

Chapter 7

How Root Structure Defines the Arbuscular Mycorrhizal Symbiosis and What We Can Learn from It?

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7.1 Introduction

7.1.1 Mycorrhizal Anatomy

Mycorrhiza is a mutualistic association between fungi and plant roots, in which the fungal partner facilitates mineral nutrient acquisition by the plant and in turn host provides carbohydrates to the fungus (Smith and Read 2008). Up to seven different mycorrhizal types have been distinguished, depending on the fungal symbiont and the type of anatomy that it forms in the root (Peterson et al. 2004; Smith and Read 2008). The arbuscular mycorrhiza (AM) formed by fungi of the division *Glomeromycota* is one of the most widespread in terrestrial ecosystems, since it is formed by almost 80 % of plants (Brundrett 2009). This is characterised by the intracellular growth of the fungal partner, forming arbuscules in the cells and, under some circumstances, vesicles. Among AM, a high variability of mycorrhizal anatomies can be distinguished, sometimes described as a continuum between the classical *Arum* and *Paris* anatomical types (Dickson 2004). However, most authors who have studied mycorrhizal anatomy classify the different plant species, genera and families as either *Arum* or *Paris* type (Smith and Smith 1997), and a reassessment considering this continuum of AM anatomical types is still pending in most cases.

The different AM types are usually highly consistent within a certain host plant and even within a certain plant family (Smith and Smith 1997; Dickson et al. 2007).

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However, the factors determining their formation are not well understood, and the AM anatomical type formed may be under host control (van Aarle et al. 2005; Ahlu et al. 2006), under AM fungal control (Cavagnaro et al. 2001a; Smith et al. 2004) or under the control of both plant species and AM fungus (Dickson 2004).

7.1.2 Root Features as “Barriers” to the Entrance or Modifiers of the Development and Anatomy of Arbuscular Mycorrhizae

The entrance of an AM fungus into a root, its development therein and formation of the mycorrhizal anatomical type and the mycorrhizal susceptibility of the different root orders present in a root system depend on many factors.

Some of the anatomical features present in roots act as barriers to the entrance of AM fungi, so that no entry points are formed. For example, the rhizodermis of some species presents extremely thickened cell walls that AM fungi cannot penetrate. Also, the presence of an exodermis or hypodermis, or thickened cells in the outer cortex, may hinder the AM fungi reaching the cortex (Brundrett and Kendrick 1990b; Brundrett et al. 1990). Modifications of the cell walls of these root tissues could determine the way in which AM fungi enter the roots. For example, in species with a dimorphic exodermis, AM fungi enter through the short cells after long cell suberisation is complete (Brundrett and Kendrick 1988). But, in the cortex, AM development may also be hampered by root anatomical features, e.g. idioblast cells that contain crystals (Brundrett and Kendrick 1990b) or aerenchyma lacunae, which clearly reduce the tissue available for colonisation (Dreyer et al. 2010).

Other root anatomical features, like the Phi-thickenings mentioned by some authors as potential barriers to fungal growth in ectomycorrhizae (see Fernández-García et al. 2014), should not act as barriers in AM as the presence of Phi-thickenings even in the innermost cortical layer in *Ginkgo biloba* did not impede the AM fungus *Glomus epigaeum* from forming arbusculate coils in these cells (Fontana 1985).

Another root anatomical feature that may influence the mycorrhizal formation is the presence of intercellular spaces. Brundrett and Kendrick (1990a) suggested that the AM anatomical type formed is determined by the presence of continuous intercellular airspaces along the root cortex. When they are present, the *Arum* type is formed; otherwise, when they are absent, the *Paris* type develops. For plant species forming discontinuous intercellular airspaces, intermediate types would be expected (Smith and Smith 1997). However, although the correlation between the size of the airspaces within the root cortex and the AM anatomy was suggested as early as 1904 with the study of Gallaud (Dickson et al. 2007), there have been no concerted attempts to quantify this, although the few studies conducted do not support this hypothesis. For example, it has been shown that a

tomato cultivar that can form either *Arum*, *Paris* or intermediate AM anatomical types, depending on the AM fungus used as inoculum, may also present discontinuous airspaces (Bago et al. 2006; Dickson et al. 2007). In addition, Li (2008) showed that the proportion of airspaces in the inner and outer cortex of Dandelion and Chive did not have any influence on the AM anatomical types (*Arum*, *Paris* and intermediate) developed. The higher abundance of intercellular hyphae in dandelion did not correlate with bigger intercellular airspaces in the cortex. However, care has to be taken with these results, as only a small number of root sections were examined and the plants were not inoculated with a known isolate of AM fungus, so that different AM fungi may have been present (Li 2008).

It has been also found that other properties of the cortex may have a considerable influence on mycorrhizal anatomy and development of the AM fungi, since in many plant species with an *Arum*-type anatomy, the intercellular hyphae with arbuscules are localised within the roots preferentially in the inner cortex (Abbott 1982; Fisher and Jayachandran 1999; Allen et al. 2006), while in plant species with a *Paris*-type anatomy, extensive intracellular hyphal coils are formed but predominantly in the outer cortex (Cavagnaro et al. 2001b). Further, AM fungi are never present in the endodermis, although these cells do not offer physical restrictions to the radial passage of fungi when they are in State I of suberisation (Brundrett and Kendrick 1990b). Other factors, like the nutrient content or gas concentration, might further determine the way in which the AM fungi spread in the root.

It has been suggested that in roots presenting physical barriers to their passage, AM fungi would penetrate the root subapical regions, from where they would colonise the entire root. However, in heterogeneous root systems, some root orders are colonised and others are not, although they all have non-differentiated root zones, in which the cell walls have no secondary modifications. Thus, there must be other reasons for the non-colonisation of some root orders, other than merely physical ones. This hypothesis is further emphasised by studies conducted with mutants that can block AM colonisation at different AM fungal developmental stages. Such studies should help unravel the events in recognition and early colonisation (Harrison 2005).

Further, root anatomical features may change in response to the presence of AM fungi or with abiotic factors like water. For example, some AM fungi are able to digest the cell wall material in order to facilitate their passage through the intercellular spaces (Bonfante and Perotto 1995), while others have no such ability.

The debate as to whether particular regions of a root are preferentially colonised by AM fungi, cells in these regions showing unique structural or physiological properties, will go further until more is known on the molecular dialogue between plants and AM fungi. It has been affirmed that the root segments are only colonisable for a limited time, after which they become permanently non-mycorrhizable (Schwab et al. 1991), although detailed analysis of AM entry points to clover and leek roots has shown that this is not the case, since a region immune to colonisation behind the root apex does not exist (Smith et al. 1992). However, it has been shown that an established arbuscular mycorrhizal symbiosis

suppresses further mycorrhization, demonstrating the presence of an autoregulatory mechanism linked with the extent of root colonisation (Vierheilig 2004).

What makes this type of study even more complicated is the highly dynamic nature of the processes involved in the establishment of a functioning AM. The growth of the root system is constant and there are always developing roots representing new colonisation sites for AM fungi. Curiously, this could be under the control of the AM fungal partner, as, among the biotic and abiotic factors that influence the root development, it has been shown that the presence of AM fungi can induce important changes in root systems (see Sect. 7.1.4).

7.1.3 Root Order Variation in Root Structure and Function and Mycorrhizal Susceptibility of Root Systems

Most plant species show a highly heterogeneous root system with a high diversity of root functions. Even the apparently homogeneous root systems of herbaceous plants show variability among root orders. For example, in maize root systems, seminal and nodal roots can be distinguished. The former play a role in the water supply and acquire less P, while the latter are for P acquisition (Hodge et al. 2009). In other plants, the real measured uptake rates suggest that only 10 % and 30 % of the total root system lengths are involved in nitrate and water uptake, respectively (Hodge et al. 2009). In trees, shrubs and other perennial plants, this variation in root function among root orders is expected to be even more marked. Unfortunately, no accompanying data have been presented on the mycorrhizal susceptibility and presence of the different AM fungal structures in these root types, although it has been repeatedly suggested that it would be highly revealing to examine root traits by root order in mycorrhizal studies (Valenzuela-Estrada et al. 2008). Many studies have shown that the root orders most susceptible to being colonised are the higher-order or ultimate roots (Janos 1977; Nadarajah 1980; Fisher and Jayachandran 1999; Dreyer et al. 2010).

The main reason for the lack of information on the mycorrhizal susceptibility of the root orders with different root functions may be the difficulty of distinguishing them. Roots, conversely to aboveground tissues, are very difficult to classify (Valenzuela-Estrada et al. 2008). Traditionally, the roots have been classified arbitrarily by their diameter, designating the ones narrower than 1 or 2 mm as ephemeral fine roots and assuming an absorptive function for them and those wider than 1 or 2 mm as perennial coarse roots with anchorage and transport functions. Recently, the characterisation of roots by their branching order has been reported as a useful approach to identify anatomical, morphological and functional differences within a root system (Pregitzer 2002; Pregitzer et al. 2002; Guo et al. 2008; Valenzuela-Estrada et al. 2008). However, care has to be taken here, as various root types of the same age and order exist in the same root system (Bagniewska-

Zadworna et al. 2012) and no study so far has determined whether branch order effectively distinguishes roots with an absorptive capacity in the entire root system.

As root functions are difficult to measure directly (Lucash et al. 2007), indirect methods, such as anatomical methods, have been used to determine root function, the assumption being that anatomy and physiology are tightly linked. The presence or absence of secondary xylem could be used as an indicator of the transition from absorptive to transport functions in temperate trees (Guo et al. 2008). Based on anatomical traits, it was estimated that 75 % and 68 % of the fine-root lengths in temperate forests were absorptive and mycorrhizal, respectively. In monocotyledonous plants such as *Arecaceae*, other anatomical and morphological features could be proposed, e.g. the absence of aerenchyma and sclerenchymatic ring, as indicators of absorptive functions (Dreyer et al. 2010).

Such studies are further complicated by the fact that not all parts of the root systems are active at the same time since tissue ageing and differentiation occur. Further, this may vary with nutrient availability. These differences have a clear impact on the pattern of AM colonisation. However, very few studies have been devoted to studying AM colonisation, from a root order point of view.

More studies are needed in which the heterogeneity of roots as regards their physiological capacity is coupled with the spatial pattern of resource availability and their interactions with soil microorganisms like AM fungi (Hodge et al. 2009), e.g. the study of Comas and Eissenstat (2009). Otherwise, the possibility exists that the data gained will remain highly confusing, because roots with different patterns and functions are being compared.

7.1.4 Arbuscular Mycorrhizal Fungi Induce Changes in the Root System Morphology and Physiology

The anatomical features of a plant root are genetically determined and no absolute change in response to biotic factors has been described. Of course, there may be minor changes, e.g. the colour change of the roots due to AM colonisation in onions (Becker and Gerdemann 1977), maize (Klingner et al. 1995) and other plants (Fester et al. 2002); enlargement of the root cortex with extra cell layers to accommodate the AM fungal structures; or minor modifications of the plant cell wall to allow the passage of AM fungi.

What may indeed be under AM fungal control is the quantity of the different root orders that constitute the highly heterogeneous root system, whose structure is determined by an interplay between the intrinsic developmental programme and external biotic and abiotic stimuli (Lynch 1995). The ability of plant root structures to adapt to the encountered environmental conditions varies greatly, depending on the plant species, soil composition and, particularly, on water and mineral nutrient availability (Malamy 2005). Much less is known about the effect of biotic factors on

the temporal-spatial distribution and structure of root systems, but one of the most important biotic interactions is that which involves AM fungi.

It has been recognised for a long time that AM colonisation could enhance root growth. Further, it has been described that nutrients could affect root morphology in non-mycorrhizal plants (Drew 1975), and it was found that the morphological changes in the root systems of mycorrhizal plants could be correlated with the improvement in nutrient acquisition that they experience. One of the first studies that showed that mycorrhizal dependency was related to root morphology was that of Baylis (1975). Since then, numerous studies have pointed out that AM fungi have an important impact on the root morphology and architecture of plants and consequently on plant physiology. This aspect has been reviewed by Atkinson et al. (1994), Berta et al. (1993), Hetrick (1991) and, more recently, Berta et al. (2002).

Morphological modifications have been divided into structural, spatial, quantitative and temporal modifications (Atkinson 1992). Analysis of the spatial morphology of *Allium porrum* using the topological method showed that the branching pattern was not affected by AM fungi despite the strong impact they had on lateral root numbers per unit of root length (Berta et al. 1993). However, in other studies, changes in the root branching pattern have been observed, with control plants presenting a herringbone-like root morphology, while mycorrhizal plants were characterised by a dichotomous root system (Atkinson et al. 1994). Conversely to these two examples, most studies have determined only the quantitative morphology of root systems. It is well known that AM fungi decrease the root biomass in relation to the aerial biomass of the host plant (Berta et al. 1990; Smith and Read 2008; Torrisi et al. 1999). However, higher root to shoot ratios have also been found in mycorrhizal plants compared to non-mycorrhizal ones like *Prunus cerasifera* (Atkinson et al. 1994), *Andropogon gerardii* (Hetrick et al. 1988), *Populus* sp. (Hooker et al. 1992), *Arachis hypogaea* and *Cajanus cajan* (Yano et al. 1996). Although the mechanisms underlying this effect are not clear, it has been observed that the addition of P to *A. porrum* plants inoculated with *G. mosseae* diminished the root to shoot ratio (Amijee et al. 1989). In non-mycorrhizal plants, the nutrient deficiency increases the root to shoot ratio (Lynch 1995). Further, it has also been suggested that plants that are less mycorrhizal dependent have a higher root to shoot ratio, although this is not supported by the study of Manjunath and Habte (1991).

Although root biomass is the root growth parameter most measured in mycorrhizal studies, studying this parameter alone, without taking into account the root morphology and architecture, can lead to important changes and differences in biomass allocation being overlooked (Hetrick 1991). Root mass can seldom be correlated with the nutrient absorption capacity, as the main roots that contribute most to total mass are those that contribute least to nutrient acquisition. For this reason, it is also important to study other morphological features of the root systems.

AM fungi have been seen to cause multiple changes in root morphology and the alteration patterns may vary highly among plant species. In most associations, AM colonisation increases the number of adventitious and lateral roots (Aguín

et al. 2004; Berta et al. 1990, 1995; Hetrick et al. 1988; Hooker et al. 1992; Torrisi et al. 1999; Yano et al. 1996) and usually results in greater branching, although this may be not a general trait of mycorrhizal roots, as a reduction in branching in *A. gerardii* has been observed (Hetrick et al. 1988). AM fungi also lead to an increase in root diameter in *Lycopersicon esculentum* (Fusconi et al. 1999), *A. gerardii* (Hetrick et al. 1988) and *P. cerasifera*, the latter only in association with *G. intraradices* but not with *G. mosseae* (Berta et al. 1995). Conversely, in *Gossypium hirsutum*, the diameter was not affected by AM colonisation (Torrisi et al. 1999).

Hooker et al. (1992) observed an increase in the branching of plants inoculated with *Glomus* sp. E3 and *G. caledonium*, but no effect was registered when they were inoculated with *Scutellospora calospora*. The results of Berta et al. (1993) showed that the root systems of *A. porrum* plants inoculated with *Glomus* E3 were more branched and contained shorter and more branched adventitious roots, with a higher proportion of roots of higher orders, with greater diameter and less specific root length. In other studies, it has been determined that the differences between mycorrhizal and control plants increased with the root order. Hooker et al. (1992) observed that inoculation with *S. calospora*, *Glomus* sp. E3 or *Glomus caledonium* of *Populus* did not affect the total root length, while the length of second- and third-order roots increased in mycorrhizal plants. The branching of second- and third-order roots was greater in mycorrhizal than in non-mycorrhizal plants. The branching of second-order roots increased with *Glomus* E3 and *G. caledonium* by 81 % and 60 %, respectively. The increase in branching of third-order roots with *Glomus* E3 and *G. caledonium* was 616 % and 500 %, respectively.

Other studies suggest that the response may not be universal. Trotta et al. (1996) found a reduction in the branching of the root system of mycorrhizal *Lycopersicon esculentum* plants in comparison with non-mycorrhizal ones. However, Vigo et al. (2000) observed no effect on root system morphology in tomato due to AM fungi. Gamalero et al. (2002) observed that AM colonisation increased total root length only in soils low in nutrients and that the branching was reduced in mycorrhizal plants, but not the number of apices.

As the AM colonisation of roots normally affects plant nutrition, the effect of AM fungi on root system morphology has been ascribed to growth effects related to nutrition (i.e. the direct effect of nutrition on plant development).

This is difficult to determine, due to the difference in growth between mycorrhizal and non-mycorrhizal plants. Using plants of similar growth, Hooker et al. (1992) showed that AM colonisation modifies the root system in a different way to the modification induced by high P levels in non-mycorrhizal plants. Conversely, Tisserant et al. (1996) indicated that the increase in branching of the root system of *Platanus acerifolia* coincided, from the fifth week after inoculation with *G. fasciculatum*, with the development of the active fungal biomass in all the lateral root orders and with a significant increase in P acquisition. Also, Amijee et al. (1989) found that high P absorption in mycorrhizal plants influenced the root geometry. Price et al. (1989) indicated that the specific root length increased with the increase in soil P concentration, but was reduced by the AM association. In

non-mycorrhizal plants of *Hordeum vulgare* cv. Proctor, Drew (1975) observed that in localised zones with a high availability of P, NO_3^- and NH_4^+ , lateral root number and branching increased. Therefore, the greater P content in mycorrhizal plants compared to non-mycorrhizal plants can at least partially explain the induced modifications in root systems of mycorrhizal plants. However, other additional or alternative effects cannot be excluded, such as modifications in the phytohormone balance or differences in mitotic index of the root apices in mycorrhizal plants due to blocked meristematic activity (Berta et al. 1990, 1991; Fusconi et al. 2000).

7.2 Case Study: The Palm *Phoenix canariensis* Chabaud

7.2.1 Some Considerations and Definitions

In case of palms, few studies about root morphology and anatomy have been carried out. Tomlinson (1990) made a brief review of these studies, while the book of von Guttenberg (1968) provides numerous examples on the root anatomy of different palm species. The most complete studies on palm root anatomy are those of Seubert (1996, 1997) devoted to the subfamilies *Calamoideae* and *Coryphoideae*. Other studies provide specific details of certain palm species; for example, the anatomy of adventitious roots of *P. canariensis* (Cabrera et al. 1990), some aspects on root morphology and anatomy of *P. dactylifera* (Oihabi 1991) and *Metroxylon sagu* (Nitta et al. 2002) or the aerial roots of different palms (de Granville 1974). Further, the root architecture of *Elaeis guineensis* has also been studied (Jourdan et al. 1995, 2000; Jourdan and Rey 1997). However, none of these studies had proposed comparing the morphology and anatomy of mycorrhizal and non-mycorrhizal root systems.

One of the prerequisites to be able to study the modifications induced by AM fungi in a given root system is to have sufficient knowledge about the morphology and anatomy of such a root system before modification has taken place. As the mycorrhizal condition is the rule in nature for most plant species, this can only be achieved under controlled conditions that allow the plant to grow without the presence of AM fungi. The modifications induced by AM fungi in the root architecture can be very extensive but difficult to identify and quantify. A detailed comparative analysis of mycorrhizal and non-mycorrhizal root systems is required, ensuring that all roots with all their intact connections to lower-order roots are sampled (Hooker et al. 1998). This was not possible to achieve for *P. canariensis* as almost all the third-order roots were loosened from the root system during the harvesting process, despite the fact that the plants were grown in silica sand to facilitate sampling and the extreme care taken in the process. Thus, of the different types of root parameters required according to Atkinson (1992) to describe root morphology or architecture (structural, spatial, quantitative and temporal morphology), we have collated data on the quantitative morphology.

Here, the term “order” is used to define the branching degree, with first-order roots being the adventitious roots, i.e. the roots originating from the shoot. Second-order roots are those originating from first-order roots, and third-order roots are the roots originating from second-order roots. Any root formed as a result of injury to the parent root (replacement roots) is designated as the same root order as the injured root from which they originated.

7.2.2 *Root Morphology and Anatomy*

The root system features of *P. canariensis* are those expected for plants with high mycorrhizal dependency (Brundrett 1991), i.e. a low specific root length (SRL), few root orders, sparse branching, absence of root hairs and protective features like the lignification of primary root structures (see below and Sect. 15.2.4 for quantitative data). Third-order roots show the thinnest diameter and the highest SRL and represent the largest proportion of total root length (TRL).

P. canariensis is characterised by a homorrhizal root system that can develop up to three root orders (Dreyer et al. 2010). An abrupt change in diameter takes place with the development of each root order (Table 7.1). The third-order roots are morphologically distinct and can be further divided into five different groups: short thick roots, mycorrhizal thickened roots, fine short roots, fine long roots and pneumatorrhizas. The short thick roots are lateral modified roots, strongly swollen, and bottle-shaped. The mycorrhizal thickened roots are also a type of swollen third-order roots but of a deep yellow colour that can be observed only in mycorrhizal root systems (Dreyer et al. 2010). It has been suggested that the precursor roots of the mycorrhizal thickened roots could be the short thick roots which, once colonised, undergo an elongation and colour change (Dreyer et al. 2010).

Along the roots of all orders, numerous pneumathodes, pneumatozones or pneumatorings are found. These zones or rings, of mealy aspect, loose tissue and bright white colour, are clearly distinguishable from the normal root segments and persist for a long time after the root abscission. In addition, the root system also presents numerous pneumatorrhizas and pneumatophores. The pneumatorrhizas are extremely short modified lateral roots, in which the loosening of the rhizodermis and the outer cortex forms a “hat” on the apex, while pneumatorings are present at their base. The pneumatophores are second-order aerial roots that develop with negative geotropic growth, with generally more than one pneumatoring at their surface.

As is to be expected, most of the anatomical aspects also vary considerably with and within each root order (Table 7.1). The first- and second-order roots consist, in general, of the same tissues, with the difference that the second-order roots are composed of fewer cell layers. The outermost root tissue is a one-layered rhizodermis consisting of large persistent cells with a thickened lignified outer cell wall. Beneath the rhizodermis lies the exodermis composed of two layers of equally thickened lignified cells. The outer cortex appears to be homogeneous, in

Table 7.1 Morphological and anatomical features of the different root orders of *Phoenix canariensis*

	Root order			
	First	Second	Third	
Diameter (mm) ^a	3.27 (2.08–4.44)	1.22 (1.01–1.57)	0.54 (0.45–0.63)	
Number of roots ^a	4.45 (3–7)	277.88 (22–713)	1598.86 (185–4461)	
Rhizodermis (µm)	240	20	24	Mycorrhizal thickened roots Fine roots ^b
Exodermis (µm)		25	40–52	24
Outer cortex (µm)		24–28		32
Inner cortex (µm)	2,080	88–110	200–360	28
Endodermis	– PC	– PC	+ PC	80
Vascular cylinder (µm)	2,040	80–130	68–120	– PC
NO. of phloem poles	34	4–16	3–4	84
Parenchymatic pith	Yes	No	No	4–8
<i>Raphia</i> -type fibres	Yes	Yes/no	No	No
Aerenchyma	Yes	Yes	No	Yes/no
AM colonisation	No	No	Yes	Yes ^c /no

^aMean of 40 plants; the diameter and number of second- and third-order roots were calculated based on 100 segments for each plant. In brackets, maximum and minimum values

^bBoth short and long fine roots together

^cIntraradical hyphae and spores were observed, but no arbuscules. +/- PC, with or without passage cells

the form of a continuous lignified sclerenchymatic ring, but is mostly composed of two zones: an outer zone consisting of thin-walled cells and an inner zone of strongly thickened cells.

The inner cortex is divided into three zones. The outer zone is built up of small cells, with few intercellular spaces; the middle zone of larger cells, with wider intercellular spaces mostly forming aerenchyma lacunae; and the inner zone of small cells, radially oriented in concentric rows, again with few intercellular spaces. The walls of all the inner cortex cells are thin, with the exception of the *Raphia*-type fibre bundles. The outermost layer of the vascular cylinder is a one-layered pericycle with equally moderately thickened walls. The rest of the vascular cylinder is formed of sclerotic tissue, in which the xylem and phloem elements are embedded. The vascular cylinder is polyarch, with a mean number of phloem and xylem poles of 34 and 6–16 in first-order roots and second-order roots, respectively (Table 7.1). In the centre, a parenchymatic pith can be observed.

The mycorrhizal thickened roots are also characterised by a one-layered rhizodermis consisting of unequally thickened lignified cells. No exodermis is present. The outer cortex is formed of two to three, more or less lignified, cell layers. The inner cortex is homogenous and no aerenchyma is present. The tertiary endodermis always displays passage cells (Table 7.1). The vascular cylinder is

triarch or tetrarch, with sclerotic pith (Table 7.1). The short thick roots show the same anatomy as the mycorrhizal thickened roots. The long fine roots are characterised by a more or less developed aerenchyma lacuna system in the inner cortex and a two-zoned outer cortex. In contrast, the short fine roots show a much reduced cortex of only four cell layers, with no division into outer and inner cortex.

7.2.3 Root Function Diversity

Based on the root anatomical features, it can be indirectly stated that the function of the first- and second-order roots, as well as of the long fine third-order roots, is anchorage and conductance. In these roots, no AM colonisation has been observed due to the presence of the sclerenchymatic ring in the outer cortex, which represents a physical barrier against AM fungal penetration, and of the aerenchyma lacunae in the inner cortex, which considerably reduce the tissue available for AM colonisation (Table 7.1; Dreyer et al. 2010). The third-order root-denominated pneumatorhizas have an aeration function, as do the pneumatorings, pneumatozones and pneumatophores. It is unclear whether an absorption function can be assigned to all other third-order roots, mycorrhizal thickened roots, short thick roots and short fine roots or just to the mycorrhizal thickened roots. However, what is clear is that only the mycorrhizal thickened roots formed mycorrhizae with arbuscules in *P. canariensis* (Dreyer et al. 2010), from which it can be deduced that only these roots harbour “functional” mycorrhizae. The AM fungal structures were found in the entire inner cortex, with the exception of the two inner layers adjacent to the stele (Dreyer et al. 2010). 89 % of the arbuscules present in transverse root sections of mycorrhizal thickened roots were succinate dehydrogenase active (Dreyer et al. 2006; Dreyer and Morte 2009). The rhizodermis and outer cortex lacked AM colonisation, except when the transverse section revealed entry points and the formation of coils. The AM anatomical type observed was characterised by the presence of intercellular hyphae and vesicles in the mature developmental stages of AM colonisation. The arbuscules were generally intercalated, although terminal arbuscules were observed as well (Dreyer et al. 2010). The arbuscules generally formed on the surface of hyphal coils and looked like arbusculate coils. Intracellular hyphae connecting two intercalated arbuscules were observed. The hyphae did not always choose the most direct way of intracellular passage, but crossed the cell wall at the corner of the cortex cells and, in transverse sections, could be distinguished as intercellular hyphae. Thus, two types of intercellular hyphae could be distinguished: those extending linearly along the roots and parallel to the root axis, called long-distance hyphae, and those that form intercalated arbuscules as a result of passing from one cell to another, called short-distance hyphae. Because of these features, the AM anatomical type of *P. canariensis* has been classified as intermediate (Dreyer et al. 2010; Fig. 7.1). Palms have been described to form *Arum*, *Paris* and intermediate AM anatomical types (Smith and Smith 1997; Fisher and Jayachandran 1999, 2005; Sengupta and Chaudhuri 2002;

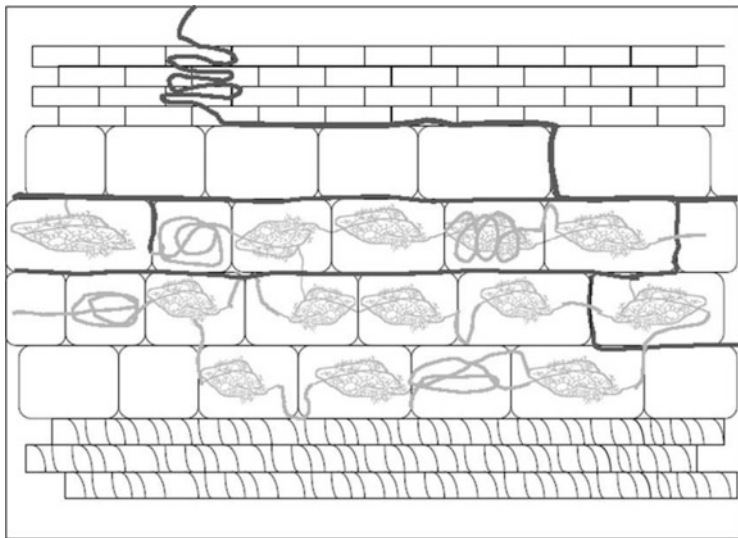


Fig. 7.1 Diagram of the intermediate AM colonisation found in *Phoenix canariensis*. The mycelium spreads intracellularly and sometimes intercellularly by long- and short-distance hyphae and forms intercalated arbuscular coils, rarely terminal

da Silva and Cardoso 2006; Ramos-Zapata et al. 2006; Dickson et al. 2007; Dreyer et al. 2010). However, in most studies, little emphasis has been placed on the aspect and formation of arbuscules, i.e. whether they were intercalated or terminal and simple or compound.

The other type of third-order roots, the short fine roots, although also colonised by AM fungi, presented only intraradical hyphae and spores or vesicles but no arbuscules (Dreyer et al. 2010). Further, it has been observed that the better aeration characteristics of the pneumatodermis in the pneumatoderms of the second-order roots seem to trigger the massive sporulation of the AM fungi, leading to the formation of a spore pseudomantle (Dreyer et al. 2010). The exact meaning of the physical separation of different AM development patterns along the *Phoenix* root system, functional colonisation in mycorrhizal thickened roots, endophytic activities in the fine roots and spore proliferation at pneumatoderms remains unclear. Muthukumar et al. (1997) suggested that mycotrophic nonfunctional plants, i.e. those with endophytic activities, may help increase the number of propagules in soils, since they observed that the association of a mycotrophic with a non-mycotrophic plant enhances arbuscular colonisation in the former plant and vesicular colonisation in the latter. *Phoenix* palms have been suggested as a good model for studying these different “endophytic” and “functional” activities of AM fungi 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 (Dreyer et al. 2010). The fine short roots and the pseudomantles could act as

inoculum reservoirs for newly developing mycorrhizal thickened roots (Dreyer et al. 2010).

Although other authors have shown that the higher-order roots in other palms are also the most susceptible to AM colonisation (Janos 1977; Nadarajah 1980; Fisher and Jayachandran 1999; Carrillo et al. 2002; Dreyer et al. 2010), they did not present the differentiation in AM function described for *Phoenix* spp. (Dreyer et al. 2010). Further, these other palms do not have the high degree of heterogeneity among the ultimate- or third-order roots that lead to different functions, such as ventilation through pneumatorhizas, which is a characteristic of *Phoenix* spp.

7.2.4 Effect of AM Colonisation on Root Morphology

In an experiment carried out with *Glomus mosseae* and the combination of *G. mosseae*, with either the isoflavonoid formononetin or the phytohormone indole-3-butyric acid (IBA), it was shown that the root diameter of the first-order roots increased in all mycorrhizal treatments in comparison with the non-mycorrhizal plants, while the diameter of second- and third-order roots did not differ (Fig. 7.2a). Conversely, the specific root length (SRL) of third-order roots, calculated by dividing the total root length by the fresh root mass, was clearly reduced by AM colonisation in all mycorrhizal treatments (Fig. 7.2b), although the total SRL for all root orders together showed a significant decrease only in the treatment with *G. mosseae* alone (results not shown).

The total root length (TRL) to shoot dry weight (SDW) ratio was much reduced by AM colonisation, showing that less biomass is invested in root development in mycorrhizal plants (Fig. 7.2c).

Specific phosphorus uptake (SPU), calculated by dividing the phosphorus acquisition efficiency ($\mu\text{g P plant}^{-1}$) by the total root length of third-order roots, was clearly increased in all mycorrhizal treatments (Fig. 7.2d).

It was supposed that formononetin enhanced the AM colonisation (Siqueira et al. 1991; Nair et al. 1991) and that this led to an increase in the number of mycorrhizal-susceptible roots (Torrise et al. 1999). An enhanced mass and total length of third-order roots were observed in mycorrhizal *P. canariensis* palms treated with formononetin compared with mycorrhizal palms without formononetin (results not shown). However, this only led to a small increase in SRL in formononetin-treated palms (Fig. 7.2b). Similarly, the application of IBA should lead to the formation of more lateral roots (Blakely et al. 1988; Muday and Haworth 1994; Reed et al. 1998; Torrey 1986). The application of IBA led to modifications in the maize root system similar to that induced by AM fungi (Kaldorf and Ludwig-Müller 2000). Conversely, mycorrhizal *P. canariensis* palms showed different morphological features, e.g. a higher SRL, when treated with IBA compared to non-treated mycorrhizal palms (Fig. 7.2b), suggesting that the mechanism inducing the morphological changes may differ between IBA and AM fungi.

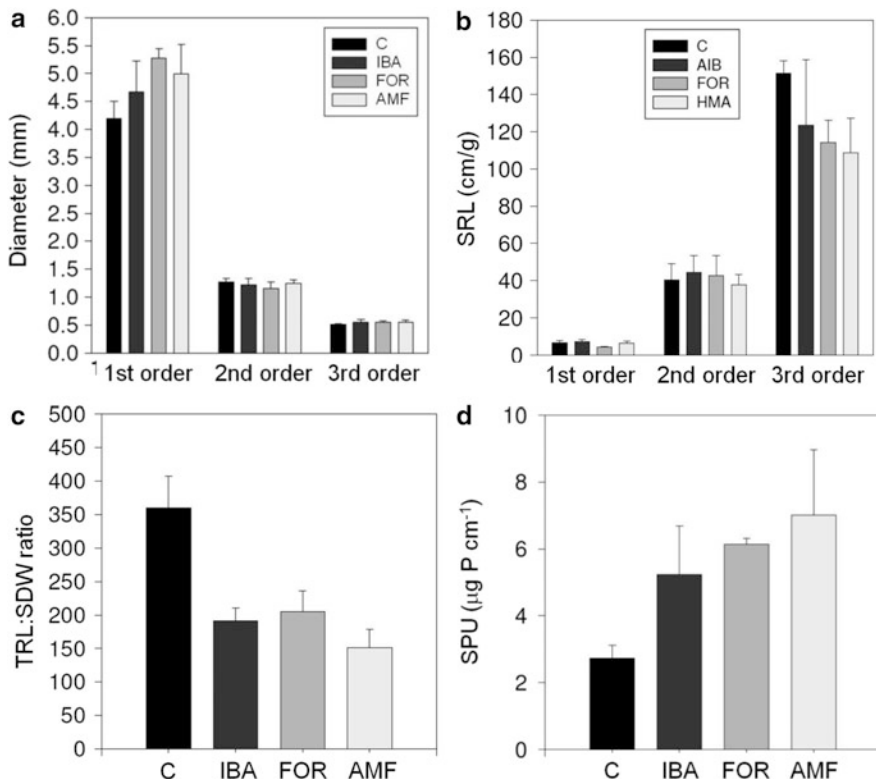


Fig. 7.2 Effect of IBA and formononetin on the root morphology of *Phoenix canariensis*, 9 months after inoculation with *Glomus mosseae*. (a) Diameter of different root orders, (b) specific root length (SRL), (c) total root length (TRL) to shoot dry weight (SDW) ratio, (d) specific P uptake (SPU). C control; AMF, *G. mosseae*; IBA, *G. mosseae* and IBA application; FOR, *G. mosseae* and formononetin application. The columns represent means of four repetitions with standard error

To study more specifically the effect of P and N on the root morphology, a further experiment was conducted, in which mycorrhizal and non-mycorrhizal *P. canariensis* palms were treated with five different fertilisation regimes. Independent of the fertilisation treatment, the AM colonisation led to a reduction in the SRL (Fig. 7.3a). The overall effect of the different fertilisation levels was similar for both mycorrhizal and non-mycorrhizal palms, with the palms treated either without P or without N showing the lowest SRLs.

The TRL:SDW ratio was also reduced by AM colonisation, and again, the results showed the same trend among fertilisation treatments (Fig. 7.3b).

A lower TRL:SDW ratio is a general feature of mycorrhizal plants (Marschner and Dell 1994) and is due to the lower investment in root development. This effect was not so evident in the root to shoot ratio (results not shown) and stresses the

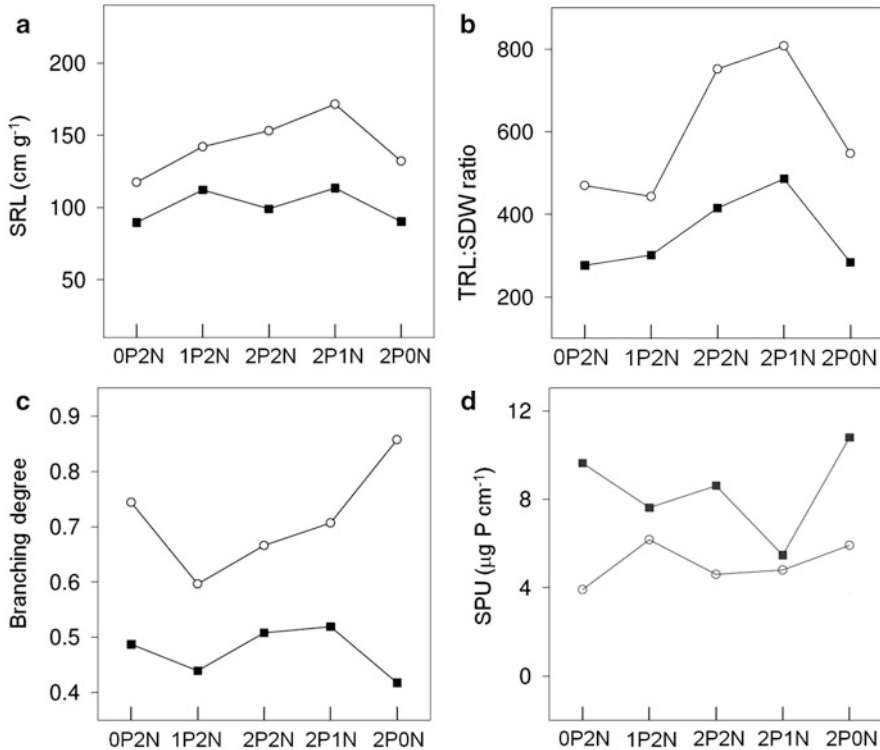


Fig. 7.3 Effect of different levels of P and N on the root morphology of *Phoenix canariensis* inoculated with *Glomus mosseae*. (a) Specific root length (SRL), (b) total root length (TRL) to shoot dry weight (SDW) ratio, (c) branching degree, (d) specific P uptake (SPU). *Hollow circles*, non-mycorrhizal; *filled squares*, mycorrhizal. Fertilisation with a Hewitt solution modified as follows: 0P2N, 0 mM P and 3.5 mM N; 1P2N, 0.67 mM P and 3.5 mM N; 2P2N, 1.33 mM P and 3.5 mM N; 2P1N, 1.33 mM and 1.75 mM; 2P0N, 1.33 mM P and 0 mM N

importance of complementing mycorrhizal studies with root morphological measures.

The branching degree of the root system, calculated by dividing the total root number by TRL, was reduced in mycorrhizal palms (Fig. 7.3c). The overall effect of the different fertilisation levels was the same in mycorrhizal and non-mycorrhizal palms, except in the case of the fertilisation without N, when the mycorrhizal palms showed the lowest branching degree, while the non-mycorrhizal palms resulted in the highest branching degree (Fig. 7.3c). The branching of root systems has been shown to decrease or be unaffected when plants are inoculated with AM fungi (Berta et al. 1995; Gamalero et al. 2002; Hetrick et al. 1988; Hooker et al. 1992; Tisserant et al. 1996; Trotta et al. 1996), although the results of the different studies are difficult to compare as branching is expressed as intensity (root numbers of order n /root numbers of order $n - 1$), degree (total root number/TRL) or frequency (root numbers of order n /root length of order $n - 1$). In palms,

branching is less in mycorrhizal root systems than in non-mycorrhizal ones, whether expressed as branching degree (Fig. 7.3c) or branching frequency (results not shown), perhaps because these plants invest more in building the external mycelium.

The lower SRL found in mycorrhizal palms compared with non-mycorrhizal ones means that more biomass is invested in the root system in mycorrhizal palms, especially in third-order roots, maybe as a strategy to harbour more AM colonisation in this moderately mycorrhizal-dependent palm. Species with high SRL have a lower cortical area available for mycorrhizal symbiosis and are normally less dependent on mycorrhizae, while plants that are more mycorrhizal dependent have a lower SRL, as they reduce the high metabolic cost of their roots by developing coarser root systems (Hetrick et al. 1988; Brundrett 1991; Hetrick 1991). Further, mycorrhizal root systems with low SRL are associated with a greater length of external AM fungal hyphae (Miller et al. 1995). The cost of producing fine roots may be superior to that involved in producing external hyphae. Extensive root systems required 20–47 % of all photosynthetic products for their production and maintenance (Smucker 1993). The maintenance of mycorrhizal root systems costs 4–20 % of additional photosynthetic products (Douds et al. 2000; Graham 2000).

It has been suggested that high SRLs are characteristic of plants grown under conditions of low P availability or, generally, in soils of low fertility (Hetrick et al. 1988). Further, Fusconi et al. (2000) found an increase in root diameter and TRL in mycorrhizal *A. porrum* plants, but only at low P concentrations. However, our results show that the *P. canariensis* palms grown without P or N presented the lowest SRLs (Fig. 7.3a). It has also been observed here that the application of P and N induced changes in the root morphology of non-mycorrhizal palms in a different way to that seen in mycorrhizal plants, as the changes induced by AM fungi were not reproducible by any of the fertilisation treatments. This has been observed in mycorrhizal *Populus* plants as well (Hooker et al. 1992). It would be important to test a higher range of nutrient concentrations in future studies with both mycorrhizal and non-mycorrhizal palms, to find the threshold, above which no additional morphological changes occur, in order to be absolutely certain about the changes induced by the AM fungi or by fertilisation (Hetrick 1991). However, this may be not so easy to do as the increase in nutrient availability may lead to an increase in lateral root numbers susceptible of being mycorrhized, which could lead also to an increase in AM colonisation and, consequently, to further morphological and physiological changes. However, this does not seem to occur as it has been shown that a high nutrient concentration, especially of P, diminished the percentage of AM colonisation. Our results show that fertilisation with a high concentration of P and N and fertilisation without N or without P have a negative impact on AM colonisation (Fig. 7.4a, b). The highest AM colonisation was achieved when the P concentration was halved, correlating with a higher number of mycorrhizal thickened roots (Fig. 7.4a). However, the palms in this treatment were not those with the highest SPU (Fig. 7.3d). Although the SPU was increased in all mycorrhizal treatments in comparison with non-mycorrhizal ones, the highest SPU was

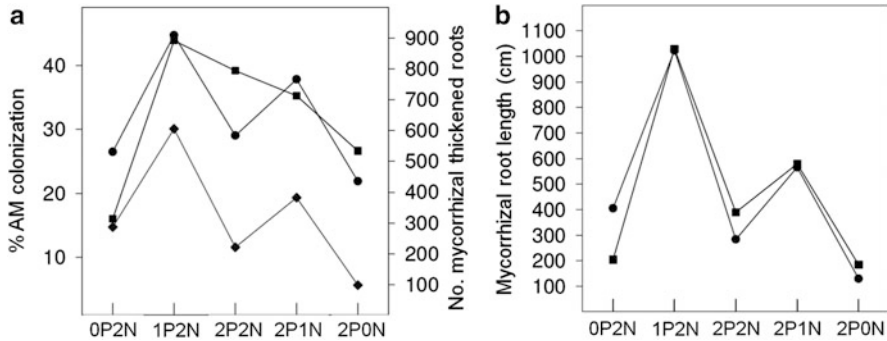


Fig. 7.4 Effect of different doses of P and N on the arbuscular mycorrhizal (AM) colonisation of *Phoenix canariensis*. (a) Percentage of AM colonisation. (b) Arbuscular mycorrhizal root length. Squares, estimation with trypan blue-stained roots; circles, direct count of mycorrhizal thickened roots; diamonds, total number of mycorrhizal thickened roots

observed in mycorrhizal palms not fertilised with N, followed by those not fertilised with P (Fig. 7.3d).

What seems to be the case is that the total number of mycorrhizal thickened roots was in agreement with their percentage in relation to all third-order roots, showing that the effect of the different fertilisation levels was similar for both third-order roots and mycorrhizal thickened roots (Fig. 7.4a). Thus, it may be that the interplay between AM fungi and the nutrient content in root cells alters the root morphology in order to adapt it to the prevailing soil conditions.

It may not be possible to achieve similar responses with nutrients and with AM fungi, because although both activate the same morphogenetic mechanisms, the intentions are different. In the case of nutrients, the production of more fine roots is a plant response to enhance nutrient absorption, while in the case of AM fungi, they increase root volume in order to accommodate more AM colonisation and, indirectly, increase nutrient absorption and interchange.

Our results show clearly that the AM fungi induce changes in the root morphology of *P. canariensis* palms and that the mechanisms underlying the morphological changes are not entirely due to improved host plant nutrition. The increase in ACP activities in roots of *P. canariensis* when inoculated with *G. mosseae* (Dreyer et al. 2008) may partially explain the increases in SPU seen here in both experiments.

7.2.5 *Inclusion of a Knowledge of Root Structure in the Methods for Evaluating AM Colonisation*

As stated above, the mycorrhizal thickened roots of *P. canariensis* presented a highly distinctive morphology and colour that could be estimated visually without the need for staining and subsequent assessment under the microscope.

It has been shown that it is possible to determine the AM colonisation level of *P. canariensis* by counting the mycorrhizal thickened roots directly (Fig. 7.4a, b). The reliability of the method can be demonstrated by comparing both methods, the visual assessment with assessment of stained roots (Fig. 7.4a, b).

The percentage of AM colonisation estimated from stained roots was in good agreement with the mycorrhizal thickened roots expressed as a percentage of the total third-order roots. Only in the treatment involving normal P and N fertilisation levels was the percentage estimated visually lower than that estimated by staining (Fig. 7.4a). The results expressed as total mycorrhizal root length estimated with both methods were even more similar (Fig. 7.4b).

It is possible that the lower AM colonisation level determined by directly counting mycorrhizal thickened roots in some treatments compared with the percentage estimated by staining is due to the fact that the whole root system was quantified in the first case, while only a subsample was subjected to staining, i.e. it was an effect caused by the sample size. Another explanation could be the time it takes since the first AM colonisation units are formed in the pre-mycorrhizal thickened roots until the synthesis of the yellow pigments is not known. For example, in maize, it has been observed that the yellow root segments are formed 1 week after the initial colonisation (Fester et al. 2002). Thus, it is possible that mycorrhizal palm roots were evaluated as non-mycorrhizal because of the lack of pigmentation. To know the limits of the visual method, the development of AM colonisation with respect to the production of the yellow pigment should be studied, e.g. by using a colorimetric method like the one used by Becker and Gerdemann (1977).

The method proposed here for *Phoenix* species is important from a practical point of view, as it could save a great deal of time in the mycorrhizal assessment of these palms and could facilitate the monitoring of the AM colonisation *in vivo*.

Regardless of the method chosen, what we have also learnt from this study is that a profound knowledge of the roots susceptible to being colonised by AM fungi is needed. Otherwise, the wrong sample, e.g. roots that will never be colonised by AM fungi, could be taken, leading to an underestimation of the AM colonisation level. This was probably the case in the first studies conducted with palms, which resulted in an extremely low AM colonisation percentage (Dreyer et al. 2001; Morte and Honrubia 2002). The contrary may also occur, as the subjective observer could be tempted to sample only mycorrhizal thickened roots as, we think, might be the case in the study of Oihabi et al. (1993), in which an AM colonisation level of 90 % was registered.

One could ask what purpose the data of the studies mentioned above serve (Dreyer et al. 2001; Morte and Honrubia 2002; Oihabi et al. 1993), besides verifying that the palms were colonised by AM fungi, if the percentages of AM colonisation levels are not related to the ratio of roots susceptible to being mycorrhizal to the total roots of the root system. Further, it is surprising that when expressing AM colonisation as mycorrhizal root length, the total root length is used for the calculations since this factor could vary among treatments, as shown by our morphological studies, and no direct relation has been demonstrated between percentage of AM colonisation and total root length. Instead, we believe that there is a relation between mycorrhizal roots and a certain type of root order. The good agreement between our results using the visual assessment method and assessment by staining was because in neither of them were the second-order and first-order roots considered; the number of mycorrhizal roots was related to the total number of third-order roots.

Although many attempts have been made to quantify the degree of mycorrhizal colonisation of root systems (Giovannetti and Mosse 1980; Trouvelot et al. 1986; McGonigle et al. 1990), none of the methods developed until now allow an accurate estimation of the high plasticity and dynamics as well as functional diversity of a given root system, which may be the main reason why in most of the studies no correlation between AM colonisation and physiological parameters was found. Thus, the tedious quantification work carried out normally ends with just the observation that the mycorrhizal system is colonised by AM fungi or not, something that could be achieved more quickly with a subjective estimation. The methods used were developed for the study of herbaceous plants, which were assumed to have homogenous root systems, and may only be accurate for them. For perennial plants, however, they are of little value, without a careful consideration of root morphological features.

7.3 What Can We Learn from These Studies?

In most mycorrhizal studies, root anatomy, root morphology and mycorrhizal anatomy are treated separately. Our results show that these three fields should be brought together to provide more information on mycorrhizal symbiosis. While *P. canariensis* may represent a very curious case, much of the information presented here would have been overlooked if an integrative approach had not been chosen.

Further, it should be remembered that the way in which root system architecture is studied has its origin in hydrology, assuming that all roots act as streams (Horton 1945). This is clearly a simplification and may be not the best option in root systems showing a high heterogeneity of roots with different functions. Studying the root system of *P. canariensis* by means of a topological model would have led the different third-order roots present being overlooked.

Different root types among root orders may also be present in other palms, but may have been overlooked due to the approach chosen. For example, Zona (1996) mentioned that the root system of *Roystonea* sp. presents tuberised roots that are mycorrhizal colonisation sites. However, 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).

Regarding the intermediate-type AM anatomy found in *P. canariensis*, we have suggested that a degree of adaptation exists to the slow growth of palms. As most palm studies have contributed little information on AM anatomy, a reassessment of this aspect may be necessary. The impression is that the intermediate and *Paris* AM anatomical types may be more widespread in palms than was formerly believed. We encourage further mycorrhizal studies in the plant family *Palmae* (*Arecaceae*) as less than 1.2 % of the existing palm species have been studied in regard to their mycorrhizal condition.

Further, the possible “autoregulation” of the soil AM inocula levels shown here for *P. canariensis* and by other authors between mycotrophic and non-mycotrophic plants should receive more attention.

And last, but not least, mycorrhizologists should rethink the way in which they evaluate mycorrhizal colonisation. Sampling of the bulk root system may be suitable for assessing the percentage of mycorrhizal colonisation in fast-growing annual plants, but in the case of tree and shrub species, the evaluation of the percentage of mycorrhizal colonisation should be preceded by a morphoanatomical study of the different root orders present.

7.4 Conclusions

The colonisation of a root system by arbuscular mycorrhizal (AM) fungi depends on different root anatomical characteristics, e.g. thickening of the cell walls of the rhizodermis, exodermis and outer cortex or the presence of aerenchyma in the inner cortex. As a result, only some root orders are susceptible of being colonised. The type of mycorrhizal anatomy formed ranges between the two extremes of a continuum, the *Paris* and *Arum* type, and it has also been suggested that this depends on features of the root anatomy.

For over two decades, it has been known that AM fungi alter the root morphology of their host plants, in most cases reducing root branching and decreasing specific root length and the total root length to shoot dry weight ratio.

Despite this knowledge in all mycorrhizal studies to date, mycorrhizal colonisation has been expressed as a percentage colonisation of the total root length and there has been no attempt to modify the methods of AM colonisation assessment. Here, the results obtained with the palm species *P. canariensis* are presented as a case study. As stated by other authors, a root order-oriented approach may expand the information gained from mycorrhizal studies. An alternative way of assessing AM colonisation for this palm species is suggested, the main objective being to

provoke a rethinking of the methods used in mycorrhizal research and to move towards a more integrative approach.

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