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ORIGINAL PAPER

Comparative study of mycorrhizal susceptibility and anatomy of four palm species

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Abstract A morphological and anatomical study of the root systems of the palm species *Brahea armata* S. Watson, *Chamaerops humilis* L., *Phoenix canariensis* Chabaud and *Phoenix dactylifera* L. has been carried out to determine possible mycorrhizal colonization sites. Furthermore, the arbuscular mycorrhizal (AM) anatomical types formed by the four palm species in association with *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe have been examined. The presence of a continuous sclerenchymatic ring in the outer cortex and aerenchyma in the inner cortex that are anatomical indicators of mycorrhizal nonsusceptibility in all four palm species is observed. The root systems of *B. armata* and *C. humilis* present only one group of third-order roots, while the third-order roots of *P. canariensis* and *P. dactylifera* may be divided into five different groups: short thick roots, mycorrhizal thickened roots, fine short roots, fine long roots, and pneumatorhizas. Third-order and some second-order roots of *B. armata* and *C. humilis* are susceptible to colonization by AM fungi, while only the mycorrhizal thickened roots form mycorrhizas with arbuscules in the *Phoenix* species. The root system of the *Phoenix* species also presents AM colonization in fine roots with only intraradical hyphae and spores, but without arbuscules, and pseudomantles of spores anchored in the pneumatorings of the second-order roots, which are described for the first time. The mycorrhizas formed by the four palm species are of an intermediate type, between the *Arum* and the *Paris* types, and are characterized by intercalary arbusculate coils and not only by intracellular but also by intercellular fungal growth. Our study suggests that a different degree of adaptation may exist among palm mycorrhizas toward the slow growth of palms and low spore numbers in the soil where they grow.

Keywords Intermediate arbuscular mycorrhizal anatomy · *Brahea armata* · *Chamaerops humilis* · *Phoenix canariensis* · *Phoenix dactylifera*

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Introduction

Although palms have long since been known to form symbiosis with arbuscular mycorrhizal (AM) fungi (St John 1988a), almost no data exist on which palm roots are susceptible to being colonized by AM fungi. Only a few authors working with palms have mentioned higher-order roots being more susceptible to AM colonization. Janos (1977) observed that the AM colonization in the root systems of *Bactris gasipaes* was low but uniformly distributed in the penultimate roots. This author did not find AM fungi in rootlets of the last order. In contrast, Carrillo et al. (2002) showed that the roots prone to colonization were second- and third-order roots in *B. gasipaes*, *Bactris mexicana*, and *Desmoncus orthacanthos*. The results of Fisher and Jayachandran (1999) on *Serenoa*

repens indicate that the roots which generally colonized were the finest roots, although sporadic colonization was also observed in all the other root orders. In relation to *Elaeis guineensis*, the third- and fourth-order roots formed mycorrhizas (Nadarajah 1980).

Information about the type of mycorrhizal anatomy formed in palms is also scarce. Two types of AM anatomy have been described in AM plants: the *Arum* type and the *Paris* type (Gallaud 1905; Smith and Smith 1997; Dickson et al. 2007). However, the factors determining their formation are not well understood. It seems that the AM anatomical type formed could be under host control (van Aarle et al. 2005), under AM fungal control (Cavagnaro et al. 2001a), or under the control of both plant species and AM fungus (Dickson 2004).

Most of the authors who have studied mycorrhizal anatomy have classified the different plant species, genera, and families as either *Arum* or *Paris* type (Smith and Smith 1997). Recently however, the existence of a continuum of mycorrhizal anatomies from *Arum* to *Paris* has been demonstrated (Dickson 2004). Accordingly, eight AM anatomical types, including four intermediate types, can be distinguished. Based on these new results, it is probably necessary to reassess many of the plant species and families which were classified in the past as either *Paris* or *Arum* type. For example, *Gingko biloba* was previously classified as *Paris* type, but rare intercellular hyphae have been identified (Fontana 1985) and should now be considered an intermediate type, as suggested by Smith and Smith (1997).

Both *Arum* and *Paris* mycorrhizal types have been described for the family Arecaceae (Smith and Smith 1997; Dickson et al. 2007). According to Nadarajah (1980), mycorrhizas formed by the palm *E. guineensis* are characterized by the presence of numerous coils and the absence of arbuscules, so it is considered a *Paris* type. *D. orthacanthos* has also been described as forming *Paris*-type mycorrhizas (Ramos-Zapata et al. 2006). Conversely, the mycorrhizas of the palms *S. repens*, *Acoelorrhaphe wrightii*, *Coccothrinax argentata*, *Pseudophoenix sargentii*, *Sabal palmetto*, and *Thrinax morrisii* have been classified as *Arum* type (Fisher and Jayachandran 1999, 2005). Bouamri et al. (2006) mentioned that the root colonization pattern of *Phoenix dactylifera* was also *Arum* type. Sengupta and Chaudhuri (2002) described the mycorrhizas of *Cocos nucifera* as *Arum* type and those of *Areca catechu*, *Borassus flabellifer*, *Nypa fruticans*, and *Phoenix paludosa* as "both types" according to Smith and Smith (1997). Da Silva and Cardoso (2006) also described the mycorrhizas of *B. gasipaes* as being both *Arum* and *Paris* types.

The palms *Brahea armata* S. Watson, *Chamaerops humilis* L., *Phoenix canariensis* Chabaud, and *P. dactylifera* L. studied herein were characterized by a very low mycorrhizal colonization level in the first experiments conducted on the effect of mycorrhizal inoculation on their growth response (Dreyer et al. 2001; Morte and Honrubia 2002). In the literature on mycorrhizal palms, the mycorrhizal colonization level shows a high degree of variability and is high (Blal et al. 1990; Oihabi et al. 1993), moderate (Bouamri et al. 2006; Rini et al. 1999), or low (Jaizme-Vega and Díaz-Pérez 1999; Janos 1977; Ramos-Zapata et al. 2006; St John 1988b), presumably depending on the palm and AM fungus species used and on the sampling method chosen. In order to rule out the possibility of an incorrect sampling procedure and to clarify the reasons for the low mycorrhizal colonization level, a detailed study was carried out into the localization of AM fungi in the palm root systems. The aim of this study is to describe the morphological and anatomical root features that determine the mycorrhizal susceptibility of the different root orders of four palm species. Another objective of this study is to characterize the type of mycorrhizal anatomy formed in the four palm species in association with *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. The only known precedent of a similar study is that of Fisher and Jayachandran (1999) on the palm *S. repens*.

Materials and methods

Biological material and growth conditions

The root systems of 2-year-old plants of the palm species *B. armata*, *C. humilis*, *P. canariensis*, and *P. dactylifera* were harvested. The two treatments applied were a control without mycorrhizal inoculation and a mycorrhizal treatment. One-year-old palms were inoculated with a bulk inoculum of *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (originally isolated by MC. Jaizme-Vega from the Canary Institute of Agricultural Research, ICIA, Tenerife, Spain) and were cultivated with silica sand as a substrate to facilitate root sampling and washing at harvest time. There were 20 replications for each treatment and palm species. The palms were grown under controlled conditions in a greenhouse, fertilized weekly with a modified Long Ashton solution (Hewitt 1952), with half phosphorus concentration, and watered when needed. At harvest, 12 months after inoculation with *G. mosseae*, the aerial parts of the palms were removed and the root systems carefully washed to avoid the loss of any roots.

Morphological and anatomical analysis

All the root material was processed fresh. The morphology of the root systems was visually examined under an Olympus SZH stereomicroscope equipped with an Olym-

pus Highlight 2000 lamp for the identification of the different root orders and other features, such as the presence of root hairs.

Numerous transverse and longitudinal sections of the different root types present in the root system of the four palm species were cut by hand with a razor blade. These root sections were stained with phloroglucinol–HCl to detect lignin, with sudan IV to visualize suberin and cutin, and with toluidine blue O to verify the nature of the cell walls. Additionally, root sections were stained with berberine/aniline and were observed under a Leica Leitz DMRD epifluorescence microscope fitted with an Hg-lamp, according to Brundrett et al. (1994).

Mycorrhizal susceptibility and anatomy

The morphoanatomical study described above was able to identify the mycorrhizal–susceptible roots in the root system of the four palm species. These were then selected and divided into four subsamples.

Bright field and epifluorescence microscopy

One subsample of roots was stained with trypan blue according to a modified version of the method described by Phillips and Hayman (1970) and was mounted on slides by carefully pressing on the cover glass to squash the root material and to allow the visualization of the AM colonization.

Another subsample of roots was cut into transverse and longitudinal sections by hand with a razor blade. One part of these root sections was stained with trypan blue and the rest assessed without staining by autofluorescence (Ames et al. 1982; Dreyer et al. 2006). All the root sections were observed under a Leica Leitz DMRD epifluorescence microscope equipped with an Hg-lamp. The stained sections were observed under bright field settings, while the autofluorescence of the AM fungal structures was observed under epifluorescence with an I3 filter cube (excitation filter BP 450-490, dichroic mirror RKP 510, barrier filter LP 515).

The third subsample of roots was cut into 2-mm segments under an Olympus SZH stereomicroscope equipped with an Olympus Highlight 2000 lamp and processed for microtome transverse sections. Root segments were fixed in 3% (*v*/*v*) glutaraldehyde in 100 mM cacodylate buffer pH 7.2 at 4°C for 24 h and washed twice for 30 min with 2% sucrose in 100 mM cacodylate buffer. Root segments were then dehydrated at room temperature in a graded ethanol series (50%, 70%, and 90%), 45 min in each ethanol solution, followed by absolute ethanol for 60 min (two changes), and finally, propylene oxide for 30 min (two changes). Root segments were then embedded in Spurr's (1969) resin and then placed in a propylene oxide/Spurr resin solution (2:1, *v*/*v*) for 30 min, in the same solution (1:1, *v*/*v*) for 2 h, and then at 1:2 (*v*/*v*) for 2 h. Then, root segments were embedded overnight in 100% Spurr resin at 4°C and polymerized at 70°C for 48 h. Blocks were sectioned on an Ultracut E Reichert Jung microtome. The thin sections were stained with 0.5% toluidine blue in 1% sodium borate and observed under an Olympus BH2 microscope.

During the examination of transverse and longitudinal sections, special attention was paid to the presence of intercellular hyphae, their length, the structure of the arbuscules, and the connection of the hyphae to the arbuscules. In this study, intercellular hyphae of a length of one cortical cell were considered short distance hyphae, while these extending over two or more cells were recorded as long distance hyphae.

Transmission and scanning electron microscopy

Some of the embedded roots (see above) were processed further for transmission electron microscopy (TEM). Blocks were cut with a Reichert Jung Ultracut E Ultramicrotome with glass blades. The fine sections were collected on EMS 200 SQO Mesrl (Veco) copper grids and were contrasted with 2% uranyl acetate and 2.5% lead citrate. Sections were observed under a Phillips TECNAI 12 Transmission Electron Microscope.

The fourth subsample of roots was processed for scanning electron microscopy (SEM). Root segments were cut into two halves transversally and longitudinally. Samples were fixed in 3% (*v*/*v*) glutaraldehyde in 100 mM cacodylate buffer pH 7.2 for 6 h and washed with 2% sucrose in 100 mM cacodylate buffer. Root samples were dehydrated in a graded acetone series, 50% acetone for 45 min, 70% acetone for 1 h, and 100% acetone for 1 h (two changes). Roots were critical point dried in CO_2 in a CPD020 model (Balzers Union). Root samples were coated with gold in a SEM coating system unit (Biolad Polaron Division). The SEM observations were made under a JEOL JSM-6100 Scanning Electron Microscope.

Results

P. canariensis and *P. dactylifera* showed the same root morphology and anatomy and shared a mycorrhizal anatomy. For this reason, the results of these two palm species are presented here together as for *Phoenix* spp.

Root morphology

The four palm species showed a homorrhizal root system, formed by the growth of equivalent first-order roots from

the stem base, from which further roots developed (Fig. 1). Up to three root orders were distinguished in the root systems of the four palm species. An abrupt change in diameter and length took place with the development of each root order (Fig. 1). No root hairs were observed in any of the root types examined.

The third-order roots were homogeneous in *B. armata* and *C. humilis*, with no special morphological differentiation. However, the third-order roots of both the *Phoenix* species were morphologically distinct and could be classified into four root types (Fig. 1): long fine roots, short fine roots, short thick roots ("root tubercles" *sensu* Seubert 1997), and pneumatorhizas (Fig. 2a–c). The short thick roots were lateral modified roots, strongly swollen, and bottle-shaped. Moreover, a fifth type of swollen third-order roots of a deep yellow color (Fig. 2b, d) was observed in the root systems of the mycorrhizal *Phoenix* plants. In this work, they are referred to as "mycorrhizal thickened roots". Normally, these roots were grouped in clusters of three to 20 roots (Fig. 2b), although they could also be found individually. Sometimes these roots were divided into two parts, a distal swollen part and a proximal or basal thin stalk, although the swollen part was directly attached to the parent root in most cases (Fig. 2b). Initially, it was thought that these roots were of limited growth, but an apical growth was observed in some of them (Fig. 2e).

Numerous aerating root structures, pneumathodes (pneumatozones or pneumatorings), were found along the roots of all the orders of the *Phoenix* spp. (Fig. 2f) and in the proximal part of the second-order roots of *B. armata* and *C. humilis*. These zones or rings of a mealy aspect, with loose tissue and a bright white color, were clearly distinguishable from the normal root segments and persisted for a long time after root abscission. In addition, the root system of *Phoenix* spp. also presented numerous aerating roots, for instance, pneumatorhizas and pneumatophores. The pneumatorhizas were extremely short modified lateral roots in which the loosening of the rhizodermis and the outer cortex formed a cap on the apex, while pneumatorings were present at the base (Fig. 2c, f). The apex was absent in advanced stages, and so vascular cylinders were frequently seen jutting out over the pneumatoring. The pneumatophores were second- or third-order aerial roots that developed with a negative geotropic growth, with generally more than one pneumatoring on their surface (Fig. 1).

Mycorrhizal susceptibility versus root anatomy

Although the palm roots were composed only of primary tissues, their composition firstly varied with the root order and secondly in the degree of dependence on the root diameter within each root order. Thus, multiple transitions were found between root orders. In general, roots consisted of the following tissues: rhizodermis, exodermis, outer cortex, inner cortex, endodermis, and vascular cylinder. The morphological and anatomical study conducted on the roots of the four palm species enabled those roots susceptible to being colonized by AM fungi to be determined.

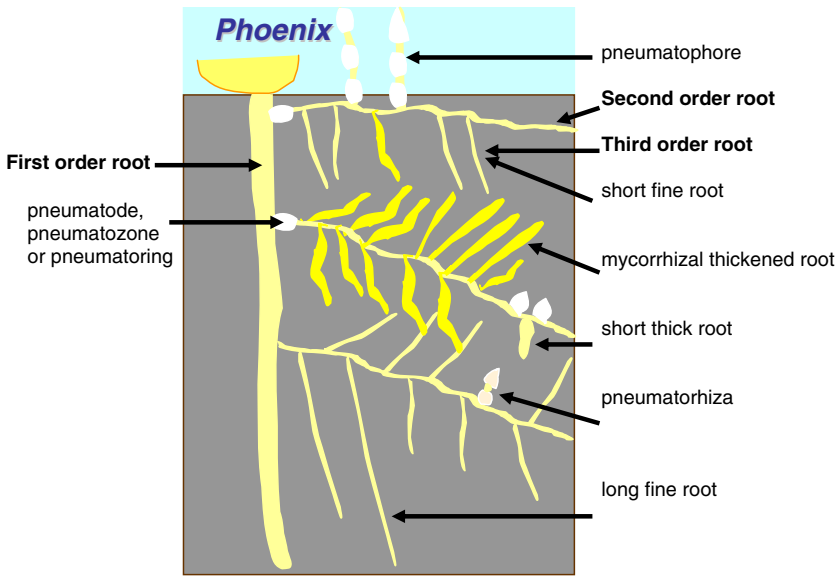


Fig. 1 Sketch of the *Phoenix* root system with the different third-order root types: long fine roots, short fine roots, short thick roots, pneumatorhizas, and mycorrhizal thickened roots. Additionally to the pneumatorhizas (short modified lateral roots with a cap on the apex and a pneumatoring in the base), other breathing roots and root structures like, for example, pneumatophores (aerial roots that develop with a negative geotropic growth, with more than one pneumatoring on their surface) and pneumathodes (zones or rings of a mealy aspect with loose tissue and a bright white color) are present

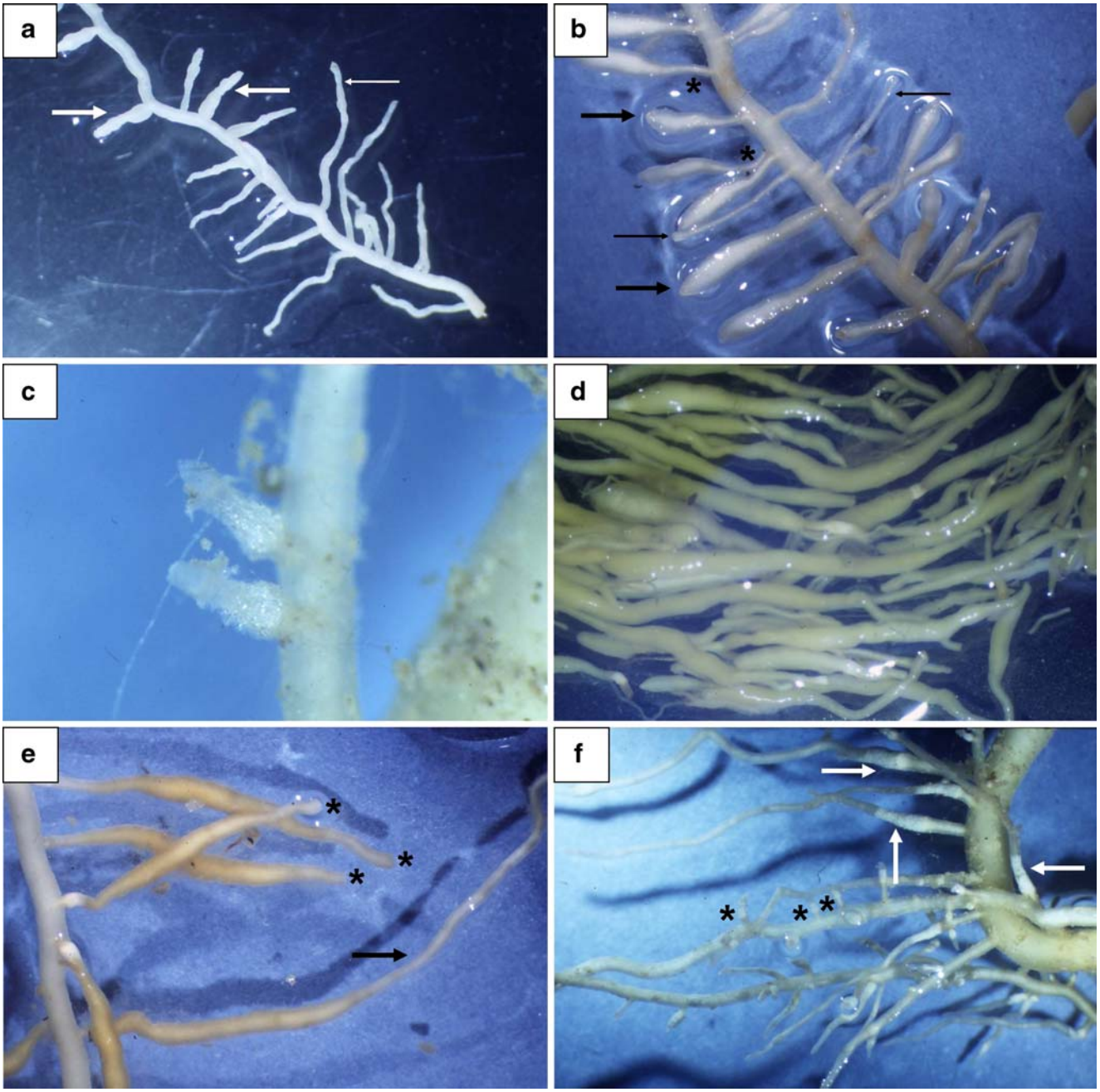


Fig. 2 Third-order root types of palms. **a** Fine long roots and short thick roots of *P. canariensis*, *fine and broad arrows*, respectively, ×7.5. **b** Young developmental stages of mycorrhizal thickened roots of *P. canariensis* (*broad arrows*), with swollen parts and with (*asterisk*) or without basal thin stalks, forming a cluster; fine short roots intercalated (*fine arrows*), ×7.5. **c** Pneumatorhizas of *P. canariensis*, ×50. **d** Mature developmental stages of mycorrhizal thickened roots of *P. dactylifera*, ×7.5. **e** Mycorrhizal thickened root of *P. canariensis* with apical growth (*arrow*); apex broken (*asterisk*) and pneumatorings at the base, ×7.5. **f** First-order root of *P. canariensis* with numerous second-order roots with pneumatorings (*arrows*) and pneumatorhizas (*asterisk*), ×7.5

The first- and almost all the second-order roots of the four palm species were not colonized by AM fungi. These roots were characterized by numerous aerenchyma lacunae in the inner cortex and by a continuous lignified sclerenchymatic ring in the outer cortex. The AM fungi of *B. armata* and *C. humilis* were seen to colonize some second-order roots only when the sclerenchymatic ring was discontinuous and the aerenchyma lacunae was not formed at all. Thus, there may be a relationship between the absence of mycorrhizal colonization and the presence of either a continuous sclerenchymatic ring in the outer cortex or aerenchyma lacunae in the inner cortex of the roots.

Those roots, which are unsusceptible to mycorrhizal colonization, were used by the AM fungi as guides along which AM fungi extended to reach the colonization sites.

All the third-order roots of the four palm species presented AM colonization, except for the pneumatorhizas and the short thick roots of the *Phoenix* spp. (Fig. 3a–e). The third-order roots of *B. armata* and *C. humilis*, as well as the mycorrhizal thickened roots of the *Phoenix* spp., were characterized by a one-layered rhizodermis consisting of unequal thickened lignified cells (Fig. 3a–c). No exodermis was present. The outer cortex was formed by two to three more or less lignified cell layers. The inner cortex was homogenous and no aerenchyma was present. The other types of third-order roots in *Phoenix* spp. displayed variations of this general pattern. The long fine roots were characterized by a more or less developed aerenchyma lacunae system in the inner cortex. In contrast, the short fine roots showed a much reduced cortex of only four cell layers, with no division into the outer and inner cortices (Fig. 3d, e).

AM colonization in all the mycorrhizal roots of the four palm species was restricted to the inner cortex (Fig. 3a–c). In the rhizodermis and the outer cortex, AM fungal structures were only observed if the root was sectioned through the part where an entry point was evident.

In the second-order roots of *B. armata* and *C. humilis*, the AM colonization units rarely occupied the whole inner cortex, but formed discrete colonization units. Conversely, the third-order roots generally showed a totally colonized inner cortex, with the exception of one or two cell layers which were directly adjacent to the vascular cylinder which were devoid of AM fungal structures (Fig. 3b, c).

As for *P. canariensis* and *P. dactylifera*, a distinction must be made between the mycorrhizal thickened roots and the fine long and short roots as only arbuscules have been observed in almost every cortical cell in the former (Fig. 3a). Only intraradical hyphae and spores formed in the fine long and short roots (Fig. 3d, e). Despite the short thick roots of *Phoenix* spp. having the same anatomy as the mycorrhizal thickened roots, they were never seen to be colonized by AM fungi.

The root anatomy at the pneumatorings level corresponded to that of the normal parts of the roots where they formed. The only difference found was that the

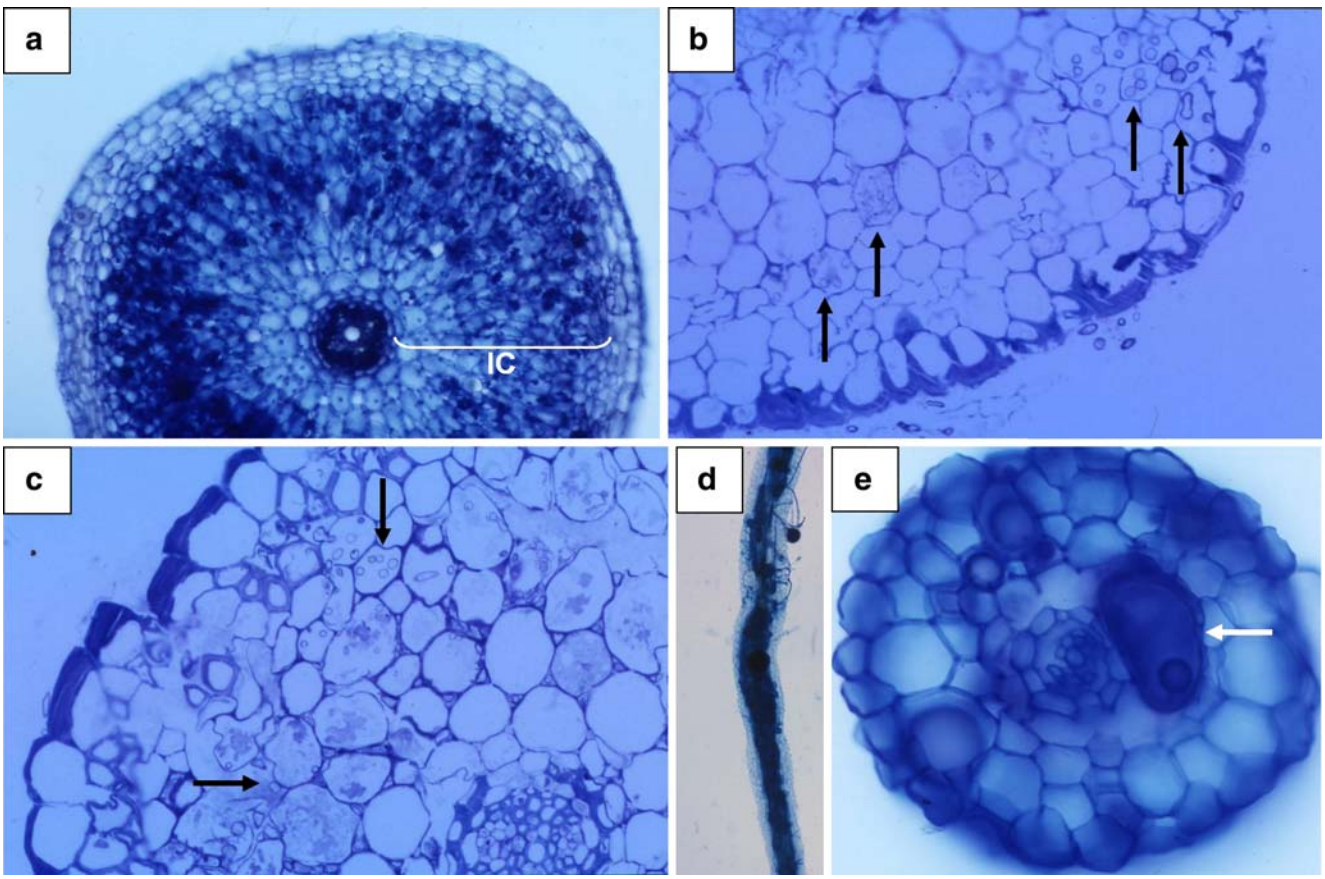


Fig. 3 Anatomy of palm roots. **a** Mycorrhizal thickened root of *P. canariensis* with a mostly complete arbuscular colonization of the inner cortex (*IC*), ×100. **b** Third-order root of *B. armata* showing AM colonization in the inner cortex (*arrows*), ×400. **c** Third-order root of *C. humilis* showing AM colonization in the inner cortex (*arrows*), ×400. **d** Fine short root of *P. dactylifera* with a very reduced cortex, showing endophytic AM fungal growth, ×40. **e** Fine short root of *P. dactylifera* showing a vesicle or spore (*arrow*), ×400

rhizodermis and the outer cortex were substituted for a loose tissue composed of round cells with multiple intercellular spaces, the pneumatodermis, which was formed through cell divisions in the outer zone of the inner cortex (Fig. 4a). Special spore group structures were observed in the parts of the pneumatorings of the second-order roots of *Phoenix* spp., where they formed pseudomantles (Fig. 4b). The pseudomantles of fungal spores were never observed to extend to the normal zones of the root. The transverse sections across these zones reveal that fungal spores only anchored in the pneumatodermis and showed no hyphal projections inside the deeper root tissues (Fig. 4c).

Mycorrhizal anatomy

The AM fungus, *G. mosseae*, colonized the roots of the four palm species by forming numerous arbuscules in the inner cortex (see above). These were found to be arbusculate coils. In all the fine transverse root sections examined, the presence of groups of two or more intracellular hyphae in each cortical cell was observed (Fig. 3b, c), presumably representing sections through hyphal or arbusculate coils. Another examination showed that they did not represent the terminal structures of hyphae, as with the typical tree form of *Arum* arbuscules, but intercallary structures instead as they were always attached to two or more hyphae, and passage hyphae from one cell to the next were frequently observed (Fig. 4d, e). The presumably previous stages to arbusculate coils, e.g., hyphal coils, were not observed in any section.

The presence of intercellular hyphae in *B. armata* occupying the plant intercellular spaces was rarely observed. Conversely, intercellular hyphae were frequently observed in almost all the intercellular spaces surrounding the cortical cells of *C. humilis* and *Phoenix* spp. (Figs. 3c, 4f). Apparently most of these intercellular hyphae were the result of the arbusculate coil hyphae growing out of one cortical cell, surrounding it and growing in the next cortical cell to form a new arbusculate coil (Fig. 4g, h), but never extending at long distances along the root axis (more than one cortical cell). Thus, they represented short distance hyphae. Moreover, in the squashed longitudinal sections of roots of *C. humilis* and *Phoenix* spp., some longitudinally extending long distance hyphae (extending over more than three cortical cells) were present (Fig. 4i). These were never observed in *B. armata*.

Vesicles were never found in the roots of *B. armata* examined and were rarely seen in those of *C. humilis* and *Phoenix* spp. On some occasions, these vesicles were attached to long distance intercellular hyphae.

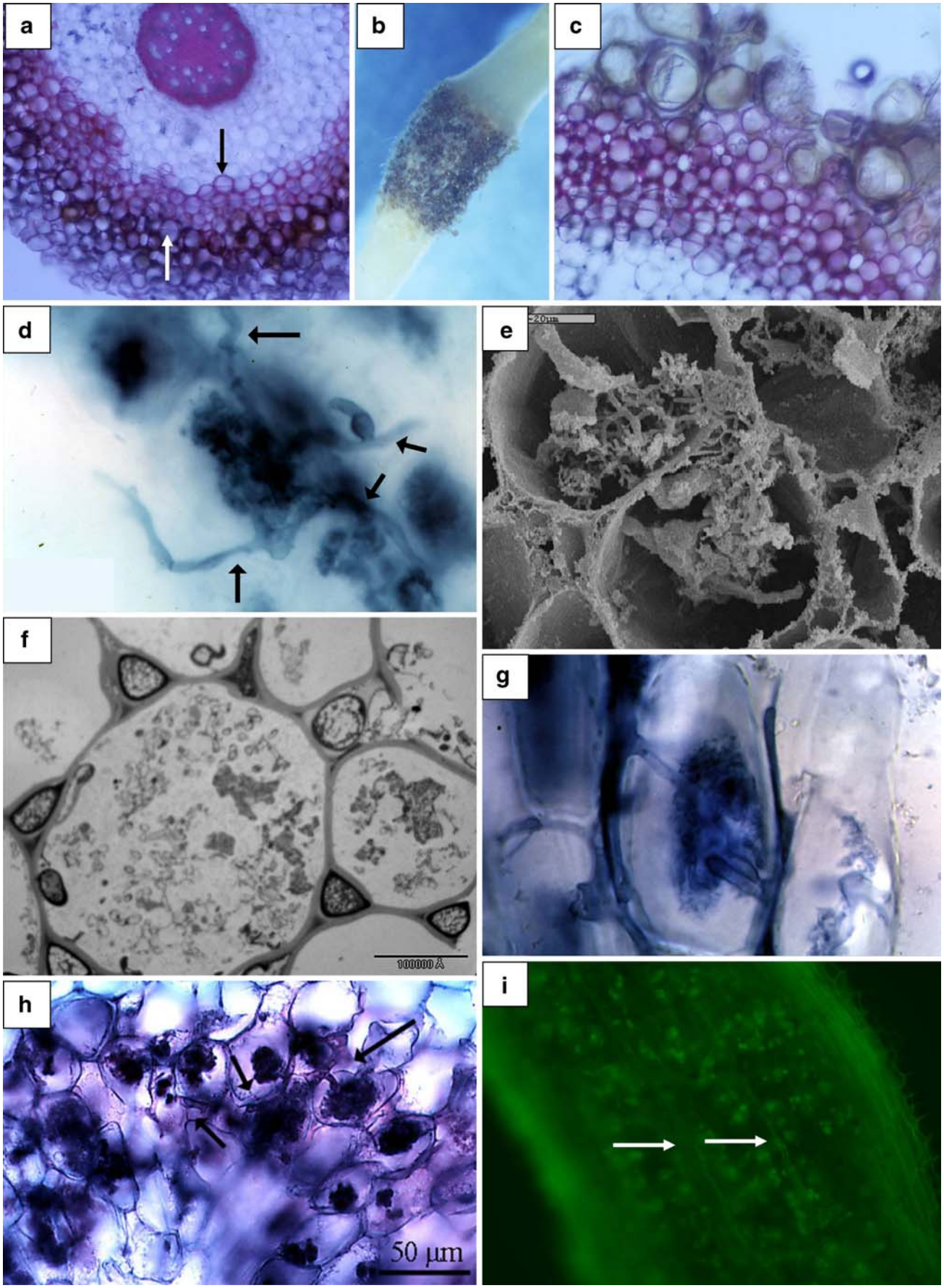
Discussion

According to Tomlinson (1990), palms can develop up to a maximum number of four root orders, although the same author recognizes that opportunistic root development also exists to a great extent. In this study, the roots of the four palm species are classified based on the structural root aspects of their interaction with AM fungi. It was not possible to distinguish more than three root orders in the root systems of the four palm species. Although the diameter and other structural aspects, e.g., width of the outer or inner cortex, vary considerably within each order, these variations were not fundamental for classifying the roots to different orders. A high number of root orders may possibly exist in adult plants. Nevertheless, Oihabi (1991) distinguished only three root orders in the root systems of adult palms of *P. dactylifera*.

Despite a few differences, the root anatomy of the four palm species is in accordance with the anatomy described for the subfamily Coryphoideae (Seubert 1997). None of the examined root orders presents piliferous zones or trichoblasts, although Seubert (1997) stated that the roots of both *Brahea* and *Phoenix* species presented root hairs. Unfortunately, this author did not mention in which root order these root hairs were present. Seubert (1997) also suggested that root hairs were a very frequent feature in some palm genera, which contradicts the finding of most of the authors who have worked with palms that states that the species of the family Arecaceae were devoid of root hairs, except the first seedling roots (Tomlinson 1990).

The results of the present study show that numerous pneumatorings were present in all the roots of *Phoenix* spp. and in the proximal parts of the second-order roots of *B. armata* and *C. humilis*. Seubert (1997) mentioned that pneumathodes were frequent in *Phoenix*, on the surface of pneumatophores and also on normal aerial and subterranean roots, but she found no pneumathodes on the roots of *Brahea* or *Chamaerops*.

At the pneumatorings level, the anatomy of all the examined roots of *B. armata*, *C. humilis*, and *Phoenix* spp. is similar, which was also described by Oihabi (1991) and de Granville (1974) for *P. dactylifera* and *Mauritia flexuosa*, respectively. The pneumathodes and other aerating structures accomplish the function of lenticells, because these, like all the other tissues derived from a cambium activity, are missing in palms. In the present study, we find that the best aeration characteristics of the pneumatodermis seem to trigger the massive sporulation of the AM fungus, leading to the formation of a spore pseudomantle. This is the first time that such a high and localized proliferation of spores has been associated with living tissue. Only in old root parts characterized by cortex death has the massive


a
b
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◀ **Fig. 4** Mycorrhizal anatomy. **a** Second-order root of *B. armata* with pneumatodermis and dividing tissue in the outer zone of the inner cortex (*arrows*), ×100. **b** Second-order root of *P. dactylifera* with spores pseudomantle on the pneumatoring, ×25. **c** Transverse section through pneumatoring in second-order root of *P. canariensis* showing spores anchored in pneumatodermis, ×200. **d** Root of *B. armata* with arbusculate coil intercalated in four hyphae (*arrows*), ×1,000. **e** SEM micrography of intercalary arbusculate coil in root of *C. humilis*, ×1,500. **f** TEM micrography of intercellular hyphae surrounding the colonized root cortical cells of *C. humilis*, ×1,450. **g** Root of *P. canariensis* with arbusculate coil intercalated between short distance intercellular hyphae, ×1,000. **h** Transverse root section of *P. dactylifera* showing arbusculate coils connected to penetrating hyphae (*arrows*), ×400. **i** Longitudinal root section of *C. humilis* showing two long distance longitudinal hyphae (*arrows*), ×200

production of spores been observed before (Fester et al. 1999).

The morphological and anatomical study conducted helps identify the roots of the four palm species which are susceptible to being colonized by AM fungi. All these roots show a rhizodermis consisting of cells with thickened outer cell walls. Thus, it is thought that the AM fungi either penetrate these roots through the younger apical zones where cell walls are still not secondary modified or are able to digest these thickened cell walls and make a way for themselves by passing inter- or intracellularly. Brundrett and Kendrick (1990b) observed that the entry points of the plant-colonizing AM fungi were close to the root apex where cell walls were still unlignified and unsuberized.

A clear relationship between the presence of both a continuous sclerenchymatic ring in the outer cortex and an aerenchyma in the inner cortex, accompanied by the absence of mycorrhizal colonization, is observed. This is logical since the sclerenchymatic ring is a physical barrier against AM fungal penetration, while the aerenchyma lacunae considerably reduce the tissue available for AM colonization. This has also been shown for other plants (Brundrett et al. 1990).

The AM fungi-colonized roots in *B. armata* and *C. humilis* are third-order roots, although some second-order roots are also colonized. In the second-order roots of these palms, a sclerenchymatic ring is present, but is always discontinuous.

In both the *Phoenix* spp., only the third-order roots present colonization. Oihabi (1991) also indicated that the first- and second-order roots of *P. dactylifera* do not harbor AM fungi. Other authors have shown that the higher-order roots in other palms are the most susceptible to AM colonization (Janos 1977; Nadarajah 1980; Fisher and Jayachandran 1999; Carrillo et al. 2002).

In terms of mycorrhizal colonization, the *Phoenix* root system presents a division of different specialized types of third-order roots. The mycorrhizal thickened roots are where the arbuscular colonization units form, while the AM fungus proliferates and sporulates, but does not form arbuscules, in the fine roots. Thus, in the same plant and without temporal, but only physical separation, the AM fungus shows two different development patterns.

We herein suggest that the precursor roots of the mycorrhizal thickened roots of the studied *Phoenix* spp. could be the short thick roots which, once colonized, undergo an elongation and color change, which is supported by the same root anatomy in both root types. The yellow coloration of roots upon AM colonization described herein for the first time in mycorrhizal thickened roots of *Phoenix* has been described before in legumes (Jones 1924, cited in Fester et al. 2002) and in many other plants (Fester et al. 2002) and has been used for the spectrophotometric quantification of the percentage of AM colonization in onion roots (Becker and Gerdemann 1977). The characteristic short thick roots of the root systems of *P. canariensis* and *P. dactylifera* have also been observed in *P. paludosa*, although these roots with tuberized aspect are not exclusive to the *Phoenix* species as they have also been found in the genera *Itaya*, *Pritchardia*, *Acoelorrhaphe*, and *Serenoa* (Seubert 1997). Seubert (1997) named this type of root, "root tubercles". However, Fisher and Jayachandran (1999) did not describe this type of root or roots similar to mycorrhizal thickened roots in *S. repens*. These authors also conducted experiments with *Acoelorrhaphe* species; once again, they observed neither of these root types (J.B. Fisher, personal communication). Seubert (1997) did not observe AM fungi inside the root tubercles and, therefore, did not explain their function. Similarly, tuberized roots have also been observed in podocarps to be the principal sites of AM colonization, and a function in retaining the AM fungus after the long roots have shed their cortex has also been suggested (Baylis et al. 1963). Short thick roots are probably very widespread and act as AM colonization sites in palms; for example, Zona (1996) mentioned that the root system of *Roystonea* sp. presents tuberized roots that were mycorrhizal colonization sites. No information on the anatomy or morphology of this type of roots in *Roystonea* is available because this observation was made by chance (S. Zona, personal communication).

The exact meaning of the physical separation of two different AM development patterns in *Phoenix*, arbuscular colonization in mycorrhizal thickened roots and vesicular colonization in the fine roots, remains unclear. Other authors refer to vesicular colonization without the presence of arbuscules as endophytic activities of the AM fungi and suggest that they may be beneficial for the fungi or may simply be a consequence of high inoculum levels in soils (Brundrett 2004). Furthermore, arbuscular colonization is regarded as functional AM colonization, defined only by the presence of the arbuscules and arbusculate coils because these are the sites of a bidirectional exchange between the symbionts. However, such an exchange could

also take place in other AM fungal intraradical structures. Muthukumar et al. (1997) suggested that mycotrophic nonfunctional plants, such as those in which root system vesicles, but not arbuscules, form, may be important and help increase the number of propagules in soils. These authors observed that the association of a mycotrophic with a nonmycotrophic plant enhances fungal colonization in both plants: the arbuscule number in the case of the former plant and the vesicle number in the latter one. *Phoenix* palms could be a good model for studying these different AM fungal activities ("endophytic" and "functional" activities) because they bring together processes in the same plant and at the same time that normally occur separately in different plants or in the same plant at different times. Apparently, *Phoenix* palms have developed a way to control sporulation since they contain roots with both arbuscular and vesicular colonizations. We believe that fine roots could act as inoculum reservoirs for newly developing mycorrhizal thickened roots.

AM fungi were observed in mycorrhizal roots in the rhizodermis or the outer cortex of the four palm species only where an entry point was present. Arbuscules and vesicles were distributed in the inner cortex of the roots. Carrillo et al. (2002) also found AM colonization in the inner cortex of the roots of the palms *B. gasipaes*, *B. mexicana*, and *D. orthacanthus*. However, Janos (1977) observed mycorrhizal colonization of the penultimate roots of *B. gasipaes* which was limited to the rhizodermis and to the outer cortex. Fisher and Jayachandran (1999) detected AM mycorrhizal structures in the outer third of the inner cortex near the sclerenchymatic ring of *S. repens* roots.

The mycorrhizal anatomy observed in the four palm species studied is of an intermediate type, corresponding to the intermediate four type described by Dickson (2004). The arbuscules present the same structure in the four palm species studied and are intercalated in the hyphae that extend intracellularly or in short intercellular hyphae. These hyphae coil to a greater or lesser extent when they penetrate the cortical cell and ramify along the whole length and resemble the arbusculate coils described in plants that form *Paris*-type mycorrhizas, e.g., *Sequoia gigantea* (figure of Gallaud (1905) reproduced in Smith and Smith 1997), *Panax quinquefolius* (Whitbread et al. 1996), *Acer saccharum* (Cooke et al. 1992; Yawney and Schultz 1990), and *Annona cherimola* (Azcón-Aguilar et al. 1994), or in plants that form near-*Paris*-type mycorrhiza, like *G. biloba* (Fontana 1985) or *Taxus baccata* (Strullu 1985). A very high density of arbusculate coils is observed; for example, *Phoenix* roots are almost totally colonized with almost every cortical cell harboring an arbusculate coil. It is rare to find coils in the inner cortex in which the arbusculate ramifications have still not developed. This contrasts not only with other studies based on plants forming *Paris*-type mycorrhizas in which, apart from the arbusculate coils, numerous hyphal coils are observed (Cavagnaro et al. 2001b; Whitbread et al. 1996) but also with the descriptions of herbaceous plants of temperate forests (Brundrett and Kendrick 1990b). The fact that practically no hyphal coils are observed in palm roots, despite the fact that these are previous stages to arbusculate coils, may be due to the very rapid transition from hyphal coils to arbusculate coils, as described by Whitbread et al. (1996) in *P. quinquefolius*. Cavagnaro et al. (2001b) described how most coils from the inner cortex transformed into arbusculate coils, while this transformation did not occur with the coils from the outer cortex. The absence of arbusculate coils in some plants could be the result of seasonal effects or environmental stresses (Brundrett and Kendrick 1990a; Whitbread et al. 1996). Therefore, it is possible that the palms studied herein were grown in optimal conditions.

Unfortunately, no emphasis has been placed on the arbuscules in other palm studies. Fisher and Jayachandran (1999, 2005) classified the mycorrhizas formed in *S. repens*, *A. wrightii*, *C. argentata*, *P. sargentii*, *S. palmetto*, and *T. morrisii* as *Arum* type, but did not describe the arbuscule structure, so it is not clear whether arbuscules were intercalary or terminal, compound, or simple. Other palms mentioned as forming *Arum*-type mycorrhizas are *P. dactylifera* (Bouamri et al. 2006) and *C. nucifera* (Sengupta and Chaudhuri 2002). Nadarajah (1980) found that the AM fungal hyphae colonized roots longitudinally, forming coils in the root cortical cells of *E. guineensis*. While these authors did not observe arbuscules, the coils probably represent the stages prior to arbusculate coils. The mycorrhizas of *D. orthacanthos* have been classified as *Paris*-type, although fungal structures other than internal hyphae represent less than 5% (Ramos-Zapata et al. 2006). Other palms have been described to have both types of mycorrhiza, e.g., *A. catechu*, *B. flabellifer*, *N. fruticans*, *P. paludosa* (Sengupta and Chaudhuri 2002), and *B. gasipaes* (Da Silva and Cardoso 2006). Yet once again, information about hyphal or arbusculate coils is lacking. More emphasis should be placed on these structures as they are considered highly relevant for nutrient transfer through the AM symbiosis in *P. canariensis* palms (Dreyer et al. 2008).

As regards intercellular hyphae, a distinction should be made between long distance and short distance hyphae. In the mycorrhizal colonization of *G. biloba*, classified as near *Paris* (Smith and Smith 1997), intercellular hyphae have been observed, although not frequently (Fontana 1985). As the author herself indicates, intercellular hyphae traverse a very short distance and then penetrate the next cortical cell intracellularly. Thus, the mycorrhizal anatomy of *G. biloba* is similar to that described herein for palms. The "rare" intercellular hyphae of *A. saccharum* (Yawney and Schultz 1990) and *T. baccata* (Strullu 1985) could also be such

short distance hyphae. For some reason, the direct cell–cell passage of the hyphae is less used by the AM fungus in palm roots than the indirect passage through the intercellular spaces. No physical barriers in the cortical cell walls that could block the most direct way of AM fungus growth have been observed. In both the *Phoenix* spp. as well as in *C. humilis*, long distance intercellular hyphae are present alongside the short distance hyphae, although these are clearly distinguished from the typical linear intercellular hyphae of the *Arum*-type mycorrhizas because no simple terminal arbuscules ramify into the cortical cells from the former one.

As regards vesicles, there is no indication that anatomical types influenced vesicle production (Dickson 2004). Indeed, the occurrence of vesicles in *Allium porrum* forming *Arum*-type mycorrhizas and in *Asphodelus fistulosus* forming *Paris*-type mycorrhizas, colonized by *Glomus intraradices*, is similar at 6% (van Aarle et al. 2005). However, we observe that vesicles are linked to the longitudinal long distance hyphae. Therefore, it would be interesting to establish whether a relationship between the development of intercellular long distance hyphae and the production of vesicles in intermediate types actually exists. This need is further supported by the fact that no intercellular long distance hyphae are observed in *B. armata*, in which no vesicles form.

One explanation for the occurrence of intermediate anatomical types in palms could be that their roots display discontinuous intercellular spaces, as stated by Smith and Smith (1997). Another suggested plant feature that may influence AM anatomy is the structure of the roots themselves given the significant variation between the different roots formed, especially in monocotyledonous plants (Dickson 2004).

Cavagnaro et al. (2001a) suggested that the intermediate types of mycorrhizas in some plants may be due to the different AM fungi present. However, the results of Dickson (2004) unequivocally indicate the fact that intermediate types form in some plants colonized by a single AM fungus. Here, it is highly improbable that the intermediate types found in palms are due to colonization by different fungi because a collection of monosporic inoculum was used.

However, a different biological explanation as to the different mycorrhizal types formed by plants exists. The *Paris*-type AM colonization expands more slowly inside the roots than the *Arum* type (Brundrett and Kendrick 1990a; Cavagnaro et al. 2001b). As the intermediate type formed by palms is nearer to the *Paris* than to the *Arum* type, this could explain the slow rate of AM development in their roots where, for instance, they take 4 months to become well established in *P. dactylifera* (Oihabi et al. 1993). Brundrett and Kendrick (1990a) suggested that the *Paris* type is the best strategy for slow-growing plants because less energy is derived from the plant. Thus, it is interesting that the most slow-growing palm and also that which most depends on the AM mycorrhiza of the four palm species examined herein, *B. armata* (Dreyer 2004), show a mycorrhizal anatomy with no long distance hyphae and are, thus, even "nearer to *Paris*" if compared with *C. humilis*, *P. canariensis*, and *P. dactylifera*, which relatively display more rapid growth.

In conclusion, not all palm roots are susceptible to colonization by AM fungi. A method based on root morphology is preferable to evaluate root colonization. Although our results are based on plantlets grown under controlled artificial conditions which cannot be extrapolated to adult palms in nature, our study suggests that a different degree of adaptation may exist among palms as regards their mycorrhizas and that special attention must be paid to the presence of short and long distance intercellular hyphae. It is not clear whether the intermediate mycorrhizal anatomical type formed here by *G. mosseae* in palm roots is also formed by other AM fungi. Moreover, the functional efficiency in terms of nutrient transfer, for example, of these arbusculate coils should be compared with that of the *Arum* arbuscules formed in other palm species. The palm species studied herein are native to arid and semiarid regions and are characterized by a very low number of AM fungal spores in soil (Dreyer 2004). A low spore number is also typical in the rhizosphere of other plants of arid or semiarid Mediterranean ecosystems (Azcón-Aguilar et al. 2003). Indeed, in these soils, the main source of inoculum is extraradical mycelium (Requena et al. 1996). The strategy developed by *Phoenix* spp. to increase propagule numbers by developing different root types and structures, such as the pseudomantle, could be regarded as a notable example of adaptation between the host and fungal partner in response to such conditions.

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