

Mycorrhizal-like interaction between gametophytes and young sporophytes of the fern *Dryopteris muenchii* (Filicales) and its fungal endophyte

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Abstract The morphology of a *Glomus*-like fungus-host interaction in chlorophyllous gametophytes and young apogamic sporophytes of *Dryopteris muenchii* A.R. Sm. was studied from ferns cultivated in laboratory, using soil as substrate. An aseptate fungus colonized the gametophytes' tissue through the rhizoids, developing vesicles. The fungus penetrated the young sporophytes primary roots by developing appressoria. It spread forming inter- and intra-cellular hyphae through the epidermis and the outermost cortical cell layers, where it formed vesicles, hyphal coils-like and arbuscules. The fungus hyphae never colonized the gametophyte-sporophyte cellular junction. The fungal structures observed on *D. muenchii* during this study, are rather similar to those reported for the plant host-arbuscular mycorrhizal fungus (AMF) interaction, where the AMF described belonged to Phylum Glomeromycota. Therefore, this study is a contribution to the scarce knowledge of the association between AMF and chlorophyllous gametophytes and young apogamic sporophytes of ferns. Rev. Biol. Trop. 56 (3): 1101-1107. Epub 2008 September 30.

Key words: Arbuscular mycorrhiza (AM), endophyte, chlorophyllous gametophyte, Dryopteridaceae, endemic fern, Mexico.

Arbuscular mycorrhizal fungi (AMF) are associated with *ca.* 80-85% of living terrestrial plants (Pirozynski 1981, Dickson 2004), and are considered to be ecologically important for most vascular plants because they improve the uptake of specific nutrients by the host, other functions attributed to the fungi include the promotion of plant growth hormones production, protection of host roots from pathogens, and favors the increase of solubility of soil minerals, among other benefits (Remy *et al.* 1994, Newsham *et al.* 1995, Merryweather and Fitter 1996, 1998a, 1998b).

Blackwell (2000) suggested that symbiotic relationships of AMF and the roots of higher plants were essential in moving plants to land. The fossil record shows that some Devonian fossil plants (genera *Aglaophyton*, *Rhynia* and *Asteroxylon*, 400 Myr) already had vesicle-like structures similar to those of *Glomus*

(Pirozynski 1981, Remy *et al.* 1994), which is consistent with the hypothesis that arbuscular mycorrhizas (AM) were instrumental in the colonization of land by ancient plants (Taylor *et al.* 1995).

Mycorrhizal associations are common in the roots of numerous fern species (sporophytic phase) and are documented in several light- and electron microscopic studies (Rayner 1927, Burgeff 1938, Boullard 1957, Fontana 1959, Hepden 1960, Cooper 1976, Mishra *et al.* 1980, Iqbal *et al.* 1981, Laferrière and Koske 1981, Berch and Kendrick 1982, Gemma and Koske 1990, Ragupathy and Mahadevan 1993, Raja *et al.* 1995, Schmid and Oberwinkler 1995, Zhi-wei 2000, Muthukumar and Udaiyan 2000, Zhang *et al.* 2004).

Fern gametophytes (haploid phase) may also be associated with endophytic fungi; in particular, the subterranean achlorophyllous

gametophytes within the Pteridophytes, which are frequently infected by fungal endophytes; hence few studies on this matter are found (Bruchmann 1904, 1906, 1908, Campbell 1907, Whittier 1977, Boullard 1979, Peterson *et al.* 1981, Schmid and Oberwinkler 1993, 1994, 1995, Kovacs *et al.* 2003, Duckett and Ligrone 2005). In contrast, there is little knowledge about fern chlorophyllous gametophytes associated with endophytic fungi. Campbell (1908) described the AM of some species of Marattiaceae and Gleicheniaceae, and of *Osmunda cinnamomea* L. Bower (1923) studied the symbiotic interaction in gametophytes of Marattiaceae and Schizaceae. Schmid and Oberwinkler (1995) studied the AM in gametophytes and young sporophytes of *Gleichenia bifida* (Willd.) Spreng. (= *Sticherus bifidus* (Willd.) Ching.), and other Gleicheniaceae, and Turnau *et al.* (2005) worked with *Pellea viridis*.

Therefore, the aim of this study is to contribute to the knowledge of the AMF-host association by describing the morphology of a *Glomus*-like fungus-host interaction in chlorophyllous gametophytes and young apogamic sporophytes of *Dryopteris muenchii* A. R. Sm. (Dryopteridaceae), a narrow endemic Mexican fern species.

Although there are studies of this type in other regions of the world, as far as we know, this is the first one done in Mexico and it is related to the knowledge of the state of *D. muenchii* wild populations.

Dryopteris muenchii in Mexico

Family Dryopteridaceae comprises 16 genera and *ca.* 400 species. In Mexico, this family is represented by eight genera: *Arachniodes*, *Didymochlaena*, *Dryopteris*, *Olfersia*, *Phanerophlebia*, *Polybotrya*, *Polystichum* and *Stigmatopteris* (Moran 1995). Genus *Dryopteris* Adans. ("shield fern") occurs in temperate and tropical regions of the world, 300-3050 m.o.s.l., with *ca.* 225 species. Its highest diversity occurs in Eastern Asia, while only 30 species are found in the New World,

13 of them occurring in Mexico (Mickel and Smith 2004).

According to Mickel and Smith (2004), *D. muenchii* A.R. Sm. is endemic to Mexico; it has only been collected in the states of Hidalgo and Chiapas; this species is found in cloud forest (2000-2850 m.o.s.l.).

MATERIALS AND METHODS

Dryopteris muenchii spores were collected from fertile sporophytes, using paper bags, from the Paraje Bonabil, state of Chiapas, Mexico (5 km on Matzab deviation, San Cristóbal de las Casas-Tenejapa road); voucher specimens are deposited at the Herbario Metropolitano-UAMIZ (A. Mendoza, R-182, R-244). Fern spores were sown, without sterilization, in 16 pots (5 cm in diam.), filled with 30 g of soil collected at 0-30 cm depth. Gametophyte cultures were maintained during nine months under laboratory conditions, inside transparent polyethylene bags for avoiding dehydration and contamination, and using a 12 h light-12 h darkness photoperiod (artificial light, 75 watts), with a daily mean temperature of 25 °C. Pots were watered every ten days with distilled water. Gametophytes, with and without young sporophytes, were carefully washed with distilled water, and fixed with FAA (formol 5%, glacial acetic acid 5%, ethylic alcohol 45%, and distilled water).

For light microscopy, 200 gametophytes and 250 gametophytes with young sporophytes were cleared with 5% KOH for 15 min and stained with acidic glycerol/trypan-blue 0.08% (Phillips and Hayman 1970, Koske and Gemma 1989). Microphotographs were taken using a light microscope.

RESULTS

D. muenchii gametophytes are chlorophyllous with a cordate-spatulate shape. The midrib, with 3-4 cells thick, projects numerous rhizoids (Figs. 1 and 2). This species is considered apogamic because, *in vitro*, it does

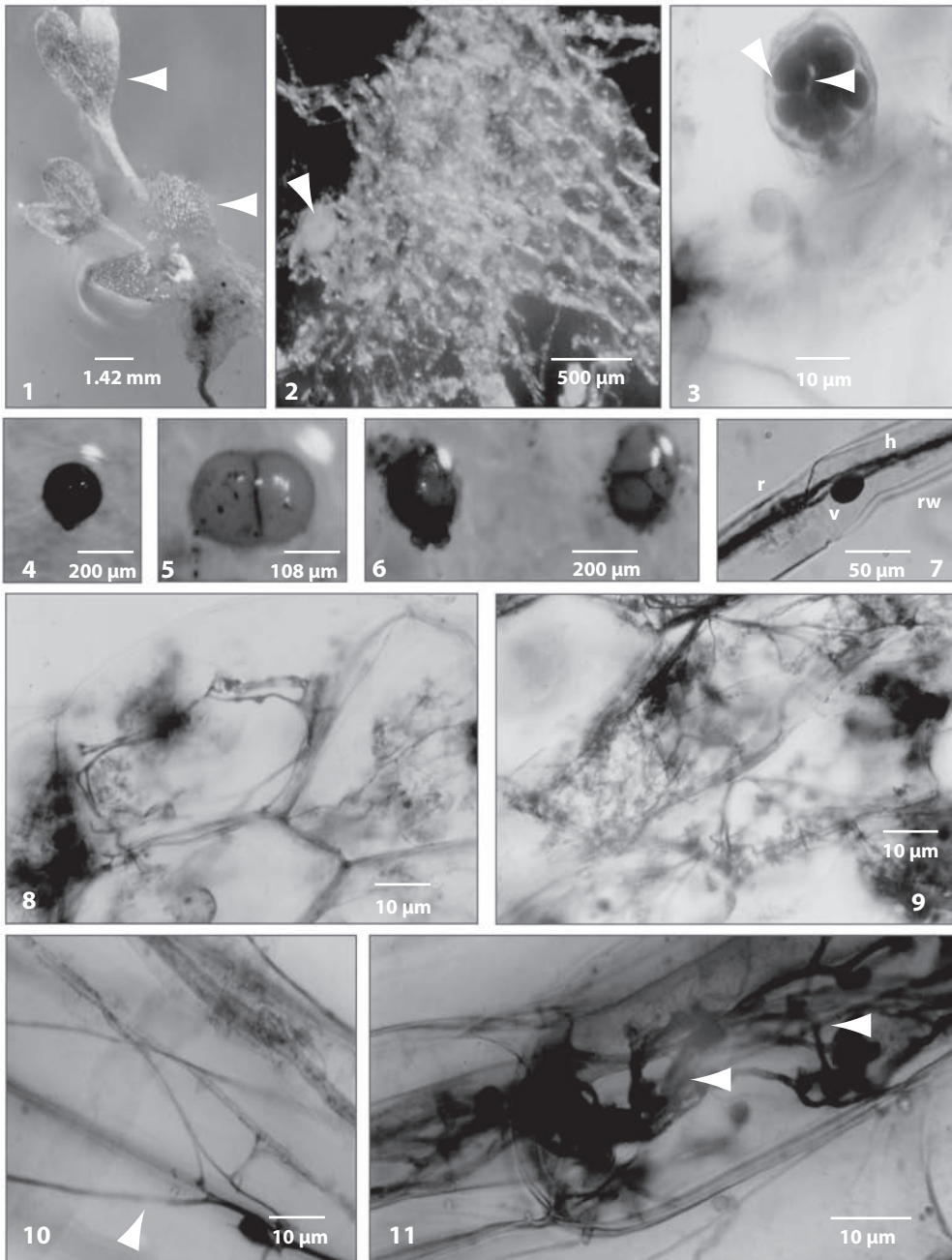


Fig. 1. *Dryopteris muenchii* gametophyte-sporophyte; **g**=gametophyte; **s**=sporophyte. Fig. 2. Chlorophyllous gametophyte with AM fungal spore; **g**=gametophyte; **s**=spore. Fig. 3. Anteridium with anterozooids; **a**=anteridium; **az**=anterozooids. Figs. 4-6. Arbuscular mycorrhizal (AM) fungal spores; **s**=spore. Fig. 7. Rhizoid with a hypha and vesicles; **h**=hypha; **r**=rhizoid; **rw**=rhizoidal wall; **v**=vesicle. Fig. 8. Gametophyte photosynthetic cell with hyphae and vesicles; **gphc**=photosynthetic cell; **h**=hypha; **v**=vesicle. Fig. 9. Gametophyte with hyphae colored with trypan-blue; **g**=gametophyte; **h**=hypha. Fig. 10. Appressorium within the sporophyte primary root; **ap**=appressorium; **spr**=sporophyte primary root. Fig. 11. Sporophytic cortical cells with an incipient arbuscule, and intra-cellular hyphal like-coil; **scc**=sporophytic cortical cell; **arb**=arbuscule; **hc**=hyphal like-coil.

not form archegonia and the sporophytes developed have an apogamic origin. The antheridia are produced in the middle region or in the margins of the prothallia (Fig. 3). From 450 gametophytes examined, 51.6% of the prothallia developed apogamic sporophytes (Fig. 1), 43% were asexual (without antheridia and archegonia), and 5% developed only antheridia without sporophytes. Only 4% of the gametophytes, with or without sporophytes, were mycorrhizal.

Very few AMF spores were reported amongst the gametophyte rhizoids, the spores were organized in groups of 5, pairs or singles, adhering to each other by common peridial hyphae. AMF spores are globose to subglobose, varying in color from orange- to red-brown (Figs. 4-6).

Gametophyte rhizoids are unicellular and hyaline with 23 μm to 50 μm in diameter. The endophytic fungus enters the host tissue through the rhizoids developing appressoria in the rhizoid wall at the penetration point. Hyphae, 2.5 to 5 μm in diameter, are found inside the rhizoids; these hyphae occur near the rhizoid apex up to the prothallial cells of the gametophyte, where they branch out. Rhizoidal hyphae are aseptate; frequently, these hyphae give rise to oval-shaped vesicles of ca. 15 x 20 μm in diameter, which are densely colored (Fig. 7).

Arbuscular mycorrhizal fungi (AMF) produced inter- and intracellular hyphae on the gametophyte midrib and wings prothallial cells; hyphae branch dispersing over a bigger surface (Figs. 8 and 9).

The meristematic region, placed in the midrib of the gametophyte, and the antheridia are regularly free of infection. The fungus never colonizes the sporophyte foot cells. The primary roots of the young sporophyte are frequently found colonized by a fungal endophyte, morphologically similar to the one described within the gametophyte. The fungus enters the host tissue through the roots developing appressoria in the rhizodermis surface at the penetration point (Fig. 10).

The fungus penetrates the primary roots and spreads forming intra-cellular linear

grouped hyphae in the rhizodermis and in the outermost cortical cell layers of the roots, developing hyphal enlargements at the distal hyphal tip, increasing the AMF surface; these structures look like incipient arbuscles (Fig. 11), which colored intensively with trypan-blue. Structures similar to hyphal coils were observed, as well, inside the inner root cortex (Fig. 11).

Finally, it is important to mention that the endophytic fungi associated to the young sporophytes, did not infected the gametophyte talus.

Discussion

Under laboratory conditions, *D. muenchii* is cordiforme, no archegonia are produced, only antheridia were reported; nevertheless, the sporophyte talus has an apogamic origin (Pérez-García *et al.* 1999, Reyes-Jaramillo and Mendoza 2004).

Apomictic plants are usually adapted to microhabitats, which could explain, in part, the fact that *D. muenchii* is a narrow endemic fern, and apomixis is also associated with polyploidy, phenomenon rather common in ferns. However, there are no studies relating both AMF and apogamic ferns, in functional and evolutionary terms.

From 450 gametophytes, only 4% of them, with or without sporophytes, were mycorrhizal. The scarce AMF spores, obtained from the pots, closely resemble to those of *Glomus globiferum*: the spores were organized in groups of 5, pairs or singles, adhering to each other by common peridial hyphae, having a globose to subglobose shape and varying in color from orange- to red-brown (Koske and Walker 1986).

We also found similar fungal structures than the ones found in *Gleichenia bifida* (Willd.) Spreng by Schmid and Oberwinkler (1995): the endophytic fungal hyphae were aseptate and spread inter- and intra-cellularly, they penetrated the host tissue through the rhizoids, developed vesicles in both the rhizoidal and the prothallial cells.

Within the gametophyte chlorophyllic cells, intracellular fungal hyphae were reported; the hyphae branched covering most of the

prothallial cells and, in some cases, the hyphae managed to trespass the cell wall, establishing inter-cellular hyphae; however, no arbuscles were registered.

In spite of this, the fungus penetrated the sporophyte primary roots and spread forming intra- and inter-cellular linear grouped hyphae in the rhizodermis and in the outermost cortical cell layers of the roots, developing "arbuscule-shaped structures" and hyphal like-coils. The fungus continued to spread by linear or coiled hyphae; this might be considered a fungal adaptation to the limited growth of both the gametophytes and the sporophytes in comparison to the root system of the vascular plants root system.

The fungal structures observed on *D. muenchii* during this study, are rather similar to those reported for the plant host-AMF interaction, where the AMF described belonged to Glomeromycota. Therefore, this study is a contribution to the scarce knowledge acquired of the association between AMF and chlorophyllous gametophytes and young apogamic sporophytes of ferns.

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RESUMEN

Se describe la morfología de un hongo endófito afín al género *Glomus*, como colonizador de gametofitos clorofílicos y de esporofitos apogámicos jóvenes del helecho *Dryopteris muenchii* A.R. Sm.; el estudio se llevó a cabo con helechos cultivados en el laboratorio y utilizando tierra como sustrato. El tejido del gametofito fue colonizado, a través de los rizoides, por un hongo micelial aseptado, el cual formó vesículas. El hongo logró penetrar las raíces primarias de los esporofitos jóvenes desarrollando apresorios. El hongo se dispersó formando hifas inter- e intra-celulares a través de la epidermis y de la capa de células corticales más externas, donde produjo vesículas, estructuras similares a ovillos y arbusculos. Las hifas del hongo nunca colonizaron las células de la unión entre el gametofito y el esporofito. Las estructuras observadas

durante este estudio en *D. muenchii*, son muy similares a las de la interacción planta hospedera-hongo micorrízico arbuscular (HMA), en donde el HMA descrito corresponde al Phylum Glomeromycota. Por lo anterior, este estudio es una contribución al escaso conocimiento que se tiene sobre la asociación entre los HMA y gametofitos clorofílicos y esporofitos apogámicos de helechos jóvenes.

Palabras clave: Micorriza arbuscular (MA), endófito, gametofito clorofílico, Dryopteridaceae, helecho endémico, México.

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