

Development of SSR markers linked to QTL reducing leaf hair density and grapevine downy mildew resistance in Vitis vinifera

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- 2 resistance in *Vitis vinifera*
- 3
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## 30 Abstract

31	Dense leaf hairs of grapevines have been known to act as a preexisting defense structure for preventing the
32	incidence of grapevine downy mildew, because the pathogen, <i>Plasmopara viticola</i> , needs water to invade
33	grapevines, and water is repelled by a hydrophobic surface due to dense leaf hairs. In the present study, we
34	performed regression analyses of downy mildew resistance with leaf hair density using hybrids of Vitis
35	labrusca origin and confirmed the effect of leaf hairs. Reducing the repelling effect of leaf hairs by detergent
36	application cancelled the effect of leaf hair, which confirmed the hypothesis. Thereafter, based on QTL
37	analyses in the population of <i>V. vinifera</i> 'Muscat of Alexandria' × the interspecific hybrid 'Campbell Early',
38	we identified a major locus in linkage group (LG) 5 of 'Muscat of Alexandria' controlling leaf hair density.
39	This locus was previously reported as a small effect QTL for downy mildew resistance, however we found
40	that the locus had high LOD scores explaining 71.9%-78.5% of the phenotypic variance of leaf hairs.
41	Moreover, this locus was detected as a QTL for downy mildew resistance. The effect of this locus was
42	confirmed in two other hybrid populations. Finally, we could successfully identify three traditional V.
43	vinifera table grapes 'Muscat of Alexandria,' 'Katta Kurgan,' and 'Parkent' as the origin of the allele linked
44	to hairlessness by defining the SSR haplotypes. The use of this locus for marker-assisted selections would

- 45 be a promising strategy for producing grapevines with resistance by preexisting defense structure.
- 46 Keywords DNA marker ·Grapevine downy mildew · Leaf hairs ·preexisting defense structure · Vitis

47 vinifera · Vitis labrusca

## 49 Introducion

51	Grapevine is a major crop cultivated for wine, fresh fruit, juice, and raisins. Vitis vinifera is the most
52	commercially important species, but it is susceptible to many diseases, such as grapevine downy mildew
53	and powdery mildew (Cahoon 1998; Di Gaspero et al. 2012; Reisch et al. 2012; Eibach and Töpfer 2014).
54	Modern grape breeding was prompted by the arrival of North American diseases and insects in Europe
55	(Reisch et al. 2012). Hedrick (1908) stated 'the comparative resistance of the American species to the
56	phylloxera, the mildews, and black-rot has been due to natural selection in the contest that has been waged
57	for untold ages between host and parasite.' Many hybrids between V. vinifera and American wild species
58	have been produced to develop disease-resistant grapevines (Reisch and Pratt 1996; Eibach and Töpfer
59	2014). So-called French hybrids have been developed in France by hybridizing V. vinifera and American
60	wild species to establish grapevines resistant to phylloxera (Daktulosphaira vitifoliae), but resistance to
61	grapevine powdery mildew (Erysiphe necator) and downy mildew (DM; Plasmopara viticola) are also
62	important reasons for producing hybrids (Cahoon 1998; Di Gaspero et al. 2012; Eibach and Töpfer 2014).
63	Interspecific hybrids with V. labrusca parentage are frequently used in the USA, Japan, and some other

- 64 countries for making juice and as table grapes (Yamada and Sato 2016), and are sometimes collectively
- 65 referred to as *V. labruscana* Bailey (Bailey and Bailey 1930).
- 66 Even today, DM is a devastating grapevine disease. Twenty-four resistance loci have been identified
- 67 (Julius Kühn-Institut 2018). The resistance genes are named Rpv (Resistance to Plasmopara viticola), and
- 68 include *Rpv1* (Merdinoglu et al. 2003; Feechan et al. 2013) and *Rpv3* (Fischer et al. 2004; Welter et al.
- 69 2007; Bellin et al. 2009). Rpv1 has been introgressed from Muscadinia (Vitis) rotundifolia after several
- 70 pseudo-backcrosses with different *V. vinifera* cultivars (Pauquet et al. 2001); this gene encodes a TIR-NB-
- 71 LRR protein (Feechan et al. 2013). *Rpv3* is frequently found in disease-resistant hybrids (Di Gaspero et al.
- 72 2012); unfortunately, the breakdown of resistance conferred by *Rpv3* has already been reported (Peressotti
- et al. 2010; Delmotte et al. 2014). Pyramiding of these resistance genes is the main strategy for producing
- new resistant cultivars (Eibach et al. 2007).
- 75 In contrast to the above genes, which provide induced resistance, leaf hairs have long been known to
- act as a physical barrier against DM because P. viticola needs water to invade grapevines (Lafon and Bulit
- 1981) and leaf hairs repel water. (Staudt and Kassemeyer 1995; Kortekamp et al. 1999). Leaf hairs are also
- called trichomes, and Ma et al. (2016) categorized non-glandular trichomes in grapevines into ribbon and

79	simple forms. Ribbon trichomes are the main structures that repel DM infection; hereafter, we use the term
80	'leaf hairs' to refer to ribbon trichomes. Leaf hairs are usually denser on shoot tips and expanding leaves
81	than on fully expanded leaves. In a broader perspective, Agrios (2005) stated that 'a thick mat of hairs on a
82	plant surface may also exert a similar water-repelling effect and may reduce infection.' Such preexisting
83	defense structure would be race non-specific and durable. Staudt and Kassemeyer (1995) mentioned that
84	resistant wild Vitis accessions have dense prostrate hairs on the lower leaf surface and hair removal renders
85	several accessions susceptible, although they did not show detailed data. Thick hydrophobic leaf hairs repel
86	water effectively, although not completely. Kortekamp et al. (1999) showed that dense pubescence on the
87	abaxial side of the leaves enhances resistance to DM. By observing V. vinifera, hybrids, and wild species,
88	including V. cinerea, V. davidii, V. girdiana, V. doaniana, V. labrusca, and M. rotundifolia, they showed that
89	less force was needed to remove water droplets from the leaves of hairy species than from those of non-
90	hairy species.
91	In this study, regression analyses of downy mildew resistance with leaf hair density using a hybrid
92	population and grapevine germplasms revealed an association between them. Detergent application to the
93	underside leaf hairs of progenies of a population and grapevine germplasms, including 'Pinot Noir' and its

94	bud sport 'Pinot Meunier,' confirmed the previously reported hypothesis that leaf hairs reduce DM infection
95	because of their repelling effect. By studying bi-parental populations and grapevine germplasms, we found
96	that downy mildew resistance and underside leaf hair density were co-localized in a QTL on LG5 in hybrid
97	populations of Vitis labrusca origin. We also found that V. vinifera table grapes harbor a major QTL whose
98	hairless allele drastically reduces the density of leaf hairs.
99	
100	Materials and methods
101	
102	Plant materials
103	
104	Grapevines (Vitis spp.) were grown in vineyards at the Grape and Persimmon Research Station, NARO,
105	Japan. The mapping population Pop AC (95 individuals) was obtained by crossing V. vinifera 'Muscat of
106	Alexandria' ('Heptalilo (synonym: Axina de tres bias)' × 'Muscat à Petits Grains Blancs (synonym:

107 'Muscat blanc à petits grains', 'Moscato Bianco'; Fig 1a)'; Cipriani et al. 2010; Vitis International Variety

108 Catalogue <http://www.vivc.de/>) and the interspecific hybrid 'Campbell Early' ('Moore Early'  $\times$ 

109	('Belvidere' × 'Muscat Hamburg'); Vitis International Variety Catalogue <http: www.vivc.de=""></http:> ; Fig 1a)
110	from 2007 to 2010. The mapping population Pop 693 (94 individuals) was obtained by crossing the
111	interspecific hybrids 626-84 and Iku 82 in 2002, and was maintained as previously described (Ban et al.
112	2014). The population Pop 777 (89 individuals) was obtained by crossing the interspecific hybrids 700-39
113	and 'Shine Muscat' (Akitsu 21 (an interspecific hybrid) × 'Hakunan') in 2012. Individuals of all three
114	populations were grafted onto Kober 5BB rootstocks before planting. Among vines listed in Supplementary
115	Table 1, the hybrids were V. labruscana (originated from V. labrusca) or its descendants, except for 'Koshu'
116	and 'Chancellor.' 'Koshu' was previously regarded as V. vinifera; however, it was recently shown to be a
117	hybrid between V. vinifera and a Chinese wild species (Goto-Yamamoto et al. 2015). 'Chancellor' is a DM-
118	resistant French hybrid and was included because it has the DM resistance gene Rpv3 (Di Gaspero et al.
119	2012).
120	
121	Isolation and maintenance of <i>P. viticola</i>
122	

123P. viticola sporangia were isolated from naturally infected 'Rizamat' ('Katta Kurgan' × 'Parkent'; Vitis

124	International Variety Catalogue <a href="http://www.vivc.de/">http://www.vivc.de/</a> ) leaves in our vineyard on 5 August 2009. 'Rizamat'
125	is a <i>V. vinifera</i> cultivar and was shown to be highly susceptible to DM (Kono et al. 2015a). Young but not
126	shiny 'Rizamat' leaves were surface-sterilized with 1/20 diluted (v/v; available chlorine final 0.25%)
127	sodium hypochlorite solution (Wako, Osaka, Japan) for 2 min, and washed with tap water three times and
128	deionized water three times. Washed leaves were kept in a plastic container with wet paper towel (Kim
129	Towel; Nippon Paper Crecia, Tokyo, Japan) with the abaxial surface upwards. Sporangia on 'Rizamat'

- 130 leaves or stored sporangia at -80 °C were suspended in sterile water, and the suspension (1 × 10<sup>4</sup>/ml) was
- 131 inoculated on the abaxial side of the washed leaves. Inoculated leaves were maintained under a 12 h light :
- 132 12 h dark photoperiod at 20 °C, and the suspension on the leaves was wiped at one day after inoculation.
- 133 We inoculated sporangia weekly to obtain sporangia in good condition. Sporangia were collected in a 50ml
- 134 plastic tubes and stored at -80 °C when 'Rizamat' leaves were not available.
- 135
- 136 Evaluation of downy mildew resistance of Pop AC in fungicide-free vineyards
- 137
- 138 Pop AC was grown in a vineyard. Grafted vines (one vine per genotype) were planted on 6 March 2014 at

139	50 cm between vines with four rows under plastic (6 m $\times$ 16 m) to prevent diseases spread by rain in
140	fungicide-free conditions. Three vines of each parent ('Muscat of Alexandria' and 'Campbell Early') were
141	planted randomly within Pop AC. Two or three short canes with two to three buds were left when the plants
142	were pruned, and ~10 g of fertilizer (N, 8%; P, 9%; K, 8%) and 100 g of cow manure were applied per vine
143	in winter. Emerging shoots were tied upward to the plastic-coated steel pipes (16 mm in diameter $\times$ 2.4 m
144	in height) during growth. A suspension of $5 \times 10^4$ sporangia/ml was prepared from the maintained <i>P. viticola</i> ,
145	and was inoculated mainly on the abaxial side of expanding leaves on 4 June 2014, 12 May 2015, and 10
146	May 2016 with a hand sprayer (DIA Spray; Furupura, Tokyo Japan). In total, 2L of the suspension was
147	inoculated to all the vines in the vineyard (~17.2 ml of inoculum per vine). When inoculated, shoots were
148	growing vigorously, but did not reach to the top of the steel pipes. Water was sprayed daily above the vines
149	from sprinklers installed under the plastic cover to ensure uniform infection. Symptoms on fully expanded
150	leaves were evaluated on 17 July 2014, 18 June 2015, and 9 June 2016 when susceptible genotypes were
151	severely infected. Almost all fully expanded leaves on each vine were evaluated and scored as shown in
152	Supplementary Table 2, and DM resistance was evaluated using the (1) incidence, defined as the ratio of
153	leaves with sporangia (the number of leaves with score 3 or more / total number of leaves examined); and

154	(2) severity, defined as the overall degree of symptoms calculated as $100 \times \Sigma$ (score × number of leaves of
155	that score) / (6 (the highest score) $\times$ total number of leaves). Fungicides were not applied until DM
156	symptoms were evaluated. After the evaluation, fungicides and pesticides were applied to control DM and
157	powdery mildew. However, a few individuals were lost owing to severe DM infection annually.
158	
159	Evaluation of downy mildew resistance of 31 grapevine germplasms in fungicide-free vineyards
160	
161	Vines listed in Supplementary Table 1 were grafted onto Kober 5BB rootstocks and were planted under
162	plastic on 8 March 2013 at 50 cm between vines with two or four vine replicates as shown in Supplementary
163	Table 1 in another plastic-covered vineyard (6 m $\times$ 12 m). Three Japanese wild grapevines were included,
164	which were possibly resistant. A suspension of DM sporangia (5 $\times$ 10 <sup>4</sup> /ml) was inoculated mainly on the
165	abaxial side of expanding leaves on 8 June 2013 and 20 May 2014 (15–20 ml per vine). Water was sprayed
166	daily above the vines from sprinklers installed under the plastic cover to ensure uniform infection.
167	Symptoms on expanded leaves were evaluated as described above on 11 July 2013 and 25 June 2014.

168 Resistance was evaluated as described for Pop AC.

169

170 Evaluation of downy mildew resistance by leaf disc assay

171

172 DM resistance of Pop AC, Pop 693, and Pop 777 were evaluated by a leaf disc assay. A leaf disc assay,

173 which provides a good estimate of the number of sporangia, was performed as previously described with a

174 slight modification (Kono et al. 2015b). Four expanding leaves (no longer translucent and shiny) were

sampled; two discs (1.5 cm diameter) were punched from each leaf with a cork borer and were placed on

176 1% agar. A sporangial suspension in water (50  $\mu$ l of 5 × 10<sup>4</sup>/ml) was inoculated on each disc. At 6 days

177 post-inoculation (dpi), symptoms on each disc were visually rated on the scale depicted in Supplementary

178 Figure 1, and the mean score was calculated.

179

180 Evaluation of leaf hair density

- 183 Supplementary Table 1 and 3 were randomly sampled from the first shoots to elongate from dormant canes;
- 184 leaves on secondary shoots were not sampled. Images of the abaxial side of leaves were taken using a digital
- 185 microscope (VH-Z20, Keyence, Osaka, Japan) at 100× magnification. The hair density of each leaf was
- 186 evaluated on a visual scale (Supplementary Fig. 2), and the mean score was calculated. The scale was
- 187 modified from Descriptor 6.1.35 (Descriptors for Grapevine; IPGRI 1997). The images in the descriptor of
- 188 the V. vinifera cultivars 'Grenache,' 'Müller-Thurgau,' and 'Clairette' and V. labrusca were used to set the
- 189 scales.
- 190 Leaf hair density was also estimated by imaging analysis in ImageJ v. 1.50b (Schneider et al. 2012)
- 191 with the commercially available 'LP\_Mouzi' plugin (LPixel Inc. Tokyo, Japan). For each image, the largest
- 192 possible area in focus was selected as the region of interest (ROI) using the Rectangular Selection tool.
- 193 Then, the area of leaf hairs within the ROI was determined manually by setting a selection threshold, and
- 194 the area was measured in pixels with the 'LP\_Mouzi' plugin. Hair density was calculated as the ratio of
- 195 'leaf hair area in pixels' / 'total ROI area in pixels.' The hair density of Pop AC was evaluated with three

196 leaf replicates in 2015 and six in 2016. Hair density of other populations and germplasms was evaluated in

197 one year with three- or four-leaf replicates per genotype.

198

199 Application of detergent to leaves

201	Two developing leaves were sampled from each of the V. vinifera 'Muscat of Alexandria,' hybrids
202	('Campbell Early,' 'Kyoho'), wild Vitis species (V. coignetiae Pulliat ex Planch. 'Y0', V. thunbergii Siebold
203	et Zucc.), and eight Pop AC individuals (17, 21–24, 26, 44, and 55) on 2 July 2015. Leaves were surface-
204	sterilized with 1/20 diluted (v/v; available chlorine final 0.25%) sodium hypochlorite solution (Wako,
205	Osaka, Japan) for 2 min, and washed with tap water three times and deionized water three times. Eight discs
206	(1.5 cm diameter) were punched from each leaf with a cork borer, and four discs were placed on 1% agar.
207	The other four discs were collected in a 15 ml tube, treated with 3 ml of 0.01% (w/v) Nonidet P-40 for 2
208	min, and washed with tap water three times and with deionized water three times. These discs were placed
209	on 1% agar. A sporangial suspension in water (50 $\mu l$ of 5 $\times$ 10 <sup>4</sup> /ml) was inoculated on each leaf disc as
210	described in Kono et al. (2015b), and the discs were maintained under a 12 h light: 12 h dark photoperiod

- above, and sporangia were counted with a disposable hemocytometer. The same experiment was repeated
- on 9 July 2015. Because water remained within the layer of leaf hairs at 6 dpi, leaf hair density of some
- 214 discs was not estimated by imaging analysis.
- 215 Ten developing leaves of *V. vinifera* 'Pinot Noir' (less hairy) and 'Pinot Meunier' (more hairy) were
- sampled on 2 May 2016. These two cultivars were shown to be bud-sports of each other (Hocquigny et al.
- 217 2004). Each leaf was halved longitudinally. One half (mock) was surface-sterilized with 1/20 diluted (v/v;
- available chlorine final 0.25%) sodium hypochlorite solution (Wako, Osaka, Japan) for 2 min and washed
- 219 with tap water three times and deionized water three times. The other half was surface-sterilized with 1/20
- diluted (v/v) sodium hypochlorite solution for 2 min, washed with tap water three times, treated with 0.01%
- 221 (w/v) Nonidet P-40 for 2 min, and washed with tap water three times and with deionized water three times.
- Example 222 Four discs (1.5 cm diameter) were punched from each leaf half with a cork borer and were placed on 1%
- agar. A sporangial suspension in water (50  $\mu$ l of 5  $\times$  10<sup>4</sup>/ml) was inoculated on each leaf disc, and the discs
- were maintained under a 12 h light: 12 h dark photoperiod at 20 °C. At 6 dpi, sporangia were counted with

225	a disposable hemocytometer.
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227 Statistical analyses

228

229	R v. 3.3.3 software (R Core Team, 2017) was used for all statistical analyses and graphical presentation of
230	results except for mapping and QTL analysis. For the variance components analysis (Snedecor and Cochran
231	1989; Crawley 2015) of leaf hair density, the proportion of leaf hair area determined by imaging analysis
232	was analyzed by one-way ANOVA with genotype as a factor. Data on the hair density estimated by imaging
233	analysis were transformed using the arcsine transformation $(\arcsin(\operatorname{sqrt}(x)))$ before ANOVA to improve
234	homogeneity of variances.
235	To determine the relationship between leaf hair density as an explanatory variable and DM resistance
236	as a response variable, we used linear models. Before linear regression, DM incidence data were arcsine-
237	transformed and sporangial counts were log10-transformed. To avoid zero counts for the log transformation,
238	we added half of the detection limit of the disposable hemocytometer to all data before transformation as

239 proposed by Yamamura (1999).

We used the non-parametric Wilcoxon rank-sum test for comparing two medians using the 'wilcox.test'

- 241 function of R. We used the Wilcoxon rank-sum test for all multiple comparisons using the
- <sup>242</sup> 'pairwise.wilcox.test' function of R with 'p.adj = 'holm' to control the family-wise error rate by the Holm
- 243 method. The Shapiro-Wilk test was performed to test the normality of the distributions of DM resistance
- and leaf hair density.
- 245

- 246 Genotyping with simple sequence repeat (SSR) markers
- 247
- 248 Genotyping with SSR markers was performed as described in Ban et al. (2014) with some modifications.
- 249 GoTaq (Promega, Madison, USA) was used for PCR amplification. Genomic DNA was extracted from
- 250 leaves with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) using a protocol modified by Yang et al.
- 251 (2016), in which polyvinylpyrrolidone (PVP)-40 (2% w/v) was added to the AP1 buffer. To reduce the
- 252 genotyping cost, we designed sequence-specific forward primers with the M13 (-21) universal sequence
- 253 (TGTAAAACGACGGCCAGT) at the 5' end, and added a FAM-labeled M13 (-21) primer to label PCR
- 254 products as described in Schuelke (2000). The reverse tail sequence (GTTTCTT) was appended to the 5'

255	end of the reverse primers for easier allele scoring. Maps were constructed with published SSR markers,
256	and SSR markers developed by Fechter et al. (2014) were used for the terminal regions of linkage groups
257	(LGs). To obtain a dense genetic map in the regions of interest and to fill gaps in genetic maps constructed
258	by using published SSR markers, we used the Tandem Repeat Finder program (v. 4.04) (Benson 1999) to
259	identify SSRs in the genome sequences of V. vinifera downloaded from the Grape Genome Browser (12X)
260	(http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/; Jaillon et al. 2007), and developed new SSR
261	markers as summarized in Supplementary Table 4.
262	
263	Map construction and QTL analysis
264	
265	The parental linkage maps and the consensus linkage map were constructed using the double pseudo-
266	testcross strategy (Grattapaglia and Sederoff 1994) with JoinMap v. 4.1 software (van Ooijen 2006). SSR
267	marker genotyping results were sorted into LGs using reference genome information, and each LG map
268	was constructed separately. Map distances were calculated by the 'Regression mapping' algorithm (Stam
269	1993) using the Kosambi mapping function (Kosambi 1944) with 95 individuals. Marker numbers used for

270	mapping are summarized in Supplementary Table 5. We found a large area of marker distortions in this
271	population, which were probably caused by lethal genes; to maximize genome coverage, we included
272	distorted markers when they were integrated with other markers at LOD (logarithm of the odds) 2.0 or more
273	by JoinMap 4.1. However, markers in LG18 of 'Campbell Early' were separated into two groups at LOD
274	2.0; hence, two separate maps, LG18a and LG18b, were constructed. Parental and consensus linkage maps
275	of Pop 693 were reconstructed in JoinMap v. 4.1 after marker additions in LG5.
276	QTL analysis was carried out using both parental and consensus maps in MapQTL v. 6 software (van
277	Ooijen 2009). QTLs were identified by interval mapping and the non-parametric Kruskal–Wallis rank-sum
278	test. The LOD threshold corresponding to the genome-wide significance level of 0.05 was determined using
279	1,000 permutations. The number of individuals used for QTL detection for each trait is shown in Table 1.
280	MapChart v. 2.3 software (Voorrips 2002) was used for the graphical presentation of genetic maps and QTL
281	positions.
282	
283	Results
284	

285 Stability of evaluation of hair density on the abaxial side of leaves

287	First, we determined the stability of the evaluation of underside leaf hair density using a hybrid population,
288	Pop AC. The parent 'Muscat of Alexandria' had a few procumbent hairs on the abaxial side, and the
289	epidermis was almost fully exposed, whereas the other parent, 'Campbell Early,' had very dense hairs (Fig.
290	1a). The density of underside leaf hairs of the hybrid population Pop AC was highly dependent on genotype;
291	hence, we analyzed the effect of genotype by ANOVA (Supplementary Table 6). The variance component
292	of genotype was estimated to high, contributing 91% of total variance. The correlation between the mean
293	hair density in 83 individuals of Pop AC in 2015 with that in 2016 was high at $r = 0.91$ .
294	
295	Regression analysis of underside leaf hair density and DM resistance in Pop AC
296	
297	To test the relationship between underside leaf hair density and DM resistance, we evaluated the
298	relationship for Pop AC (Fig. 1b; Supplementary Fig. 3a). Leaf hair density was evaluated by imaging
299	analysis (Fig. 1b) and a visual rating (Supplementary Fig. 3a) for two years. DM resistance was evaluated

300	by incidence and severity (see Materials and Methods) for three years. Linear regression analysis between
301	the 2-year mean of leaf hair density and 3-year mean of DM resistance showed that all the slopes were
302	significant ( $P < 0.001$ ) and leaf hair density had an effect on DM resistance as evaluated by incidence ( $r^2 =$
303	0.64, imaging analysis; $r^2 = 0.67$ , visual rating) and severity ( $r^2 = 0.53$ , imaging analysis; $r^2 = 0.56$ , visual
304	rating), suggesting that leaf hairs have a significan and substantial effect on DM resistance, although they
305	did not completely prevent infection.
306	
307	Regression analysis of underside leaf hairs and DM resistance using 31 grapevine germplasms
308	
309	To confirm this relationship in a wider range of genotypes, we evaluated leaf hair density and DM resistance
310	of 31 grapevine germplasms, which included cultivars, selections, and wild grapevines (Supplementary
311	Table 1). We found high variation in hair density among hybrids (Fig. 1c; Supplementary Fig. 3b). Vitis
312	vinifera 'Rizamat' had no hairs and 'Muscat Hamburg' had a few hairs, whereas 'Campbell Early' and the
313	American hybrid 'Concord' had very dense hairs such that their epidermis was not visible under the
314	microscope (Supplementary Fig. 4). The hairs on these latter two cultivars were very long and heavily

entangled with each other to form a felt-like surface. The relationship between hair density and mean DM

- resistance in this 2-year evaluation (Supplementary Fig. 5) is shown in Figure 1b and Supplementary Fig.
- 317 3b in black. Linear regression analysis between leaf hair density and DM resistance showed that all the
- slopes were significant (P < 0.001) and leaf hair density had an effect on DM resistance as evaluated by
- incidence ( $r^2 = 0.45$ , imaging analysis;  $r^2 = 0.39$ , visual rating) and severity ( $r^2 = 0.53$ , imaging analysis;  $r^2$
- 320 = 0.51, visual rating). 'Chancellor' and *V. flexuosa* had almost no leaf hairs (Supplementary Fig. 4) but were
- 321 highly resistant to DM. Two Japanese wild Vitis species were also resistant and had dense leaf hairs
- 322 (Supplementary Fig. 4). By excluding grapevines that were not used as parents in the table grape breeding
- 323 program in Japan (open circles in Fig. 1c and Supplementary Fig. 3b), we analyzed only table grapes and
- 324 their parents (Fig. 1c and Supplementary Fig. 3b in red). Linear regression analysis between leaf hair density
- and DM resistance for this group showed that all the slopes were significant (P < 0.001) and leaf hair density
- had a greater effect on DM resistance as evaluated by incidence ( $r^2 = 0.75$ , imaging analysis;  $r^2 = 0.74$
- 327 visual rating) and severity ( $r^2 = 0.72$ , imaging analysis;  $r^2 = 0.75$ , visual rating).
- 328

329 Regression analysis of underside leaf hairs and previously reported DM resistance by Terai and Yano (1977)



<sup>344</sup> To test whether the effect of leaf hairs was derived from their water-repelling effect, we applied the non-

345	ionic detergent Nonidet P-40 to leaves to abolish the water-repelling effect and tested the DM resistance.
346	First, we prepared leaf discs from developing leaves of 13 grapevines, including wild Vitis, and then
347	inoculated a DM sporangia suspension onto discs pre-treated with or without Nonidet P-40 (Fig 2a). Linear
348	regression analysis between underside leaf hair density and sporangia number per leaf disc without Nonidet
349	P-40 treatment showed that all the slopes were significant ( $P < 0.001$ ) and underside leaf hair density had
350	an effect on sporangia number on discs ( $r^2 = 0.71$ , visual rating; $r^2 = 0.64$ , imaging analysis), whereas there
351	was no relationship between leaf hairs and infection when Nonidet P-40 was applied since no slopes were
352	significant ( $P > 0.05$ ; Fig. 2b). Sporangia were produced even on hairy genotypes, such as 'Campbell Early',
353	V. thunbergii, and V. coignetiae treated with the detergent.
354	Next, we performed a similar experiment using V. vinifera 'Pinot Noir' and 'Pinot Meunier,' which are
355	traditional cultivars and were shown to be bud-sports of each other (Hocquigny et al. 2004). Even though
356	the two cultivars are genetically almost identical, 'Pinot Meunier' has far denser leaf hairs (Fig 2c). If DM
357	resistance of the two cultivars are different and the difference is canceled by detergent application, it would
358	provide additional evidence that leaf hairs provide DM resistance. The results showed that the two cultivars
359	differed significantly in the number of sporangia on discs when the detergent was not applied (Fig. 2d),

360	whereas detergent application abolished the difference. Because the detergent broadened the area of
361	infection after inoculations even on 'Pinot Noir,' detergent application significantly increased the number
362	of sporangia.
363	
364	Segregation of DM resistance and underside leaf hair density in Pop AC
365	
366	To determine the loci controlling leaf hair density and DM resistance in Pop AC, we analyzed the DM
367	resistance of Pop AC and hair density. The segregation of leaf hair density in Pop AC was clearly bimodal
368	between the parents (Supplementary Fig. 6), and the normality of the distribution was rejected by the
369	Shapiro-Wilk test ( $P < 0.001$ ). No individual had hairs as dense as 'Campbell Early. 'Muscat of Alexandria'
370	was always susceptible and 'Campbell Early' was resistant whether DM incidence or severity was evaluated.
371	The distribution of resistance as evaluated by the leaf disc assay was bimodal and the normality was rejected
372	(P < 0.001). However, the normality of the distribution of resistance by incidence and severity was not
373	rejected ( $P = 0.08$ , incidence; $P = 0.50$ , severity), suggesting the continuous distribution (Supplementary

374Fig. 6).

- 376 QTL analysis of Pop AC for leaf hair traits and DM resistance
- 377

378	We constructed genetic maps of each parent and the consensus map (Supplementary Fig. 7 and
379	Supplementary Table 5) with 338 markers and performed QTL analysis. We found a QTL located at the top
380	of LG5 of 'Muscat of Alexandria,' which explained 37.7%-49.3% (incidence), 34.5%-43.2% (severity),
381	and 49.2%–76.3% (leaf disc assay) of phenotypic variation in DM resistance (Table 1 and Fig. 3a). The
382	QTL was detected in all 3 years with almost the same effect (Table 1). Because the QTL was detected in
383	'Muscat of Alexandria,' which is susceptible to DM, it confers DM susceptibility. A distinct minor QTL
384	affecting severity was detected in LG18 of 'Campbell Early,' but only in 2014. A QTL on LG7 was detected
385	in 2014 and 2015 by leaf disc assay; this QTL explained approximately 15% of phenotypic variation. In a
386	non-parametric Kruskal–Wallis test, the statistic K* values of all three QTLs were highly significant.
387	QTL analysis for leaf hair traits identified the same locus as that for DM resistance (with Nifts5-50363
388	as the nearest marker). This QTL had a very high LOD score (71.9%-78.5% of phenotypic variance
389	explained; Table 1). Because of its large effect on leaf hair density, we designated the locus Leaf Hairs 1

- 390 (LH1). A minor QTL explaining 14.9%–22.8% of phenotypic variance was detected in LG7 of 'Campbell
- 391 Early.' Both loci were detected in 2015 and 2016. Both QTLs were supported by the Kruskal–Wallis test
- 392 (Table 1).
- 393 To analyze the effect of alleles of the *LH1* locus, we compared the mean values of the genotypes of
- the Nifts5-50363 marker (Fig. 3c). 'Campbell Early' is homozygous not only for Nifts5-50363 (188/188
- 395 bp; Table 2) but also for several closely linked markers in this region, and 'Muscat of Alexandria' is
- heterozygous (212/225 bp). Progeny were genotyped as either 188/212 or 188/225. Plants harboring the
- 397 212-bp allele had fewer leaf hairs, whereas those with the 225-bp allele had significantly denser hairs. By
- leaf disc assay, the 188/212 genotype was more susceptible to DM than the 188/225 genotype, and the
- 399 188/212 genotype showed higher incidence and severity of DM under fungicide-free conditions. The mean
- 400 incidence and severity of DM for the 188/212 genotype were 1.24× and 1.27×, respectively, that of those
- 401 of the 188/225 genotype.

403 Validation of the QTLs in two other populations

404

- 406 populations, Pop 693 and Pop 777. A genetic map had already been constructed for Pop 693 (Ban et al.
- 407 2014); we performed QTL analysis using the map and added two newly developed markers (Nifts5-50304
- 408 and 50363) near the LH1 locus. The same QTL was detected at the top of LG5 of both parents (626-84 and
- Iku 82) and explained 15.4% and 17.0% (leaf disc assay), respectively, and 22.0% and 33.7% (leaf hairs),
- 410 respectively, of phenotypic variation (Table 1). Because the genotypes of the parents at Nifts5-50363 were
- 411 heterozygous (188/201; Table 2), the progeny genotypes were 188/188, 188/201, or 201/201. The combined
- 412 effect of the locus was detected in the consensus map (Fig. 3b; Table 1), and the explained phenotypic
- 413 variance was as high as 54.4% (leaf disc assay) and 88.4% (leaf hairs). The effect of the QTL at the locus
- 414 (Nifts5-50363) in Pop 693 is shown in Fig. 3d. Progeny harboring the 201-bp allele had significantly fewer
- 415 leaf hairs than the 188/188 plants. Surprisingly, the 201/201 genotype resulted in almost no hairs and
- therefore the leaf hair scores of the 188/201 and 201/201 genotypes were significantly different by the
- 417 pairwise non-parametric Wilcoxon rank-sum test (P = 0.003). The 188/188 genotype leaf discs had
- 418 significantly fewer sporangia than the other genotypes, but there was no significant difference between the
- 419 188/201 and 201/201 genotypes.

- 420 No genetic map was available for Pop 777, and therefore we analyzed the effect of Nifts5-50363 by
- 421 using only the leaf hair score and leaf disc assay (Fig. 3e). Because the parents are heterozygous with three
- 422 alleles (700-39, 188/212; 'Shine Muscat,' 188/225; Table 2), there are four possible progeny genotypes
- 423 (188/188, 188/212, 188/225, 212/225). Plants harboring the 212-bp allele had significantly fewer leaf hairs
- than the 188/188 and 188/225 genotypes. Accordingly, the 188/188 and 188/225 genotype leaf discs had
- 425 significantly fewer sporangia than the genotypes carrying the 212-bp allele (Fig. 3e).
- 426
- 427 *Vitis vinifera* origin of the hairless allele
- 428

429	Because the size of the hair	ess allele of Nifts5-	-50363 in Pop	o 693 differed fr	om that in the other	populations,
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- 430 their origins might be different. We traced them back to the original cultivars by genotyping the ancestors
- 431 of the three populations with the closely linked SSR markers Nifts5-50304, 50363, and 50437 near LH1
- 432 (Table 2; Supplementary Fig. 8). A SSR haplotype, 206/201/235, was derived from V. vinifera 'Katta
- 433 Kurgan.' Another haplotype, 206/212/235, was found in V. vinifera 'Muscat of Alexandria' and 'Parkent.'
- 434 We could not genotype *V. vinifera* 'Heptakilo,' a parent of 'Muscat of Alexandria.' However, it might have

435	the latter haplotype because the other parent, V. vinifera 'Muscat à Petits Grains Blancs,' does not have the
436	haplotype. 'Muscat of Alexandria' and 'Parkent' have the same haplotype, whereas the haplotype found in
437	Pop AC came from 'Muscat of Alexandria' and that found in Pop 777 came from 'Parkent' (Supplementary
438	Fig. 7). In accordance with the genotype, all three V. vinifera—'Katta Kurgan,' 'Muscat of Alexandria,' and
439	'Parkent'—had a few or no hairs (Fig. 1a; Supplementary Fig. 4).
440	
441	Discussion
442	
443	We have quantitatively shown that underside leaf hairs are associated with resistance to DM by regression
444	analyses of Pop AC and other germplasms, and detergent application to the underside of the leaf supported
445	the hypothesis that the underside leaf hairs function to impart preexisting structural defense to DM. This
446	hypothesis was proposed previously by Kortekamp and Zyprian (1999), and Divilov et al. (2018) also found
447	that leaf hairs had an effect on disease resistance in their F1 family. In this study, we also confirmed that
448	the effect of leaf hairs could be completely canceled by detergent application using our progenies, as well
449	as 'Pinot Noir' and its bud sport 'Pinot Meunier.' 'Pinot Meunier' could be regarded as a hairy mutant of

451 by detergent application. This is additional new evidence that leaf hairs function to create preexisting

452 structural defense.

- 453 This resistance is not qualitative or true resistance, and disease progression cannot be completely
- 454 prevented, but DM incidence and severity in less haired individuals in Pop AC were significantly higher
- 455 (1.24× and 1.27×, respectively) than those of haired individuals. Leaf hairs provide typical 'quantitative
- 456 disease resistance,' and the LH1 locus is an example of 'quantitative resistance loci,' which confer
- 457 quantitative disease resistance (Poland et al. 2009). This locus was a major QTL for DM resistance in this
- 458 population because all the values for phenotypic variance explained (Table 1) exceeded the threshold (20%–
- 459 25%) defined by St. Clair (2010).
- 460 We observed significant differences in leaf hair density among cultivars, selections, and species. Leaf
- 461 hair density on fully expanded leaves was evaluated stably as shown by the variance component analysis
- 462 of Pop AC. Accordingly, hairs on the lower leaf surface are often important in identification of Vitaceae
- 463 species (Gerrath et al. 2015). These hairs are described as 'prostrate hairs between main veins on lower side
- 464 of blade' (Characteristic 28) in the DUS (Distinctness, Uniformity and Stability) guidelines of UPOV

(International Union for the Protection of New Varieties of Plants); this is also the Descriptor 6.1.35 for

- 466 Grapevine in IPGRI (1997), in which V. labrusca is indicated as a grapevine with the densest hairs. 467 Gerrath et al. (2015) mentioned that V. labrusca plants have dense brownish or whitish prostrate hairs 468 covering the lower leaf surface; the hairs are so dense that they obscure the lower leaf surface except for 469 veins. This species was an important parent of American hybrids. The exact genetic mechanisms that 470increase leaf hair density are not clear. In the Japanese grapevine breeding program, American hybrids, 471 especially 'Steuben' and 'Campbell Early,' are important parents. One of their ancestors is 'Concord,' which 472is a hybrid between 'Catawba' and V. labrusca; 'Catawba' is a hybrid between V. vinifera 'Sémillon' and V. 473labrusca (Huber et al. 2016; Vitis International Variety Catalogue <a href="http://www.vivc.de/">http://www.vivc.de/</a>), suggesting a 474strong V. labrusca background. Cadle-Davidson et al. (2008) compared their data with several previous 475studies and concluded that 'Concord' remains the only consistently resistant or moderately resistant cultivar 476 among 89 cultivars. This suggests that 'Concord' has a broad-spectrum, durable foliar resistance, which is 477 rarely found in cultivated Vitis. The present study suggests that thick leaf hairs might be a mechanical cause 478of the durable resistance of 'Concord,' and that this trait originated from a wild species, V. labrusca, during
- 479long-term interaction between this species and downy mildew in North America.

480	Kortekamp and Zyprian (1999) suggested that V. labrusca and V. cinerea might have defense
481	mechanisms different from leaf hairs. The presence of such mechanisms was implied in our regression
482	analyses as variation from the regression lines. Because $r^2$ explains the variance derived from leaf hair
483	density, $1 - r^2$ explains the unexplained variances, including those caused by other resistance mechanisms
484	and experimental error. As $1 - r^2$ was 0.33 to 0.47 (Fig. 1b; Supplementary Fig. 3a), considerable variance
485	may result because of other resistance mechanisms, although their effect was smaller than that of leaf hairs
486	in this population. However, we found that 'Chancellor' and V. flexuosa had almost no hairs but were highly
487	resistant to DM (Supplementary Fig. 5). 'Chancellor' has the Rpv3 gene (Di Gaspero et al. 2012), but the
488	resistance genes in <i>V. flexuosa</i> are unknown. These are examples of germplasm with high resistance to DM
489	unrelated to leaf hairs.
490	In Pop 777, no significant difference in leaf hair density was detected between the 188/188 and
491	188/225 genotypes (Fig. 3e), suggesting that QTL linked to the 225-bp allele has the same effect as the 188-
492	bp allele, and both are recessive to the hairless 212-bp allele. Throughout the analysis of the locus, the
493	hairless 201- and 212-bp alleles at Nifts5-50363 were dominant to other alleles and significantly reduced

the density of underside leaf hairs. We used SSRs closely linked to the *LH1* locus (Nifts5-50304, 50363,

496	found that the haplotype 206/201/235 originated from 'Katta Kurgan' and 206/212/235 originated from
497	'Muscat of Alexandria' and 'Parkent' (Table 2; Supplementary Fig. 8). 'Muscat of Alexandria' and its parent
498	'Heptakilo' (a probable source of the hairless allele; Cipriani et al. 2010) originated in Greece, whereas
499	'Katta Kurgan' and 'Parkent' originated in Uzbekistan (Vitis International Variety Catalogue
500	<a href="http://www.vivc.de/">http://www.vivc.de/</a> ). These V. vinifera cultivars are important parents in the Japanese breeding program
501	(Yamada and Sato 2016), hence the hairless traits might have been reducing the resistance of resultant
502	populations to DM. More thorough investigation of V. vinifera genotypes is necessary, but our data suggest
503	the presence of at least two distinct hairless alleles that originated from southeastern Europe and central
504	Asia. Because wine grape populations are not included in this study, we do not know whether the hairless
505	alleles found in this study prevail in <i>V. vinifera</i> wine grapes. The isolation of gene(s) responsible for the
506	hairless trait and the explanation of the evolutionary benefit of this trait for the table grape V. vinifera are
507	the main challenges.
508	A DM resistance locus with small effects, designated as Rpv11, was previously identified in LG5

509 (Fischer et al. 2004; Schwander et al. 2012). The QTL was detected based on the leaf disc assay, and the 1-

LOD confidence interval was 14.6-22.8 cM on the consensus map of Gf.Ga-52-42 ('Bacchus' × 'Villard

- 511 blanc') × 'Solaris' ('Merzling' × Geisenheim 6493), which corresponds to the region between the VMC3B9
- and VRZAG79 markers (Schwander et al. 2012). Almost the same region was found by Bellin et al. (2009)
- 513 by the observation of mesophyll invasion of DM, and the confidence interval (1-LOD) was 10.6-39.2 cM
- on the 'Chardonnay' map. The confidence interval (1.5-LOD) of LH1 defined by leaf hair density was on
- 515 the top of LG5 (0-2.3 cM of the 'Muscat of Alexandria' map; Table 1), and it does not overlap with the
- region between VMC3B9 and VRZAG79. Hence, *Rpv11* and *LH1* could be regarded as different loci.
- 517 Recently, QTL for leaf hair density was identified on LG5 from a hybrid grapevine 'Horizon' (Divilov et
- al. 2017; Divilov et al. 2018). The percentage of variance explained by the sporulation trait on the leaf disc
- assay was 11.27%, and the 95% credible interval is 0.844-5.511 Mbp (Divilov et al. 2018). The interval
- 520 includes *LH1* because the locus is located around Nifts5-50363 (0.877 Mbp). However, Divilov et al. (2018)
- 521 performed another analysis, the multiple phenotype Bayesian network analysis, and found the physical
- 522 locations of QTL on LG7, 8, and 15, but not LG5, for the leaf trichome (Lt) phenotype. Thus, they did not
- 523 designate QTL on LG5. In this study, however, we successfully located the QTL on LG5 by analyzing
- 524 hybrid populations originating from *V. labrusca* and identified the origin of the hairless alleles in traditional

- 526 detect the hairless genotypes at the *LH1* locus.
- 527 We also found a small effect QTL in LG7 on the 'Campbell Early' map, and its confidence interval
- 528 (1.5-LOD) was 25.8-41.7cM (the largest interval by visual rating, 2015), and the nearest marker was Nifts7-
- 529 59300 (9.903Mbp). *Rpv7* (Bellin et al. 2009), *Rpv9* (Moreira et al. 2011), and *Rpv21* (Divilov et al. 2018)
- are located near the QTL. Another QTL found in LG18 of the 'Campbell Early' map was close to Rpv3
- 531 (Fischer et al. 2004; Welter et al. 2007; Bellin et al. 2009), however, it was detected only in one year (2014).
- 532 Owing to the large confidence intervals of these two QTLs on LG7 and 18, further work is necessary to
- 533 determine which *Rpv* genes are equivalent to the QTLs on LG7 and 18, and whether they are the same
- 534 genes or not.
- 535 Durability of resistance is always the concern when resistance genes are used for breeding, and
- 536 resistance is sometimes effective for just a few years (Parlevliet 2002). This is consistent with the emergence
- 537 of aggressive *P. viticola* isolates that overcame the resistance of 'Bianca' (Peressotti et al. 2010) and 'Regent'
- 538 (Delmotte et al. 2014). Delmotte et al. (2014) stated that the erosion of 'Regent' resistance may have

539 occurred in less than 5 years and at least three times independently. Both cultivars harbor the *Rpv3* gene

540	(Di Gaspero et al. 2012). Gene pyramiding (Eibach et al. 2007) and the use of quantitative resistance with
541	a different mechanism would be promising strategies for producing cultivars with durable resistance. As
542	Kortekamp and Zyprian (1999) suggested, breeding 'hairy' grapevines could be an alternative to the use of
543	R-gene-mediated resistance. In this respect, the LH1 on LG5 would be an important locus and a promising
544	DNA marker that could be effectively used in grapevine breeding for discarding hairless genotypes.
545	
546	Compliance with ethical standards
547	
548	Conflict of interest
549	
550	The authors declare that they have no conflict of interest.
551	
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562	
563	Author's contributions
564	
565	A. K. wrote the manuscript. A. K., Y. B., K. S., A. A., N. O., and A. S. designed the experiments. A. A.
566	contributed to the experiments using 'Pinot Meunier.' A. K. and A. S. contributed to statistical data analyses.
567	A. K. performed the experiments. A. K., Y. B., N. M., and S. S. genotyped Pop AC. Y. B. and N. M.
568	developed populations. H. F. extracted SSRs from the grapevine reference genome.
560	

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712	
713	Figure captions
714	
715	Fig. 1 (a) Leaf hairs on the abaxial side of fully expanded leaves of 'Muscat of Alexandria' (left) and
716	'Campbell Early' (right). Bars, 250 μm. (b) Regression of mean incidence and severity of DM (2014–2016)
717	in Pop AC on leaf hair density. The latter was evaluated as the proportion of leaf hair area by imaging

analysis. The linear regression equations are  $y = 1.45^{***} - 0.73^{***}x$  (incidence) and  $y = 69.9^{***} - 33.6^{***}x$ 

- (severity). \*\*\*P < 0.001. (c) Regression of mean incidence and severity of DM (2013, 2014) in 31 grapevines.
- Black, all data. The linear regression equations are  $y = 1.05^{***} 0.82^{***}x$  (incidence) and  $y = 55.1^{***} 33.7^{***}x$
- 721 (severity). \*\*\*P < 0.001.; Red, excluding 'Chancellor' and 'Koshu,' V. coignetiae, V. thunbergii, and V.
- flexuosa ( $\bigcirc$ ), which are not used in our table grape breeding. The linear regression equations are y =
- 723  $1.16^{***} 0.93^{***}x$  (incidence) and  $y = 58.1^{***} 36.2^{***}x$  (severity).  $*^{***}P < 0.001$ . Leaf hair density was
- evaluated by imaging analysis. (d) Regression of mean incidence and severity of DM in 27 cultivars and
- selections evaluated by Terai and Yano (1977). The linear regression equations are  $y = 1.45^{***} 1.31^{***}x$
- (incidence) and  $y = 44.8^{***} 62.5^{***}x$  (severity). \*\*\* P < 0.001. Leaf hair density was evaluated by imaging
- 727 analysis. \*\*\*P < 0.001
- 728
- Fig. 2 (a) Inoculated leaf discs at 1 dpi. Note that inoculum on a detergent (Nonidet P-40)-treated disc of
- 730 'Campbell Early' was absorbed within the layer of leaf hairs, whereas that on the untreated disc was clearly
- repelled by thick hairs. (b) Regression of number of sporangia on leaf discs treated with or without detergent

733	halved leaf. The linear regression equations are $y = 5.31^{***}$ - $0.56^{***}x$ (leaf hair score/without detergent), y
734	= $3.97^{***}$ - $2.22^{***}$ x (proportion of leaf hair area/without detergent), y = $4.30^{***}$ - $0.00^{ns}$ x (leaf hair score/with
735	detergent), and y = $4.47^{***}$ - $0.22^{ns}x$ (proportion of leaf hair area/with detergent). *** $P < 0.001$ ; ns, not
736	significant ( $P > 0.05$ ). (c) Underside hairs on leaf discs prepared from young developing leaves of 'Pinot
737	Noir' and 'Pinot Meunier.' Bars, 250 $\mu$ m. (d) Number of sporangia on discs prepared from 10 leaves of
738	'Pinot Noir' and 'Pinot Meunier' treated with or without Nonidet P-40. Bars labeled with the same letter
739	are not significantly different (pairwise Wilcoxon rank-sum test; $\alpha = 0.01$ ). Error bars denote SE
740	
741	Fig. 3 (a) QTL-LOD profiles of leaf hair density and downy mildew resistance traits in Pop AC. QTLs for
742	mean incidence and severity under fungicide-free conditions (2014-2016) and mean leaf hair density (the
743	proportion of leaf hair area by imaging analysis; 2015, 2016) on the 'Muscat of Alexandria' map are shown.
744	The proportion of leaf hair area were arcsine-transformed. (b) QTL-LOD profiles of leaf hair density and
745	downy mildew resistance traits in Pop 693. QTLs for mean scores of leaf disc assay (2016) and mean leaf

- represent 1.5-LOD confidence interval. (c) Effect of the Nifts5-50363 locus on downy mildew resistance
- and leaf hair trait in Pop AC. Mean score of leaf disc assay (2013–2015), mean incidence and severity under
- fungicide-free conditions (2014–2016), and leaf hair density (2015, 2016) were compared between different
- 750 genotypes at the locus. (d, e) Effect of the Nifts5-50363 locus on downy mildew resistance and leaf hair
- trait in (d) Pop 693 and (e) Pop 777. Mean scores of leaf disc assay (2015, Pop 777; 2016; Pop 693) and
- leaf hair density by visual rating (2015) were compared between different genotypes at the locus. (c-e) Bars
- 153 labeled with the same letter are not significantly different (pairwise Wilcoxon rank-sum test;  $\alpha = 0.01$ ).
- Error bars denote SE