CAROLINE HAWERROTH

# INFECTION PROCESS OF *Gaeumannomyces graminis* var. *graminis* ON THE ROOTS AND CULM OF RICE

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Magister Scientiae*.

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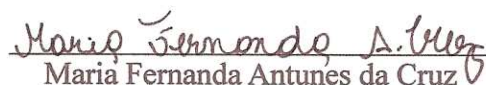
Maria Fernanda Antunes da Cruz

Fabrício de Ávila Rodrigues
(Orientador)

*Aos meus pais, Sebastião Hawerroth (in memoriam),*

*e Renita Dirksen Hawerroth,*

*que sempre acreditaram nos meus sonhos,*

*incentivaram meus estudos*

*e me apoiaram nessa caminhada.*

*DEDICO*

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## BIOGRAFIA

Caroline Hawerroth, filha de Sebastião Hawerroth e Renita Dirksen Hawerroth, nasceu em 05 de dezembro de 1989, em São Bonifácio, Estado de Santa Catarina.

Em 2008, ingressou no curso de Agronomia da Universidade Federal de Santa Catarina (UFSC) e em julho de 2013, graduou-se Engenheira Agrônoma. Nesta instituição, foi bolsista de Iniciação Científica em Fitopatologia, atuando na área de indução de resistência em plantas à patógenos sob orientação do Prof. Marciel João Stadnik.

Em março de 2014, iniciou o curso de Mestrado em Fitopatologia na Universidade Federal de Viçosa sob orientação do Prof. Fabrício de Ávila Rodrigues.

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# RESUMO

HAWERROTH, Caroline, M.Sc., Universidade Federal de Viçosa, Fevereiro de 2016. **Processo infeccioso de *Gaeumannomyces graminis* var. *graminis* em raízes e colmo de arroz.** Orientador: Fabrício de Ávila Rodrigues. Coorientador: Leonardo Araújo.

O mal-do-pé, causado pelo ascomiceto *Gaeumannomyces graminis* var. *graminis*, infecta as raízes e a base do colmo de arroz causando a maturação precoce dos grãos, morte dos perfilhos e redução do rendimento. Considerando a pouca informação para a interação arroz-*G. graminis* var. *graminis* a nível microscópico, este estudo teve como objetivo obter novas percepções em relação ao processo infeccioso deste importante patógeno de solo em raízes e colmo de plantas de arroz usando microscopia de luz e microscopia eletrônica de varredura. Nas raízes, o fungo inicialmente colonizou as células da epiderme, exoderme e esclerênquima. Aos 15 dias após a inoculação (dai), proeminentes hifas fúngicas colonizaram o córtex e grupos de peritécios foram observados nas raízes. Aos 20 dai, o fungo atingiu o cilindro central. Houve intensa colonização fúngica na base do colmo das plantas que resultou na formação de um "tapete" micelial em ambas as superfícies adaxial e abaxial das bainhas. Aos 25 dai, o crescimento fúngico foi observado nas células do parênquima, nos feixes vasculares e nos aerênquimas. Peritécios emergiram através da base do prófilo e da primeira bainha aos 30 dai. Os resultados deste estudo fornecem uma nova visão sobre o processo infeccioso de *G. graminis* var. *graminis* em arroz e podem contribuir para o desenvolvimento de medidas de controle mais eficazes para redução do mal-do-pé a campo.

# ABSTRACT

HAWERROTH, Caroline, M.Sc., Universidade Federal de Viçosa, February, 2016. **Infection process of *Gaeumannomyces graminis* var. *graminis* on the roots and culm of rice.** Advisor: Fabrício de Ávila Rodrigues. Co-advisor: Leonardo Araújo.

Crown sheath rot, caused by the ascomycete *Gaeumannomyces graminis* var. *graminis*, infects the roots and the base of the culm of rice and causes early grains maturation, the death of the tillers and reduced yield. Considering the little information for the interaction rice-*G. graminis* var. *graminis* at the microscopical level, this study aimed to gain novel insights regarding the infection process of this important soilborne pathogen on the roots and culm of rice plants by using both light and scanning electron microscopy. In the roots, the fungus initially colonized the epidermal, exodermal and sclerenchyma cells. At 15 days after inoculation (dai), prominent fungal hyphae colonized the cortex as well as clusters of perithecia were noticed into the roots. At 20 dai, the fungus reached the central cylinder. There was intense fungal colonization in the culm-base of the plants that resulted in the formation of a mycelial mat on both adaxial and abaxial surfaces of the leaf sheaths. At 25 dai, fungal growth was noticed in the parenchyma cells, the vascular bundles and the air spaces. Perithecia emerged through the base of prophyllum and the first leaf sheath at 30 dai. The results of this study provide new insight into the infection process of *G. graminis* var. *graminis* on rice plants and may contribute to the development of more effective control measures to reduce the crown sheath rot in the field.

# 1. INTRODUCTION

Brazil is the ninth largest rice-producing country with 2,3 million hectares planted annually (GRiSP, 2013, FAOSTAT, 2015). The crown sheath rot, caused by the fungus *Gaeumannomyces graminis* (Sacc.) von Arx & D. Olivier var. *graminis*, was reported for the first time in some Brazilian states in the 1997/1998 growing season (Prabhu & Filippi, 2002). The disease can cause early grains maturation, reduce the number of grains per panicle and even the death of the tillers depending on the rice growth stage that the fungal infection takes place (Ou, 1985; Peixoto et al., 2013; Prabhu & Filippi, 2002). Curiously, most of the information available in the literature regarding the biology of the genus *Gaeumannomyces* and the epidemiological aspects of the disease caused by this pathogen and its control methods have been extensively reported for wheat in contrast to rice.

The fungus colonizes the roots and the base of the culm of rice plants by producing two types of hyphae known as runner and infectious hyphae (Peixoto et al., 2013). The runner hyphae are brown, septated, with thick walls and often grow on the surface of roots and culm forming mycelium strands; the infectious hyphae are hyaline and with thin cell walls and are located within the host tissue (Peixoto et al., 2013). The mycelium that grows over the base of the infected culm abundantly produce hyphopodia which are flat, hyaline or brown, and lobed structures and play the functions of fungal fixation and further penetration (Walker, 1981). Perithecia produced by *G. graminis* var. *graminis* are dark, spherical and contain many unitunicated, elongated and clavated asci with an apical ring (Walker, 1975, 1981).

In rice, the crown sheath rot symptoms begin as dark brown or black lesions on the sheaths, at the culm base as well as on the first and second nodes and internodes (Prabhu & Filippi, 2002). Roots became dark in colour due to the intense tissue necrosis

caused by fungal infection (Prabhu & Filippi, 2002). The fungus *G. graminis* var. *graminis* can survive in infected rice stubbles and on several alternative hosts (Datnoff et al., 1997; Peixoto et al., 2013, 2014). Crown sheath rot management is quite difficult due to the absence of resistant cultivars and the availability of efficient fungicides (Nunes, 2008; Prabhu & Filippi, 2002; Peixoto et al., 2014).

Considering the little information, to the best our knowledgment, for the interaction rice-*G. graminis* var. *graminis* at the microscopical level, the present study aimed to gain novel insights regarding the infection process of this important soilborne pathogen on the roots and culm of rice plants by using both light and scanning electron microscopy.

## 2. MATERIAL AND METHODS

### Plant growth

Rice seeds (cultivar BRS Primavera) were surface sterilized in 0.5% (vol/vol) NaOCl for 3 min and a total of fifty seeds were sown per plastic tray filled with autoclaved sand. At 15 days after seedlings emergence, they were fertilized with a nutrient solution (50 mL per plastic tray) containing the following in g/L: 6.4 KCl, 3.48 $K_2SO_4$, 5.01 $MgSO_4.7H_2O$, 2.03 $(NH_2)_2CO$, 0.009 $NH_4MO_7O_{24}.4H_2O$, 0.054 $H_3BO_3$, 0.222 $ZnSO_4.7H_2O$, 0.058 $CuSO_4.5H_2O$, 0.137 $MnCl_2.4H_2O$, 0.27 g/L $FeSO_4.7H_2O$ and 0.37 g/L EDTA bisodic (Xavier Filha et al., 2011). The nutrient solution was prepared using deionized water. Plants were fertilized weekly and watered as needed.

### Inoculum production and plant inoculation

The isolate of *G. graminis* var. *graminis* (UFV-DFP *Ggg*212) was grown in Petri dishes containing potato-dextrose-agar (PDA) medium for four weeks and kept in an incubator (25°C, photoperiod of 12 hours of light and 12 hours of darkness). The inoculum was produced in rice seeds according to Datnoff et al. (1997) with a few modifications. Briefly, a total of 40 g of rice seeds were carefully washed in distilled water, soaked overnight, drained and autoclaved for 25 min at 120°C on each of two consecutive days. The PDA medium containing fungal mycelium was chopped into small pieces, mixed with the seeds and incubated for 4 weeks at 25°C and with 12 hours photoperiod.

Four-weeks old plants were transplanted to plastic tubes filled with substrate composed of a 1:1:1 mixture of pine bark, peat and expanded vermiculite (Tropstrato®, Vida Verde, Mogi Mirim, São Paulo, Brazil) and autoclaved sand in the proportion of 3:1 (vol/vol). The substrate was infested with inoculum of *G. graminis* var. *graminis* in the proportion of 20 g of inoculum per 100 g of substrate (vol/vol). The control treatment

corresponded to plants growing on substrate that received only autoclaved rice seeds. After transplanting, a plastic tray containing wet sand was place below the support of the plastic tubes to allow high humidity on the base of the substrate.

**Disease assessment**

Disease development on the roots and on the culm of rice plants was assessed at 5, 15 and 30 days after inoculation (dai). Roots and culms fragments exhibiting symptoms of crown sheath rot were photographed at × 5.6 and × 11.2, respectively, at each sampling time using a stereoscopic microscope (Stemi 2000-C; Carl Zeiss, Jena, Thuringia, Germany) coupled to a digital camera (Canon PowerShot A640, Canon Inc., Tokyo, Japan).

**Processing the infected roots and culm fragments for the microscopical observations**

A total of 50 fragments (≈ 5 mm) of roots and culms were obtained from the plant of each replication at 5, 10, 15, 20, 25 and 30 dai. Roots and culms fragments of non-inoculated plants were also sampled and served as the control treatment. Culms fragments were obtained from the two outer sheaths of each plant exhibiting symptoms of crown sheath rot. The fragments were carefully transferred to glass vials containing 10 mL of a fixative composed of 3% (vol/vol) glutaraldehyde and 2% (vol/vol) paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). Vials were covered with aluminum foil and stored at 4°C for one month until being processed for the microscopical observations.

## Processing the infected culms fragments for differential interference contrast microscopy

Culms fragments collected at 3, 5 and 10 dai were taken from ethanol 70% (vol/vol) and cleared for three weeks in saturated choral hydrate solution (300 g/mL) (Sigma-Aldrich, São Paulo, Brazil) (Rodrigues et al., 2005). Cleared culms fragments were mounted adaxial side up on glass slides containing 3 drops of modified Hover's mounting medium (Rodrigues et al., 2005). Images of the culms fragments colonized by *G. graminis* var. *graminis* were acquired digitally (Axio Cam HR, Carl Zeiss, Jena, Thuringia, Germany) using a Carl Zeiss Axio Imager A1 microscope (Carl Zeiss, Germany) equipped with differential interference contrast and further processed using the AXION VISION v. 4.8.1 software.

## Processing the infected roots and culms fragments for light microscopy

Roots and culms fragments collected at 5, 10, 15, 20, 25 and 30 dai were taken from the fixative and washed with 0.1 M sodium cacodylate buffer, subsequently dehydrated through a graded alcohol series (10, 30, 50, 70, 85, 95 and 100%) and then embedded in methacrylate resin (Historesin, Leica Microsystems®, Nussloch/Heidelberg, Germany) (Araujo et al., 2014, 2015). The fragments were placed in a vacuum chamber for 2 hours during the pre-infiltration and infiltration steps twice a day, respectively, for three weeks to allow better resin infiltration into the roots and culms fragments. The fragments were stored at 4°C after each vacuum procedure. A total of six blocks of resin, each one containing two roots or culms fragments, were obtained for each treatment at each sampling time. A total of 36 longitudinal and transversal serial sections (4 μm thick), which were cut from each block using a Leica RM 2245 rotary

microtome (Leica Microsystems®, Nussloch/Heidelberg, Germany), were randomly divided and placed on three glass slides and stained with 1% toluidine blue in 2% sodium borate for 5 min. Toluidine blue is a metachromatic dye commonly used for staining plant tissue sections. When the specimen samples stained with toluidine blue are viewed under the light microscope, distinct different cell components produce different colors: DNA is bluish-green; RNA is violet; the middle lamella is red; non-lignified cell walls and soluble phenolics are red-violet, blue violet, blue, or purple; and polymerized phenolics such as lignin become green or bluish-green (Vermerris & Nicholson, 2006). The images of the details regarding the infection process of *G. graminis* var. *graminis* were acquired digitally using a Carl Zeiss Axio Imager A1 microscope in the bright-field mode and further processed as described previously.

**Processing the infected roots and culms fragments for scanning electron microscopy**

Roots and culms fragments were collected at 3, 10, 15 and 30 dai, washed with sodium cacodylate buffer (0.1 M), dehydrated in an alcohol series, subjected to critical point drying in $CO_2$ using the 'Critical Point Dryer' device (Bal-tec, model CPD 030; Electron Microscopy Sciences [EMS], Hatfield, PA) and mounted on aluminum stubs. Additionally, some roots fragments were longitudinally sectioned with a scalpel and a few culms fragments were open by pressing a stub covered with double-sided tape over the stub already containing the mounted culms fragments. Fractured roots and culms fragments were post-fixed for 20 min at room temperature with 1% (wt/vol) osmium tetroxide prepared in sodium cacodylate buffer (0.1 M) before dehydration. Stubs were sputter coated with gold (Balzers Union, model FDU 010; EMS, Hatfield, PA). A LEO scanning electron microscope operating at 10 Kv and with a working distance of 10 to

15 nm was used to obtain the photomicrographs. For each sample time, one stub with three fragments of roots or culms was examined by SEM.

### Experimental design

An experiment consisting of two treatments (non-inoculated and inoculated plants) was arranged in a completely randomized design with five replications. Each replication corresponded to a plastic tube with one plant. The experiment was repeated twice.

# 3. RESULTS

## Symptoms of crown sheath rot on the roots and culms-base

Necrosis on the roots was first noticed at 5 dai (Fig. 1A and C) and on the culms-base at 15 dai (Fig. 1D and E). On both roots and on the culms-base of the plants, the necrosis became more intense at 30 dai (Fig. 1G). Runner hyphae abundantly grew over the first leaf sheath and formed many hyphopodia at 5 and 15 dai (Fig. 1B and E). Several perithecia were noticed on the first necrotic leaf sheath at 30 dai (Fig. 1H). Clusters of perithecia of different sizes and formats were observed on the roots at 15 and 30 dai (Fig. 1F and I), respectively.

## Light microscopy and scanning electron microscopy of the infected roots

Runner hyphae abundantly colonized the surface of the roots at 3 dai (Fig. 2A). At 5 dai, infectious hyphae colonized the epidermal and exodermal cells (Fig. 3A). Infectious hyphae reached the sclerenchyma (Fig. 3B) and also the cortex (Fig. 3C and D) at 10 and 15 dai, respectively. At 15 dai, the intense *G. graminis* var. *graminis* colonization on the epidermis, exodermis, sclerenchyma and cortex of the roots caused profound cells disorganization (Fig. 3C). At 15 dai, runner and infectious hyphae were noticed on the roots surface and in the cortex, respectively, (Fig. 3F) and clusters of perithecia of different sizes and formats were found in the cortex region at 15 (Fig. 2B; 3E) and 30 dai (Fig. 2C). At 20 dai, the central cylinder was displaced as the perithecia increased in size as well as due to the intense fungal growth as noticed by the presence of stroma (Fig. 3G). Weft mycelia were noticed on the surface of perithecia (Fig. 2D). Asci containing ascospores and many fungal hyphae were found inside the perithecia (Fig.

2E, F and G). Perithecium showed its neck formed toward the cortex region at 30 dai (Fig. 3H).

**Light microscopy, differential interference contrast microscopy and scanning electron microscopy of the infected culms-base**

Runner hyphae grew over the first leaf sheath and produced hyaline and pigmented hyphopodia as well as welf of mycelium at 3 dai (Fig. 4A, B and C). Several pigmented hyphopodia and mycelial strands were observed at 5 dai (Fig. 4D and E). At 10 dai, runner hyphae abundantly covered both adaxial and abaxial surfaces of the leaf sheath (Fig. 5A and B). Necrotic parenchyma cells were noticed at 10 dai (Fig. 4F). Infectious hyphae abundantly colonized the parenchyma cells, the vascular bundles and the air spaces at 25 and 30 dai (Figs. 4G, H and I; 5C). Perithecia in formatiobn were noticed inside the parenchyma cells at 25 dai (Fig. 4I). Profuse fungal colonization formed a layer of mycelium over the adaxial surface of the first leaf sheath at 30 dai (Fig. 5D). Clusters of perithecia were observed inside the prophyllum and on the first leaf sheath at 30 dai (Fig. 5E and F).

## 4. DISCUSSION

The present study brings, to the best of our knowledge, novel insights regarding the microscopic details of the infection process of *G. graminis* var. *graminis* on the roots and culms of rice plants. Hyphae of *G. graminis* var. *graminis* were abundantly noticed on both the surface and inside of the rice roots and caused the symptoms of crown sheath rot. By contrast, Peixoto et al. (2013) did not find mycelium of *G. graminis* var. *graminis* on rice roots. Hyphopodia of *G. graminis* var. *graminis* were not observed on the rice roots surface. This suggests that fungal penetration probably took place directly by infectious hyphae similarly to what has reported for *Ophiobolus graminis* infecting wheat roots (Weste, 1972). Fungal hyphae of *G. graminis* var. *graminis* fully colonized the cortex and reached the central cylinder indicating, therefore, a radial colonization on the rice roots. The *Fusarium culmorum*, which also is a root pathogen, colonized the rhizodermal layer and cortex of wheat roots, but was inefficient to reach the endodermis and the stele (Beccari et al., 2011). Although *G. graminis* var. *graminis* is not considered to be a vascular pathogen, fungal hyphae widely colonized the cortex and the central cylinder of rice roots. Such colonization resulted in root tissue degradation, thus affecting the water and nutrients uptake and the plant development.

After fungal fixation and further penetration by lobed hyphopodia, runner hyphae extensively colonized both the adaxial and abaxial surfaces of the leaf sheath. This finding is in agreement with the mycelial mat formed between the rice leaf sheaths and the tufts of fan shaped mycelium in rice plants infected by *G. graminis* var. *graminis* (Ou, 1985; Peixoto et al., 2013). Infectious hyphae profusely colonized the parenchyma cells and the perithecia formation appears to start from these cells in the leaf sheaths.

Ascocarp formation by *O. graminis* is influenced by environmental conditions such as nutrition, temperature and pH and glucose is the favorite carbon source for perithecia production *in vitro* (Moore-Landecker, 1992). The preference for the perithecia to form on the parenchyma cells of rice leaf sheaths may be associated with the availability of nutrients in these cells.

Cluster of perithecia of *G. graminis* var. *graminis* were abundantly found in the roots and at the base of the culm of rice plants. Initially, perithecia were first observed on the roots and later on they became evident on the prophyllum as well as on the base of the leaf sheaths. Perithecia are often produce by some ascomycetes fungi such as *Magnaporthe grisea* on the rice culm and *G. incrustans* sp. nov. on the roots of wheat and other grasses (Silue & Notteghem, 1990; Landschoot & Jackson, 1989). However, the present study is the first to report the occurrence of perithecia of *G. graminis* var. *graminis* on rice roots. The orientation of the neck of the perithecia followed the same pattern on the leaf sheaths while on the roots the neck of the perithecia showed different orientations besides having part of their body being completely outside of the roots. Weste (1972) provided information on the ascosporic infection by *O. graminis* on the roots of wheat and oat plants. However, for take-all disease on wheat, the infection mediated by ascospores is of less importance and seems to depend on other factors such as the soil microbial activity and the macro hyphae growing on the root surface or in wheat stubble is consider a more effective inoculum source (Brooks, 1965). Peixoto et al. (2013) reported the occurrence of ascosporic infection by *G. graminis* var. *graminis* in rice plants grown in greenhouse when were exposed to infected rice stubbles. Ascospores of *G. gramninis* var. *graminis* are forcibly ejected into the air from the perithecia produced on the leaf sheaths (Ou, 1985). The mechanism of ascospores release from perithecia formed on rice roots is unknown; however, they can serve as a

source of inoculums that will allow the fungus to survive in rice debris between the growing seasons.

Considering the unavailability of literature providing the details of the infection process of *G. graminis* var. *graminis* on both roots and culms of rice, the present study brings novel information regarding this important host-pathogen interaction that will allow to better understand how the disease develops and consequently to define new control strategies that will be reduce the occurrence of epidemics in the field.

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# 6. LIST OF FIGURES

**Figure 1.** Symptoms of crown sheath rot in rice plants at 5 (A, B and C), 15 (D, E and F) and 30 (G, H and I) days after inoculation with *Gaeumannomyces graminis* var. *graminis*. **A, D and G,** Symptoms of crown sheath rot on roots and on the culms-base of the plants. Arrows in G indicate groups of perithecia. **B**, Runner hyphae colonizes the base of the culm and abundantly produces hyphopodia. **C**, Roots were abundantly colonized by fungal hyphae and showed early tissue necrosis (double arrow). **E**, Fungal hyphae grew abundantly on the culms-base and formed a weft of mycelium (asterisk). **F**, Development of perithecia in the necrotic roots. **H**, Perithecia (arrowheads) were noticed inside the leaf sheath. Double arrow indicates a perithecium releasing a mass of ascospores. **I**, Group of pertithecia in the necrotic roots.

**Figure 2.** Scanning electron micrographs of roots of rice plants at 3 (A), 15 (B) and 30 (C-G) days after inoculation with *Gaeumannomyces graminis* var. *graminis*. **A,** Fungal hyphae grew abundantly over the roots. **B,** A cluster of perithecia was observed on the roots. **C,** A cluster of perithecia with different neck (asterisks) orientations were noticed on the roots. **D,** A perithecium was noticed outward of the root. **E and F,** Fractured perithecium containing asci (arrowheads) and paraphyses. **G,** Ascospores (arrows) inside the asci (arrowheads) were found in abundance in a fractured perithecium. Fungal hyphae (fh), lateral root (lt), paraphyses (pa), perithecium (p) and root (r). Scale bars: A-D = 100 µm, D1 = 10 µm and E-G = 20 µm.

**Figure 3.** Light micrographs of transverse (A, B, C, D and G) and longitudinal (E, F and H) sections of rice roots colonized by *Gaeumannomyces graminis* var. *graminis* at 5 (A), 10 (B), 15 (C-F), 20 (G) and 30 (H) days after inoculation. **A,** Fungal hyphae (arrows) colonized the epidermis and the exodermis. **B,** Fungal hyphae (arrows) grew in the direction to the endodermis and some runner hyphae (arrowheads) were noticed outer of the roots. Epidermis and exodermis became disorganized due to intensive fungal colonization. **C,** Fungal hyphae (arrows) completely colonized the cortex. A few perithecia-like structures (asterisks) were noticed. **D,** Fungal hyphae (arrows) colonized the cortex, but were not noticed in the epidermis and exodermis. Fungal hyphae formed aggregates (double arrowhead). **E,** Cluster of perithecia of different sizes and orientations was formed within the cortex. **F,** Perithecia of different sizes and format were noticed in the colonized cortex. **G,** Fungal hyphae (arrows) reached the central cylinder of the roots. Fungal stroma filled part of the cortex tissue. **H,** A perithecium with its base outward of the root. Aerenchyma (arc), asci (a), central cylinder (cc), cortex (cx), endodermis (end), epidermis (ep), exodermis (ex), infectious hyphae (ih), lateral root (lr), paraphyses (pa), perithecium (p), runner hyphae (rh), sclerenchyma (es) and stroma (st). Scale bars: A-C = 10 μm and D-H = 20 μm.

**Figure 4.** Differential interference contrast (A, B, C, D, E and F) and light micrographs (G, H and I) of the adaxial surface and longitudinal sections, respectively, of rice leaf sheath at 3 (A-C), 5 (D and E), 10 (F) and 25 (G-I) days after inoculation with *Gaeumannomyces graminis* var. *graminis*. **A,** Runner hyphae (arrow) grew over the leaf sheath. Arrowhead indicates a hyaline hyphopodia. **B,** Pigmented hyphopodia (arrowheads) and runner hyphae (arrows) were noticed over the leaf sheath. **C,** Runner hyphae (arrow) formed a welf of mycelium. **D,** Pigmented hyphopodia (arrowheads) were abundantly formed over the leaf sheath. **E,** Runner hyphae (arrow) was organized as a strand. **F,** Necrotic parenchyma cells (double arrow) were noticed below the hyphopodia (arrowhead). **G and H,** Infectious hyphae (double arrowheads) colonized the parenchyma cells, the vascular bundle and the air space. **I,** Perithecia in formation (asterisks) within the parenchyma cells. Air space (as), leaf sheath (ls), parenchyma cell (p), runner hyphae (rh) and vascular bundle (vb). Scale bars: 10 µm.

**Figure 5.** Scanning electron micrographs of culms-base of rice plants at 10 (A and B) and 30 (C-F) days after inoculation with *Gaeumannomyces graminis* var. *graminis*. **A,** Runner hyphae (arrows) grew over the adaxial surface of the leaf sheath and abundantly produced lobed hyphopodia (arrowheads). **B**, Runner hyphae (arrow) grew over the abaxial surface of the leaf sheath forming mycelial strands. **C,** Infectious hyphae (arrowheads) colonized the parenchyma cells on a fractured leaf sheath. **D,** Runner hyphae (arrow) grew abundantly over the abaxial surface of the leaf sheath and formed a weft of mycelium. **E,** Perithecia were extruded from the abaxial surface of the leaf sheath. **F,** Cluster of perithecia was formed on the adaxial surface of a prophyllum. A double arrow indicates an ostiole of a perithecium. Leaf sheath (ls), parenchyma cell (p), perithecium (pe) and prophyllum (pr). Scale bars: A and C = 20 µm and B, D, E and F = 100 µm.

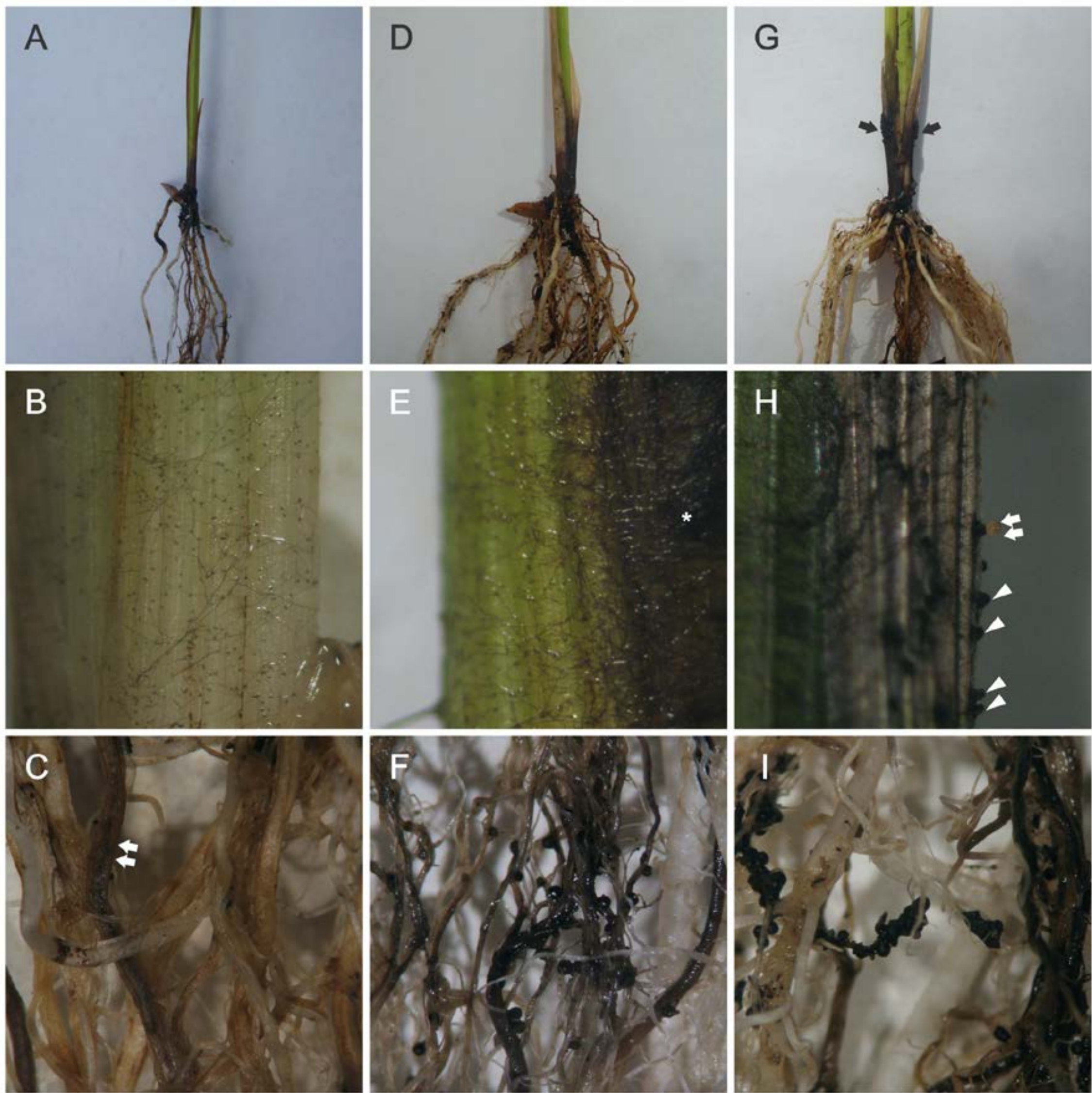


**Figure 1**

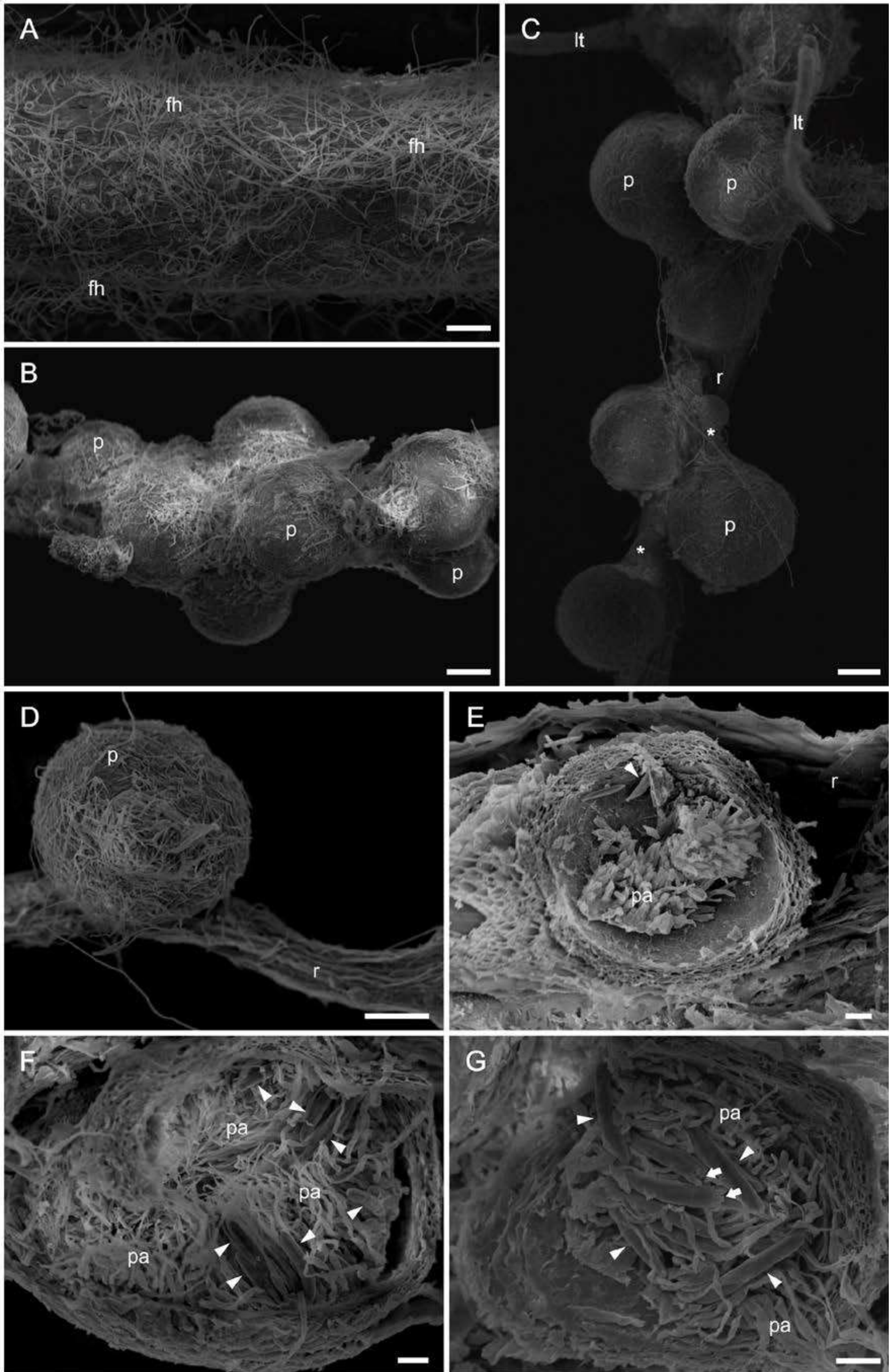


**Figure 2**

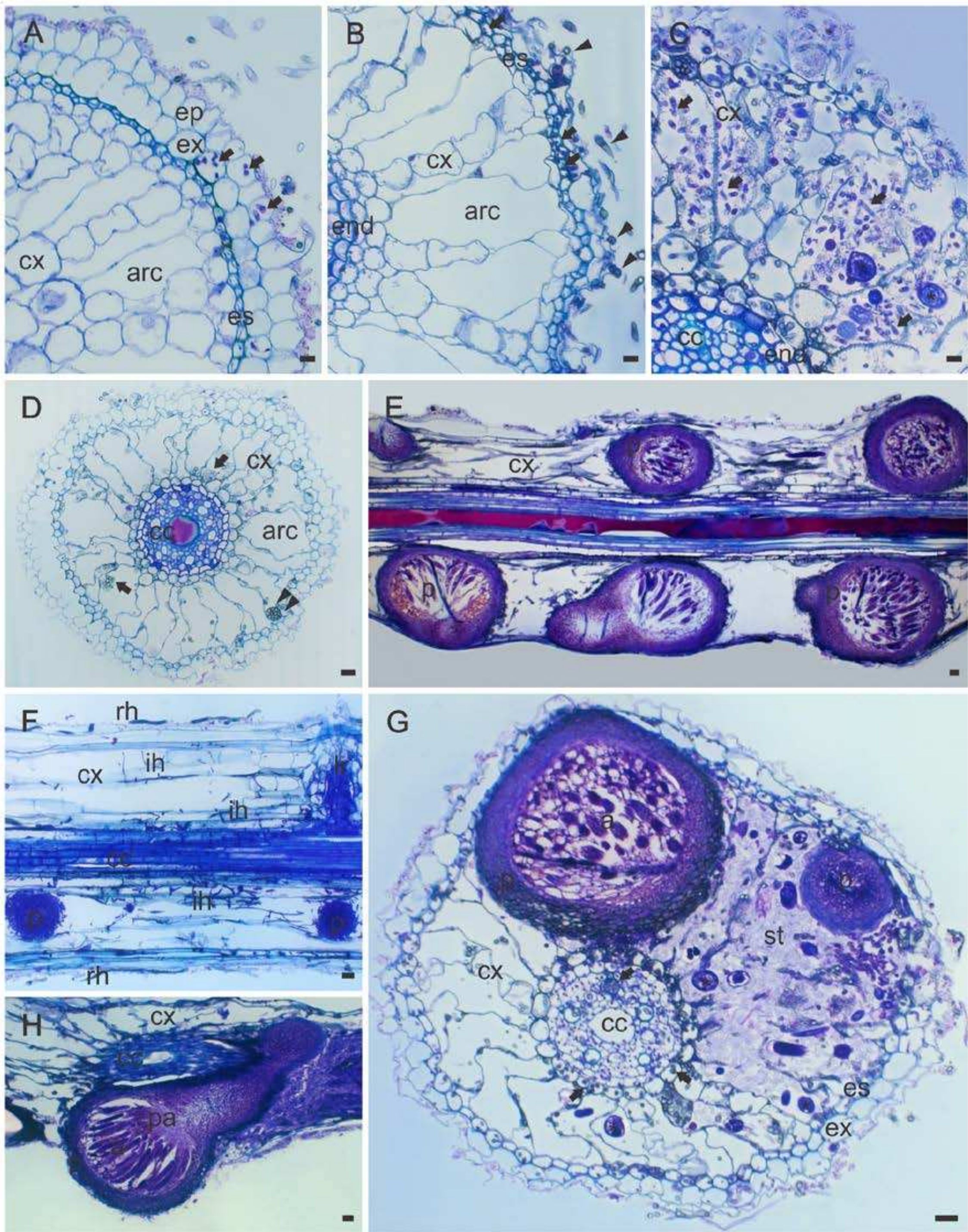


**Figure 3**

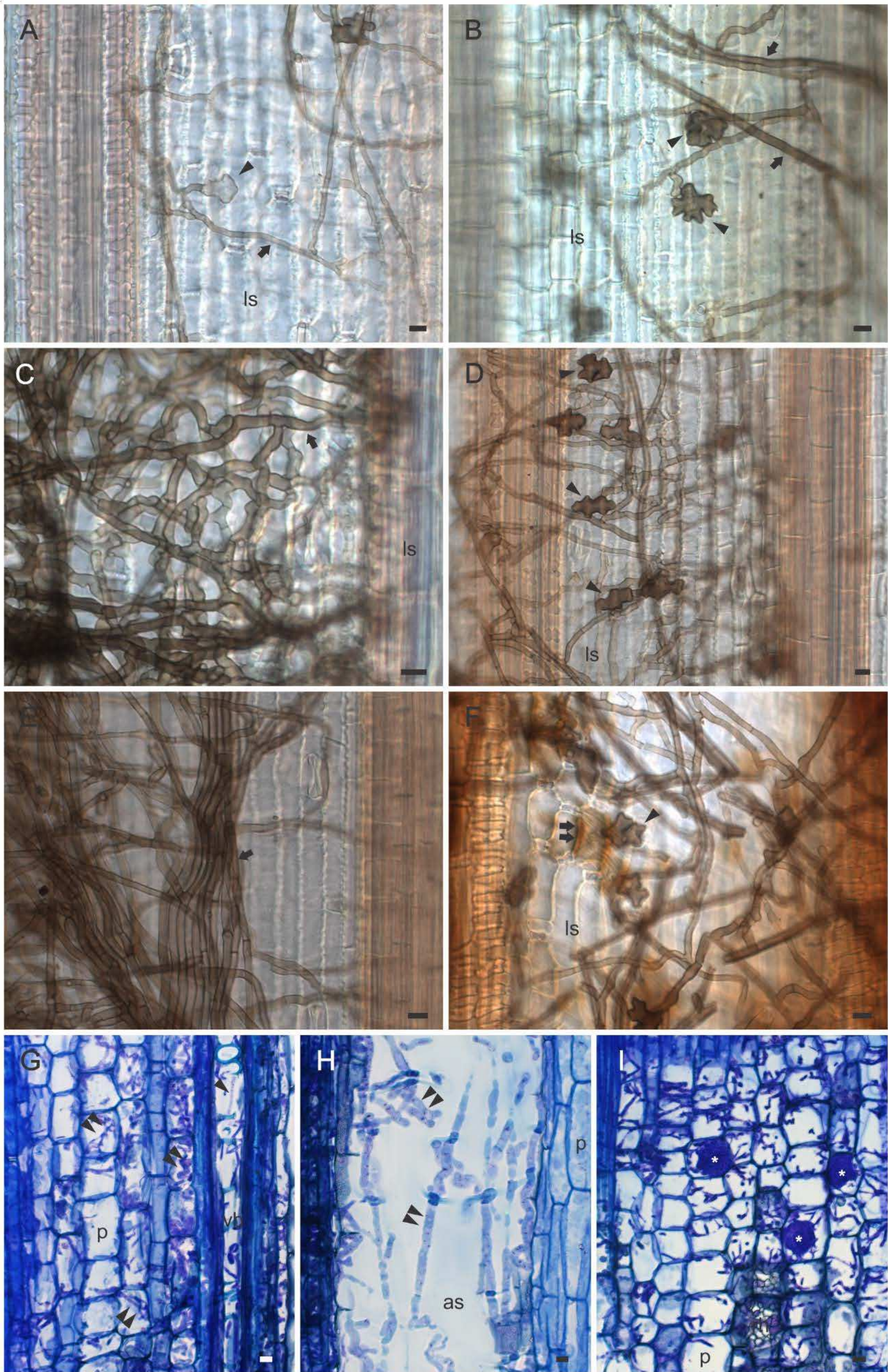


**Figure 4**

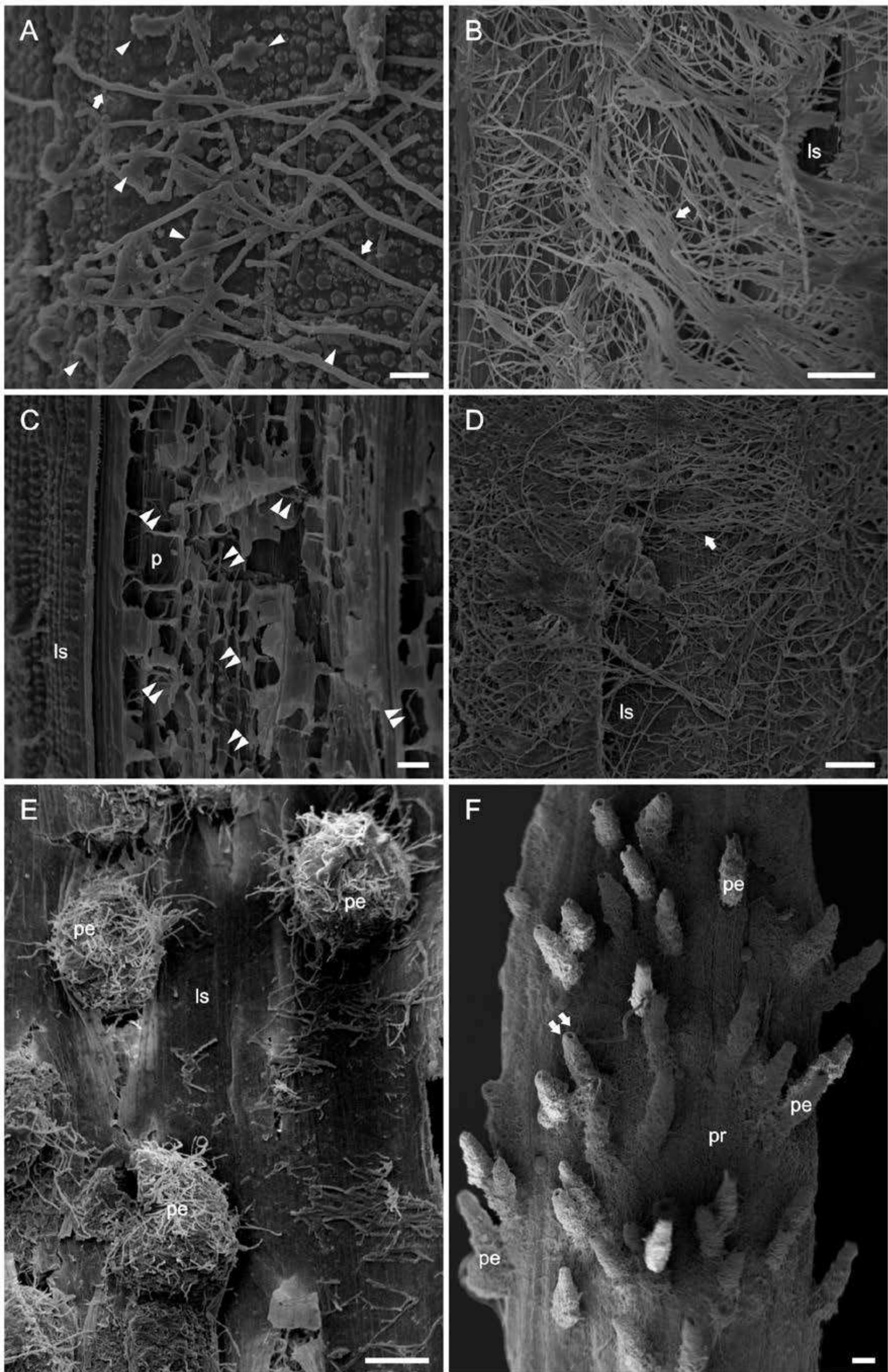


**Figure 5**