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Original Article

Histological description of *Exobasidium vexans* infection on tea leaves (*Camellia sinensis*)

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Abstract

Exobasidium vexans Massee is a parasitic fungus that causes tea blister blight. Tea blister blight is characterized by swelling of infected spots on tea leaves. To date, there is limited information on the cellular alterations that occur during *E. vexans* infection. This study aimed to observe the histological changes on tea leaves infected by *E. vexans*. Diseased and healthy leaves were thin-sliced by a cryostat microtome and examined by light microscopy. Observations on the thin sections revealed the mode of hymenium development in *E. vexans* in which the basidia protruded between the epidermal cells. The infection caused hypertrophy as the size of the cells at the infected spots became double the size of the healthy cells. We also report the first description of venous infections of *E. vexans*. The proliferation of the fungus on the vein resulted in the formation of hymenium on both the lower and upper sides of the leaves and there was complete disruption of the vascular bundle in the leaf veins. Blister blight disease was found to be moderately severe at the tea plantation in this study. Morphological and molecular identification confirmed that the fungus isolated from the symptomatic leaves was *E. vexans*.

Keywords: disease severity, leaf galls, cell differentiation, mesophyll, phloem, plant parasite

1. Introduction

Tea is one of the most frequently consumed drinks in the world. The leaves of the tea plant (*Camellia sinensis* (L.) Kuntze) are processed and dried for subsequent brewing to prepare the drink. *Exobasidium vexans* Massee is a pathogenic plant fungus that infects tea plants and causes tea blister blight. The spread of this disease has brought tremendous loss to the tea production industry and it has become a serious threat to all tea plantations across Asia including India, Sri Lanka, Japan, and Indonesia (Baby, 2002). As the number one tea producer to the global market, India has about 5 million hectares of land dedicated to tea plantations (Boriah, 2002). The country which had contributed up to 23% of the total world tea production suffered a great loss due to tea blister blight as the pathogen attacks harvestable young shoots resulting in up to 40% yield losses (Basu, Bera, & Rajan, 2010; Gulati, Gulati, Ravindranath, & Chakrabarty, 1993). In addition, tea blister blight inflicted a severe crop loss in Sri Lanka in which 33% of yield reduction was recorded in unprotected areas compared to fields which were sprayed with chemicals (de Silva, Murugiah, & Saravapavan, 1997). A transgenic tea plant resistant to *E. vexans* infection was developed in which the clone expresses a higher amount of chitinase enzyme to hydrolyse the cell wall of the invading fungus (Jayaswall *et al*., 2016; Jeyaramraja, Pius, Manian, & Nithya Meenakshi, 2005).

Symptoms of *E. vexans* infection include the swelling and formation of conspicuous white hymenium on the infected spots that will eventually necrotize. Several studies have been done on the histology of plants infected by other *Exobasidium* species. According to Mims and Richardson (20 07) and Lee, Lee, Shin, Park, and Sieber (2015), the infection of *Exobasidium gracile* (Shirai) Syd, and P. Syd on *Camellia sasanqua* Thunb. was characterized by the formation of a distinct pseudoparenchymatous layer of intercellular hyphae which initially developed several layers above the lower epidermis. Eventually the lower epidermis layer sloughed off to give rise to the white hymenium on the underside of the leaf (Mims & Richardson, 2007; Lee *et al*., 2015). Meanwhile, Li

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and Guo (2008) described a different mode of hymenium development in the infection caused by *Exobasidium ovalifoliae* Z. Y. Li and L. Guo in which the basidia protruded between epidermal cells to form continuous white hymenium upon reaching the final stage of infection. Gadd and Loos (1949) reported that the formation of hymenium in *E. vexans* progressed in a similar manner but they only provided an illustration to support their observation.

At the cellular level, the enlargement of the infected spots caused by an infection of *Exobasidium* species is either due to hypertrophy or hyperplasia – an increase in the number of the cells. For example, Graafland (1960) reported that both hypertrophy and hyperplasia occurred in the infection of *Exobasidium japonicum* Shirai on the leaves of Azaleas. Meanwhile, the flowers of *Lyonia ferruginea* (Walter) Nutt. that were infected with *Exobasidium ferrugineae* Minnis, A.H. Kennedy & N.A. Goldberg only became hypertrophied but this observation was less common in the leaves of the plant (Kennedy, Goldberg, & Minnis, 2012).

Several methods have been developed to measure the severity of fungal diseases on tea plants. Webster and Park (1956) first evaluated the severity of *E. vexans* infection based on the disease incidence from a pool of sampled leaves. Meanwhile Balasuriya (2003) assessed blister blight infection according to the number of blistered lesions on tea leaves. Recently, Sinniah, Wasantha Kumara, Karunajeewa, and Ranatunga (2016) developed an improvised assessment key to evaluate the severity of *E. vexans* infection on tea leaves that accounted for both host reactions and the sequential stages of symptom development.

To date, there have been no detailed descriptions on the histological aspects of *E. vexans* infection on *C. sinensis* except the illustration provided by Gadd and Loos (1949). Hence, the aim of this study was to provide a deeper understanding on the histological changes that occur in *C. sinensis* leaf cells upon *E. vexans* infection. The mode of hymenium development and the effect of *E. vexans* infection on the architectural state of the leaf cells were examined. In addition, microscopic descriptions of the venous infection are also presented. The disease severity of *E. vexans* infection in the tea plantation of this study was assessed and the morphology of basidia and basidiospores of this fungus are described. Finally, a phylogenetic tree was constructed based on the internal transcribed spacer (ITS) gene to assess the position of *E. vexans* relative to other *Exobasidium* species.

2. Materials and Methods

2.1 Histology of *E. vexans* infection

Healthy and infected shoots of tea leaves were collected from MARDI Agro-technology Park, Cameron Highlands, Malaysia in August 2016 and preserved in formalin acetic acid solution. The vein and lamina of both healthy and infected spots were embedded in Jung Tissue Freezing Medium (Leica Microsystems, Nussloch, Germany). The samples were then transversely thin-sliced by a cryostat microtome at -20 °C at 10 µm thicknesses (Leica CM 1850 UV, Nussloch, Germany). The thin sections were viewed under light microscope (Olympus BX53, Tokyo, Japan) equipped with a camera (Olympus DP72, Tokyo, Japan). The cell sizes of healthy and infected leaves were measured and statistically analyzed by Mann-Whitney U test.

2.2 Disease severity

Disease severity of *E. vexans* infection at a tea plantation in MARDI Agro-technology Park, Cameron Highlands, Malaysia was assessed according to the assessment key developed by Sinniah *et al.* (2016). Thirty tea shoots showing symptoms of *E. vexans* infection were collected and the severity of infection on the third leaf of each shoot was rated according to the following assessment key. A 0 to 6 point scoring system was used according to the following conditions: (0) No translucent spots (TL) or no disease; (1) all TL showed hypersensitive reaction; (2) TL covered <2% of the leaf area; (3) blisters or necrosis covering or both <5% of the total leaf area or TL covered 2-15% of the leaf area; (4) blisters or necrosis or both covered 5-15% of the total leaf area or TL covered >15% of the leaf area; (5) blisters or necrosis or both covered 15-30% of the leaf area; and (6) blisters or necrosis or both covered >30% of the leaf area or stem infections (Sinniah *et al*., 2016).

2.3 Morphological and molecular identification

Morphological observation of the basidia and basidiospores of *E. vexans* was done on fresh specimens according to Nagao, Akimoto, Kishi, Ezuka, and Kakishima (2003a). Hymenia that had formed on blistered spots were scratched and mounted in Shear's solution on a glass slide. The basidia and basidiospores were observed and measured under a light microscope.

Molecular identification was done on a culture of *E. vexans* that was established according to the method described by Nagao *et al*. (2003a). In brief, the infected spot on a tea leaf was cut into 2 mm^2 pieces and stuck onto a 10 mm^2 agar block on the lid of a petri dish. The hymenium of the infected spot was set to face the potato dextrose agar (PDA) surface. The petri dish was incubated at 22 °C in the dark, overnight to collect the released basidiospores. The basidiospores were then allowed to germinate and the *E. vexans* colony formed after 1 month of culturing. DNA extraction was adapted from the method by Suyama *et al*. (1996). The extraction solution was composed of 0.01% SDS, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% proteinase K, and 10 mM Tris-HCL (pH 8.3 at 20 °C). Small clumps of *E. vexans* colony were added to 20 µL of the extraction solution and incubated for 60 min at 37 °C, followed by heating at 95 °C for 10 min. After the incubation process, the extraction solution that contained the extracted DNA was used as the template for downstream polymerase chain reaction (PCR).

Two genes were amplified in this experiment. The large subunit (LSU) of the nuclear ribosomal RNA gene was amplified using the NL1 (5'- GCA TAT CAA TAA GCG GAG GAA AAG -3') and NL4 (5'- GGT CCG TGT TTC AAG ACG G-3') primers (O'Donnell 1992, 1993). Amplification was done according to the following cycle: Initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 60 °C for 1 min, extension at 72 °C for 90 s, and a final extension at 72 °C for 10 min. Meanwhile, the ITS gene was amplified using the ITS1F (5'- CTT

GGT CAT TTA GAG GAA GTA A-3') and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3') primers according to the following cycle: Initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min (White, Bruns, Lee, & Taylor, 1990; Gardes & Bruns, 1993; Virtudazo, Nakamura, & Kakishima, 2001). The PCR mixture to amplify the two genes consisted of 20 mM of each dNTP, 1.5 mM $MgCl_2$, 4 µL of extracted DNA, and 0.2 µM of each ITS1F/ITS4 and NL1/NL4 primers. PCR products were sent to a service provider for sequencing.

A phylogenetic tree was constructed using the ITS sequence of *E. vexans* obtained from this study and other ITS sequences of *Exobasidium* species available in GenBank (Table 1). The sequences were aligned and neighbor-joining (NJ) tree was constructed using the MEGA 7 software (Kumar, Stecher, & Tamura, 2016). The stability of clades was evaluated by generating 1000 bootstrap replicates and distance analyses were computed using the Maximum Composite Likelihood method. Gaps and missing data were completely deleted from the dataset.

3. Results

3.1 Histology of *E. vexans* infection on tea leaves

Leaves with mature *E. vexans* infection appeared swollen and the presence of white-velvety hymenium was observed in the lower epidermis. Meanwhile, if the infection occurred on the veins, the hymenium could be observed on both lower and upper epidermis (Figures 1A and 1B). The leaf lamina that was close to the infected veins also had the presence of the hymenium in both lower and upper epidermis. Infection by *E. vexans* was normally localised and eventually caused necrosis of the infected spots.

In the case of the hymenium developing on the underside of the leaf (Figure 1B), the structure of the lower epidermis was completely disrupted and filled with masses of slender hyphae and basidia (Figure 2B). The air cavities that are normally present in a healthy leaf were filled with networks of intercellular hyphae in the infected leaf (Figure 2B). As the infection reached its final stage, intracellular hyphae could also be observed in the infected leaf cells. The Mann-Whitney U test revealed that the sizes of the cells in the spongy mesophyll layer were significantly greater in the infected leaf (Mdn=38.56 µm) than the healthy leaf (Mdn=19.23 µm), U=510 ($P<0.05$). However, the structure of the upper epidermis and the palisade mesophyll of the infected leaf (Figure 2B) appeared similar to the ones in the healthy leaf (Figure 2A).

Infection on the vein of the leaf resulted in the proliferation of hymenium on both lower and upper side of the leaf (Figures 1A and 1B). In this case, both lower and upper epidermis of the leaf were completely absent and filled with exposed, slender hyphae which developed to become basidia that bore basidiospores (Figures 3A and 3B). The sclerenchyma layer in the infected vein (Figure 4B) was completely ruptured. Meanwhile, the normally organised and intact structure of the xylem and phloem became disrupted and the intercellular spaces were tightly packed with the proliferation of intercellular hyphae (Figures 4A and 4B).

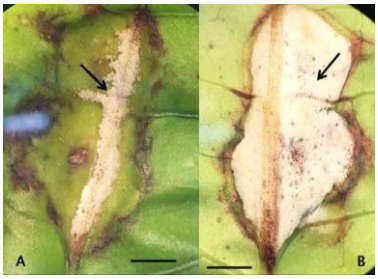

Figure 1. *E. vexans* infection on *C. sinensis* leaf. A. Upper side of the leaf with white and velvety hymenium (arrow) formed on the vein. B. Lower side of the same leaf in (A) with hymenium (arrow) formed on both of the vein and the leaf lamina. Bar = 5 mm

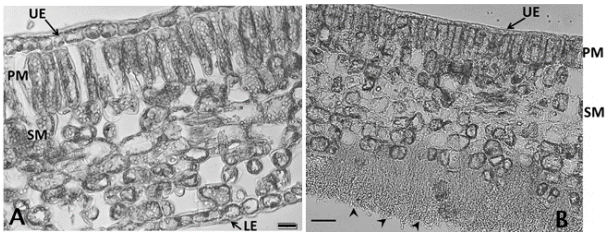

Figure 2. Transverse section of healthy and infected leaf of *C. sinensis*. A. Healthy leaf with normally differentiated palisade (PM) and spongy mesophyll (SM) layer. Lower epidermis (LE) and air cavities are present and intact. Bar = 20 µm. B. Infected leaf with normally differentiated palisade mesophyll but hypertrophied spongy mesophyll cells that were double the size of healthy spongy mesophyll. Lower epidermis were absent and basidia were protruding between the cells (arrowhead). Bar = 50 µm.

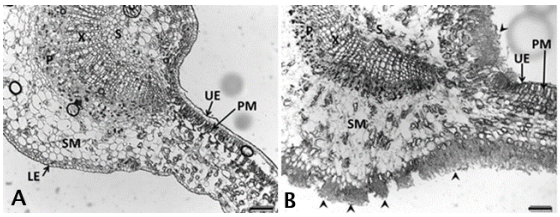

Figure 3. Transverse section of healthy and infected vein of *C. sinensis*. A. Healthy vein with intact and normally organized sclerenchyma (S), xylem (X), and phloem (P). B. Infected vein with hymenium (arrowhead) proliferation on upper epidermis (UE) and lower epidermis (LE). The structure of sclerenchyma, xylem, and phloem were disrupted. Spongy mesophyll (SM), palisade mesophyll (PM). Bar = 100 µm.

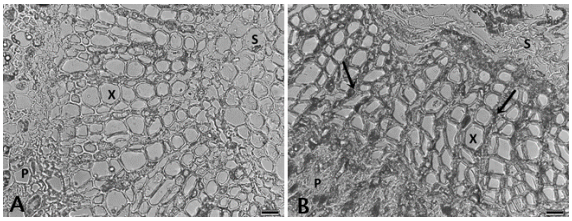

Figure 4. Enlarged images of healthy and infected vascular bundle of *C. sinensis* in Figure 3. A. Intact and normally organized sclerenchyma (S), xylem (X), and phloem (P). B. Infected vascular bundle with collapsed sclerenchyma (S) and phloem (P) with intercellular hyphae (arrow) present in the xylem (X). Bar = 20 µm.

Table 1. List of *Exobasidium* species used to construct the phylogenetic tree with their respective hosts, isolate/strain, locations, GenBank accession numbers, and sources.

Species	Isolate/ strain	Host	Location	Source	GenBank ITS accession number
E. vexans	MC32016	*C. sinensis*	Cameron Highlands Malaysia	This study	MG827276
E. vexans	GZZCB	*C. sinensis*	China	GenBank, submitted by L. Peng in 2013	KC442794.1
E. gracile (Shirai) Syd. & P. Syd	CB	Camellia oleifera Abel.	China	GenBank, submitted by L. Peng in 2010	HQ398622.1
E. camelliae Shirai	MAFF 238578	*Camellia japonica* L.	Japan	GenBank, submitted by H. Nagao in 2004	AB180317.1
E. reticulatum S. Ito & Sawada	IFO30393	*C. sinensis*	Japan	Nagao *et. al* in 2004	AB180369.1
E. nobeyamense Nagao & Ezuka	MAFF 239439	*Rhododendron wadanum* Makino	Japan	GenBank, submitted by H. Nagao in 2004	AB180375.1
E. cylindrosporum Ezuka	MAFF 238663	*R. x mucronatum*	Japan	GenBank, submitted by H. Nagao in 2004	AB180357.1
E. japonicum Shirai	MAFF 238591	*Rhododendron obtusum var. kaempferi* Planch.	Japan	GenBank, submitted by H. Nagao in 2004	AB180326.1
E. woronchinii Nagao	MAFF 238610	*Rhododendron brachycarpum* D. Don ex G. Don	Japan	GenBank, submitted by H. Nagao in 2004	AB180342.1
E. otanianum Ezuka emend. Nagao	MAFF 238613	*Rhododendron reticulatum* D.Don ex G.Don f. *glabrescens* (Nakai & H.Hara) T.Yamaz.	Japan	GenBank, submitted by H. Nagao in 2004	AB180345.1
E. shiraianum Henn.	MAFF 238602	*Rhododendron degronianum* Carrière	Japan	GenBank, submitted by H. Nagao in 2004	AB180336.1
E. rhododendrii (Fuckel) C.E. Cramer	Voucher RBG Kew K(M)102494	-	England	GenBank, submitted by P.M. Brock in 2009	EU784219.1
E. symploci japonicae Kusano & Tokubuchi var. *carpogenum* Nagao & S. Ogawa	MAFF 238620	*Symplocos lucida* (Thunb.) Siebold & Zucc.	Japan	GenBank, submitted by H. Nagao in 2004	AB180351.1
E. symploci-japonicae Kusano & Tokubuchi	MAFF 238811	*Symplocos lucida* (Thunb.) Siebold & Zucc.	Japan	GenBank, submitted by H. Nagao in 2004	AB180678.1
E. kishianum Nagao & Ezuka	MAFF 238623	*Vaccinium hirtum* Thunb. var. *pubescens* (Koidz.) T. Yamaz.	Japan	GenBank, submitted by H. Nagao in 2004	AB180353.1
E. rostruspii Nannf.	CNJ2-3	*Vaccinium macrocarpon* Ait.	USA	GenBank, submitted by J. E. Stewart in 2015	KR262425.1
E. bisporum Sawada ex Ezuka	IFO9942	*Eubotryoides grayana* (Maxim.) H. Hara var. *glabra* (Komatsu) H. Hara	Japan	GenBank, submitted by H. Nagao in 2004	AB180364.1
E. vacinii (Fuckel) Woronin	MAFF 238668	*Vaccinium vitis-idaea* L.	Japan	GenBank, submitted by H. Nagao in 2004	AB180362.1
E. inconspicuum Nagao & Ezuka	MAFF 238616	*Vaccinium hirtum* Thunb. *var. pubescens* (Koidz.) T. Yamaz	Japan	GenBank, submitted by H. Nagao in 2004	AB180347.1
Tilletiopsis washingtonensis Nyland	PB 383 18S	contaminant from an *Exobasidium* infection on *Vaccinium uliginosum* L.	Germany	GenBank, submitted by H. Doering in 2005	DQ025483.1

3.2 Disease severity

Eighty percent of the sampled leaves in this study showed apparent symptoms of *E.* vexans infection which scored ≥3 points according to the assessment key by Sinniah *et al*. (2016). Meanwhile, 16.7% of the samples scored 6 points as the necrotic spots were observed to cover >30% of the leaf area (Table 2). The overall disease severity index (DSI) for tea plantation in this study was 2.07 according to the equation by Sinniah *et al*. (2016).

Table 2. Number of symptomatic host leaves as rated according to the blister blight severity assessment key by Sinniah *et al*. (2016). TL = Translucent spots

Disease severity score	Number of leaves
0 (No TL or no disease)	1
1 (all TL show hypersensitive reaction)	2
2 (TL cover <2% leaf area)	4
3 (Blisters and/or necrosis covering <5% of total leaf area, or TL covering 2-15% leaf area)	7
4 (Blisters and/or necrosis covering 5-15% of total leaf area or TL covering >15% leaf area)	4
5 (Blisters and/or necrosis covering 15-30% leaf area)	7
6 (Blisters and/or necrosis covering >30% leaf area or stem infections)	5

3.3 Morphological identification

The hymenium of *E. vexans* consisted of clavate to cylindrical basidia (46.98-86.42 µm × 4-5 µm) that generally had 2 sterigmata. Basidiospores (12.82-16.07 µm × 3.3-3.8 µm) were hyaline, aseptate, and ellipsoid. Upon maturation, basidiospores can develop a single septation.

3.4 Molecular identification and phylogenetic tree

Amplification of the LSU and ITS genes of *E. vexans* yielded PCR products of size 556 bp and 586 bp respectively. The LSU and ITS sequences were deposited in GenBank database with accession numbers MG827275 and MG827276, respectively. BLAST search for the LSU sequence showed 99% similarity with several *E. vexans* sequences (AB180380.1, AB262789.1). Meanwhile the ITS sequence matched 100% (KC493154.1) and 99% (KY038487.1) with *Exobasidium reticulatum*. However, the ITS sequence obtained from this study did not match with an *E. vexans* ITS sequence that was readily available in the database (KC442 794.1).

The NJ tree in Figure 5 was constructed based on the ITS sequence analysis. The NJ tree of *Exobasidium* species was divided into four clades according to specific host groups. *E. vexans* isolated from this study belonged in the *Camellia* clade together with *E. reticulatum* S Ito & Sawada, *E. gracile* and *Exobasidium camelliae* Shirai. The separation of this clade from the other clades (*Rhododendron, Vaccinium*, and *Symploci*) was supported by a high NJ bootstrap value of 84%. Nonetheless, the '*E. vexans* KC442794.1' sequence did not belong in the *Camellia* clade, instead it was found to be clustered with the outgroup of this NJ tree (*Tilletiopsis washingtonensis*).

4. Discussion

Observations on the mode of hymenium development during *E. vexans* infection on *C. sinensis* were coherent with the illustration provided by Gadd and Loos (1949) in which basidia protruded through the intercellular spaces of the lower epidermis. This finding was similar to the mode of hymenium development in the majority of other *Exobasidium* species including *E. ovalifoliae* and *Exobasidium tengchongense* Z.Y. Li and L. Guo (Li & Guo, 2008). Meanwhile, Wolf and Wolf (1952) reported that the hymenium of *E. gracile* developed differently than the rest of the *Exobasidium* species including *E. vexans* in which the hymenium layer first formed beneath the lower epidermis and became exposed when the epidermal layer were sloughed off. This observation was further confirmed by Mims and Richardson (2007) and Lee *et al*. (2015).

In the case of hymenium formation only on the lower side of the leaf, the morphology of the palisade layer remained cylindrical indicating that the infection did not disrupt the cellular differentiation. Similarly, Li and Guo (2009) also reported a clear differentiation of palisade and mesophyll cells in the leaf of *C. sinensis* infected by *Exobasidium yunnanense* Z.Y. Li and L. Guo. This observation was different from the infection of *E. gracile* and *E. ovalifoliae* on *C. sasanqua* and *Lyonia ovalifolia* var. elliptica (Siebold & Zuccarini) Handel-Mazzetti, respectively, in which the palisade layers in the diseased leaves were not able to differentiate properly (Li & Guo, 2008; Mims & Richardson, 2007).

In addition, the prominent hypertrophied condition primarily in the spongy mesophyll layer of *E. vexans* infected leaf was similar to the infection of several other *Exobasidium* species on their respective hosts (Chlebicki & Chlebická, 20 07; Gómez P & Kisimova-Horovitz, 1998; Kennedy *et al*., 20 12). However, the effect of *Exobasidium* infection was not only limited to hypertrophy. Akai (1938) reported that the infection of *E. camelliae* on *Camellia japonica* L. resulted in both hypertrophy and hyperplasia of the leaf cells. Several researchers also reported the occurrence of both hypertrophy and hyperplasia in the infection of other *Exobasidium* species on the leaf (Graafland, 1960; Lee *et al*., 2015; Li & Guo, 20 08) and different plant organs such as flower (Kennedy *et al*., 2012) and fruit (Nagao, Ogawa, Sato, & Kakishima, 2003b).

Gadd and Loos (1949) reported that *E. vexans* could invade *C. sinensis* by forming appressoria through either lower or upper sides of the leaf but eventually, the white hymenium still proliferated on the lower side of the leaf regardless of the entry side. In contrast, the observation in this study showed that the infection of *E. vexans* could result in the formation of hymenium on both the lower and upper sides of the leaf primarily on the leaf midrib and vein. Venous infection of *E. vexans* could relatively enhance the spread of the infection to other tissues. The sclerenchyma and phloem components of the vascular bundle in the infected vein had completely collapsed and were filled with intracellular hyphae which were similar to the histological appearance of strawberry petiole infected with *Colletotrichum fragariae* (Milholland, 1982).

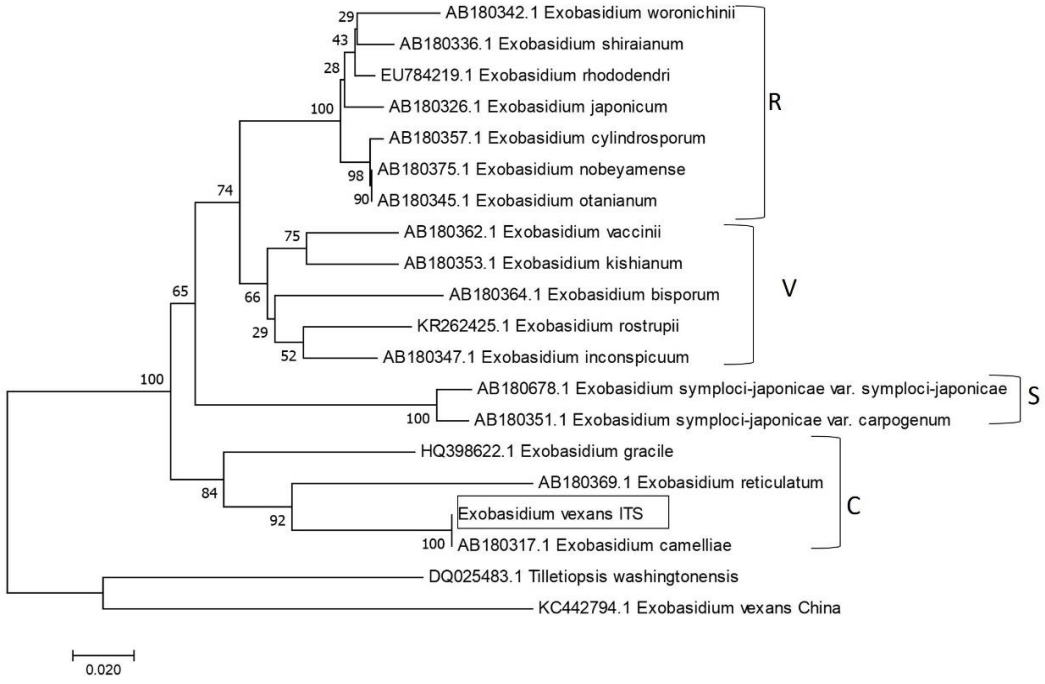

Figure 5. Neighbor-joining tree of *E. vexans* relative to other *Exobasidium* species constructed based on the ITS sequences with *Tilletiopsis washingtonensis* as the outgroup. Values shown at each node were obtained from 1000 bootstrap replicates, meanwhile the evolutionary distances were computed using the Maximum Composite Likelihood method. The sum of branch length is 1.0190 and a total of 420 nucleotides were used in the final dataset. The letters represent the clustering of *Exobasidium* species according to the host group. R = *Rhododendron*, V = *Vaccinium*, S = *Symploci*, C = *Camellia*.

Meanwhile, the cellular structure of the xylem became disorganised and the intercellular spaces were filled with *E. vexans* hyphae. Based on the descriptions of the different symptoms of *Exobasidium* infection provided by Nannfeldt (1981), *E. vexans* infection could be categorised as circumscribed and monocarpic in which the fungus usually shows localised infection spots and dies after sporulation.

Assessment of the disease severity on the tea plantation in this study showed that *E. vexans* infection almost consistently ends with necrosis of the infected spots or stems as shown by the high percentage of scores ≥3. Some of the resistant tea strains infected by *E. vexans* would show hypersensitive reactions or smaller blisters with less sporulation (Sinniah *et al*., 2016). Histological observation in this study also supported this result as *E. vexans* infection on tea leaves led to the formation of hymenium to allow for sporulation and eventually, the symptomatic spots would necrotise. Susceptibility of the specific tea strain towards blister blight infection plays an important role in the scoring of the disease severity. The overall DSI score for the tea plantation in this study was 2.07 which could be considered as moderately resistant compared to a highly resistant tea strain that was reported to have a DSI score of 0.6 (Sinniah *et al*., 2016). However, it is crucial to note that the weather conditions during the sampling period also played a huge role in the disease development. The overall DSI score might increase as the weather becomes more favorable for *E. vexans* proliferation. For example, the monsoon season from October to March in Malaysia could worsen the disease as a combination of high relative humidity and less sunshine results in severe blister blight infection (Homburg, 1955; Reitsma & Van Emden, 1950).

Extensive studies on the phylogenetics of *Exobasidium* species were done by using different genes including ITS and LSU (Begerow, Bauer, & Oberwinkler, 2002; Blanz & Doring, 1995; Piatek, Lutz, & Welton, 2012). Nonetheless, *E. vexans* has never been included in any of the analyses involving ITS gene because the ITS sequence of *E. vexans* is not available in GenBank except for KC442794.1. Based on the ITS sequence obtained in this current study, *E. vexans* is clustered with other *Exobasidium* species that are parasitic to their respective hosts belonging in the *Camellia* clade. This result is coherent with the analysis by Blanz and Doring (1995) in which the *Exobasidium* species that are highly host-specific cospeciate with their respective hosts. *E. vexans* was found to be in the same sub-clade with *E. reticulatum* as these fungi are pathogens of tea plants. Nonetheless, an *E. reticulatum* infection on tea plants causes tea net blister blight which is a disease with distinctive symptoms that are different from tea blister blight. The surface of the infected tea leaves appeared to be covered by networks of hymenium, but the infected spots are not swollen which is the prominent characteristics of *E. vexans* infection instead (Chen & Chen, 1982). In addition, the symptoms of tea net blister blight by *E. reticulatum* have never been observed in tea plantations in Malaysia. Only the ITS gene was used to construct the NJ tree in this study as the LSU sequence was found not able to resolve the phylogenetic relationship of *Exobasidium* species as reported by Piatek *et al*. (2012).

Interestingly, the *E. vexans* ITS sequence KC44279 4.1 that is available in the GenBank database was clustered with the outgroup of the NJ tree (*T. washingtonensis*). Further analysis of this sequence revealed that it was 99% similar to *Tilletiopsis pallascens* with 100% query coverage. Hence, it is important to point out that the KC442794.1 sequence could be contaminated by co-occurring *Tilletiopsis* species that are common plant endophytes. The contamination of *Tilletiopsis* is common when a voucher specimen is used in the DNA extraction of *Exobasidium* species as reported by several other researchers (Begerow *et al*., 2002; Boekhout, 1995; Peatek *et al*., 2012). Thus, this current study eliminated the possibility of contamination by isolating the basidiospore and culturing *E. vexans* on artificial media even though the culturing of *Exobasidium* species is known to be difficult (Begerow *et al*., 2002). In addition, the sequencing of two genes (LSU and ITS) and the thorough description of the infection histology further confirmed that the fungus isolated in this study was indeed *E. vexans*.

5. Conclusions

The findings from this study confirmed and expanded the observation provided by Gadd and Loos (1949). Several new observations were made including the occurrence of venous infection of *E. vexans* on *C. sinensis*. Phylogenetic analysis of the ITS gene revealed that *E. vexans* belonged in the same clade with other *Exobasidium* species whose host plants are in the genus *Camellia*. For future work, it would be interesting to study whether the *E. vexans* infection on vascular bundles could cause an infection in other parts of the plant since an infection on the petioles and internodes of *C. sinensis* shoots are common.

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