Correlation between total hypha length and haustoria number of *Pseudoidium neolycopersici* in type I trichome cells of tomato leaves

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**Abstract**

Powdery mildew haustoria are easier to be observed by light microscopy in trichome cells compared to epidermal cells of infected leaves. The objective of this study was to explore the relationship between the hyphal length and the number of haustoria in type I trichome cells of tomato (*Solanum lycopersicum* Mill.) leaves. The trichome cells of tomato cv. Moneymaker were inoculated with conidia of tomato powdery mildew (*Pseudoidium neolycopersici* L. Kiss), isolate KTP-04. On these cells, the *P. neolycopersici* isolate produced a maximum of four vigorously elongated hyphae per conidium. At 12 days after inoculation, KTP-04 formed two to five haustoria per conidium. Field-emission scanning electron microscopy showed that the haustorium consisted of a haustorial body and several lobes embedded in an extrahaustorial matrix. The number of haustoria per hypha and hyphal length on trichomes were positively correlated. Also, the tips of one to four hypha per conidium (excluding germ tubes and primary appressoria) were injured using a minute glass needle installed on micromanipulator under a high-fidelity digital microscope, and their total hyphal lengths were compared. Wounded hyphae possessed the same number of haustoria in trichome cells as non-wounded hyphae, and total hyphal lengths were similar between treatment groups. In this study, a new model was developed to study the infection mechanisms of powdery mildews that will be useful in future gene expression studies.

**Keywords** Conidial germination · Haustorial formation · Hyphal elongation · *Solanum lycopersicum* · Tomato powdery mildew

**Introduction**

Powdery mildew infections of tomato are caused by three species: *Pseudoidium neolycopersici* L. Kiss and *Leveillula taurica* (Lév.) G. Arnaud, two pathogens known from many parts of the world (White et al. 1997; Kiss et al. 2001; Zheng et al. 2013), and *Golovinomyces lycopersici* (Cooke & Massee) L. Kiss, reported only from Australia (Kiss et al. 2001; Braun et al. 2019). All these species are obligate biotrophs that infect leaves and stems, and reduce the yield of the infected tomato plants. In Japan, *P. neolycopersici* occurs regularly on hydroponically cultured tomato (*Solanum lycopersicum* Mill.) (Matsuda et al. 2001; Shimizu et al. 2007; Nonomura et al. 2008). Since 1998, we isolated five *P. neolycopersici* isolates from infected leaves collected in our greenhouse (Kashimoto et al. 2003; Nonomura et al. 2010, 2013; Seifi et al. 2012). The infection cycle of *P. neolycopersici* begins with conidial germination, elongation of an appressorial germ tube and formation of an appressorium after the conidium landed on a plant leaf surface. The appressorium helps the adhesion to plant leaf surface and fungal penetration of the plant cell wall. Matured haustorium forms after penetration into the host epidermal cell; it begins
to draw nutrients and water from the host cell, and new epi-
phytic hyphal growth leads to an expansion of the colony on
the leaf surface (Jones et al. 2001; Kashimoto et al. 2003;
Jacob et al. 2008). Thus, the establishment of functional
haustoria is essential for successful fungal colonisation
on the leaf surface; effector molecules must be delivered from
fungal haustoria to host cells to suppress plant defences and
promote fungal establishment (Lindegren et al. 2012).

The leaves of wild and cultivated Solanum species have
morphologically distinct trichomes (types I to VII) (Lemke
and Sorensen 1985; Duffey 1986; Peter and Shanower 1998;
Kessler and Baldwin 2001; Pichersky and Gershenzon 2002;
Simmons and Gurr 2005; Nonomura et al. 2009b; Kang et al.
2010; Tooker et al. 2010). Morphological characteristics
of leaf type I trichomes of tomato cv. Moneymaker (MM) were
1.5–2.5 mm in length on a multicellular base with a small
glandular tip, and abundant and larger/larger than other types
of trichomes. In a previous study, we used the leaf type I
trichomes of MM to elucidate the interaction between tri-
chomes and P. neolycopersici, as well as the infection pro-
cesses of powdery mildew pathogens on trichomes, by high-
fidelity digital microscopy (DM) (Suzuki et al. 2018). We
found that trichome cells induce the same cytological re-
sponses as leaf epidermal cells following inoculation with
P. neolycopersici conidia, and demonstrated that trichomes
provide experimental material to be used as infection sites
for powdery mildews. MM leaf epidermal and trichome cells
induce hypersensitive cell death (HR) following invasion by
the barley powdery mildew pathogen (Blumeria graminis F.
sp. hordei Marchal race1) and the melon powdery mildew
pathogen (Podosphaera xanthii Pollacci), and produce
papilla-like structures at red clover powdery mildew pathogen
(Erysiphe trifoliorum Greville) invasion sites (Matsuda et al.
2005; Nonomura et al. 2010; Seifi et al. 2012; Takikawa et al.
2011a, 2015). We also demonstrated that the hyphal develop-
ment of specific tomato powdery mildew isolates (P. neolycopersici KTP-03 and -04) was not suppressed in the
cells of trichomes on powdery mildew-resistant wild to-

toate lines (S. peruvianum LA2172). Therefore, we compared
the resistance and virulence of isolates using type I trichomes
of tomato powdery mildew-susceptible and resistant lines, by
simultaneously observing the infection processes of powdery
mildews on leaf trichomes and the cytological responses of
powdery mildew-invaded trichome cells.

Generally, chemical treatments such as chlorophyll remov-
al (discoloration), fixation, and staining are required for clear
observation of HR or papillae in epidermal cells by epifluorescent microscopy (EM) (Li et al. 2007; Nonomura
et al. 2010), and cell haustoria by light microscopy (LM)
(Huang et al. 1998; Dyki 2003; Li et al. 2012). In contrast,
we were able to easily and directly observe cytological re-
sponses and mature haustoria in powdery mildew-invaded
trichomes by microscopy without chemical treatment, because
trichomes consist of transparent cells growing from the leaf
epidermis (Suzuki et al. 2018). Therefore, trichomes were
excellent and advantageous sites for morphological and cyto-
logical analysis of the interactions between plant cells and
pathogens under natural environmental conditions. The phys-
iological relationships among infection structures of tomato
powdery mildew pathogens, in or on trichome cells, have
not yet been analysed and reported. In this study, our main
objectives were to clarify (1) the morphological characteristics
of functional haustoria of tomato powdery mildew pathogens
in both leaf epidermal and trichome cells using field-emission
scanning electron microscopy (FE-SEM), (2) the relationships
between total hypha length and haustorium number per hypha
on trichome cells, and (3) the trichome infection processes of
intact and wounded hyphae. To our knowledge, this is the first
study described about developments of a new method to study
haustorial formation of powdery mildew fungi in leaf tri-
chome cells with light microscopy, without chemical
treatment.

Materials and methods

Plant materials

MM tomato seeds were germinated on water-soaked filter
papers in a Petri dish for 3 days in a growth chamber (LH-
240N; Nippon Medical & Chemical Instruments, Osaka,
Japan) under continuous illumination (19.8–40.3 µmol m
−2
s
−1; 400–700 nm) from white (full-spectrum) fluorescent
lamps (FL40SS W/37; Mitsubishi, Tokyo, Japan) at 25 ± 2
°C. Seedlings at the cotyledon stage were placed into polyure-
thane cubic sponge supports (3 x 3 x 3 cm³). Sponge supports
containing seedlings were inserted into 30-mL cylindrical
plastic cases (diameter 3 cm, length 5 cm), each containing
20 mL hydroponic nutrient solution (4.0 mM KNO₃, 1.5 mM
Ca(NO₃)₂, 1.0 mM MgSO₄, 0.66 mM NH₄H₂PO₄, 0.057 mM
FeEDTA, 0.048 mM H₂BO₃, and 0.009 mM MnSO₄), and
then incubated for 14 days in a temperature-controlled room
under the following conditions: 25 ± 2 °C, 50–70% relative
humidity (RH) and continuous illumination at 22.2 µmol m
−2
s
−1. MM seeds were obtained from their self-pollinated prog-
ey in our greenhouse.

Fungal materials

Isolate KTP-04 of tomato powdery mildew (P. neolycopersici) was used in this study (Nonomura et al.
2013). Mature conidia were collected from fungal mycelia on
infected leaves using an electrostatic spore collector, as previously described (Nonomura et al. 2009a), and transferred onto true leaves of 14-day-old tomato healthy seedlings by high-fidelity DM (KH-2700; Hirox, Tokyo, Japan). Inoculated seedlings were maintained for 14 days in growth chambers at 25 ± 1 °C and 50–70% RH under continuous illumination at 22.2 µmol m−2 s−1 (Nonomura et al. 2013).

**Dynamic analysis of powdery mildew infection on tomato leaf type I trichomes**

Conidia of KTP-04 were inoculated onto leaf epidermal cells and type I trichomes of 14-day-old MM plants. More than 100 conidia were used for inoculation in one experiment. Powdery mildew-inoculated plants were incubated in the growth chamber for 2–12 days at 25 ± 1 °C, 70–90% RH under continuous illumination at 22.2 µmol m−2 s−1. The infection processes of powdery mildew isolates on leaves and type I trichomes were observed using the KH-2700 DM. KTP-04 hyphal development was photographed at 2–12 days after inoculation of a single conidium onto a type I trichome using the ½” Interline transfer charge-coupled device (CCD) camera of the KH-2700 DM. Micrographs were analysed using the Adobe Photoshop image-processing software (ver. 5.0; Adobe Systems, San Jose, CA, USA) to improve image contrast without changing the original information. For rates of germination and appressorium formation, data were presented as means and standard deviation (SD) of five replicates (where 20 times was equal to one replicate).

**Microscopic observation of mature haustoria in tomato powdery mildew-invaded type I trichomes**

We collected 25 leaf segments (approximately 1 × 1 cm² in area) from five powdery mildew-inoculated tomato plants. The experiments were conducted three times (total of 75 leaf segments). Samples were directly observed under the KH-2700 DM, and under a light and epifluorescence microscope (BX-60; Olympus, Tokyo, Japan) without chemical treatment. Other samples were fixed and their chlorophyll removed in a boiling alcoholic lactophenol solution (10 mL glycerol, 10 mL phenol, 10 mL lactic acid, 10 mL distilled water, and 40 mL 99.8% ethanol) for 1–2 min, and then stained with 0.1% Aniline Blue (Nacalai Tesque, Tokyo, Japan) dissolved in distilled water, as previously described (Sameshima et al. 2004). Samples were then observed under the BX-60 light and epifluorescence microscope with a dichroitic mirror at 400 nm (maximum excitation, 330–385 nm; barrier filter, 420 nm).

Tomato leaf pieces (1 × 1 mm²) with tomato powdery mildew-inoculated trichomes were prefixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (0.1 M NaH₂PO₄ and 0.1 M Na₂HPO₄, pH 7.4) at 4 °C for 3 days, and postfixed with 1.0% buffered osmium tetroxide (Nisshin EM, Tokyo, Japan) for 1 h. After fixation, leaf pieces were washed for 5 min three times in ultrapure water, dehydrated in a graded ethanol series from 30–100%, and embedded in Quetol 651 resin mixture (Nisshin EM). Serial semithin sections (200 nm thickness) were cut from the resin blocks using a diamond knife, mounted on cover glasses (13 mm diameter), stained with 1.0% uranyl acetate for 20 min, and counterstained with lead solution (Sigma Aldrich, Japan) for 5 min. The sections were coated with 1.0 nm thick osmium using an osmium coater (Neoc-Pro; MeiwaFosis, Tokyo, Japan), and then observed under a FE-SEM (SU8220; Hitachi, Tokyo, Japan) using a backscattered electron (BSE) detector with an accelerating voltage at 2.0 kV, as previously described by Koga et al. (2015).

**Length measurement of hyphae on powdery mildew-inoculated type I trichomes**

Mature KTP-04 conidia were inoculated onto young leaves and type I trichomes of 20 14-day-old MM plants. Powdery mildew-inoculated plants were incubated in the growth chamber for 2–12 days at 25 ± 1 °C, 70–90% RH under continuous illumination at 22.2 µmol m−2 s−1. We examined 20 conidia from the isolate. The lengths of one to four colony-forming hyphae formed from conidia were measured separately using the KH-2700 DM and summed. Data were presented as means and SD of 20 measurements.

**Relationship between haustorium number and length in hyphae from conidia on tomato leaf type I trichomes**

Mature KTP-04 conidia were inoculated onto young leaves and type I trichomes of 60 14-day-old MM plants. Powdery mildew-inoculated plants were incubated in the growth chamber for 2–12 days under the conditions described above. The tips of hyphae (excluding germ tubes and appressoria) elongated from 60 conidia were injured by pricking with a tiny glass needle installed in a micromanipulator. We conducted five experiments to explore conidium infection processes, varying the number (zero to four) of injured colony-forming hyphae. We observed the haustoria in type I trichomes under a BX-60 LM instrument (Olympus), and counted the haustoria. The lengths of one to four colony-forming hyphae formed from each of the 60 conidia were measured separately using the KH-2700 DM and summed. Data were presented as means and SD of 20 samples (zero injured hyphae) and 10 samples (one to four injured hyphae). Standard curves were prepared by plotting total haustoria number against total hyphal length (zero to four injured hyphae).
Results

Comparable infection process of powdery mildew on both tomato leaf epidermal cells and type I trichomes

We monitored the process of KTP-04 infection on MM leaf epidermal and type I trichome cells. On the surface of both cells, KTP-04 conidia showed very high germination rates, exceeding 98% of inoculated conidia, and 40–50% of germ tubes grew downwards producing primary appressoria. The remaining conidia (50–60%) germinated upwards and germ tube elongation ceased completely within 24 h after germination, without forming primary appressoria on either epidermal cells (Fig. 1A) or type I trichomes (Fig. 1B). Next, we compared downward growing germ tubes. All conidia (100 conidia per plant cell) germinated on epidermal and type I trichome cells at 3–5 h and then produced primary appressoria at 6–8 h after inoculation, with germination rates of 97.8 ± 2.1% on epidermal cells and 98.1 ± 1.8% on type I trichomes. The rate of appressorium formation was 44.5 ± 5.1% on epidermal cells and 45.1 ± 4.7% on type I trichomes. Fungi produced elongating colony-forming hyphae from the conidia within 48 h after inoculation, and then successfully infected epidermal cells and type I trichomes. From a single conidium, and before hyphal development ceased completely at 12 days after inoculation, a maximum of five hyphae (containing germ tubes) formed on epidermal cells (Fig. 1C), whereas a maximum of four hyphae formed on trichomes (Fig. 1D). Conidiophores were produced on hyphae growing on epidermal cells (Fig. 1C), whereas no conidiophores formed on type I trichomes during the experimental period (Fig. 1D).

Powdery mildew haustoria in type I trichomes

KTP-04 haustoria were observed in trichome cells using the KH-2700 DM (Figs. 1D and 2A) and BX-60 LM instruments (Fig.
We then observed papilla-like structures produced beneath primary and hyphal appressoria in type I trichomes using the BX-60 EM instrument (arrows in Fig. 2C), and the functional haustorium formed in them using the SU8220 FE-SEM; the transparent globular structure of trichome cells is shown in Fig. 2A. As shown in Fig. 2D, trichome cells were penetrated through penetration pegs arising from hyphal appressoria. These were surrounded by papillae consisting of thinner, electron-dense and much thicker, electron-lucent callose-like materials. Penetration pegs continued their intracellular development and gave rise to haustoria. The fully developed haustorium consisted of a central body and several lobes embedded in a haustorial matrix surrounded by an extrahaustorial membrane. Large spherical structures (up to 1–1.5 µm in diameter) resembling vacuoles were seen within the central bodies and lobes of haustoria. The first functional haustoria were observed in trichome cells at 24–36 h after inoculation.

Length of powdery mildew hyphae on type I trichomes

The lengths of all hyphae formed from conidia were measured under the KH-2700 DM when hyphal growth completely stopped on type I trichomes after inoculation with KTP-04 conidia. The average total hyphal length was approximately 2,637.5 ± 1,157.0 µm at 12 days after inoculation (Table 1). Hyphal injury was performed as shown in Fig. 3. There was no significant difference in either hyphal length or haustorium number among elongated hyphae produced by injury and non-injury treatments (Fig. 4A–E; Table 1). When all four hyphal tips were injured, a new (fifth) hypha appeared from the conidium (Fig. 4G), or occasionally from a germ tube (Fig. 4H) or appressorium (Fig. 4I), at rates of 93, 5, and 2%, respectively. Breaking the tip of the fifth hypha by the same method did not result in the appearance of a sixth hypha from the conidium (Fig. 4F).

![Fig. 2](image-url)
Relationships between hyphal length and haustorium number on type I trichomes

When zero hyphae were injured, KTP-04 formed 3–14 functional haustoria in type I trichomes. As the number of injured hyphae increased, the total number of haustoria in trichomes generally decreased, with 4 to 10, 2 to 10, 1 to 9, and 2 to 6 haustoria produced following injury to one to four hyphae, respectively (Table 1). Total haustorium number was correlated with total hyphal length in each of the four injury experiments (Fig. 5). Hyphal length per haustorium was similar between injured and non-injured hyphae (Table 1).

**Discussion**

In this study, we observed germination of tomato powdery mildew isolate KTP-04 conidia on MM type I trichomes, and successfully analysed the infection processes on leaf epidermis and trichome cells. In our previous study (Takikawa et al. 2011b), we reported that the direction of germ tube projection from conidia was potentially determined after inoculation of tomato powdery mildew conidia onto host and non-host plant leaves, as well as artificial membranes (e.g. Parafilm). Suzuki et al. (2018) reported that the direction of germ tube projection is a unique characteristic among powdery mildew fungi; tomato and red clover powdery mildew pathogens have non-catenated conidia, and barley and melon powdery mildew pathogens have catenated conidia. These characteristics are important for successful host plant infection by powdery mildews. We found that among tomato powdery mildew pathogens that germinated downwards onto type I trichomes, nearly all conidia formed non-lobed appressoria on the trichome cells (see Fig. 2A), successfully infecting cells upon the first penetration attempt by appressoria (Suzuki et al. 2018). Thus, we confirmed that tomato powdery mildews were capable of producing appressoria and primary haustoria in type I trichomes, successfully infecting the cells and vigorously elongating hyphae by repeated cell invasion.

Only a few histological studies have been conducted to fully observe the steps of the infection processes of tomato

| Number of hyphae injured | Number of hyphae produced from conidia | Number of conidiophores | Number of haustoria | Total hyphal length (µm) | Hyphal length per haustorium (µm) |
|--------------------------|----------------------------------------|-------------------------|---------------------|--------------------------|-----------------------------------|
| 0                        | 4.0 ± 0.6<sup>a</sup>                  | 0<sup>a</sup>           | 7.6 ± 2.9<sup>a</sup> | 2637.5 ± 1157.0<sup>a</sup> | 342.4 ± 73.0<sup>a</sup>          |
| 1                        | 3.9 ± 0.3<sup>a</sup>                  | 0<sup>a</sup>           | 6.6 ± 2.1<sup>a</sup> | 2862.5 ± 1098.0<sup>a</sup> | 429.4 ± 68.4<sup>a</sup>          |
| 2                        | 3.9 ± 0.3<sup>b</sup>                  | 0<sup>a</sup>           | 5.8 ± 2.3<sup>ab</sup>| 2311.0 ± 1214.3<sup>ab</sup>| 388.1 ± 109.1<sup>ab</sup>        |
| 3                        | 4.0 ± 0.6<sup>b</sup>                  | 0<sup>b</sup>           | 3.2 ± 2.6<sup>b</sup> | 1134.6 ± 975.9<sup>b</sup>  | 371.8 ± 124.2<sup>b</sup>         |
| 4                        | 4.5 ± 0.5<sup>b</sup>                  | 0<sup>b</sup>           | 4.0 ± 1.4<sup>b</sup> | 1765.4 ± 865.5<sup>ab</sup> | 430.1 ± 116.8<sup>b</sup>         |

Different letters indicate a significant difference (<0.05, Tukey’s method).
Fig. 4 Process of KTP-04 infection on type I trichome cells observed under a KH-2700 DM instrument. Tips of hyphae (excluding germ tubes and appressoria) elongating from conidia on type I trichome cells were injured as shown in Fig. 3. Six treatment levels were set: (A) four uninjured colony-forming hyphae, (B) one of four hyphae injured, (C) two of four hyphae injured, (D) three of four hyphae injured, (E) all four hyphae injured, and (F) five hyphae injured. G, H, and I show the appearance of a fifth hypha (arrow) from a conidium, germ tube, and appressorium, at 5 days pi with KTP-04 conidia, respectively. The emergence of a fifth hypha from a germ tube or appressorium was much rarer. In A–E, hyphae vigorously elongated on the trichome until 12 days pi, then ceased hyphal elongation without forming conidiophores. In F, fungi did not form a sixth hypha from a conidium. Ap, appressorium; Co, conidium; Gt, germ tube. Bars represent 20 µm.
powdery mildew pathogens. These earlier studies focused on the morphology of pathogen features at the tomato leaf surface, including appressoria (Nonomura et al. 2010; Lebeda et al. 2014) and conidiophores (Whipps et al. 1998; Dyki 2003; Oichi et al. 2004, 2006). In the present study, we examined haustorial formation in powdery mildew-infected trichomes, because these are the most important infection structures for nutrient uptake from plant cells. To our knowledge, no studies of tomato powdery mildew haustoria have described their shapes and characteristics within infected plant cells in detail. Braun (1987) and Zeller (1995) reported that powdery mildew pathogen haustorial types are broadly divided into two groups, globose and digitate. Saenz and Taylor (1999) described *Erysiphe* haustoria as globose. LM observations of haustorial shapes have revealed round complexes 15–24 µm in diameter (Dyki and Staniaszek 1997; Li et al. 2012), circular complexes (LaMondia et al. 1999), and spherical to sac-like complexes 5–25 µm in diameter (Whipps et al. 1998). Segarra et al. (2009) examined micrograph sections of tomato powdery mildew (*Erysiphe polygoni*) haustorial bodies within tomato plant (cv. Roma) adaxial epidermal cells using TEM. However, they did not describe the haustorial shapes or characteristics in detail. In the present study, we applied DM, LM, EM and SEM to study infected trichomes, and confirmed previous reports of *P. neolycopersici* haustoria in trichomes at 24–36 h post-inoculation. Our results support the TEM findings of Segarra et al. (2009). Interestingly, tomato powdery mildew fungi formed functional mature haustoria (primary haustoria beneath appressoria and haustoria beneath hyphal appressoria) in a single trichome cell. Dyki and Staniaszek (1997) observed up to three haustoria within one cell. We observed up to six functional haustoria within a single trichome cell (data not shown); thus, appressorial formation allows fungi to produce functional haustoria within trichomes. Recently, Micali et al. (2011) demonstrated that papillae produced in epidermal cells of *Arabidopsis thaliana* infected with the epiphytic powdery mildew *Golovinomyces orontii* contain callose (β-1,3-glucan). Because the papillae examined in this work were morphologically similar to those observed in the *Arabidopsis–Golovinomyces* interaction (see Fig. 2D), we surmise that electron-lucent materials observed around penetration pegs are also made of callose. Also similar to the *Arabidopsis–Golovinomyces* interaction, membranes and vesicular structures were observed in the callose layer surrounding penetration pegs, as well as callose encasement of haustoria (see Fig. 2D); however, this structure was not as prominent as that seen in *Arabidopsis* epidermal cells invaded by *G. orontii* haustoria (Micali et al. 2011).

In our previous studies, we clarified that tomato leaf type I trichome and epidermal cells were able to induce the same response to fungal invasion i.e. papilla in response to *E. trifoliorum* from red clover and HR in response to *P. xanthii* from melon (Suzuki et al. 2018). We simultaneously monitored powdery mildew infection processes and trichome cytological responses using the KH-2700 DM and BX-60 LM instruments without histochemical staining (Suzuki et al. 2018). Following these observations, we suggest that trichome cells invaded by non-pathogenic and pathogenic powdery mildews are useful for the study of molecular interactions between plants and powdery mildews at the scale of a single trichome cell. It was much easier to collect only powdery mildew-inoculated single trichome cells at different powdery mildew developmental stages than on leaf epidermal cells. Little is known of the genetic factors and molecular mechanisms associated with non-host resistance, plant immunity to potential pathogens (Ellis 2006), and host...
susceptibility. Our knowledge of non-host resistance is based on the zigzag model of plant immunity (Jones and Dangl 2006). Schweizer (2007) subsequently proposed two models for non-host resistance. The first of these postulates the absence of fungal effectors, leading to a non-compromised basal defence response due to the interaction between plant pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs); the second postulates non-host resistance (R)-genes and host susceptibility caused by fungal effectors. To further investigate the molecular mechanisms of non-host resistance and host susceptibility, dynamic cellular changes should be studied at the transcriptomic and/or proteomic level. In such studies, it is crucial to avoid intracellular contamination of differently responding plant cells and fungal materials. Therefore, the isolation of intracellular contents using powdery mildew-inoculated trichome cells by micropipette manipulation (Fujita et al. 2004) and laser microdissection (Chandran et al. 2010) will facilitate analysis of the molecular mechanisms of powdery mildew infections, such as differential gene expression during the establishment and proliferation stages of infection. Techniques developed for foliar trichomes can thus be used to study plant–pathogen interactions and communication at the level of individual trichome cells.

It is impossible to directly observe haustoria in tomato leaves without removing chlorophyll from leaf epidermal cells to confirm whether haustoria were formed beneath the hyphal appressoria. However, using the microscopic techniques applied in this study, we successfully observed haustoria in trichome cells. We also reported, for the first time, the relationship between the number of powdery mildew haustoria and total hyphal length, and demonstrated no significant effect of hyphal injury on hyphal length per haustorium. Our results indicate that trichome cells can be used to further study tomato host responses to pathogenic powdery mildews and host susceptibility to powdery mildew fungal effectors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Braun U (1987) A monograph of the Erysiphales (powdery mildews). Beih Nova Hedwig 89:1–700
Braun U, Shin HD, Takamatsu S, Meeboon J, Kiss L, Lebeda A, Kittner M, Götz M (2019) Phylogeny and taxonomy of Golovinomyces orontii revisited. Mycol Prog 18:335–357
Chandran D, Inada N, Hather G, Kleindt CK, Wildermuth MC (2010) Laser microdissection of Arabidopsis cells at the powdery mildew infection site reveals site-specific processes and regulators. Proc Natl Acad Sci U S A 107:460–465
Duffey SS (1986) Plant glandular trichomes: their partial role in defense against insects. In: Juniper BE, Southwood TE (eds) Insects and the plant surface. Arnold, London, pp 151–172
Dyk B (2003) Morphogenesis of pathogen causing powdery mildew in Polish cultivation of tomato. Veg Crop Res Bull 59:131–138
Dyk B, Staniszeck M (1997) Infection of tomato by Oidium lycopersicum (Cook & Massere, emend. Noordeloos & Loerakker). Phytopathol Pol 13:13–17
Ellis J (2006) Insights into nonhost disease resistance: can they assist disease control in agriculture? Plant Cell 18:523–528
Fujita K, Matsuda Y, Wada M, Hirai Y, Mori K, Morimura N, Nonomura T, Kakutani K, Toyoda H (2004) Powdery mildew pathogens can suppress the chitinase gene expression induced in detached inner epidermis of barley coleoptile. Plant Cell Rep 23:504–511
Glover BJ (2000) Differentiation in plant epidermal cells. J Exp Bot 51:497–505
Huang CC, Groot T, Meijer-Dekens F, Nikes RE, Lindhout P (1998) The resistance to powdery mildew (Oidium lycopersicum) in Lycopersicon species is mainly associated with hypersensitive response. Eur J Pl Pathol 104:399–407
Jacob D, David DR, Sztjenberg A, Elad Y (2008) Conditions for development of powdery mildew of tomato caused by Oidium neolycopersici. Phytopathology 98:270–281
Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329
Jones H, Whipp JM, Gurr SJ (2001) The tomato powdery mildew fungus Oidium neolycopersici. Mol Plant Pathol 2:303–309
Kang J-H, Liu G, Shi F, Jones AD, Beaudry RM, Howe GA (2010) The tomato odorless-2 mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. Plant Physiol 154:262–272
Kashimoto K, Matsuda Y, Matsutani K, Sameshima T, Kakutani K, Nonomura T, Okada K, Kusakari S, Nakata K, Takamatsu S, Towamura H (2003) Morphological and molecular characterization for a Japanese isolate of tomato powdery mildew Oidium neolycopersici and its host range. J Gen Plant Pathol 69:176–185
Kennedy GG, Sorenson CF (1985) Role of glandular trichomes in the resistance of Lycopersicon hirsutum f. glabratum to Colorado potato beetle (Coleoptera: Chrysomelidae). J Econ Entomol 78:547–551
Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141–2144
Kiss L, Cook RTA, Saenz GS, Cunninjgh JT, Takamatsu S, Pascoe I, Bardin M, Nicot PC, Sato Y, Rossman AY (2001) Identification of two powdery mildew fungi, Oidium neolycopersici sp. nov. and O. lycopersici, infecting tomato in different parts of the world. Mycol Res 105:684–697
Koga D, Kusumi S, Shodo R, Dan Y, Ushiki T (2015) High-resolution imaging by scanning electron microscopy of semithin sections in correlation with light microscopy. Microscopy 64:387–394
LaMondia JA, Smith VL, Douglas SM (1999) Host range of Oidium lycopersicum on selected Solanaceous species in Connecticut. Plant Dis 83:341–344
Lebeda A, Mieslerová B, Petřivalský M, Luhová L, Spudová M, Sedlářová M, Nožková-Hlaváčková V, Pink DAC (2014) Resistance mechanisms of wild tomato germplasm to infection of Oidium neolycopersici. Eur J Pl Pathol 138:569–596
Lemke CA, Mutschler MA (1984) Inheritance of glandular trichomes in Lycopersicon esculentum and L. pennellii. J Am Soc Hort Sci 109:592–596
Li C, Bonnema G, Che D, Dong L, Lindhout P, Visser R, Bai Y (2007) Biochemical and molecular mechanisms involved in monogenic...
resistance responses to tomato powdery mildew. Mol Plant Microbe Interact 20:1161–1172

Li C, Faino L, Dong L, Fan J, Kiss L, de Giovanni C, Lebeda A, Scott J, Matsuda Y, Toyoda H, Lindhout P, Visser RGF, Bonnema G, Bai Y (2012) Characterization of polygenic resistance to powdery mildew in tomato at cytological, biochemical and gene expression level. Mol Plant Pathol 13:148–159

Lindeberg M, Cunnac S, Collmer A (2012) *Pseudomonas syringae* type III effector repertoires: last words in endless arguments. Trends Microbiol 20:199–208

Matsuda Y, Kashimoto K, Takikawa Y, Aikami R, Nonomura T, Toyoda H (2001) Occurrence of new powdery mildew on greenhouse tomato cultivars. J Gen Plant Pathol 67:294–298

Matsuda Y, Sameshima T, Morura N, Inoue K, Nonomura T, Kakutani K, Nishimura H, Kusakari S, Takamatsu S, Toyoda H (2005) Identification of individual powdery mildew fungi infecting leaves and direct detection of gene expression by single conidium polymerase chain reaction. Phytopathology 95:1137–1143

McDowell ET, Kapteyn J, Schmidt A, Li C, Kang JH, Descour A, Shi F, Micali CO, Neumann U, Grunewald D, Panstruga R, OConnell R (2011) Comparative functional genomic analysis of *Solanum* glandular trichome types. Plant Physiol 155:524–539

Micali CO, Neumann U, Grunewald D, Panstruga R, OConnell R (2011) Biogenesis of a specialized plant-fungal interface during host cell internalization of *Golovinomyces orontii* haustoria. Cell Microbiol 13:210–226

Nonomura T, Matsuda Y, Kakutani K, Takikawa Y, Toyoda H (2008) Physical control of powdery mildew (*Oidium neolycopersici*) on tomato leaves by exposure to corona discharge. Can J Plant Pathol 30:517–524

Nonomura T, Matsuda Y, Xu L, Kakutani K, Takikawa Y, Toyoda H (2009a) Collection of highly germinative pseudochain conidia of *Oidium neolycopersici* from conidiophores by electrostatic attraction. Mycol Res 113:364–372

Nonomura T, Xu L, Wada M, Kawamura S, Miyajima T, Nishitomi A, Kakutani K, Takikawa Y, Matsuda Y, Toyoda H (2009b) Trichome exudates of *Lycopersicon pennellii* form a chemical barrier to suppress leaf-surface germination of *Oidium neolycopersici* conidia. Plant Sci 176:31–37

Nonomura T, Nishitomi A, Matsuda Y, Soma C, Xu L, Kakutani K, Takikawa Y, Toyoda H (2010) Polymorphic change of appressoria to detect leaf-surface germination. Mycoscience 52:204

Nonomura T, Matsuda Y, Yamashita S, Akahoshi H, Takikawa Y, Kakutani K, Toyoda H (2013) Natural woody plant, *Malloittus japonicus*, as an ecological partner to transfer different pathogenic conidia of *Oidium neolycopersici* to greenhouse tomatoes. Plant Prot Sci 49:S33–S40

Oichi W, Matsuda Y, Sameshima T, Nonomura T, Kakutani K, Nishimura H, Kusakari S, Toyoda H (2004) Consecutive monitoring for conidiogenesis by *Oidium neolycopersici* on tomato leaves with a high-fidelity digital microscope. J Gen Plant Pathol 70:329–332

Oichi W, Matsuda Y, Nonomura T, Toyoda H, Xu L, Kusakari S (2006) Formation of conidial pseudochains by tomato powdery mildew *Oidium neolycopersici*. Plant Dis 90:915–919

Peter AJ, Shanower TG (1998) Plant glandular trichomes: Chemical factories with many potential uses. Resonance 3:41–45

Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles; perfumes for pollinator attraction and defense. Curr Opin Plant Biol 5:237–243

Saenz GS, Taylor JW (1999) Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. Can J Bot 77:150–168

Sameshima T, Kashimoto K, Kida K, Matsuda Y, Nonomura T, Kakutani K, Nakata K, Kusakari S, Toyoda H (2004) Cytological events in tomato leaves inoculated with conidia of *Blumeria graminis* f. sp. *hordei* and *Oidium neolycopersici* KTP-01. J Gen Plant Pathol 70: 7–10

Schweizer P (2007) Nonhost resistance of plants to powdery mildew – new opportunities to unravel the mystery. Physiol Mol Plant Pathol 70:3–7

Segura G, Reis M, Casanova E, Trillas MI (2009) Control of powdery mildew (*Erysiphe polygoni*) in tomato by foliar applications of compost tea. J Plant Pathol 91:683–689

Seifi A, Nonomura T, Matsuda Y, Toyoda H, Bai Y (2012) An avirulent tomato powdery mildew isolate induces localized acquired resistance to a virulent isolate in a spatiotemporal manner. Mol Plant Microbe Interact 25:372–378

Shimizu K, Matsuda Y, Nonomura T, Ikeda H, Tamura N, Kusakari S, Kimbara J, Toyoda H (2007) Dual protection of hydroponic tomatoes from rhizosphere pathogens *Ralstonia solanacearum* and *Fusarium oxysporum* f. sp. radicis-lycopersici and airborne conidia of *Oidium neolycopersici* with an ozone-generative electrostatic spore precipitator. Plant Pathol 56:987–997

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. Agric For Entomol 7:265–276

Suzuki T, Murakami T, Takizumi Y, Ishimaru H, Kudo D, Takikawa Y, Matsuda Y, Kakutani K, Bai Y, Nonomura T (2018) Trichomes: interaction sites of tomato leaves with biotrophic powdery mildew pathogens. Eur J Pl Pathol 150:115–125

Takikawa Y, Kakutani K, Nonomura T, Matsuda Y, Toyoda H (2011a) Conidia of *Erysiphe trifoliorum* attempt penetration twice during a two-step germination process on non-host barley leaves and an artificial hydrophobic surface. Mycoscience 52:204–209

Takikawa Y, Xu L, Kakutani K, Nonomura T, Sameshima T, Matsuda Y, Toyoda H (2011b) Conidia of the tomato powdery mildew *Oidium neolycopersici* initiate germ tubes at a predetermined site. Mycoscience 52:198–203

Takikawa Y, Nonomura T, Miyamoto S, Okamoto N, Murakami Y, Kakutani K, Kida K, Toyoda H (2015) Digital microscopy analysis of developmental process of conidiogenesis by powdery mildew pathogens isolated from melon leaves. Phytoparasitica 43:517–530

Tooker JF, Peiffer M, Luthe DS, Felton GW (2010) Trichomes as sensors detecting activity on the leaf surface. Plant Signal Behav 5:73–75

Whipp JM, Budge SP, Fenlon JS (1998) Characteristics and host range of tomato powdery mildew isolate induces localized acquired resistance to powdery mildew pathogens. Phytoparasitica 26:199–209

White JF Jr, Johnston SA, Wang CL, Chin CK (1997) First report of powdery mildew in greenhouse-grown tomatoes in New Jersey. Plant Dis 81:227

Zeller KA (1995) Phylogenetic relatedness within the genus *Erysiphe* estimated with morphological characteristics. Mycologia 87:525–531

Zheng Z, Nonomura T, Bóka K, Matsuda Y, Visser RGF, Toyoda H, Kiss L, Bai Y (2013) Detection and quantification of *Leveillula taurica* growth in pepper leaves. Phytopathology 103:623–632

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