semiglossy was completed in the greenhouse in 2012. Plants were allowed to mature during July and August in the greenhouse, after which bulbs were dipped in a solution of azoxystrobin fungicide (Quadris; Syngenta, Greensboro, NC) and allowed to air-dry on the greenhouse bench for 24 h. Bulbs were stored at 5 °C until Apr. 2013 and then planted in the field at the UW Horticulture Research Station near Arlington, WI. As a result of storage conditions or unfavorable weather, numerous F₂ bulbs rotted in the field. In June 2013, two samples were collected from different leaves from each of 86 plants for a total of 172 samples. In July 2013, two samples were collected from different leaves from each of 74 plants for a total of 148 samples. In total, five samplings were taken from the same F₂ plants across two environments to yield a total of 1520 samples. All leaf samples were prepared for gas chromatography–mass spectrometry (GC-MS) as described by Damon et al. (2014) to identify and quantify epicuticular waxes. Identification was made by GC-MS solution post-run software (Shimadzu, Kyoto, Japan) comparing mass spectra with a library to reveal the closest match. Quantities of specific waxes were estimated by the area under peaks as previously described by Damon et al. (2014). All peak areas were normalized to an internal standard (docosane) by dividing by the docosane peak area.

STATISTICAL ANALYSIS AND QTL MAPPING. A mixed model analysis of variance (ANOVA) with the repeated measures option was performed in SAS (Version 9.3; SAS Institute, Cary, NC) to generate restricted maximum likelihood estimates of variance components for the calculation of phenotypic and genotypic correlations among waxes (Holland, 2006). Variance components were also used to calculate broad-sense heritability on an entry mean basis for each epicuticular wax. Phenotypic variances for heritability and correlations were calculated by dividing genotype by environment variance by number of environments and dividing error variance by number of environments multiplied by number of samplings. For QTL mapping, mixed-model ANOVAs with repeated measures were performed in SAS to compute peak area least square means for each F₂ plant. Individual plants were experimental units and the three samplings in 2012 and two samplings in 2013 were considered as repeated measures. GC-MS data for each wax was considered as unique dependent variables. Genotypes and samplings were considered as fixed and environments and subsamples as random variables. Departure from normality of model residuals was corrected by square root transformation.

DNAs from 119 F₂ plants were used to genotype of 324 SNPs using the KASPar platform (LGC Genomics, Beverly, MA) as described by Duangjit et al. (2013). Linkage maps were created using the Joinmap software (Van Ooijen and Voorrips, 2001) with the Kosambi function. For phase-unknown SNPs, both phases were analyzed and the phase that placed the marker on the map with flanking markers was retained. Cosegregating markers were removed and data from 255 SNPs were combined with least squared means of GC-MS data from 119 plants for composite interval mapping using the R software (Broman et al., 2003). Two covariates and a window size of 10 cM were used in the composite interval model for each of eight waxes. The first covariate was a marker just outside the assigned

window next to the QTL; the second covariate was a genomic position with a non-significant effect [i.e., not above the 0.05 logarithm-of-odds (LOD) threshold]. One thousand permutations of each analysis were run to determine the LOD threshold at the 0.05 significance level (Churchill and Doerge, 1994). For each QTL detected, percent of variance explained, maximum LOD score, 1.5 LOD confidence intervals, and additive and dominance effects were calculated.

Results and Discussion

GENETIC MAP. Genetic mapping of 324 SNPs using DNAs from 119 F₂ progenies of ‘DehyA’ × B5351C yielded eight linkage groups. Linkage groups were aligned with genetic maps from two other onion mapping populations (OH1 × 5225 and BYG15-23 × AC43) described by Duangjit et al. (2013), allowing for the assignment of groups to the eight chromosomes of onion (Supplemental Table 1). Of the 324 segregating SNPs, 308 fit the expected 1:2:1 segregation ratio and 16 showed significant segregation distortion ($P < 0.05$); this level of segregation distortion is at the level expected by random chance. SNPs with segregation distortion at $P < 0.0001$ were discarded. SNPs with distorted segregation ratios between 0.05 and 0.0001 were concentrated in four areas on chromosomes 1, 4, 5, and 8 (Supplemental Table 1). Of the 324 SNPs, 214 also segregated in the OH1 × 5225 population and 142 were in common with the BYG15-23 × AC43 map (Duangjit et al., 2013). Here we report positions of 66 previously unmapped SNPs (Supplemental Table 1), adding to the 713 previously reported by Duangjit et al. (2013). As a result of missing parental genotypes or heterozygosity in the parental lines, 23 SNPs remained as phase-unknown and were added to the linkage map in both phases, keeping the phase for which the marker was assigned to a linkage group. Total length for the genetic map from ‘DehyA’ × B5351C was 7.8 Morgans.

MAPPING OF EPICUTICULAR WAX DIFFERENCES. Damon et al. (2014) detected significantly different amounts of eight waxes

(hentriacontanone-16, heptacosane, heptadecanol-1, hexacosanol-1, octacosanol-1, 1-ethenyloxy octadecane, 2-methyl octacosane, and triacontanol-1) on the foliage of semiglossy and waxy onions. These same eight waxes were identified on the foliage of F₂ progenies from ‘DehyA’ by B5351C (Table 1). Because onion is biennial, we sampled the same F₂ plants during the bulb (2012) and seed (2013) production years. Although the mapping population performed poorly in the 2013 field environment resulting in a reduced data set, coefficients of variation for the two most prevalent waxes were relatively low (less than 20%) and comparable between the 2 years (13.2 in 2012 and 15.4 in 2013 for hentriacontanone-16 and 19.8 in 2012 and 16.4 in 2013 for octacosanol-1). Significant phenotypic and genotypic correlations were detected among waxes (Table 2). Hexacosanol-1, octacosanol-1, and triacontanol-1 were positively correlated with each other and negatively correlated with heptacosane. Hentriacontanone-16 was positively correlated with heptadecanol-1 and both of these had little or no correlation with octacosanol-1 or triacontanol-1. Broad-sense heritabilities on an entry-mean basis ranged from 0.26 to 0.86 for the eight waxes (Table 1). The high heritabilities for amounts of most waxes likely contributed to our ability to measure significant differences in the 2013 field environment despite reduced progeny numbers.

QTL were detected controlling amounts of the eight epicuticular waxes, explaining from 17% to 65% of the phenotypic variation (Table 1). Maximum LOD scores ranged from 4.7 to 31.3 and 1.5 LOD confidence intervals ranged from 3 to 10 centiMorgans (cM). Hentriacontanone-16 and triacontanol-1 showed the highest heritability, percent of variance explained, and LOD scores (Table 1). Amounts of hentriacontanone-16 and 1-ethenyloxy octadecane mapped to a region on chromosome 5 (22 to 28 cM) with higher amounts from the waxy ‘DehyA’ parent (Fig. 1; Table 1). Amounts of three fatty alcohols (triacontanol-1, octacosanol-1, hexacosanol-1) and 2-methyl octacosane were controlled by a region on chromosome 2 from 68 to 75 cM (Fig. 1; Table 1) with variation from the semiglossy

Table 1. Broad-sense heritabilities (H^2), positions in centiMorgans (cM) on chromosomes (Chr) of quantitative trait loci (QTL), percent of variance explained, additive and dominance effects, maximum logarithm-of-odds (LOD) scores, 0.05 LOD significance thresholds, and single nucleotide polymorphisms (SNPs) flanking 1.5-LOD confidence intervals of QTL affecting amounts of epicuticular waxes in onion and visual phenotypes in the F₂ family from ‘DehyA’ crossed with B5351C.

Trait	H^2	QTL position [Chr (cM)]	Variance explained (%)	Additive effect ^z	Dominance deviation ^y	LOD	0.05 LOD significance threshold	SNPs flanking 1.5 LOD confidence interval ^x
Hentriacontanone-16	0.82	5 (22)	56	17.7	-6.6	25.3	4.1	c00142_122 i31628_1166
Heptacosane	0.59	2 (68)	49	-1.2	0.7	18.5	4.1	i38736_505 i17237_4883
Heptacosane		5 (28)	10	-0.6	-0.5	5.2	4.1	c00142_122 i31628_1166
Heptadecanol-1	0.80	2 (74)	10	0.7	-2.4	4.7	4.1	i33533_568 i30495_450
Heptadecanol-1		5 (25)	45	3.7	0.4	17.3	4.1	c00142_122 i31628_1166
Hexacosanol-1	0.27	2 (70)	33	0.8	-0.7	11.9	4.2	i39908_252 i31965_1307
Octacosanol-1	0.59	2 (69)	41	6.9	-6.4	17.5	4.1	i38736_505 i31965_1307
2-methyl octacosane	0.26	2 (75)	19	-0.4	-0.2	5.8	4.1	i33533_568 i30495_450
1-ethenyloxy octadecane	0.47	5 (25)	17	0.1	-0.1	6.3	4.0	c00142_122 i31628_1166
Triacontanol-1	0.86	2 (69)	65	7.8	-5.9	31.3	4.1	i19682_515 i17237_4883
Visual phenotype		5 (28)	27	2.2	0.4	9.7	4.0	c00142_122 i31628_1166

^zPositive additive effects indicate the substitution of the chromosome region from the waxy parent in micrograms of wax per gram of fresh leaf tissue.

^yPositive dominant deviations are from the waxy parent; negative are from semiglossy parent. Large negative dominance deviations compared with positive additive effects indicate dominance of the region from the semiglossy over the waxy parent.

^xSNPs are listed in Supplemental Table 1 and were described by Duangjit et al. (2013).

Table 2. Significance of genotypic (above diagonal) and phenotypic (below diagonal) correlations among eight epicuticular waxes on onion foliage from the segregating family from 'DehyA' crossed with B5351C.

	C1 ^z		C2		C3		KE		OH1		OH2		OH3		OH4	
C1	—		0.08		0.80	***	0.11		0.08		0.33	***	-0.83	***	-0.49	***
C2	0.23	***	—		-0.94	***	0.99	***	0.99	***	0.67	***	0.58	***	0.32	***
C3	0.63	***	-0.28	***	—		-0.34	***	-0.38	***	-0.69	***	-0.99	***	-0.60	***
KE	0.23	***	0.89	***	-0.11	*	—		0.99	***	0.48	***	0.26	**	0.00	
OH1	0.16	***	0.83	***	-0.09	*	0.88	***	—		0.50	***	0.16		0.08	
OH2	0.35	***	0.47	***	-0.18	***	0.32	***	0.24	***	—		0.88	***	0.82	***
OH3	-0.02	***	0.48	***	-0.43	***	0.31	***	0.24	***	0.67	***	—		0.99	***
OH4	-0.10	*	0.22	***	-0.38	***	0.06		0.10	*	0.59	***	0.86	***	—	

^zC1 = 2-methyl octacosane; C2 = 1-ethenyl octadecane; C3 = heptacosane; KE = hentriacontanone-16; OH1 = heptadecanol-1; OH2 = hexacosanol-1; OH3 = octacosanol-1; OH4 = triacontanol-1.

*, **, and *** Significance at $P < 0.05$, 0.01 , and 0.001 , respectively.

parent reducing amounts of fatty alcohols and increasing the amount of 2-methyl octacosane. No interactions were detected between the regions chromosomes 2 and 5, indicating that QTL independently affect concentrations of hentriacontanone-16 and 1-ethenyl octadecane (chromosome 5) vs. triacontanol-1, octacosanol-1, hexacosanol-1, and 2-methyl octacosane (chromosome 2). Amounts of heptacosane and heptadecanol-1 mapped to both regions on chromosomes 2 and 5 (Table 1). Higher amounts of heptacosane at both regions were conditioned by the semiglossy parent, whereas higher amounts of heptadecanol-1 at both regions were from the waxy parent. There was no evidence of epistasis or interaction between QTL on chromosomes 2 and 5 controlling amounts of heptadecanol-1 and heptacosane. Because three genotypic classes were present in the F_2 population, additive and dominance effects were estimated (Table 1). QTL affecting amounts of hentriacontanone-16, heptadecanol-1, and 1-ethenyl octadecane on chromosome 5 were mainly additive. QTL for amounts of hexacosanol-1, octacosanol-1, triacontanol-1, and heptacosane on chromosome 2 showed dominance toward the lower values of the semiglossy (B5351C) parent (Table 1).

Visual scores of F_2 plants as waxy vs. semiglossy revealed the same position as QTL on chromosome 5, but not on chromosome 2 (Table 1). Mapping of the visual phenotype to the same region on chromosome 5 as amounts of hentriacontanone-16 supports the conclusion of Damon et al. (2014) that crystals of hentriacontanone-16 are primarily responsible for the visible waxy phenotype. The additive effect of the QTL on chromosome 5 controlling amounts of hentriacontanone-16 (Table 1), and therefore the visual phenotype, is consistent with greater waxiness of progenies between waxy and semiglossy parents.

Studies of the biosynthetic pathway for epicuticular waxes in plants have revealed that ketones (such as hentriacontanone-16) are derived from the decarbonylation pathway with aldehydes and alkanes as intermediates (Kunst and Samuels, 2003). The *MAH1* gene in *Arabidopsis thaliana* (L.) Heynh. is a midchain alkane hydroxylase involved in the production of secondary alcohols and ketones in the decarbonylation pathway; *mah1* mutants are deficient in these waxes and have increased amounts of alkanes (Greer et al., 2007). The QTL on onion chromosome 5 may be associated with the decarbonylation pathway with the semiglossy region reducing production of hentriacontanone-16. The mainly additive effects of the QTL on chromosome 5 support partial blockage of the decarbonylation pathway in heterozygous plants, possibly as a result

of production of both functional and non-functional gene products.

Primary alcohols such as hexacosanol-1, octacosanol-1, and triacontanol-1 are derived from the acyl-reduction pathway (Kunst and Samuels, 2003). The *CER4* gene in *A. thaliana* has been characterized as a fatty acyl-coenzyme A reductase involved in the production of primary fatty alcohols and *cer4* mutants were deficient in primary alcohols with slightly elevated levels of other waxes (Rowland et al., 2006). Because the QTL on chromosome 2 from the semiglossy parent are associated with reduced amounts of primary alcohols, this region may encode an enzyme involved in the acyl-reduction pathway. However, the large dominance effect of the QTL on chromosome 2 from the semiglossy parent suggests that this region may encode a regulatory factor affecting genes located elsewhere in the genome (Pu et al., 2013).

Unlike observations in *A. thaliana*, alkane accumulation in onion appears to correspond more closely with obstruction of the acyl-reduction pathway than the decarbonylation pathway. Jenks et al. (1995) showed that a reduction in primary alcohols resulted in an increase in aldehydes and hypothesized the blockage of an aldehyde reductase in the acyl-reduction pathway. However, Vioque and Kolattukudy (1997) suggested that fatty acids are converted immediately to primary alcohols without release of an aldehyde intermediate. Kunst and Samuels (2003) suggested that the increase in aldehydes resulted from an increased flux of fatty acyl precursors into the decarbonylation pathway. In the semiglossy B5351C parent, such a flux of acyl precursors toward decarbonylation may be associated with the additional blockage of the decarbonylation pathway, resulting in accumulation of heptacosane. The odd chain length of heptacosane should result from decarbonylation (Greer et al., 2007); however, the lack of a significant interaction between regions on chromosomes 2 and 5 suggests that the increase in heptacosane is independent of the step(s) in the decarbonylation pathway affected by the QTL on chromosome 5.

The association of heptadecanol-1 with the chromosome 5 region is not easily explained, because primary alcohols are expected to result from the acyl-reduction pathway. However, this alcohol differs from those associated with the QTL on chromosome 2 by being shorter and having an odd (C17) chain length; odd chain length is typical of secondary alcohols (Kunst and Samuels, 2003). These observations suggest that mechanisms other than the acyl-reduction pathway may be involved in heptadecanol-1 production and, as a result of the highly significant association with chromosome 5, the decarbonylation

Fig. 1. Quantitative trait loci (QTL) on chromosomes 2 and 5 affecting amounts of epicuticular waxes on onion foliage as determined by gas chromatography–mass spectrometry and visual phenotyping. Logarithm-of-odds (LOD) thresholds at $P < 0.05$ ranged from 3.98 to 4.15 for the various waxes and the horizontal line indicates the most stringent threshold at 4.15.

pathway is implicated. Interestingly, amounts of another unusually short wax, 1-ethenyloxy octadecane (C20), was also significantly associated with chromosome 5.

This study provides strong evidence that two chromosome regions in onion independently and significantly affect the two branches of the biosynthetic pathway for epicuticular waxes. The lack of interaction between these two regions suggests that the QTL do not control early steps in the biosynthesis of epicuticular waxes such as fatty acid elongation and are not involved in any broad regulatory control of the pathway. Therefore, characterization of genes associated with these QTL would likely contribute to a greater understanding of subsequent steps in the pathway, where precursor fatty acids are used to produce diverse groups of epicuticular waxes.

Onion cultivars with semiglossy foliage are commonly grown in many arid and semiarid regions of the world; waxy cultivars predominant in more temperate areas. Backcross conversion of waxy onions to the semiglossy phenotype is complicated because crosses of semiglossy and waxy plants produce visually waxy progenies. Because onion is biennial, introgression of QTL affecting amounts and types of epicuticular waxes will be expedited by molecular tags to avoid selfing plants to reveal less waxy genotypes. Three SNPs on chromosome 5 (i26178_547, i29989_644, and i32901_1333) and two on chromosome 2 (i28284_1005 and i33533_568) are located within the 1.5 LOD confidence interval of the highly significant QTL controlling amounts of epicuticular waxes in onion (Table 1). If amounts of hentriacontanone-16 were a major factor affecting parasitism by onion thrips (Damon et al., 2014), one could select for reduced amounts of hentriacontanone-16 (QTL on chromosome 5) and increased amounts of fatty alcohols (QTL on chromosome 2) to potentially produce plants with adequate amounts of epicuticular waxes to perform well in the field and show resistance to onion thrips, which would be useful toward integrated management of this important insect pest.

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Supplemental Table 1. Positions, observed segregations, and probabilities for fits to expected 1:2:1 ratio for single nucleotide polymorphisms (SNPs) segregating in the F₂ family from DehyA by B5351C.

Locus ^z	Chrom.	cM	Observed			Probability
			DehyA	Heterozygous	B5351C	
isotig20159_957*	1	0.0	31	56	30	0.906
isotig30863_958	1	1.8	30	58	28	0.971
isotig31993_1423	1	13.5	33	58	28	0.812
isotig21293_730	1	13.9	33	57	27	0.744
isotig26412_1215*	1	13.9	33	58	27	0.761
isotig26855_718	1	15.3	32	57	27	0.818
isotig30061_1069	1	20.4	36	56	24	0.323
isotig25793_1638	1	22.7	36	59	24	0.360
isotig32795_827	1	23.1	37	58	24	0.294
isotig33340_1003*	1	24.8	37	57	24	0.280
isotig33160_1074*	1	26.6	36	60	22	0.241
isotig32065_872	1	32.2	37	58	23	0.241
isotig27517_440	1	36.7	36	59	23	0.297
isotig27537_659	1	40.7	38	59	21	0.125
isotig32775_1170*	1	43.4	39	62	18	0.041
isotig29455_1102	1	43.4	38	62	18	0.050
isotig29462_1710*	1	43.4	39	61	18	0.040
isotig35370_364	1	44.2	37	62	16	0.026
isotig31226_709	1	44.7	36	65	17	0.045
isotig30515_1420	1	47.2	38	63	18	0.050
isotig34151_1136	1	47.7	38	62	19	0.072
isotig31196_1500*	1	50.0	37	58	21	0.149
isotig32512_171*	1	54.7	34	61	22	0.319
isotig40255_572	1	58.2	36	58	24	0.350
isotig26446_844*	1	65.0	39	56	23	0.140
isotig31077_1018	1	67.6	34	62	22	0.312
isotig30950_933	1	68.9	34	60	25	0.562
isotig36938_811*	1	69.7	32	62	25	0.648
isotig26470_847	1	72.8	31	63	25	0.652
isotig32772_1034	1	76.0	33	61	23	0.440
isotig36532_752	1	79.8	33	60	24	0.535
isotig28615_1377	1	80.2	34	59	25	0.559
contig00172_269	1	81.5	33	61	25	0.616
isotig36110_940	1	86.3	28	67	23	0.333
isotig39274_732	1	86.3	28	68	23	0.302
isotig31382_1702	1	88.0	28	67	22	0.268
isotig37265_852	1	92.0	33	62	23	0.429
isotig40554_114	1	92.0	33	63	23	0.415
isotig43680_368	1	93.2	31	64	24	0.532
isotig29661_1491	1	95.7	32	58	25	0.688
contig00676_1004	1	96.2	33	58	27	0.761
isotig36087_421	1	101.1	28	63	27	0.789
isotig18500_243*	1	102.8	29	62	28	0.909
isotig37348_328*	1	102.8	29	61	28	0.937
isotig28777_484	1	104.5	30	62	26	0.783
isotig32287_1090*	1	105.0	32	60	25	0.676
isotig39789_529	1	111.2	30	63	25	0.664
isotig37419_329	1	111.2	31	61	25	0.702
isotig34723_664	1	114.7	31	61	27	0.865
isotig31626_574	1	116.5	31	61	27	0.865
isotig37443_625	1	117.7	33	58	28	0.812
isotig40509_271	2	0.0	31	56	32	0.835
isotig19136_223*	2	1.7	32	56	31	0.835
isotig36268_410	2	1.7	32	56	31	0.835
isotig27567_733	2	3.0	32	55	32	0.751
isotig39106_393	2	3.0	32	55	32	0.751

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Supplemental Table 1. Continued.

Locus ^z	Chrom.	cM	Observed			Probability
			DehyA	Heterozygous	B5351C	
isotig32786_424	2	6.0	32	51	32	0.528
isotig30687_1336	2	6.0	32	54	32	0.698
isotig40534_246*	2	6.0	33	54	32	0.648
isotig38484_281*	2	10.0	32	55	32	0.751
isotig41662_224	2	12.1	31	58	30	0.962
isotig30461_1472	2	13.4	31	60	27	0.879
isotig36256_344*	2	19.7	30	64	23	0.449
isotig32787_1241*	2	20.5	30	63	25	0.664
isotig31443_295	2	28.2	28	63	25	0.645
isotig34894_810	2	30.0	30	62	26	0.783
isotig33051_1252	2	30.0	30	61	27	0.885
isotig30946_1170*	2	31.3	29	61	29	0.969
isotig28947_218	2	31.3	29	61	28	0.937
isotig31641_515	2	32.1	30	59	30	0.996
isotig34066_1226	2	40.4	30	61	28	0.942
isotig36775_363	2	44.3	30	58	31	0.962
isotig35805_506*	2	46.1	29	56	34	0.705
isotig15060_964*	2	48.7	27	59	31	0.886
isotig39908_252	2	50.8	27	60	31	0.879
isotig34382_910	2	50.8	27	61	31	0.865
isotig38736_505	2	63.0	26	59	34	0.634
isotig19682_515	2	66.9	29	58	30	0.989
isotig28284_1005	2	67.9	30	57	30	0.968
isotig33533_568	2	68.3	30	59	30	0.996
isotig17237_4883	2	70.3	31	58	28	0.933
isotig31346_1020	2	71.4	31	60	27	0.879
isotig31965_1307	2	73.1	31	64	24	0.532
isotig33705_468	2	74.0	32	61	24	0.572
isotig34671_610	2	74.4	31	63	25	0.652
isotig30715_1591	2	75.7	30	62	25	0.697
isotig34815_1050*	2	75.7	30	61	26	0.812
isotig33142_579	2	75.7	31	61	26	0.789
isotig29186_1830	2	75.7	31	62	26	0.767
isotig38339_429*	2	77.0	31	61	27	0.865
isotig30495_450	2	94.2	36	59	24	0.360
isotig12268_1682	2	94.6	37	58	24	0.294
isotig32416_685	2	105.6	39	55	24	0.158
isotig30919_911*	2	107.3	39	54	25	0.171
isotig30821_1151	2	107.8	39	54	23	0.118
isotig33538_1298	2	113.7	40	58	21	0.076
isotig33275_395	3	0.0	21	69	28	0.167
isotig29680_142	3	1.3	22	67	28	0.268
isotig29139_1024	3	1.7	22	67	28	0.268
isotig39498_201	3	2.6	21	67	30	0.223
isotig30333_269	3	3.0	20	69	28	0.125
isotig33783_1096	3	7.4	23	67	29	0.350
isotig30724_1666	3	7.4	22	67	29	0.280
isotig33531_1155	3	7.8	24	65	29	0.498
isotig26524_1202*	3	10.4	25	68	26	0.358
isotig13565_195	3	19.6	30	66	23	0.390
isotig28347_304	3	19.6	28	64	22	0.357
isotig31015_812	3	20.5	31	63	23	0.466
isotig32375_266	3	24.0	30	66	23	0.390
isotig32139_534	3	36.5	33	61	25	0.616
isotig33166_330	3	47.3	35	56	27	0.555
isotig30817_727*	3	70.4	34	52	31	0.505

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Supplemental Table 1. Continued.

Locus ^z	Chrom.	cM	Observed			Probability
			DehyA	Heterozygous	B5351C	
isotig35352_760	3	72.2	35	53	30	0.498
isotig36199_943	3	78.0	28	58	31	0.933
isotig42494_328	3	78.4	30	58	30	0.986
isotig37167_658	3	78.4	30	58	30	0.986
isotig36487_789	3	78.4	29	59	30	0.993
isotig21577_861	3	78.4	30	58	30	0.986
isotig29460_868	3	79.2	32	56	29	0.855
isotig31657_1599	3	79.7	31	56	29	0.915
isotig30352_1460	3	81.8	31	59	29	0.969
isotig34054_350	3	82.2	30	60	29	0.989
isotig28422_1226	3	82.2	30	59	29	0.993
isotig35180_158	3	82.2	30	60	29	0.989
isotig29185_1241	3	86.6	28	56	33	0.760
contig00187_695	3	86.6	27	56	33	0.721
isotig27896_600	3	87.1	28	57	34	0.710
isotig33746_1093	3	87.1	26	56	34	0.586
isotig43826_494	3	87.1	28	57	34	0.710
isotig37687_135*	3	87.1	28	57	34	0.710
isotig35214_301	3	89.6	28	61	30	0.942
isotig33232_1046	3	89.6	27	61	30	0.885
isotig32041_215	3	89.6	28	61	30	0.942
isotig26005_1583	3	90.5	28	61	29	0.937
isotig29570_2011*	3	93.5	27	66	26	0.547
isotig29415_721*	3	95.2	28	66	25	0.517
isotig22876_134	3	96.1	27	64	26	0.638
isotig28607_1011	3	96.1	27	65	25	0.524
isotig18967_973	3	96.5	28	65	26	0.634
isotig36406_986	3	97.3	27	66	24	0.412
isotig33810_581	3	98.6	25	69	24	0.236
isotig32926_115	3	100.3	27	68	24	0.338
isotig20406_751	3	100.8	26	69	24	0.272
isotig29299_806	3	103.3	28	64	26	0.679
isotig33971_1040	3	103.8	28	63	27	0.789
isotig32390_1451	3	106.0	23	67	25	0.248
isotig29163_2080	4	0.0	30	59	30	0.996
isotig32380_191	4	0.0	29	58	30	0.989
isotig28982_1927	4	8.2	27	62	29	0.854
isotig31593_1051	4	8.2	27	63	28	0.789
isotig31588_1120	4	10.3	29	59	30	0.993
isotig19074_815	4	16.6	29	61	28	0.937
isotig40592_178	4	16.6	29	62	28	0.909
isotig28762_702	4	20.7	26	65	28	0.634
isotig37158_542	4	40.5	25	68	26	0.358
isotig26526_748	4	50.0	26	67	24	0.338
isotig35345_1067	4	53.2	27	68	24	0.338
isotig28612_1057	4	57.7	27	61	28	0.868
isotig29590_1187	4	58.6	28	64	27	0.746
isotig34783_896	4	60.4	27	62	29	0.854
isotig30027_971	4	61.6	28	61	27	0.868
isotig32123_1465	4	62.5	28	63	28	0.841
isotig30914_1690	4	62.9	28	62	29	0.909
isotig38830_623	4	65.1	29	59	31	0.969
isotig29175_343	4	65.1	29	58	31	0.958
isotig37023_265	4	65.9	31	57	31	0.916
isotig31370_1070	4	65.9	31	57	30	0.937
isotig33399_1211	4	66.8	31	57	31	0.916

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Supplemental Table 1. Continued.

Locus ^z	Chrom.	cM	Observed			Probability
			DehyA	Heterozygous	B5351C	
isotig19574_601	4	67.2	30	55	32	0.812
isotig29854_973	4	68.0	29	58	31	0.958
isotig32829_1057	4	71.5	33	55	29	0.744
isotig26045_1046	4	73.2	31	56	30	0.906
isotig21519_664	4	74.1	32	55	31	0.789
isotig35268_1082	4	75.4	33	55	31	0.730
isotig31392_1360	4	75.8	32	55	31	0.789
isotig09889_661	4	77.9	33	54	30	0.697
isotig31879_649	4	78.4	32	55	31	0.789
isotig38440_478*	4	80.1	30	59	29	0.993
isotig36493_410	4	84.7	28	62	29	0.909
isotig28561_1513	4	93.6	29	62	25	0.700
isotig36793_769	4	98.6	27	64	27	0.698
isotig16136_1083	4	102.2	26	64	26	0.586
isotig40309_475*	4	102.2	26	65	27	0.592
isotig36318_875*	4	102.2	27	64	27	0.698
isotig29353_406*	4	103.9	26	64	28	0.679
isotig33886_426	4	103.9	25	63	27	0.614
isotig32333_1322	4	103.9	26	65	28	0.634
isotig45610_340*	4	106.5	28	63	28	0.841
isotig43376_492	4	118.8	26	77	12	0.001
isotig28081_488	4	118.8	26	81	12	0.000
isotig32307_1162	5	0.0	34	51	33	0.396
isotig41553_294	5	0.9	34	50	34	0.312
isotig39428_415*	5	4.9	36	51	31	0.333
isotig25097_2357*	5	4.9	37	51	30	0.280
contig00142_122	5	19.4	33	57	29	0.818
isotig26178_547	5	24.0	36	53	29	0.419
isotig29989_644*	5	25.3	36	53	28	0.403
isotig32901_1333*	5	25.3	37	53	28	0.333
isotig31628_1166	5	32.0	41	49	29	0.076
isotig29167_1843	5	32.8	40	49	30	0.104
isotig33680_1027	5	32.8	40	48	30	0.086
isotig35383_182	5	35.9	42	47	29	0.038
isotig32271_1015*	5	35.9	40	48	29	0.083
isotig28226_139	5	36.7	39	49	29	0.129
isotig37670_180	5	41.1	37	53	28	0.333
isotig28787_1516*	5	43.3	34	59	24	0.480
isotig34198_886*	5	45.4	33	56	30	0.789
isotig38424_376*	5	49.8	33	58	28	0.812
isotig14741_247	5	53.3	30	58	30	0.986
isotig29282_688	5	53.8	30	59	29	0.993
isotig31406_1529	5	56.8	32	58	29	0.909
isotig34069_110	5	56.8	32	57	29	0.885
isotig28803_1689	5	59.2	30	61	28	0.942
isotig33059_107	5	59.6	30	60	28	0.958
isotig26595_878	5	69.7	28	63	28	0.841
isotig28889_2528*	5	70.1	28	62	29	0.909
isotig28536_1567	5	71.4	28	62	28	0.879
isotig19361_480	5	72.7	29	63	26	0.745
isotig31727_1490	5	73.9	28	67	24	0.404
isotig14721_448*	5	83.7	24	60	34	0.481
isotig42464_471	5	86.3	26	57	35	0.528
isotig33965_554	5	86.3	26	58	35	0.547
isotig35535_407	5	86.7	26	56	36	0.429
isotig32376_621	5	88.0	25	58	35	0.481

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Supplemental Table 1. Continued.

Locus ^z	Chrom.	cM	Observed			Probability
			DehyA	Heterozygous	B5351C	
isotig29284_408	5	93.8	26	53	38	0.224
isotig34113_654	5	93.8	26	53	40	0.138
isotig31477_611*	5	94.2	26	52	41	0.092
isotig25797_639	5	96.0	26	54	39	0.198
isotig40014_661	5	97.3	27	53	38	0.250
isotig31278_1407	5	98.5	26	52	37	0.253
isotig37209_549	5	102.6	29	53	37	0.350
isotig33663_1163	6	0.0	30	55	32	0.812
isotig33493_1045	6	0.0	30	57	31	0.937
isotig35592_657	6	1.7	31	55	33	0.730
isotig29703_1814	6	1.7	30	54	32	0.765
isotig30464_1503	6	1.7	31	55	33	0.730
isotig31425_789	6	2.1	30	56	33	0.789
isotig45729_183	6	2.6	31	55	32	0.789
isotig28812_2285	6	4.7	28	58	33	0.812
isotig29120_2137*	6	8.2	26	60	33	0.705
isotig39121_540*	6	13.5	28	60	31	0.935
isotig34210_322*	6	14.4	28	58	31	0.933
isotig25085_1155*	6	24.0	26	62	30	0.783
isotig34547_833*	6	26.2	24	63	28	0.561
isotig41991_298*	6	32.2	25	66	28	0.517
isotig33185_668	6	32.6	25	65	29	0.583
isotig30163_1343	6	32.6	25	65	28	0.559
isotig35291_456	6	32.6	25	64	29	0.623
isotig30880_1388	6	32.6	25	64	29	0.623
isotig31550_508	6	33.9	25	62	30	0.697
isotig31528_193	6	33.9	25	64	30	0.630
isotig31017_1099*	6	35.4	24	51	24	0.955
isotig10023_2319*	6	36.5	26	62	31	0.767
isotig40098_630	6	36.5	26	62	31	0.767
isotig28451_1808	6	57.3	31	56	30	0.906
isotig28330_2105	6	64.7	30	55	33	0.745
isotig30657_853	6	64.7	30	55	33	0.745
isotig29476_1380	6	73.6	35	54	30	0.547
contig00298_108	6	78.9	33	56	28	0.760
isotig32739_152	6	83.7	28	64	27	0.746
isotig36782_698	6	83.7	28	63	27	0.789
isotig37512_264	6	83.7	27	63	27	0.744
isotig34246_1009*	6	85.7	29	62	26	0.783
isotig33019_124	6	86.7	30	63	26	0.751
isotig29293_1435*	6	89.5	30	54	28	0.909
isotig33031_559	7	0.0	18	64	31	0.110
isotig41937_218	7	0.0	18	66	33	0.085
isotig39918_357	7	3.2	20	62	36	0.140
isotig30655_190	7	5.1	20	63	36	0.138
isotig28239_126	7	5.6	20	62	37	0.119
contig00293_452	7	7.3	19	64	33	0.136
isotig29209_2397*	7	7.3	19	66	33	0.121
isotig34313_545	7	9.5	20	67	32	0.163
isotig28716_113	7	9.6	16	55	23	0.135
isotig36042_298	7	9.9	20	64	33	0.187
isotig34753_1109	7	9.9	20	66	32	0.176
isotig40501_344	7	14.3	23	60	36	0.302
isotig26063_406	7	14.3	23	59	34	0.401
isotig29101_1894	7	15.1	21	59	37	0.154
isotig28488_2757	7	18.1	22	61	36	0.243

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Supplemental Table 1. Continued.

Locus ^z	Chrom.	cM	Observed			Probability
			DehyA	Heterozygous	B5351C	
isotig35010_835*	7	19.0	22	61	36	0.243
isotig31939_448*	7	21.6	21	65	33	0.236
isotig30988_471	7	22.4	21	64	33	0.248
isotig29530_152	7	23.7	19	66	34	0.112
isotig28398_1732	7	34.0	22	60	37	0.203
isotig30110_847	7	35.7	23	58	35	0.343
isotig28365_1152	7	36.1	22	59	37	0.199
isotig34666_385	7	44.5	26	54	35	0.450
isotig33898_339	7	46.9	29	55	34	0.664
isotig39752_384	7	47.3	28	56	35	0.595
isotig26033_1589*	7	48.1	28	54	35	0.520
isotig34668_1112	7	51.7	30	54	32	0.765
isotig42387_377	7	53.4	31	56	32	0.835
isotig29157_1006*	7	56.5	30	59	30	0.996
isotig29331_101*	7	74.6	29	52	37	0.312
isotig35302_619*	7	75.0	29	54	36	0.462
isotig38248_370	7	78.1	27	58	33	0.761
isotig22339_503	8	0.0	42	47	30	0.040
isotig30020_476	8	2.1	42	47	29	0.038
isotig28911_1986	8	2.1	41	47	29	0.051
isotig32102_1515	8	2.1	42	47	29	0.038
isotig30907_420	8	3.9	43	45	30	0.018
isotig34070_1138	8	4.3	43	44	31	0.014
isotig38873_388	8	4.7	43	45	30	0.018
isotig20266_1040	8	8.2	42	49	26	0.041
isotig20235_630*	8	10.4	39	53	27	0.199
isotig36797_307	8	11.2	39	51	27	0.154
isotig31683_1344	8	11.2	39	52	27	0.176
isotig37323_396	8	16.0	35	57	26	0.528
isotig33589_1176	8	16.9	36	56	27	0.475
isotig30323_747	8	16.9	36	56	27	0.475
isotig37010_277	8	18.6	38	54	27	0.278
isotig43294_202	8	18.6	38	52	27	0.223
isotig32346_843*	8	19.4	39	51	27	0.154
isotig28432_1104	8	19.4	38	51	28	0.212
isotig31913_1153*	8	20.7	36	53	29	0.419
isotig12913_342*	8	21.1	37	51	30	0.280
isotig31511_1415	8	33.3	36	55	26	0.403
isotig37822_830	8	37.5	34	57	28	0.710
isotig31608_1092	8	37.9	33	54	28	0.688
isotig33645_542	8	39.2	33	57	29	0.818
isotig28367_2185	8	48.4	29	60	29	0.986
isotig30151_1442	8	48.4	29	61	29	0.969
isotig31261_1350	8	51.5	27	60	31	0.879

^zLocus names followed by * indicate SNPs not previously reported by Duangjit et al. (2013).