

## REVIEW

# Leaf Trichomes as an Effective Structure for Disease Resistance: The Case of Grapevine Downy Mildew

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### Abstract

Leaf trichomes confer pest resistance. Dense trichomes could also present a physical barrier to microorganisms, such as grapevine downy mildew caused by *Plasmopara viticola* on grape. Zoospores of *P. viticola* swim in water and enter stomata. Some wild *Vitis* accessions such as *V. labrusca* and *V. cinerea* have highly hydrophobic, dense, prostrate trichomes on the lower leaf surface that repel water and enhance resistance to downy mildew. Quantitative trait loci for leaf trichome density have been identified on LGs 5, 7, 8, and 15 of grapevine. A major locus on LG 5 was found in *V. vinifera* ‘Muscat of Alexandria,’ which has few leaf trichomes. Hairless alleles on LG 5 in historical *V. vinifera* cultivars reduce trichome density in the progeny of *V. labrusca* origin. Applying information on these loci in breeding programs would allow the introduction of this natural physical barrier against pathogenic microorganisms into cultivars.

**Discipline:** Horticulture

**Additional key words:** preexisting disease resistance, *Plasmopara viticola*, *Vitis labrusca*, *Vitis vinifera*

## Introduction

Leaf hairs are termed trichomes from the Greek word for hair—*trichos* (Hülkamp 2004). Trichomes are historically well-known for conferring pest resistance (Levin 1973), such as against the potato leafhopper (*Empoasca fabae*) (Poos 1929, Poos & Smith 1931). The hooked trichomes of *Passiflora adenopoda* act as a highly efficient defense mechanism against butterfly larvae by hooking into the larval prolegs (Gilbert 1971). In grapevines, the abundance of the predatory mite *Typhlodromus pyri* was significantly associated with a quantitative trait locus (QTL) having a major effect on the density of leaf trichomes (Barba et al. 2019). Trichomes can also protect plants against UV-B irradiation and freezing (Hülkamp 2004, Johnson 1975, Karabourniotis et al. 1995, Łaźniewska et al. 2012). Trichomes could also play a role in plant-microbe interactions. This review

focuses on the effect of leaf trichomes on resistance to grapevine downy mildew (DM) and on QTLs for leaf trichome density, with examples of other crops.

## Grapevine downy mildew

Grapevine is an important crop with worldwide economic importance. *Vitis vinifera* L. is commercially the most important. It is derived from *V. vinifera* L. ssp. *sylvestris*, a wild species indigenous to Transcaucasia (Olmo 1976) and the Mediterranean region (Arroyo-García et al. 2006, Grassi et al. 2003). During the 19th century, several grape pathogens, notably DM and powdery mildew, were introduced from North America into Europe, where those pathogens caused serious losses in *V. vinifera* production (Muganu & Paolocci 2013, Reisch et al. 2012). Many crosses between *V. vinifera* and wild species have been made to develop disease-resistant

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cultivars (Eibach & Töpfer 2014, Reisch & Pratt 1996). Among them, interspecific hybrids with *V. labrusca* parentage produced in the United States, Japan, and other countries can be grown successfully in a wide area of Japan (Yamada & Sato 2016). These hybrids are sometimes collectively referred to as *V. labruscana* Bailey (Bailey & Bailey 1930).

Among the pathogens of grapevines, grapevine DM (Fig. 1 A, B) is one of the most economically damaging diseases and causes serious production losses in Japan (Watauchi et al. 2015). *Plasmopara viticola* is the causal organism of grapevine DM, and the infection process always takes place in water (Lafon & Bulit 1981). The oomycete produces zoospores, which swim to stomata on leaves or other green parts. At least 27 DM resistance loci named *Rpv* (Resistance to *Plasmopara viticola*) have been identified (Julius Kühn-Institut 2019). In addition, some Pathogenesis-related (PR) genes are induced after DM infection. The defense-related gene *PR-2* was induced in ‘Shine Muscat’ and the rootstock cultivar ‘Kober 5BB,’ but not in the susceptible ‘Katta Kurgan’ (Shimizu et al. 2019), and the synthesis of most PR-10 defense-related proteins increased significantly 24 to 96 h after inoculation (Milli et al. 2012). In contrast to these induced resistance responses, leaf trichomes provide a preexisting resistance mechanism against grapevine DM.

### Trichomes as an effective structure for disease resistance

A thick mat of hairs may reduce infection by repelling water (Agrios 2005). Such dense non-glandular trichomes could present a physical barrier to pathogenic fungi or bacteria (Karabourniotis et al. 2019, Łażniewska et al. 2012). In grapevines, stomata are formed on the lower surface of leaves (Dürinig 1980), where DM infection takes place exclusively. Two main types of trichomes in *Vitis* are documented: non-glandular and glandular. Among non-glandular trichomes, ribbon and simple trichomes are found on different vegetative organs (Ma et al. 2016). Trichomes on the lower leaf surface are often important in species identification in the family Vitaceae (Gerrath et al. 2015). Ribbon trichomes, the main structures that prevent DM infection, are twisted, greatly elongated, and unicellular. Thick hydrophobic trichomes on the lower surface repel water effectively, and Staudt & Kassemeyer (1995) showed that wild species with dense trichomes lost resistance after removal of the trichomes. Kortekamp et al. (1999) proposed that these dense trichomes enhance resistance to DM and showed that less force was necessary for removing water droplets from hairy wild species, such as *V. labrusca* and

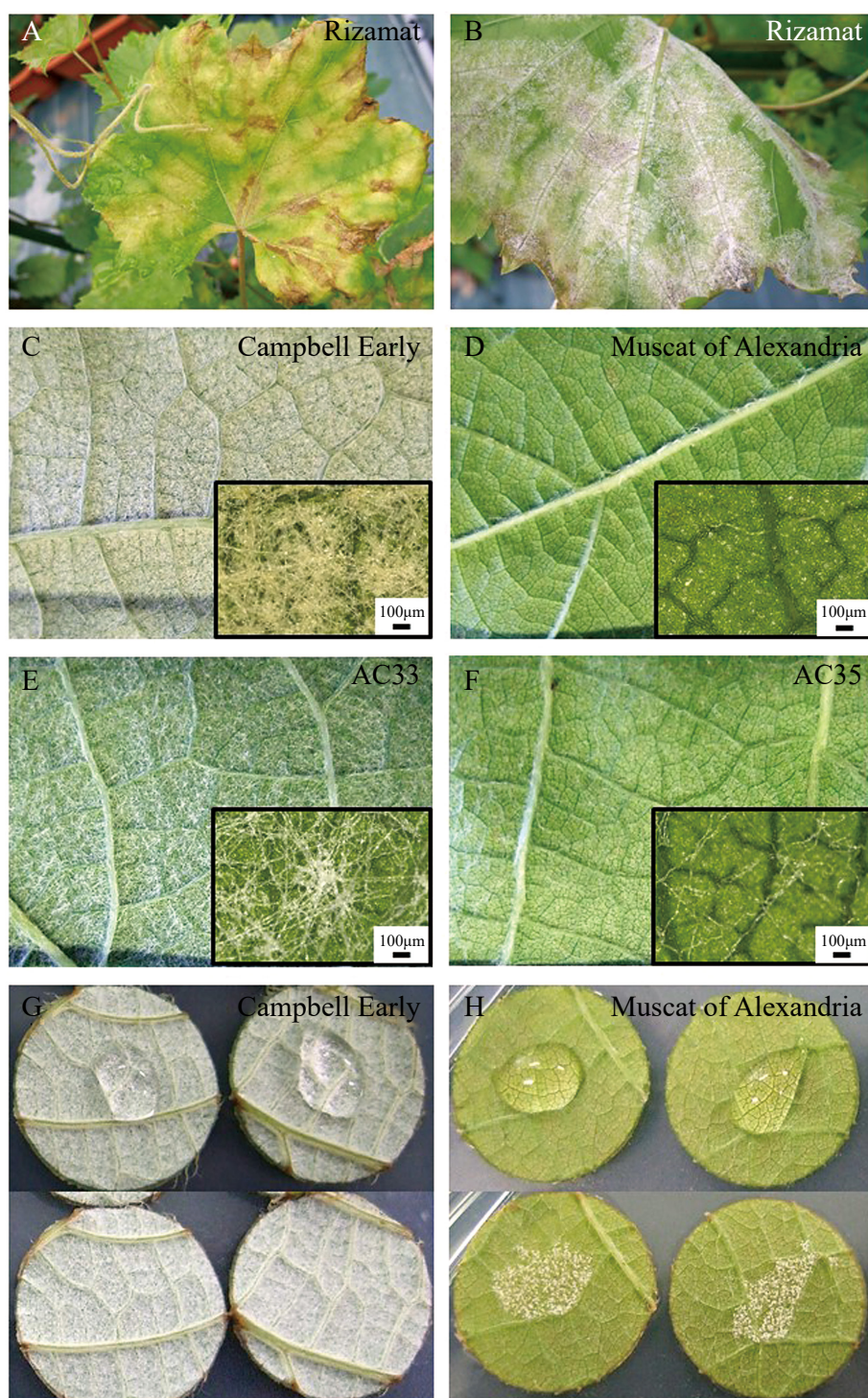
*V. cinerea*, than from non-hairy grapevines (Kortekamp et al. 1999).

*Vitis labrusca* is one source of the dense trichomes in hybrid grapes, and its trichomes make its lower leaf epidermis almost invisible (Gerrath et al. 2015). Its *V. labruscana* descendants, usually with more leaf trichomes than *V. vinifera*, are important cultivars for the breeding of table grapes in Japan (Yamada & Sato 2016). In Figure 1, C-H show leaf trichomes and their effects on DM infection of a hairy *V. labruscana* (‘Campbell Early’) and a glabrous *V. vinifera* (‘Muscat of Alexandria’), and two descendants. This relationship between leaf wettability and pathogen invasion has been noted in other plant-pathogen interactions, such as maize (*Zea mays*) – *Puccinia polysora* and strawberry (*Fragaria × ananassa*) – *Phytophthora cactorum* (Hollier & King 1985, Reynolds et al. 1989). *Vitis labrusca* and *Vitis cinerea* are suggested to have other defense mechanisms as well (Kortekamp & Zyprian 1999). In contrast, sparse trichomes are associated with susceptibility to bacteria or fungi (Kim 2019). Anthracnose of tea (*Camellia sinensis*) is caused by *Discula theae-sinensis*, which invades tea plants through dispersed foliar trichomes (Hamaya 1982, Yamada & Sonoda 2014).

### Inheritance of leaf trichome density and QTLs

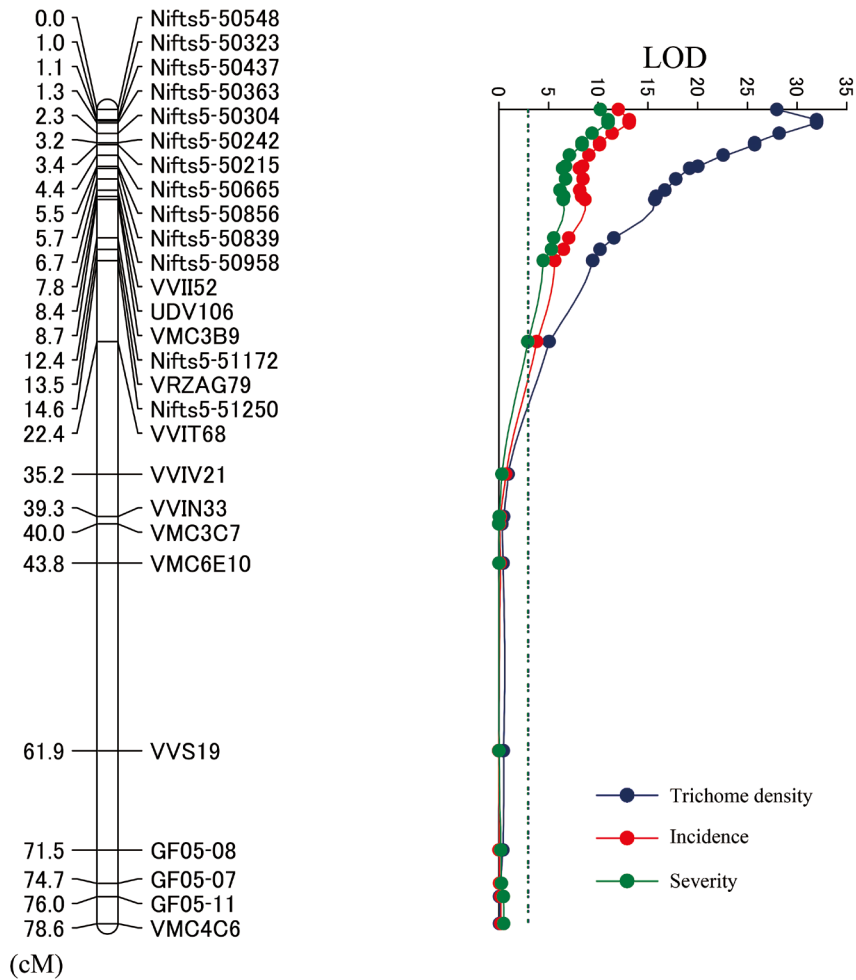
Trichome inheritance has been studied in the race-nonspecific resistance of the common bean (*Phaseolus vulgaris*) to *Uromyces appendiculatus* var. *appendiculatus* (Shaik 1985), and genetic variation in leaf trichomes on the lower surface was detected among cultivars and lines (Zaiter et al. 1990). Segregation for hairiness versus hairlessness indicates that trichome density is determined by a single major gene or by duplicate recessive epistatic genes, depending on the cross used.

Recently, the inheritance of grapevine leaf trichomes and the associated QTLs were revealed. QTLs for leaf trichome density were identified on linkage groups (LGs) 5 and 8 from a hybrid wine grape, ‘Horizon’ (Divilov et al. 2017). Divilov et al. (2018) performed a detailed analysis using two F<sub>1</sub> families, *Vitis rupestris* × ‘Horizon’ and ‘Horizon’ × *V. cinerea*, and showed that the physical locations of QTLs on LGs 7, 8, and 15, but not LG 5, had an effect on leaf trichome density. A major QTL for leaf trichome density on LG 5, named *Leaf Hairs 1 (LH1)*, explaining 71.9%-78.5% of the phenotypic variance, was identified in a population of *V. vinifera* ‘Muscat of Alexandria’ × *V. labruscana* ‘Campbell Early’ (Kono et al. 2018; Fig. 2 A). Its effect was confirmed in two other populations, and the historical *V. vinifera* cultivars ‘Muscat of Alexandria,’ ‘Katta Kurgan’ and ‘Parkent’

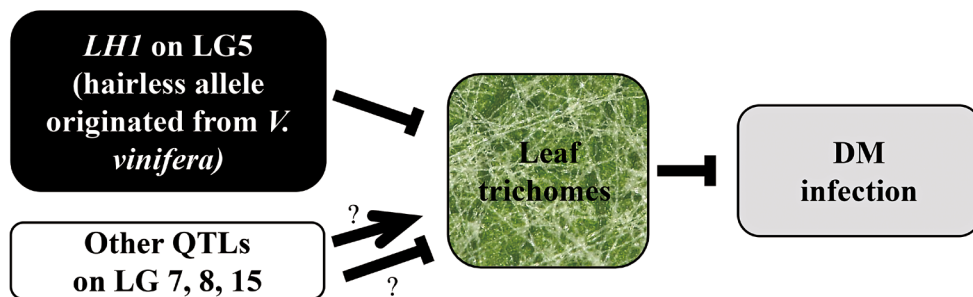


**Fig. 1. Symptoms of grapevine downy mildew and underside leaf trichomes in grapevines**  
 A, B: Severely infected leaf of *Vitis vinifera* 'Rizamat': (A) upper and (B) lower surface of the same leaf. Sporangia are visible only on the lower surface.  
 C-F: Lower side of fully expanded leaves of (C) *Vitis labruscana* 'Campbell Early,' (D) *V. vinifera* 'Muscat of Alexandria,' and their descendants, (E) AC33, and (F) AC35. Insets show magnified views.  
 G, H: Leaf discs of expanding leaves of (G) 'Campbell Early' and (H) 'Muscat of Alexandria.' Upper images show discs inoculated with DM sporangia suspension one day after inoculation. Lower images show the same discs six days after inoculation. Note that (G) no sporangia emerged on the discs with dense leaf trichomes, whereas (H) sporangia almost exactly matched the coverage of the inoculum (upper panel) on the discs with sparse trichomes.

A



B



**Fig. 2. Symptoms of grapevine downy mildew and underside leaf trichomes in grapevines**

A: QTL-LOD profile of leaf hair density and downy mildew resistance traits in a population by crossing *V. vinifera* ‘Muscat of Alexandria’ and ‘Campbell Early.’ QTLs for downy mildew resistance (mean incidence and severity) under fungicide-free conditions and mean leaf trichome density on the ‘Muscat of Alexandria’ map are shown. Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Kono et al. (2018) *Molecular Breeding*, 38, 138.

B: Schematic diagram of interactions of QTLs for leaf trichome density and resultant downy mildew resistance in grapevines.

were shown to have the hairless allele, which drastically reduces the density. A minor QTL was detected on LG 7 of ‘Campbell Early’ (Kono et al. 2018). A DM resistance locus with small effects (designated *Rpv11*) was identified on LG 5 (Fischer et al. 2004, Schwander et al. 2012). The relationship between *LHI* and nearby QTLs remains to be resolved. Figure 2 B shows a schematic diagram of interactions of QTLs for leaf trichome density and resultant DM resistance.

## Future perspectives

In terms of the berry quality, *V. vinifera* table grape cultivars are preferable to *V. labruscana*. ‘Muscat of Alexandria’ and ‘Katta Kurgan’ are important old *V. vinifera* cultivars frequently used in Japanese table grape breeding (Yamada & Sato 2016), but are susceptible to DM. *Plasmopara viticola* has already become resistant to some fungicides in Japan (Watauchi 2015), France, and Italy (Heaney et al. 2000). Furthermore, the partial resistance to DM in ‘Regent’ carrying the *Rpv3* gene was overcome by some *P. viticola* isolates in less than five years in Europe (Delmotte et al. 2014). Even though the resistance conferred by trichomes cannot completely protect grapevines from DM, the dense trichome trait offers a feasible and promising mechanism for the sustainable control of DM in vineyards. If the trait is adopted as a breeding target, attention should be paid to avoid hairless alleles at the *LHI* locus in breeding programs.

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