

Genetic Analysis of the Unique Epicuticular Wax Profile of ‘Odourless Greenleaf’ Onion

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ABSTRACT. The amounts and types of epicuticular waxes on onion (*Allium cepa*) leaves affect the severity of feeding damage by onion thrips (*Thrips tabaci*), a serious insect pest of onion. Onion plants with light green leaves are referred to as “glossy” and accumulate less epicuticular wax relative to the blue–green (“waxy”) foliage of wild-type onion. The onion cultivar Odourless Greenleaf (OGL) has visually glossy foliage, shows resistance to thrips feeding damage, and has the unique profile of accumulating waxes with 28 or fewer carbons. Plants of glossy OGL were crossed with the glossy inbred B9885 and waxy inbred lines DH2107, DH066619, and B8667. Hybrid progenies from glossy OGL by waxy plants had waxy foliage, indicating recessiveness of the glossy OGL phenotype relative to the waxy phenotype. Hybrids from the cross of glossy OGL with glossy B9885 were also waxy, revealing different genetic bases for the glossy phenotype in these two onions. Hybrid plants were self-pollinated and segregations in F₂ families from OGL × waxy crosses fit the expected 3:1 ratio for the single locus at which the homozygous recessive genotype conditions glossy foliage. Segregations in F₂ families from crosses of glossy 9885 × glossy OGL fit the 9:7 ratio, supporting two independently segregating loci, where the recessive genotype at either locus conditions the glossy phenotype. Amounts and types of epicuticular waxes on leaves of F₂ progenies from crosses of OGL × waxy B8667 and glossy B9885 × OGL were determined using gas chromatography-mass spectrometry. Single-nucleotide polymorphisms were genotyped and genetic maps were constructed. The visually glossy phenotype from OGL and its unique profile of epicuticular waxes were conditioned by one locus on chromosome 6, for which we propose the name *gl^{og}*. Onion populations such as OGL with unique epicuticular wax profiles will be important germplasms for the development of onion cultivars that suffer less feeding damage from onion thrips compared with waxy onion.

The epicuticular waxes on onion (*Allium cepa*) foliage are primarily composed of fatty alcohols, alkanes, and a ketone (Damon et al., 2014). The foliage of wild-type onion has a blue–green color, accumulates relatively high amounts of epicuticular waxes, and is referred to as “waxy.” The waxy appearance on leaves of wild-type onion is likely due to large amounts of the ketone 16-hentriacontanone (H16) relative to the other waxes that build as platelets on the leaf surface (Damon et al., 2014). The “glossy” phenotype of onion is characterized by a light green leaf color and low amounts of epicuticular waxes (Damon et al., 2014; Molenaar, 1984). The leaf color and amounts of epicuticular wax on the leaf surface affect feeding damage by onion thrips (*Thrips tabaci*), which are a serious

insect pest of onion worldwide. Onion plants with a light green leaf color and/or different amounts and types of epicuticular waxes support fewer onion thrips per plant and suffer significantly less feeding damage relative to waxy foliage (Boateng et al., 2014; Cramer et al., 2014; Coudriet et al., 1979; Damon et al., 2014; Diaz-Montano et al., 2010; Jones et al., 1934; Munaiz et al., 2020a).

Jones et al. (1944) studied the genetics of glossy plants selected from the onion cultivars Australian Brown and White Persian. For plants selected from ‘Australian Brown’, the glossy phenotype was conditioned by the homozygous recessive genotype at one locus named *gl* (Jones et al., 1944). For glossy plants from ‘White Persian’, segregations did not fit a single recessive model because too few glossy progenies were observed in families (Jones et al., 1944). Molenaar (1984) completed a genetic study using glossy plants descended from the same selections from ‘White Persian’ identified by Jones et al. (1934) and observed that segregation ratios fit a single recessive model. Munaiz and Havey (2020) mapped the glossy phenotype from ‘White Persian’ to chromosome 8 and named the *gl^{wp}* locus at which the homozygous recessive genotype conditions glossy foliage. The *gl^{wp}* locus interacts with a region on chromosome 1 to increase the numbers of waxy progenies (Munaiz and Havey, 2020), consistent with the observation by Jones et al. (1944) of too few glossy progenies in segregating families.

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Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Fig. 1. Phenotypes of waxy B8667 (left), glossy ‘Odourless Greenleaf’ (center), and glossy B9885 (right) onion at the stage of leaf sampling for gas chromatography-mass spectrometry.

The onion cultivar Odourless Green Leaf (OGL) has a light green leaf color, is less attractive to thrips relative to waxy onion (Cramer et al., 2014), and has a unique profile of epicuticular waxes (Munaiz et al., 2020b). During this research, we determined the genetic basis of the wax profile and glossy phenotype of OGL, and we showed that it is inherited independently from the *gt^{wp}* locus (Munaiz and Havey, 2020). Germplasms such as OGL with unique wax profiles may be useful for the development of onion cultivars that suffer less damage by onion thrips compared with waxy onion.

Materials and Methods

Seed of OGL was obtained from the U.S. Department of Agriculture (USDA) germplasm collection as PI 289689. Inbred B9885 has glossy foliage (Damon et al., 2014) and inbred B8667 has waxy foliage (Havey and Bohanec, 2007), and both were from the USDA onion-breeding program. Waxy doubled haploids (DH) 2107 and 066619 were described by Hyde et al. (2012). Seeds of these accessions were planted at the Dean Kincaid Farm (Palmyra, WI) and bulbs were produced under normal production conditions. After harvest, bulbs were stored for at least 4 months at 7 °C for vernalization, and planted in greenhouses with 25 °C days and 20 °C nights, and supplemental lighting for 12 to 13 h for OGL or 14 to 15 h for B8667, B9885, DH2107, and DH066619. Individual flowering plants of OGL were paired with plants of the other parents and covered with mesh cages, and houseflies (*Musca domestica*) were introduced for crossing. Seed was harvested from OGL plants and planted in the greenhouse. Progenies were grown under 12-h days and hybrids were identified by the presence of waxy foliage. At the six-leaf stage, hybrid plants were transferred to a cold room at ≈4 °C with 12 h lighting for at least 4 months. In late April, hybrid plants were transplanted to field plots at the University of Wisconsin Horticulture Research Farm (Arlington, WI) under naturally increasing daylengths. Umbels on individual hybrid plants were covered with mesh cages and blue-bottle flies (*Calliphora* sp.) were introduced to produce F₂ families.

Plants of parents, hybrids, and F₂ progenies were grown in a soilless mix (Pro-Mix HP Mycorrhizae; Premier Tech Horticulture, Quakertown, PA) in 11.4-cm pots in a greenhouse with 25 °C days, 20 °C nights, and supplemental lighting for 14 h. Pots were randomly arranged on benches, watered daily, and fertilized with 0.5× Hoagland’s solution (Hoagland and Arnon, 1950) once per week. Eight weeks after the planting of seed, plants had approximately six true leaves and foliage was visually scored as glossy vs. waxy (Fig. 1). Two leaf segments ≈9 cm were collected from the middle region of the fourth or fifth fully expanded leaf from five plants each of OGL, B9885, B8667, and their hybrid progenies, and from individual F₂ progenies from the OGL × B8667 and B9885 × OGL families. Each leaf segment was placed in a 16- × 100-mm glass tube (Thermo Fisher Scientific, Waltham, MA) and ≈20 mL of chloroform [high-performance liquid chromatography (HPLC) grade; Sigma-Aldrich, St. Louis, MO] was added to the tube for 1 min; after which, leaf pieces were removed and oven-dried at 80 °C for 5 d, and dry weights were measured. Then 25 μL of docosane (Sigma-Aldrich) dissolved in HPLC-grade chloroform at a stock concentration of 1 mg·mL⁻¹ was added to each tube. The chloroform with docosane was allowed to dry in a fume hood for ≈2 weeks. To prepare for gas chromatography-mass spectrometry (GCMS), dried wax extracts were dissolved in 250 μL chloroform, 300 μL acetonitrile (HPLC grade; Thermo Fisher Scientific), and 105 μL N,O bis(trimethylsilyl) trifluoro-acetamide (HPLC/GC grade; Sigma-Aldrich) and incubated for 30 min at 80 °C. Identification and quantification of waxes involved the GCMS conditions described by Damon et al. (2014) and Khosa et al. (2020).

DNA were isolated from parents, hybrids, and F₂ progenies from the B9885 × OGL and OGL × B8667 crosses using a mini-preparation (Nucleospin 96 Plant II DNA extraction kit; Macherey-Nagel, Bethlehem, PA). DNA concentrations were determined spectrophotometrically (NanoDrop ND-1000; Thermo Fisher Scientific), and quality was assessed by electrophoresis through 1% agarose gels. Two-hundred fifty single-nucleotide polymorphisms (SNPs) evenly spread across the eight chromosomes of onion were genotyped using the KASPar assay (Duangjit et al., 2013; Havey and Ghavami, 2018). Joinmap 5 (Van Ooijen, 2018) was used to assess goodness-of-fit to the expected ratios and for genetic mapping using a logarithm of odds (LOD) of linkage at 6.0.

Individual peak areas from GCMS were adjusted by dividing by the dry weights of leaf pieces. Peak areas from the two samples from each leaf were averaged and used for all analyses. Analyses of variance of the amounts of waxes were calculated using R Studio (R Foundation for Statistical Computing, Vienna, Austria). Visually scored foliar phenotypes were analyzed using the binary model and the amounts of individual and total waxes were analyzed using composite interval mapping (CIM) with the R/qrtl package (Broman and Sen, 2009; Broman et al., 2003). Haley Knott regression with 10 marker covariates within a window size of 10 cM was used to determine significant associations (Haley and Knott, 1992) and a 95% significance LOD threshold was used after 1000 permutations. Multiple quantitative trait loci (QTLs) were detected using the stepwiseqrtl function dropping one marker at a time with parameters of 0.05 significance level and 1.5-LOD score. Effects of the candidate QTLs were refined using makeqrtl, refineqrtl, and fitqrtl, and the percentages of the phenotypic variation explained by the QTLs were estimated.

Table 1. Mean peak areas adjusted for the leaf dry weight of epicuticular waxes detected by gas chromatography-mass spectrometry on onion leaves of 'Odourless Green Leaf' (OGL), glossy inbred B9885, waxy inbred B8667, and hybrids from crosses of OGL with B8667 and B9885 with OGL. Means are from the measurements of five plants.

Parent or hybrid	Fatty alcohols ^z			Alkanes ^z			Ketone ^z			Total ^z	HOH/Total ^z
	Hex (C26)	Oct1 (C28)	Tri (C30)	Heps (C27)	Nons (C29)	Hent (C31)	H16 (C31)	Total ^z			
OGL	112,518,268 a ^y	292,805,104 ab	3,167,082 a	8,668,491 a	2,440,847 a	13,287,838 a	14,876,951 a	447,764,582 a	0.870 a		
B9885	25,569,396 b	158,010,723 b	77,332,849 b	3,666,911 b	8,244,238 ab	23,919,378 ab	146,576,283 a	443,319,778 a	0.396 b		
B8667	33,045,112 b	194,937,377 b	109,615,415 bc	4,585,642 b	19,949,546 c	38,708,441 c	499,276,403 b	900,117,936 b	0.224 d		
OGL×B8667	38,360,966 b	245,760,797 ab	97,995,745 bc	5,063,366 b	12,798,110 bc	27,269,672 bc	442,821,672 b	870,070,328 b	0.296 cd		
B9885×OGL	41,172,329 b	336,171,686 a	124,290,013 c	5,646,896 b	16,288,675 bc	39,842,987 c	494,270,777 b	1,057,683,363 b	0.318 c		

^zEpicuticular waxes are Hex = 1-hexacosanol; Oct1 = 1-octacosanol; Tri = 1-triacontanol; Heps = heptacosane; Nons = homacosane; Hent = hentriacontane; and H16 = 16-hentriacontanone; Total = sum of all waxes; and HOH/Total = sum of amounts of Hex, Oct1, and Heps over total wax. C(number) = number of carbons.

^yMeans in a column followed by the same letter are not significantly different using least significant differences with the Bonferroni adjustment at $P = 0.05$.

Results

EPICUTICULAR WAXES ON THE FOLIAGE OF OGL. GCMS analyses of epicuticular waxes revealed that the foliage of OGL accumulated significantly greater amounts of the fatty alcohol 1-hexacosanol (Hex) with 26 carbons (C) and the alkane heptacosane (Heps; C27) and lower amounts of most of the other waxes and total wax relative to waxy B8667 and glossy B9885 (Table 1, Fig. 2). Amounts of Oct1 (C28) were not significantly different for OGL, glossy B9885, waxy B8667, and their hybrids (Table 1). Therefore, the foliage of OGL has the unique phenotype of accumulating C26 and C28 fatty alcohols (Hex and Oct1, respectively) and a C27 alkane (Heps) and significantly lower amounts of waxes with more than 28 carbons compared with waxy onion.

GENETIC ANALYSES OF SEGREGATING FAMILIES FROM GLOSSY OGL BY WAXY CROSSES. Crosses of OGL plants with waxy B8667, DH2107, and DH066619 produced hybrids with waxy foliage, indicating that the glossy phenotype from OGL is recessive relative to waxy foliage. For 19 of 22 F₂ families from the cross of glossy OGL with waxy plants, visual segregations of waxy vs. glossy foliage fit the 3:1 expected ratio ($P > 0.05$) for a single locus at which the homozygous recessive genotype conditions the glossy phenotype (Table 2). F₂ families 2, 3, and 11 (Table 2) had excesses of glossy progenies and fit the expected 3:1 only at P between 0.05 and 0.01. After confirming the homogeneity of errors across families, segregation for waxy vs. glossy foliage summed across families from the crosses of OGL with waxy male parents fit the 3:1 ratio [1049 waxy: 373 glossy ($P = 0.284$)]. Genetic mapping was completed using 131 SNPs and linkage groups were constructed at an LOD of 6.0. Visual segregations for glossy vs. waxy foliage mapped as a single locus at position 113 cM on chromosome 6 (Supplemental Table 1).

We calculated the ratio of summed amounts of Hex, Oct1, and Heps (HOH) over total wax (HOH/Total), and OGL was significantly different from B9885, B8667, and hybrids from crosses (Table 1, Fig. 2). A scatterplot of this ratio for F₂ progenies from the cross of OGL × waxy B8667 (family 4 in Table 2) showed discrete segregation (Fig. 3) that fit the expected ratio for a single locus at which the homozygous recessive genotype conditioned the glossy OGL phenotype [68 progenies with ratio <0.7: 22 progenies with ratio > 0.7 ($P = 0.903$)]. Quantitative mapping of the visual phenotype as a binary trait and CIM of the HOH/Total ratio revealed a highly significant QTL at the same position on chromosome 6 at 111 cM, explaining 85% (LOD 37) and 80% (LOD 31.5) of the phenotypic variation, respectively (Table 3). QTL at or near the same position on chromosome 6 were detected and conditioned high amounts of Hex and Heps, and the chromosome region of wild-type waxy B8667 showed significant additive and dominance effects that decreased amounts. Significant QTL conditioning low amounts of the other individual waxes and total wax were detected at or near the same position on chromosome 6, and the chromosome region of B8667 increased amounts (Table 3). Although the amounts of Oct1 were not significantly different between OGL and B8667 (Table 1), CIM revealed a QTL (LOD 3.7) just above the significance threshold (LOD 3.3) on chromosome 5 at 57 cM, explaining 17% of phenotypic variation (Table 3). No QTL were detected for the amounts of the alkane Non in the segregating family from OGL × B8667.

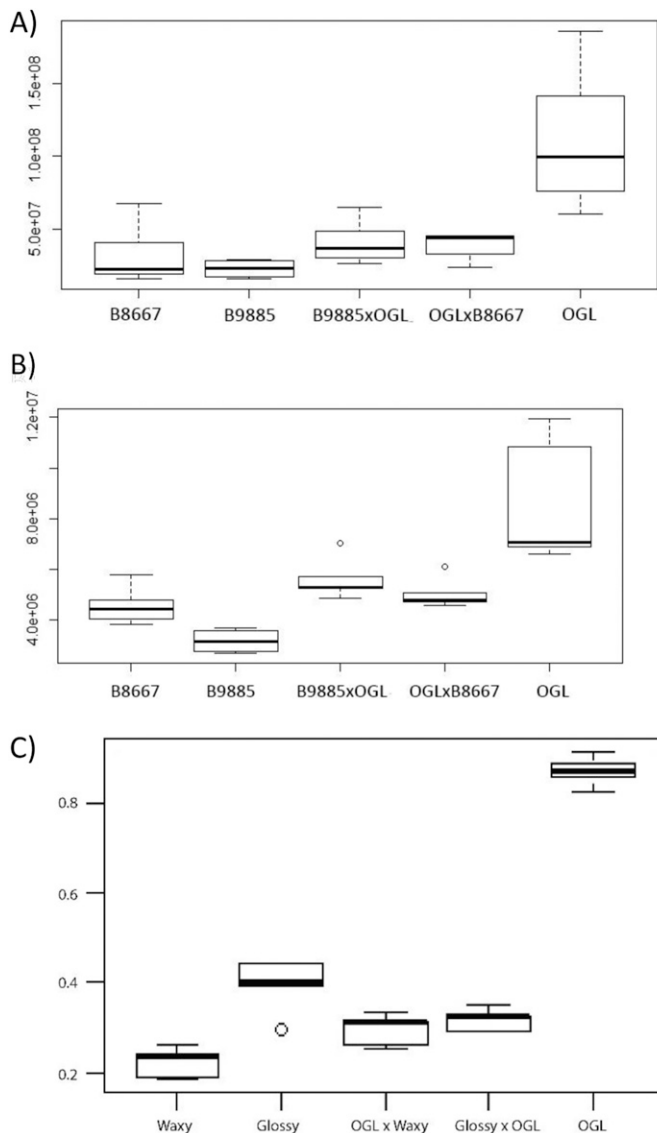


Fig. 2. Boxplots for the amounts of (A) fatty alcohol 1-hexacosanol, (B) alkane heptacosane, and (C) ratio (HOH/Total) of the summed amounts of 1-hexacosanol, 1-octacosanol, and heptacosane (HOH) over the sum total of all waxes (Total wax) on foliage of five plants each of glossy 'Odourless Greenleaf' (OGL), waxy B8667, glossy B9885 onion, and hybrid progenies. Values on Y-axes in (A) and (B) are peak areas adjusted for leaf dry weights.

GENETIC ANALYSES OF SEGREGATING FAMILIES FROM GLOSSY B9885 × GLOSSY OGL CROSSES. Crosses of glossy inbred B9885 × glossy OGL produced hybrids with waxy foliage, indicating that the glossy phenotype from OGL is not conditioned by recessive alleles at the gl^{wp} locus on chromosome 8 from B9885 (Munaiz and Havey, 2020). For F_2 families from the cross of B9885 × OGL, visual segregations of waxy vs. glossy foliage fit the expected 9:7 ratio for two independently segregating loci where the homozygous recessive genotype at either locus conditions glossy foliage (families 23–25 in Table 2). After confirming homogeneity of errors, segregations summed across F_2 families from the cross of B9885 × OGL fit a 9:7 ratio [132 waxy: 102 glossy ($P = 0.730$)]. This result indicates that the glossy OGL phenotype segregates independently of the gl^{wp} locus from glossy B9885 (Munaiz and Havey, 2020).

Table 2. Observed (Obs) segregations and probabilities of goodness-of-fit to expected (Exp) ratios for the numbers of progenies with glossy (Gl) versus waxy (Wx) foliage in F_2 families from crosses of glossy 'Odourless Greenleaf' (OGL) with glossy (B9885) or waxy (B8667, DH2107, and DH066619) inbred lines of onion.

Cross	Family no.	Obs (no.)		Exp	P
		Wx	Gl		
OGLxB8667	1	49	26	3:1	0.053
OGLxB8667	2	38	23	3:1	0.022
OGLxB8667	3	59	31	3:1	0.039
OGLxB8667	4	112	38	3:1	0.925
OGLxB8667	5	58	15	3:1	0.380
OGLxDH2107	6	43	12	3:1	0.586
OGLxDH2107	7	58	25	3:1	0.281
OGLxDH2107	8	52	19	3:1	0.732
OGLxDH2107	9	54	16	3:1	0.679
OGLxDH2107	10	77	16	3:1	0.083
OGLxDH2107	11	45	27	3:1	0.014
OGLxDH2107	12	35	18	3:1	0.132
OGLxDH2107	13	47	14	3:1	0.712
OGLxDH2107	14	60	20	3:1	1.000
OGLxDH2107	15	29	7	3:1	0.441
OGLxDH2107	16	38	8	3:1	0.233
OGLxDH2107	17	69	16	3:1	0.188
OGLxDH066619	18	27	10	3:1	0.776
OGLxDH066619	19	27	10	3:1	0.776
OGLxDH066619	20	40	12	3:1	0.749
OGLxDH066619	21	45	16	3:1	0.824
OGLxDH066619	22	63	16	3:1	0.330
B9885×OGL	23	45	30	9:7	0.513
B9885×OGL	24	93	92	9:7	0.101
B9885×OGL	25	47	32	9:7	0.561

Genetic mapping was completed using 129 SNPs and DNA from F_2 family 24 (Table 2), and linkage groups were constructed at an LOD of 6.0 (Supplemental Table 2). Mapping of glossy vs. waxy foliage as a binary trait revealed two QTL, one on chromosome 6B at 21 cM (LOD = 3.6) and one on chromosome 8 at 26 cM (LOD = 9.0), agreeing with the 9:7 segregation (Tables 2 and 4). The amounts of Hex, Tri, and Heps were significantly different on leaves of glossy OGL and glossy B9885 (Table 1). The CIM of amounts of fatty alcohols Hex and Tri detected a QTL on chromosome 6B at 21 cM (LOD 10.4 and 11.6), explaining 40% and 44% of the phenotypic variation, respectively (Table 4). The chromosome region from OGL increased the amounts of Hex and decreased the amounts of Tri, consistent with the results from the OGL × waxy B8667 family described. Regarding the amounts of the alkane Heps, significant QTL were detected on chromosomes 6B at 21 cM (LOD = 9.7) and 8 at 39 cM (LOD = 8.9), explaining 18% and 13% of the phenotypic variation, respectively (Table 4). Both chromosome regions from OGL had significant additive effects that increased the amounts of Heps (Table 4). The scatterplot of the HOH/Total ratio for F_2 progenies from family 24 (Table 2) showed discrete segregation of the phenotype (Fig. 3) that fit a 3:1 ratio [71 progenies < 0.7: 22 progenies > 0.7 ($P = 0.765$)]. The CIM of the HOH/Total ratio revealed one significant (LOD = 12.1) QTL on chromosome 6B at 21 cM, explaining 45% of the phenotypic variation (Table 4).

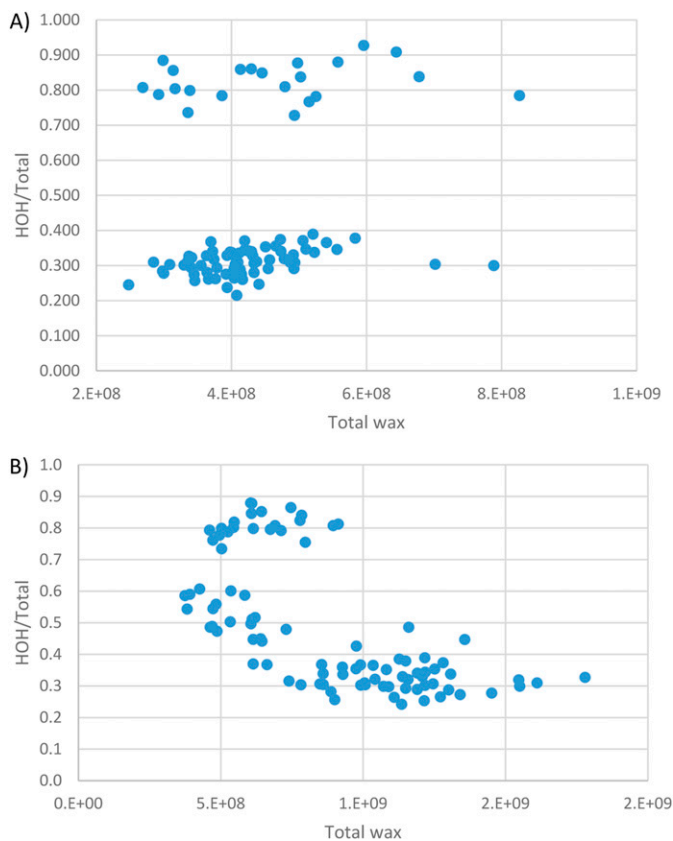


Fig. 3. Scatterplots of the ratio of the summed amounts of 1-hexacosanol, 1-octacosanol, and heptacosane (HOH) over the sum total of all waxes (HOH/Total) for F₂ progenies from the crosses of glossy OGL with waxy B8667 (A) or glossy B9885 (B) onion. Values were calculated using peak areas adjusted for the leaf dry weights.

Although OGL and B9885 were not significantly different for amounts of the alkanes Hent and Non, the ketone H16, and total wax, significant QTL were detected on chromosomes 6B and 8. Regarding the amounts of Hent and Non, significant QTL

on chromosome 6B at 21 cM (LOD = 9.1 and LOD = 10.3) explained 36% and 24% of the phenotypic variation, respectively, and the chromosome region from OGL decreased the amounts of both alkanes (Table 4). Regarding the amounts of H16, significant QTL were detected on chromosomes 6B at 21 cM (LOD = 10.7) and chromosome 8 at 36 cM (LOD = 13.0), explaining 22% and 32% of the phenotypic variation, respectively (Table 4). The chromosome region on chromosome 8 from OGL increased the amounts of H16, whereas the region on 6B from OGL decreased the amounts of H16. Regarding total wax, a QTL was detected on chromosome 8 at 36 cM (LOD 8.2), explaining 24% of the phenotypic variation (Table 4). The location and effect of QTL controlling the amounts of H16 and total wax are consistent with the glossy phenotype conditioned by the *gl^{wp}* locus (Munaiz and Havey, 2020). No significant QTL were detected for the amounts of Oct1 in the B9885 × OGL family.

Discussion

The foliage of OGL onion accumulates the fatty alcohols Hex (C26) and Oct1 (C28), the alkane Heps (C27), and low amounts of waxes with more than 28 carbons (Table 1). Wax profiles similar to OGL have been reported for other plants conditioned by the *cer19* mutant of *Arabidopsis thaliana* (Rashotte et al., 2001), *gl3* mutant of *Zea mays* (Avato et al., 1987), and *gl2* and *gl5* mutants of the *Brassica oleracea* (Macey and Barber, 1970); all of those do not accumulate significant amounts of waxes with more than 28 carbons. In plants, the synthesis of epicuticular waxes begins with the production of long-chain (>C26) fatty acids that enter the acyl reduction and decarbonylation pathways (Millar et al., 1999; Samuels et al., 2008). In the acyl reduction pathway, fatty acids with even numbers of carbons are used to produce fatty alcohols such as Hex (C26), Oct1 (C28), and Tri (C30). In the decarbonylation pathway, fatty acids lose one carbon, generating waxes with odd numbers of carbons such as the alkanes Heps (C27), Non (C29), and Hent (C31) and the ketone H16 (C31). The accumulation of two fatty alcohols and one alkane on the

Table 3. Chromosome (Chr) and position (Pos) in centimorgans of the most significant single-nucleotide polymorphism (SNP), SNPs flanking the 1.5 logarithm of odds (LOD) confidence interval, percent variation (Var) explained, observed (Obs) LOD with threshold (Thresh) LOD from permutation analysis, and additive (Add) and dominance (Dom) effects of chromosome region from wild-type waxy B8667 for quantitative trait loci detected by composite interval mapping for the visual (glossy versus waxy) phenotype and amounts of epicuticular waxes on foliage of F₂ progenies from the cross of glossy ‘Odourless Greenleaf’ by waxy B8667 onion.

Trait ^z	SNP ^y	Chr		Interval	Var (%)	LOD		Effect			
		Pos (cM)				Obs	Thresh	Add ^x	Dom ^x		
Visual phenotype	i32152_614	6 at 111		i40098_630 to i32739_152	84.9	37.0	6.3	0.49	*	0.51	*
Hex	i32152_614	6 at 111		i40098_630 to i32739_152	44.4	11.5	8.1	-53,690,372	*	-43,791,048	*
Oct1	i37258_745	5 at 57		i37670_180 to i28803_1689	17.3	3.7	3.3	47,135,079	*	-18,456,782	
Tri	i32152_614	6 at 111		i40098_630 to i32739_152	75.8	27.7	7.9	59,237,113	*	36,110,170	*
Heps	i32152_614	6 at 111		i40098_630 to i32739_152	40.3	10.1	8.0	-1,365,333	*	-1,408,744	*
Hent	i32152_614	6 at 111		i40098_630 to i32739_152	34.2	8.2	8.1	13,276,883	*	5,413,987	
H16	i32152_614	6 at 111		i40098_630 to i32739_152	57.3	16.6	7.7	282,924,218	*	214,704,602	*
HOH/Total	i37258_745	6 at 111		i40098_630 to i32739_152	80.0	31.5	7.8	-0.25	*	-0.24	*
Total	i32152_614	6 at 111		i40098_630 to i32739_152	50.7	13.8	7.8	3.89E+08	*	2.71E+08	*

^zEpicuticular waxes are Hex = 1-hexacosanol; Oct1 = 1-octacosanol; Tri = 1-triacontanol; Heps = heptacosane; Hent = hentriacontane; and H16 = 16-hentriacontanone; Total = sum of all waxes; and HOH/Total = summed of amounts of Hex, Oct1, and Heps over total wax.

^ySNPs were described by Duangjit et al. (2013); i = isotig.

^xVisual phenotype was analyzed as a binary trait; values for individual waxes represent peak areas adjusted for leaf dry weight.

*Significant at $P < 0.05$.

Table 4. Chromosome (Chr) and position (Pos) in centimorgans of the most significant single nucleotide polymorphism (SNP), SNPs flanking the 1.5 logarithm of odds (LOD) confidence interval, percent variation (Var) explained, observed (Obs) LOD with threshold (Thresh) LOD from permutation analysis, and additive (Add) and dominance (Dom) effects of chromosome region from glossy ‘Odourless Greenleaf’ (OGL) for quantitative trait loci detected by composite interval mapping for the visual (glossy versus waxy) phenotype, and amounts of epicuticular waxes on foliage of F₂ progenies from the cross of glossy B9885 by glossy OGL onion.

Trait ^z	SNP ^y	Chr		Interval	Var (%)	LOD		Effect			
		Pos (cM)				Obs	Thresh	Add ^x		Dom ^x	
Visual phenotype	i28330_2105	6B at 21		i32739_152 to i28330_2105	11.3	3.6	2.1	-0.18	*	0.22	*
Hex	i20235_630	8 at 26		i30907_420 to i37010_277	31.8	9.0	2.1	0.24	*	0.47	*
Hex	i28330_2105	6B at 21		i32739_152 to i28330_2105	40.2	10.4	8.0	38,887,212	*	-24,785,034	*
Tri	i28330_2105	6B at 21		i32739_152 to i28330_2105	43.6	11.6	7.5	-38,270,996	*	27,324,331	*
Heps	i32346_843	8 at 39		i20235_630 to i32346_843	13.4	8.9	8.0	988,503	*	-396,939	*
Heps	i28330_2105	6B at 21		i32739_152 to i28330_2105	18.3	9.7	8.0	1,127,141	*	-886,631	*
Nons	i28330_2105	6B at 21		i32739_152 to i28330_2105	24.4	10.3	7.9	-4,463,089	*	4,604,888	*
Hent	i28330_2105	6B at 21		i32739_152 to i28330_2105	36.2	9.1	8.1	-10,195,341	*	9,408,788	*
H16	i33589_1176	8 at 36		i20235_630 to i37010_277	31.8	13.0	8.2	186,229,634	*	99,297,412	*
H16	i28330_2105	6B at 21		i32739_152 to i28330_2105	22.1	10.7	8.2	-126,601,508	*	159,976,507	*
HOH/Total	i28330_2105	6B at 21		i32739_152 to i28330_2105	45.1	12.1	7.7	0.16	*	-0.14	*
Total	i33589_1176	8 at 36		i20235_630 to i37010_277	23.6	8.2	8.0	232,302,412	*	115,887,338	*

^zEpicuticular waxes are Hex = 1-hexacosanol; Oct1 = 1-octacosanol; Tri = 1-triacontanol; Heps = heptacosane; Nons = nonacosane; Hent = hentriacontane; and H16 = 16-hentriacontanone; Total = sum of all waxes; and HOH/Total = sum of amounts of Hex, Oct1, and Heps over total wax.

^ySNPs were described by Duangjit et al. (2013); i = isotip.

^xVisual phenotype was analyzed as a binary trait; values for individual waxes represent peak areas adjusted for leaf dry weight.

*Significant at $P < 0.05$.

foliage of OGL indicates that both the acyl reduction and decarbonylation pathways are functioning, and that the availability of the C30 fatty acid (triacontanoic acid) as the substrate for synthesis of waxes with more than 28 carbons may be limiting in OGL leaves.

Results from segregation analyses of the OGL × B8667 and B9885 × OGL families supported a single region on chromosome 6 controlling the glossy foliage and unique wax profile of OGL. SNP i32739_152 mapped within the LOD-1.5 interval for these traits in both the OGL × B8667 and B9885 × OGL families (Tables 3 and 4, Supplemental Tables 1 and 2). Because the same region on chromosome 6 was mapped in two segregating families, we propose the locus name gl^{og1} at which the recessive allele from OGL conditions its glossy foliage and unique epicuticular wax profile.

The glossy phenotype from ‘White Persian’ and OGL suffer significantly less feeding damage from onion thrips relative to waxy onion (Cramer et al., 2014; Damon et al., 2014; Jones et al., 1934). However, the glossy phenotype from ‘White Persian’ accumulates significantly lower amounts of total epicuticular waxes compared with waxy onion (Damon et al., 2014; Molenaar, 1984) (Table 1) and shows susceptibility to foliar pathogens (Mohan and Molenaar, 2005). The selection of onion for unique wax profiles, such as high amounts of fatty alcohols and low amounts of H16 (Damon et al., 2014; Munaiz and Havey, 2020), may produce populations that support fewer thrips and suffer less feeding damage from the insect (Munaiz et al., 2020a) but still accumulate adequate amounts of total epicuticular wax for commercial production. Onion populations with unique wax profiles, such as OGL, will also be useful for more basic studies of genes affecting specific profiles of epicuticular waxes.

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Supplemental Table 1. Loci, chromosome (Chrom), position (Pos) in centiMorgans, observed (Obs) segregations, and probabilities of goodness-of-fit to the expected (Exp) segregation ratios for the F₂ family from the cross of glossy ‘Odourless Greenleaf’ with waxy B8667 onion.

Locus ^z	Chrom		Obs (no.) ^x			Exp	P ^w
	no. ^y	Pos (cM)	a	h	b		
c13740_1338	1	0.0	28	44	18	1:2:1	
i27537_659	1	14.4	24	49	17	1:2:1	
i35370_364	1	17.3	22	50	18	1:2:1	
i31226_709	1	17.8	22	51	17	1:2:1	
i29498_587	1	22.0	25	48	17	1:2:1	
i36337_305	1	55.8	0	74	16	1:2:1	
i30950_933	1	70.8	21	53	16	1:2:1	
i36938_811	1	73.1	21	53	16	1:2:1	
i26470_847	1	74.8	22	52	16	1:2:1	
i32772_993	1	76.0	22	50	18	1:2:1	
i34779_398	1	78.3	22	50	18	1:2:1	
c00172_269	1	81.2	24	49	17	1:2:1	
i30185_1091	1	81.2	24	49	17	1:2:1	
i33099_885	1	99.2	27	44	19	1:2:1	
i43680_368	1	105.2	26	44	20	1:2:1	
i44346_390	1	107.6	25	44	20	1:2:1	
i37348_328	1	121.0	26	46	18	1:2:1	
i32466_361	1	123.9	29	45	16	1:2:1	
i32287_1090	1	126.2	29	43	18	1:2:1	
i32558_1386	1	126.2	29	43	18	1:2:1	
i38094_355	1	140.6	23	52	15	1:2:1	
i39789_529	1	144.1	24	52	14	1:2:1	
i37419_329	1	144.7	24	51	15	1:2:1	
i37443_625	1	149.5	21	53	15	1:2:1	
i38484_281	2	0.0	24	43	23	1:2:1	
i30461_1472	2	4.8	21	45	24	1:2:1	
i32787_1241	2	17.8	20	45	24	1:2:1	
i34894_810	2	31.0	16	52	22	1:2:1	
i28947_218	2	33.3	15	52	23	1:2:1	
i33051_1252	2	33.3	15	52	23	1:2:1	
i34497_853	2	46.9	17	50	23	1:2:1	
i36775_363	2	50.5	15	54	21	1:2:1	
i15060_964	2	53.4	14	55	21	1:2:1	
i31233_1053	2	53.4	14	55	21	1:2:1	
i36511_657	2	63.0	15	49	25	1:2:1	
i19682_515	2	64.2	15	50	25	1:2:1	
i28284_1005	2	65.9	13	53	24	1:2:1	
i17237_4883	2	67.6	12	56	22	1:2:1	*
i12617_836	2	70.5	12	55	23	1:2:1	*
i30715_1591	2	70.5	12	55	23	1:2:1	*
i32987_979	2	70.5	12	55	23	1:2:1	*
i33705_468	2	70.5	12	55	23	1:2:1	*
i34671_610	2	70.5	12	54	23	1:2:1	*
i26438_1359	2	89.1	11	58	21	1:2:1	**
i33282_718	3	0.0	22	42	25	1:2:1	
i31068_1490	3	2.9	22	44	24	1:2:1	
i29611_2140	3	4.1	22	44	24	1:2:1	
i33343_325	3	14.7	22	41	25	1:2:1	
i42601_220	3	26.1	24	40	26	1:2:1	
i32139_534	3	26.7	23	41	26	1:2:1	
i34107_268	3	33.4	25	40	25	1:2:1	
i30817_727	3	55.9	25	44	21	1:2:1	
i33909_1031	3	58.2	22	46	21	1:2:1	
i35352_760	3	58.8	24	44	21	1:2:1	
i39258_399	3	64.2	23	44	23	1:2:1	
i21577_861	3	67.1	24	43	23	1:2:1	

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Supplemental Table 1. Continued.

Locus ^z	Chrom no. ^y	Pos (cM)	Obs (no.) ^x			Exp	P ^w
			a	h	b		
i28422_1226	3	70.6	23	43	24	1:2:1	
i29185_1241	3	74.7	23	42	25	1:2:1	
i33232_1046	3	89.0	22	45	22	1:2:1	
i26005_1583	3	96.0	30	36	23	1:2:1	
i29415_721	3	107.4	32	36	22	1:2:1	
i22876_134	3	107.9	31	37	22	1:2:1	
i28607_1011	3	107.9	31	37	22	1:2:1	
i18967_973	3	109.7	32	38	20	1:2:1	
i33810_581	3	113.2	32	39	17	1:2:1	*
i20406_751	3	120.0	29	45	15	1:2:1	
i35022_816	4	0.0	21	67	0	1:2:1	
i40592_178	4	24.7	18	47	23	1:2:1	
i19902_922	4	36.1	24	44	22	1:2:1	
i26526_748	4	63.1	23	37	29	1:2:1	
i35345_1067	4	69.9	24	41	25	1:2:1	
i32123_1465	4	74.7	22	43	25	1:2:1	
i37023_265	4	80.1	23	39	27	1:2:1	
i33399_1211	4	81.2	22	40	28	1:2:1	
i19574_601	4	82.3	24	38	28	1:2:1	
i26045_1046	4	89.0	22	41	27	1:2:1	
i31392_1426	4	91.9	23	36	31	1:2:1	
i35268_1082	4	91.9	23	36	31	1:2:1	
i38440_478	4	98.0	24	40	26	1:2:1	
i36493_410	4	102.1	22	43	25	1:2:1	
i37821_780	4	118.0	26	36	27	1:2:1	
i27765_337	4	131.2	28	36	26	1:2:1	
i32307_1162	5	0.0	23	35	31	1:2:1	
i35248_671	5	25.9	21	44	25	1:2:1	
c00142_122	5	34.8	23	44	23	1:2:1	
i29167_1843	5	45.1	23	43	23	1:2:1	
i33680_1027	5	45.6	22	45	23	1:2:1	
i37670_180	5	52.4	22	45	22	1:2:1	
i37258_745	5	57.2	23	46	21	1:2:1	
i28803_1689	5	66.8	21	51	18	1:2:1	
i30732_1102	5	84.2	22	45	20	1:2:1	
i34184_1205	5	96.0	23	45	22	1:2:1	
i28460_1862	5	107.0	23	42	25	1:2:1	
i32285_805	5	109.9	24	41	25	1:2:1	
i21529_706	5	130.4	22	43	25	1:2:1	
i37209_549	5	133.3	21	44	25	1:2:1	
i33663_1163	6	0.0	18	47	25	1:2:1	
i31425_789	6	2.3	17	47	26	1:2:1	
i33111_603	6	2.9	17	48	25	1:2:1	
i45729_183	6	5.2	15	50	25	1:2:1	
i28812_2285	6	9.4	13	49	28	1:2:1	
i27132_551	6	37.0	12	43	35	1:2:1	***
i30880_1388	6	64.3	19	43	27	1:2:1	
i28276_1535	6	65.7	21	40	28	1:2:1	
i23831_442	6	67.1	22	42	26	1:2:1	
i40098_630	6	80.5	22	46	22	1:2:1	
i32152_614	6	110.9	23	46	21	1:2:1	
Visual phenotype	6	113.1	23	—76—		1:3	
i32361_962	6	132.4	27	41	22	1:2:1	
i32739_152	6	132.4	27	41	22	1:2:1	
i29293_1435	6	135.9	27	39	24	1:2:1	
i25801_1760	7	0.0	25	47	18	1:2:1	

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Supplemental Table 1. Continued.

Locus ^z	Chrom no. ^y	Pos (cM)	Obs (no.) ^x			Exp	P ^w
			a	h	b		
i22582_277	7	4.1	24	46	20	1:2:1	
i34313_545	7	7.0	24	47	19	1:2:1	
i28716_113	7	7.6	23	46	20	1:2:1	
i34753_1109	7	8.1	23	47	20	1:2:1	
i35897_949	7	8.1	23	47	20	1:2:1	
i32140_1181	7	23.4	19	45	26	1:2:1	
i41653_558	8	0.0	27	57	6	1:2:1	**
i30323_747	8	2.9	27	57	4	1:2:1	***
i43294_202	8	8.3	26	59	5	1:2:1	***
i33645_542	8	23.1	24	54	11	1:2:1	*
i28367_2185	8	24.8	25	55	10	1:2:1	**
i35099_237	UN		19	45	25	1:2:1	
i22339_503	UN		21	48	21	1:2:1	
i25497_1235	UN		24	47	19	1:2:1	
i30821_1151	UN		17	55	18	1:2:1	
i30863_958	UN		27	44	19	1:2:1	
i31015_812	UN		25	41	22	1:2:1	
i31315_598	UN		20	48	22	1:2:1	
i34783_896	UN		24	42	24	1:2:1	
i36002_905	UN		17	55	18	1:2:1	

^zi = isotig, c = contig as described by Duangjit et al. (2013).

^yUN = unlinked.

^xHomozygotes for the maternal (a) or paternal (b) allele or heterozygous (h).

^wAsterisks indicate significant deviation from expected ratio at * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$.

Supplemental Table 2. Loci, chromosome (Chrom), position (Pos) in centiMorgans, observed (Obs) segregations, and probabilities of goodness-of-fit to the expected 1:2:1 segregation ratios for the F₂ family from the cross of glossy B9885 with glossy 'Odourless Greenleaf' onion.

Locus ^z	Chrom (no.) ^y	Pos (cM)	Obs (no.) ^x			P ^w
			a	h	b	
i25497_1235	1	0.0	30	39	23	
i30863_958	1	8.6	27	43	22	
i21293_730	1	23.9	25	43	25	
i33340_1003	1	39.2	26	43	23	
c13740_1338	1	40.3	26	41	24	
i27537_659	1	59.1	20	45	28	
i35370_364	1	59.7	21	44	28	
i29498_587	1	66.2	21	45	27	
i32512_171	1	67.8	21	44	28	
i36938_811	1	82.2	26	40	27	
i26470_847	1	84.4	26	42	25	
i32772_993	1	85.5	25	44	24	
i34779_398	1	86.6	25	46	22	
c00172_269	1	88.3	24	49	19	
i30185_1091	1	90.6	24	53	15	
i36337_305	1	102.1	26	67	0	
i17703_462	1	131.8	22	52	19	
i33099_885	1	131.8	22	52	18	
i44346_390	1	141.4	26	49	16	
i32466_361	1	153.3	25	50	18	
i37348_328	1	153.3	25	50	18	
i32558_1386	1	158.6	27	49	17	
i38094_355	1	170.0	28	50	14	
i37419_329	1	175.9	28	51	14	
i34723_664	1	177.6	28	50	15	
i37443_625	1	180.4	26	51	16	
i40509_271	2A	0.0	26	44	23	
i27567_733	2A	2.8	27	45	21	
i34018_317	2A	8.7	28	43	22	
i30687_1336	2A	12.1	26	44	22	
i35692_495	2A	12.1	26	44	22	
i41662_224	2A	20.6	20	49	24	
i36256_344	2A	33.9	20	40	32	
i28947_218	2A	45.6	22	44	27	
i34497_853	2A	66.4	29	42	21	
i31233_1053	2A	73.6	25	46	21	
i15060_964	2A	74.2	25	48	20	
i36511_657	2A	90.5	21	48	23	
i17237_4883	2A	97.0	16	55	22	
i12617_836	2A	98.7	17	52	24	
i32987_979	2A	98.7	17	51	24	
i34671_610	2A	98.7	17	51	24	
i26438_1359	2A	115.2	15	54	24	
i36002_905	2B	0.0	21	44	28	
i30821_1151	2B	4.6	23	42	27	
i33538_1298	2B	12.0	26	38	26	
i35099_237	3	0.0	28	45	19	
i29139_1024	3	7.9	27	43	23	
i33783_1096	3	24.8	22	40	31	
i30724_1666	3	25.4	22	39	32	
i33282_718	3	30.0	21	42	29	
i31068_1490	3	32.2	20	45	28	
i29611_2140	3	33.3	20	45	28	
i31015_812	3	45.4	22	41	29	
i33343_325	3	47.0	23	41	29	
i32139_534	3	56.2	22	44	27	

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Supplemental Table 2. Continued.

Locus ^z	Chrom (no.) ^y	Pos (cM)	Obs (no.) ^x			P ^w
			a	h	b	
i42601_220	3	56.2	22	44	27	
i34107_268	3	60.2	25	39	29	
i33909_1031	3	84.5	19	43	30	
i39258_399	3	92.4	18	45	30	
i21577_861	3	92.9	18	46	29	
i29460_868	3	94.0	18	48	27	
i28422_1226	3	99.9	18	48	27	
i27896_600	3	106.4	14	57	22	*
i22876_134	3	125.8	16	50	27	
i28607_1011	3	125.8	16	50	27	
i32926_115	3	131.0	17	51	25	
i20406_751	3	132.1	17	51	25	
i40592_178	4	0.0	26	48	18	
i19902_922	4	17.1	24	53	15	
i37158_542	4	35.0	22	51	20	
i35345_1067	4	48.0	24	43	26	
i27809_396	4	49.1	22	43	26	
i34783_896	4	49.6	22	46	25	
i33399_1211	4	53.6	23	42	27	
i37023_265	4	53.6	23	43	27	
i26045_1046	4	60.7	20	47	26	
i31392_1426	4	62.4	22	46	25	
i36493_410	4	75.7	22	47	23	
i36793_769	4	95.5	18	48	27	
i16136_1083	4	106.9	25	42	26	
i27765_337	4	118.2	27	44	22	
i31628_1166	5	0.0	31	46	16	
i35819_544	5	3.4	32	48	13	*
i37258_745	5	5.0	34	44	14	*
i14741_247	5	8.4	36	42	14	**
i34069_110	5	10.7	35	43	15	*
i28803_1689	5	11.2	34	44	15	*
i30732_1102	5	19.7	39	36	18	***
i28536_1567	5	20.2	37	37	17	**
i34184_1205	5	38.6	33	40	20	*
i28460_1862	5	49.2	28	45	17	
i32285_805	5	50.3	28	45	20	
i31477_611	5	58.8	28	43	22	
i21529_706	5	63.4	26	45	22	
i40014_661	5	63.4	26	45	22	
i31278_1407	5	63.9	26	44	23	
i23831_442	6A	0.0	16	61	15	**
i28276_1535	6A	0.6	16	59	16	*
i27132_551	6A	27.1	21	49	23	
i28812_2285	6A	47.9	25	42	26	
i33111_603	6A	52.5	26	43	23	
i29293_1435	6B	0.0	24	36	33	*
i32361_962	6B	7.8	27	35	31	*
i32739_152	6B	8.9	25	37	31	
c00298_108	6B	14.1	22	40	31	
i28330_2105	6B	21.2	19	48	26	
i42645_520	7	0.0	23	51	19	
i41937_218	7	13.8	23	48	22	
i39918_357	7	18.4	22	50	21	
i22582_277	7	20.1	22	50	20	
i25801_1760	7	20.1	22	51	20	

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Supplemental Table 2. Continued.

Locus ^z	Chrom (no.) ^y	Pos (cM)	Obs (no.) ^x			<i>P</i> ^w
			a	h	b	
i28716_113	7	20.1	22	51	20	
i29209_2397	7	20.1	22	51	20	
i34753_1109	7	20.1	22	51	20	
i35897_949	7	20.1	22	51	20	
i40501_344	7	30.1	24	53	16	
i32140_1181	7	38.0	25	56	12	*
i31315_598	8	0.0	26	43	24	
i22339_503	8	7.2	28	40	24	
i30020_476	8	11.8	27	41	25	
i30907_420	8	16.4	25	45	23	
i20235_630	8	26.2	28	41	24	
i30323_747	8	36.1	30	35	27	
i33589_1176	8	36.1	29	37	27	
i37010_277	8	38.9	30	35	27	
i32346_843	8	39.4	29	37	27	
i35248_671	UN		27	44	22	
i38248_370	UN		22	47	24	

^zi = isotig, c = contig as described by Duangjit et al. (2013).

^yLetters indicate linkage groups for the same chromosome at LOD = 6.0. UN = unlinked.

^xHomozygotes for the maternal (a) or paternal (b) allele or heterozygous (h).

^wAsterisks indicate significant deviation from expected ratio at **P* < 0.05, ***P* < 0.01, or ****P* < 0.001.