Arbuscular Mycorrhizal Fungi Associated With Rhizosphere of *Casuarina* in Morocco

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Abstract

The presence and diversity of arbuscular mycorrhizal fungi (AMF) in the rhizosphere of *Casuarina* trees was studied in four regions of Morocco. The results showed that all the sampled roots were mycorrhizal and various AMF structures were evident (arbuscules, vesicles, hyphae, spores, and non-specialized hyphae) in all regions. AMF colonization and diversity varied among regions, but all regions had a relatively high level. A total of 83 morphotypes belonging to 14 genera and 10 families were isolated and documented. *Glomus* was the most common and widespread genus found. Understanding the association of AMF with this important restoration species has implications for nursery production and outplanting strategies.

Introduction

The *Casuarinaceae* family comprises 86 species of trees and shrubs distributed in 4 genera (*Allocasuarina, Casuarina, Ceuthostoma,* and *Gymnostoma*) (Steane et al. 2003). These species are actinorhizal plants forming nitrogen-fixing nodules with the actinomycete *Frankia* (Dommergues et al. 1999). The *Casuarina* genus belongs to tropical and subtropical trees from Australia, Southeast Asia, and the Pacific Islands (Sougoufara et al. 1992). *Casuarina* are characterized by a conifer-like appearance with articulated and needle-shaped foliage that gradually reduce to tiny green-twined teeth (Zhong et al. 2010). The vegetative and floral parts develop with considerable scleromorphism (Midgley et al. 1983, Pinyopusarerk et al. 1996, Subba-Rao and Rodriguez-Barrueco 1995).

Casuarina sp. trees are widely used as shelterbelts (Castle 2017, Poynton 1995) and are planted along coasts, mobile dunes, and eroded slopes for controlling erosion. *Casuarina* sp. are also used for improving soil fertility due to their nitrogen-fixing ability and production of organic litter (Parrotta 1993, Zhong et al. 2010). Additionally, these species have been used as ornamental trees and for timber (Beadle 1981, Castle 2017, El-Lakany 1983, Kondas 1983, Midgley et al. 1983, Turnbull 1990).

In Morocco, *Casuarina* trees are planted in all regions, especially *Casuarina cunninghamiana* Miq, for windbreaks and shelterbelts (Ducousso et al. 2003). Several studies have reported the symbiotic association between roots of *Casuarina* sp. and *Frankia* as well as mycorrhizal fungi (Diagne et al. 2013). Arbuscular mycorrhizal fungi (AMF) is a type of endomycorrhizae which form a symbiotic association with plants (Redecker et al. 2000, Schübler et al. 2001). AMF are the most common mycorrhizal fungi (Wipf 2014) and are associated with 80 percent of green plants (Béreau et al. 2003). AMF are characterized by the formation of several structures (arbuscules, vesicles, spores, and non-specialized hyphae) (Béreau et al. 2003, Tommerup 1984, Wipf 2014).

The symbiotic associations between AMF and the host plant contribute to nitrogen fixation at similar rates to those of nodulated legumes (Zhong 1993). *Casuarina* trees with AMF have significantly improved mineral nutrition and increased tolerance to drought, flooding, and salt stress. Thus, this association enhances the host plant's ability to thrive in challenging environments (Elumalai and Raaman 2009, Evelin et al. 2009, Osundina 1997, Zhong et al. 2010) which

can be vital for replanting forest species in their natural environment, especially during the first few months after outplanting (Nouaim and Chaussod 1994). AMF can also improve seedling quality in the nursery by improving rooting and initial growth and thus make it possible to compensate for stress after outplanting (Bousselmame et al. 2002).

Research on microorganisms of *Casuarina* sp. in Morocco is limited. Ducousso et al. (2003) noted that the frequency and intensity of AMF are generally low in *Casuarina* sp. but can be high in *C. cunninghamiana* growing in nurseries. Tellal (2008) reported AMF spore morphotypes of *C. cunninghamiana* and *C. glauca* Siebold ex Spreng. belonging to *Acaulospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. Touati et al. (2016) found proteoid roots in *Casuarina* sp. with or without an endomycorrhizal inoculum.

The objective of our study was to evaluate the diversity of AMF and their development in the rhizosphere of *Casuarina* sp. in four regions of western Morocco.

Materials and Methods

Sites and Sampling

Surveys were carried out in four regions (Allal Tazi, Had Kourt, Kenitra, and Sidi Slimane) in western Morocco (figure 1). These regions have a flat geography with average elevations reaching 60 m, the height of the border dunes to the west (El Jihad et al. 2014). The Mediterranean climate is characterized by alternating wet seasons (October to April) and dry, hot seasons (May to September) (Anonymous 2013). In each region, three sites were selected for soil collection (figure 2). At each site, fine roots and soil samples were collected from three *Casuarina* trees (1 kg soil/tree) from 0 to 20 cm depth.

Root Staining for the Evaluation of AMF Root Colonization

Roots were evaluated for AMF colonization using the technique described by Phillips and Hayman (1970). The roots were washed with tap water and then cut into fragments approximately 1-cm long. These fragments were bleached with a solution of 10-percent potassium hydroxide for 45 min at 90 °C and then whitened for 5 min by adding four drops of 33-percent hydrogen peroxide. Next, the root fragments were rinsed with distilled water and stained with a solution of brilliant cresyl blue for 15 min at 90 °C in a water bath. Following staining, roots were rinsed with distilled water and observed using a microscope to determine the proportion of mycorrhizal roots in each sample.

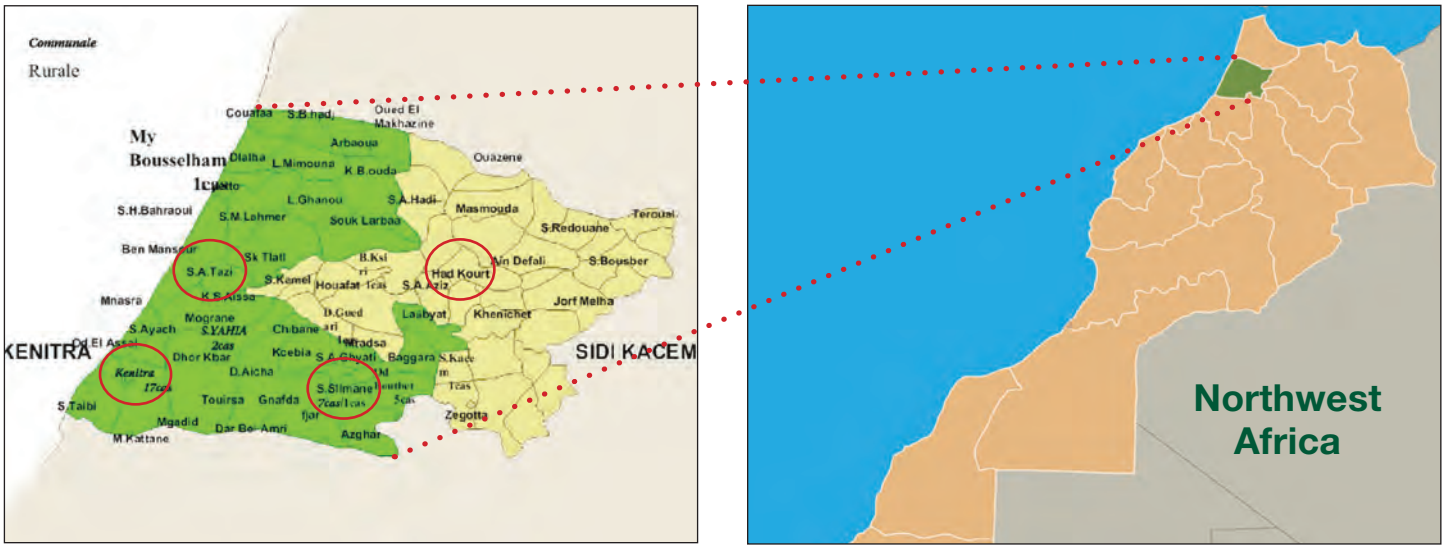


Figure 1. Samples were collected in four regions (Allal Tazi, Had Kourt, Kenitra, and Sidi Slimane) (Belomaria et al. 2007) in Morocco (Source: https://fr.wikinews.org/wiki/Fichier:Gharb-Chrarda-B%C3%A9ni_Hssen.svg).

Figure 2. Typical sampling site for the study. (Photo by N. Hibilik, 2015)

Evaluation of the Mycorrhization Rate

Mycorrhization parameters were evaluated by assessing 30 fragments from each region as described by Trouvelot et al. (1986) and Amir and Renard (2003). Root fragments were observed at 100 and 400 magnifications. Mycorrhizal intensity (MI), arbuscule content (A), and vesicle content (V) were measured by assigning an index of mycorrhization from 0 to 5 (Derkowska et al. 2008) as follows: 0=none, 1=trace, 2=less than 10 percent, 3=11 to 50 percent, 4=51 to 90 percent, and 5=more than 91 percent.

Mycorrhizal frequency (MF) reflects the colonization percentage of the root system:

$$MF = 100 \times (N - n0)/N$$

Where:

N = total number of root fragments

n0 = number of nonmycorrhizal root fragments

MI estimates the proportion of colonization in the entire root system:

$$MI = (95n5 + 70n4 + 30n3 + 5n2 + n1)/N$$

Where:

n = number of fragments with the index 0, 1 2, 3, 4, or 5 of colonization (according to the scale developed by Derkowska et al. 2008)

N = total number of root fragments

A estimates the proportion of the root cortex containing arbuscules:

$$A = (100\ mA3 + 50\ mA2 + 10\ mA1)/100.$$
$$mA = (95\ n5A + 70\ n4A + 30\ n3A + 5\ n2A + n1A)/N$$

Where:

n and N are determined as above for MI

A1: 1 to 10 percent, A2: 11 to 50 percent, A3: 51 to 100 percent

nA denotes the number of root fragments for a given n and A (e.g., n4A3 is the number of fragments denoted 4 with A3)

V estimates the proportion of the root cortex containing vesicles and is calculated in the same way as for A:

$$V = (100\ mV3 + 50\ mV2 + 10\ mV1)/100.$$
$$mV = (95\ n5V + 70\ n4V + 30\ n3V + 5\ n2V + n1V)/N$$

Spore Collection

AMF spores were extracted from *Casuarina* rhizosphere soil samples from each region using the wet sieving method described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100 g of each composite soil sample was immersed in 0.5 L of tap water and stirred for 1 min with a spatula. After 10 to 30 seconds of decantation, the supernatant was passed through four superposed sieves with a decreasing mesh size (500, 200, 80, and 50 microns). This operation was repeated twice. The contents recovered after passing through the different sieves were divided into two tubes and centrifuged for 4 min at 9,000 rpm. The supernatant was discarded, and a viscosity gradient was created by adding 20 ml of a 40-percent sucrose solution to each centrifuge tube (Walker et al. 1982). The mixture was rapidly stirred, and the tube was returned to the centrifuge for 1 min at 9,000 rpm. In contrast to the first centrifugation step, the supernatant was poured into the sieve with a mesh size of 50 microns. The resulting

substrate was rinsed with distilled water to remove sucrose and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with a little distilled water in a flask.

The number of spores in soil was estimated by counting the spores in 1 ml of supernatant which was proportionate to the total spore number in 100 ml. If no spores were detected, the supernatant was concentrated to 1 ml and observed again. The characteristics (color, shape, size, and number of separation membranes) of spores were observed using an optical microscope.

Spore identification was based on the criteria developed by Berch (1986), Dalpé (1994, 1995), Ferrer and Herrora (1981), Hall (1984), Morton and Benny (1990), Mukerji (1996), Schenck and Perez (1987), Schenck and Smith (1982), Walker (1992), and available information in different databases (INVAM 2017).

Species richness was determined based on the total number of observed species per collection site. The frequency of occurrence corresponds to the percentage of sites where a species was detected.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) for a completely randomized design. Significant differences among the four regions were determined using the least significant difference test at the 5 percent threshold. Data were analyzed using Statistica software (Stat Soft Inc.).

Results

In all four regions, *Casuarina* trees were associated with AMF, and characteristic AMF structures were observed (figure 3). The Had Kourt site tended to have the highest mycorrhizal colonization (MF, MI, A, and V) compared with the other three regions, and the Sidi Slimane site tended to have higher colonization compared with the Kenitra and Allal Tazi sites, though all four regions had relatively high AMF levels (figure 4). Average AMF spore densities and species richness followed the same pattern among regions (figure 5).

Spore identification revealed a total of 83 morphotypes present in the rhizosphere of *Casuarina* trees (table 1, figure 6). Dominant arbuscular mycorrhizal fungi varied among regions (table 1). Based on Oehl et al. (2011), morphotypes were divided into 14 genera (*Acaulospora, Ambispora, Cetraspora, Claroideoglomus, Dentiscutata, Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Pacispora, Paraglomus, Rhizoglomus, Scutellospora, Septoglomus*) occurring within 10 families (Acaulosporaceae, Ambisporaceae, Dentiscutataceae, Diversisporaceae, Entrophosporaceae, Gigasporaceae, Glomaceae, Pacisporaceae, Racocetraceae, Scutellosporaceae) and 5 within orders (Archeosporales, Diversisporales, Gigasporales, Glomerales, Paraglomerales).

Discussion

Our analyses show that *Casuarina* trees in four regions of western Morocco were associated with AMF. Tellal (2008) also found AMF associated with this species. We found characteristic structures including arbuscules, vesicles, internal hyphae, and external hyphae. The presence of arbuscules reveals it is a mycotrophic plant. Arbuscules are sites of nutrient exchange between symbionts (Smith and Read 1997). Differences in colonization and spore density among regions may be attributable to influences of seasons, edaphic factors (pH level and soil moisture), dormancy period, and the distribution of AMF in soil (Lugo et al. 2008).

AMF has also been found in the rhizosphere of other plant species in Morocco. In the western region of Morocco, AMF spores have been found associated with sugarcane (*Saccharum officinarum* L.) (Selmaoui et al. 2017), citrus (*Citrus aurantium* L.) (Artib et al. 2016), and olive (*Olea europaea* L.) (Chliyeh et al. 2014, Msairi et al. 2020). In south Morocco, AMF has been found in association with argan (*Argania spinosa* L.) (Nouaim and Chaussod 1994, Ouallal et al. 2018, Maazouzi et al. 2021), date palm (*Phoenix dactylifera* L.) (Bouamri and Dalpé 2006, Sghir et al. 2014), and carob (*Ceratonia siliqua* L.)(El Asri et al. 2014).

Glomus was the most widespread genus in our soil samples and is typically the most encountered genus in Moroccan soils. This genus has been reported in several studies in tropical and rainforest areas such as Latin America (Cruz 1989, Lopes et al. 1983), China (Zhao et al. 2001), and Mexico (Guadarrama and Alvarez-Sanchez 1999). The genus has also been found in arid and semi-arid areas such as Ethiopia (Jefwa et

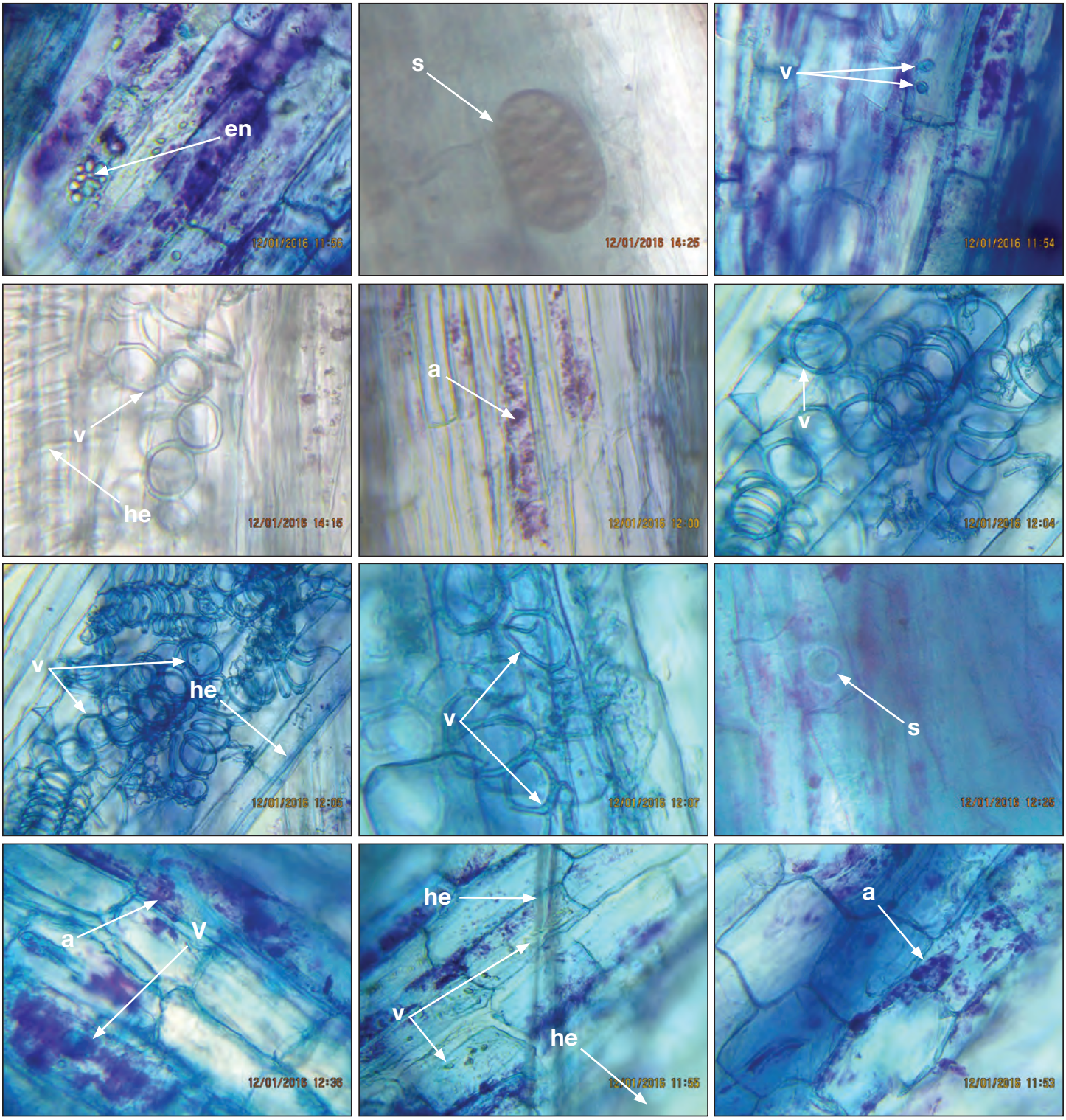


Figure 3. Different structures of arbuscular mycorrhizal fungi observed in the roots of *Casuarina* trees included (a) arbuscules, (h) extracellular hyphae, (s) spores, (v) vesicles, and (e) non-specialized hyphae (G× 400). (Photos by N. Hibilik, 2015)

Table 1. Morphological characteristics and regional distribution of endomycorrhizal fungi isolated from the *Casuarina* rhizosphere in four Morroco regions (see also figure 6).

Number	Name	Form	Color	Spore size (µm)	Wall size (µm)	Hypha length	Spore surface	Number of spores per 100 g of soil in each region			
								Had Kourt	Sidi Slimane	Kenitra	Allal Tazi
1	*Acaulospora alpina*	Globular	Yellow	119.88			Grainy	5	1	-	-
2	*Acaulospora capsicula*	Oval	Orange	173.16	3		Grainy	4	-	-	-
3	*Acaulospora cavernata*	Subglobose	Orange	139.86			Grainy	-	3	-	-
4	*Acaulospora colossica*	Globular	Yellow green	99.90	2.1		Grainy	4	-	10	-
5	*Acaulospora delicata*	Globular	Yellow	103.23	1		Grainy	5	-	-	-
6	*Acaulospora denticulata*	Oval	Dark yellow	126.54	1.3		Grainy	4	11	2	2
7	*Acaulospora elegans*	Globular	Brown	73.26	1	99.90	Grainy	-	-	2	-
8	*Acaulospora excavata*	Globular	Yellow	129.87	1		Grainy	14	-	-	-
9	*Acaulospora gedanensis*	Globular	Yellow	116.55	1	73.26	Smooth	-	4	-	-
10	*Acaulospora gerdemanii*	Globular	Brown	106.56	3		Grainy	-	-	2	-
11	*Acaulospora koskei*	Subglobose	Dark yellow	213.12	1		Grainy	-	4	-	-
12	*Acaulospora lacunose*	Subglobose	Yellow	116.55	1	33.3	Grainy	5	-	-	-
13	*Acaulospora laevis*	Globular	Orange	99.90	2	49.95	Smooth	1	-	4	4
14	*Acaulospora longula*	Globular	Brown	133.20	1		Smooth	-	-	1	-
15	*Acaulospora mellea*	Globular	Yellow	106.56	1	33.30	Smooth	-	-	2	-
16	*Acaulospora morrowiae*	Oval	Brown	103.23	2		Grainy	2	8	2	-
17	*Acaulospora nicolosonii*	Globular	Orange	166.50	1.3		Grainy	4	7	-	-
18	*Acaulospora polonica*	Globular	Yellow	133.20	1.8		Grainy	4	4	3	-
19	*Acaulospora reducta*	Globular	Yellow	109.89	1	49.95	Grainy	1	-	-	-
20	*Acaulospora rehmii*	Subglobose	Light yellow	186.48	0.1		Grainy	-	-	2	-
21	*Acaulospora scrobiculata*	Globular	Yellow	113.22			Grainy	10	5	5	-
22	*Acaulospora* sp1	Globular	White	116.55	2		Grainy	1	-	-	-
23	*Acaulospora* sp2	Subglobose	Yellow green	209.79	1.2	66.60	Grainy	2	-	-	-
24	*Ambispora callosa*	Subglobose	Yellow green	119.88	1	73.26	Grainy	3	4	-	-
25	*Cetraspora helvetica*	Globular	Yellow	66.60	1.5	66.60	Grainy	1	-	-	-
26	*Claroideoglomus etunicatum*	Globular	Beige	119.88	1.2	99.90	Smooth	4	3	1	
27	*Dentiscutata reticulata*	Globular	Beige	103.23			Grainy	-	-	1	-
28	*Diversispora epigea*	Globular	Beige	103.23			Grainy	4	-	-	-
29	*Diversispora omani*	Globular	Brown	133.20			Smooth	-	1	-	-
30	*Entrophospora infrequens*	Subglobose	Yellow	103.23			Grainy	7	5	3	-
31	*Entrophospora kentinensis*	Globular	Yellow	93.24		66.60	Grainy	6	5	1	1
32	*Funneliformis caledonius*	Subglobose	Dark orange	76.59	3		Smooth	-	13	-	-
33	*Funneliformis mossae*	Globular	Yellow	99.90	1	3.33	Grainy	-	8	-	-
34	*Gigaspora albida*	Globular	Orange	153.18	1.2		Smooth	4	3	-	-
35	*Gigaspora margarita*	Globular	Dark yellow	99.90	2.1	43.29	Smooth	5	4	-	-
36	*Gigaspora* sp1	Subglobose	Yellow Green	113.22	1		Grainy	-	3	-	-
37	*Gigaspora* sp2	Globular	Green	166.50			Grainy	5	-	-	-
38	*Glomus aggregatum*	Globular	Dark yellow	99.90	2		Smooth	5	4	3	-
39	*Glomus albidum*	Globular	Dark yellow	119.88	1.5		Grainy	-	6	-	-
40	*Glomus arenarium*	Subglobose	Brown	193.14	1	33.30	Smooth	-	-	-	2
41	*Glomus aureum*	Globular	Dark yellow	86.58	1.2	39.96	Smooth	-	5	-	-
42	*Glomus boreale*	Subglobose	Dark yellow	159.84	2.3		Grainy	-	7	4	-

Number	Name	Form	Color	Spore size (µm)	Wall size (µm)	Hypha length	Spore surface	Number of spores per 100 g of soil in each region			
								Had Kourt	Sidi Slimane	Kenitra	Allal Tazi
43	*Glomus botryoides*	Globular	Orange	106.56	2.8	99.90	Grainy	-	1	-	-
44	*Glomus caesaris*	Globular	Yellow	109.89	1	3.33	Smooth	8	-	-	-
45	*Glomus callosum*	Globular	Yellow	106.56	1		Smooth	-	-	-	1
46	*Glomus clarum*	Globular	Yellow	109.89		99.90	Grainy	8	4	3	2
47	*Glomus constrictum*	Globular	Orange	139.86	3		Smooth	4	3	-	-
48	*Glomus coronatum*	Globular	Yellow	113.22	1		Grainy	-	-	-	3
49	*Glomus deserticola*	Globular	Dark orange	136.53	2.2		Grainy	4	5	2	-
50	*Glomus etunicatum*	Globular	Beige	109.89	2		Grainy	8	6	-	3
51	*Glomus fasiculatum*	Globular	Yellow	66.60	2		Grainy	5	-	-	-
52	*Glomus fecundisporum*	Globular	Beige	99.90	0.1	43.29	Grainy	4	-	-	-
53	*Glomus formasum*	Globular	Brown	126.54	1		Grainy	2	-	-	-
54	*Glomus geosporum*	Globular	Dark orange	119.88	2	116.55	Smooth	4	-	-	2
55	*Glomus globiferum*	Globular	Dark orange	103.23	1		Grainy	-	5	-	-
56	*Glomus heterosporum*	Globular	Yellow	133.20	1		Smooth	-	6	-	1
57	*Glomus intraradices*	Oval	Yellow	99.90	1	43.29	Smooth	7	5	2	8
58	*Glomus lamellosum*	Globular	Green	119.88	2.5		Grainy	-	-	1	-
59	*Glomus macrocarpum*	Globular	Brown	106.56	1.5	33.30	Smooth	-	4	-	2
60	*Glomus manihoti*	Globular	Orange	119.88			Grainy	16	-	-	-
61	*Glomus monosporum*	Globular	Dark yellow	96.57	2		Grainy	4	-	7	-
62	*Glomus mossae*	Globular	Yellow	99.90	1	33.3	Grainy	6	7	-	3
63	*Glomus radiatus*	Globular	Orange	133.20			Grainy	4	-	1	-
64	*Glomus rubiformis*	Globular	Yellow	163.17	2.3	119.88	Smooth	-	-	3	--
65	*Glomus* sp1	Subglobose	Dark yellow	206.48	1.2		Grainy	-	5	-	-
66	*Glomus s*p2	Globular	Gray	69. 93	0.1		Smooth	-	-	-	2
67	*Glomus tetrastratosum*	Globular	Dark yellow	103.23	2		Grainy	-	5	-	-
68	*Glomus verruculosum*	Globular	Dark yellow	99.90	2.1	43.29	Smooth	1	-	-	-
69	*Glomus versiforme*	Oval	Yellow	113.22	1		Grainy	3	5	-	6
70	Multicolored *Glomus*	Globular	Orange	139.86	2.3		Smooth	8	-	-	-
71	*Pacispora boliviana*	Globular	Yellow	119.88	1.5	3.33	Grainy	_	-	3	-
72	*Pacispora scintillans*	Globular	Yellow	103.23	0.1	186.48	Smooth	4	4	-	2
73	*Paraglomus pernambucanum*	Globular	Yellow	156.51	1	33.30	Grainy	-	-	1	3
74	*Rhizoglomus fasiculatum*	Subglobose	Orange	113.22	1.2	76.59	Smooth	-	8	-	-
75	*Scutellospora armeniaca*	Globular	Dark orange	99.90	1.2	76.59	Smooth	-	3	-	-
76	*Scutellospora biornata*	Globular	Beige	99.90	1	200	Grainy	3	2	-	-
77	*Scutellospora calospora*	Globular	Beige	46.62	166.50		Smooth	2	-	-	-
78	*Scutellospora dipapillosa*	Subglobose	Yellow	106.56	1	66.6	Grainy	3	2	-	-
79	*Scutellospora nigra*	Globular	Black	99.90			Smooth	6	4	1	-
80	*Scutellospora pellucida*	Oval	Light yellow	149.85	1		Grainy	4	-	2	1
81	*Scutellospora scutata*	Globular	Yellow green	133.20	0.1	49.95	Smooth	-	-	-	2
82	*Septoglomus constrictum*	Globular	Dark yellow	99.90	1	76.59	Smooth	4	-	-	-
83	*Septoglomus deserticola*	Globular	Dark yellow	106.56	66.60		Smooth	6	-	-	-

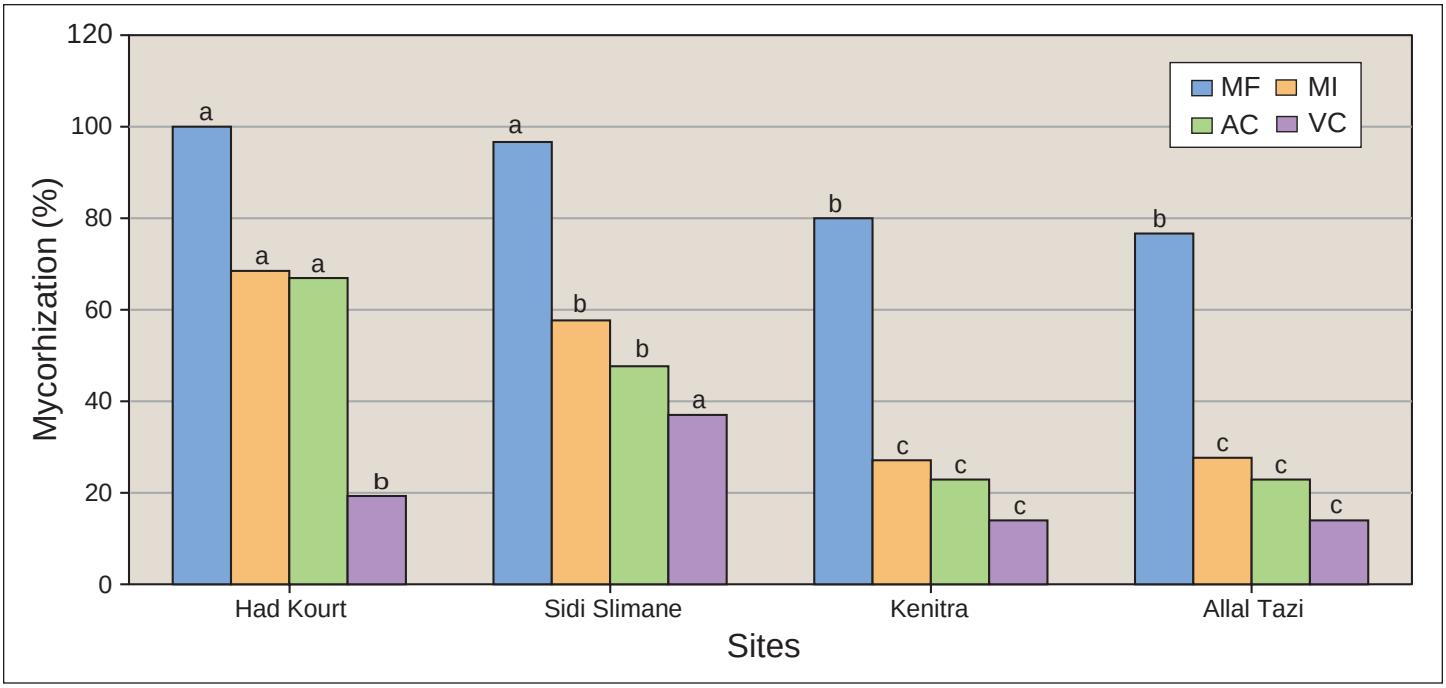


Figure 4. Mycorrhizal intensity (MI), mycorrhizal frequency (MF), arbuscular content (A), and vesicle content (V) varied among regions but were relatively high overall. For each variable, bars with the same letter are not significantly different at the 5 percent level.

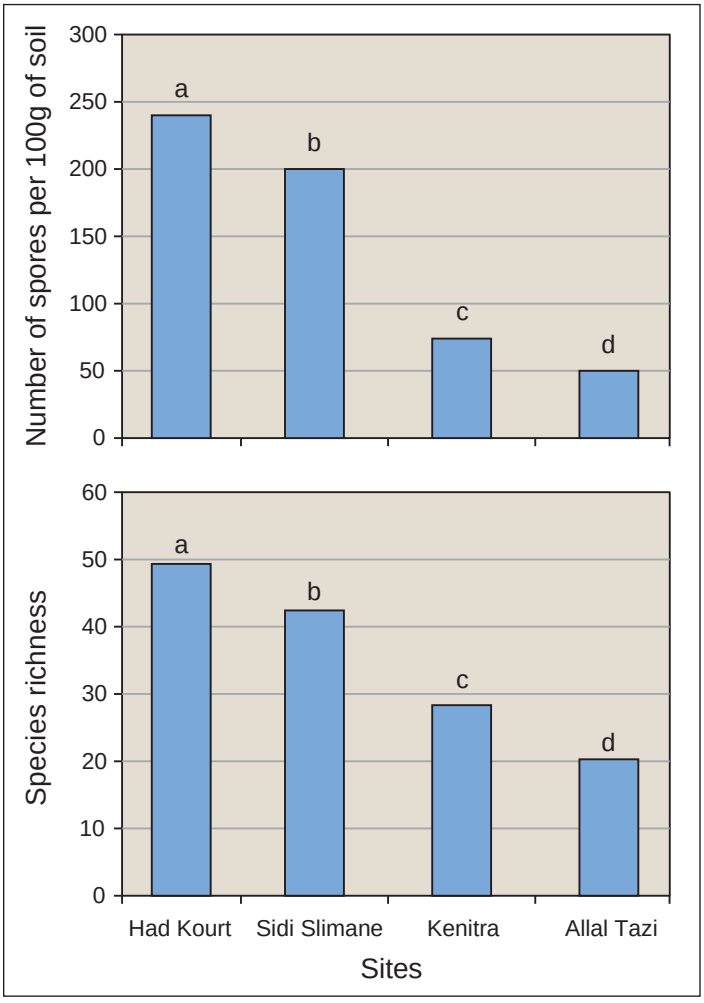
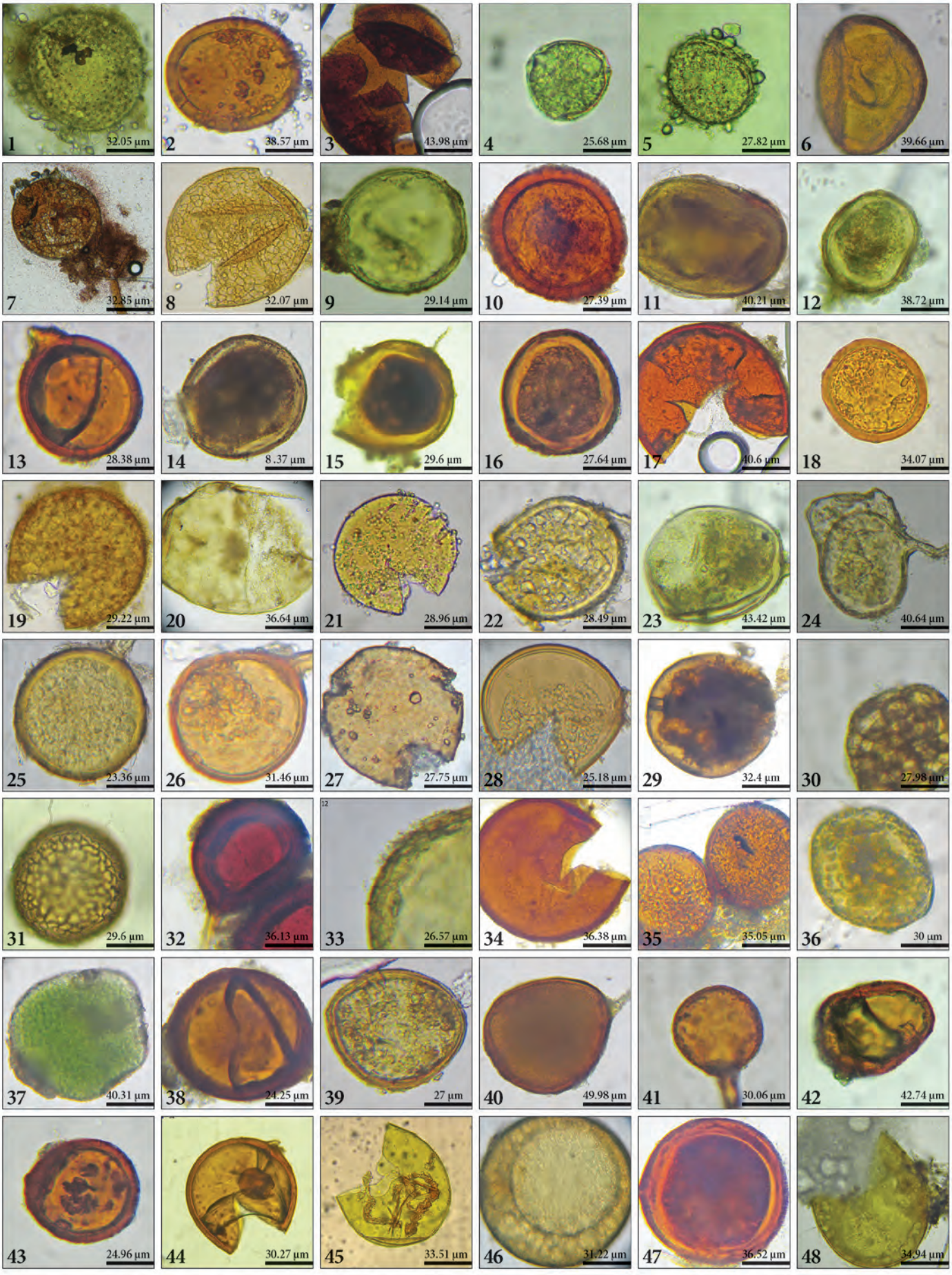


Figure 5. The (a) density of AMF spores and (b) species richness in the rhizosphere of *Casuarina* sp. differed significantly among all four sites.

al. 2009, Muleta et al. 2008, Tesfaye et al. 2004), Jordan (Mohammad et al. 2003), and several coastal dune areas (Bergen and Koske 1984, Hatimi and Tahrouch 2007, Giovannetti et al. 1983, Nicolson and Johnston 1979).

Casuarina mycorrhizae greatly improve plant growth and survival in difficult environments (Potgieter et al. 2014). Mycorrhizae have also been found to improve nutrient uptake (Abbott and Robson 1982) and to promote the symbiosis of *Frankia* in *Casuarina*, thereby increasing nitrogen fixation (He and Critchley 2008). This symbiosis also increases tolerance to drought (Abdelmoneim et al. 2013), flooding (Osundina 1997), acid soils (Diem et al. 2000), salt stress (Evelin et al. 2009), and disease (Akhtar and Siddiqui 2008, Liu et al. 2007). In a study on *Casuarina equisetifolia* L., a triple inoculation with endomycorrhizae, ectomycorrhizae, and *Frankia* significantly increased root and shoot AMF colonization (Elumalai and Raaman 2009).

Tacon et al. (1997) concluded that trees cannot survive without mycorrhizae in forest ecosystems. The interaction of the AMF and host plant must be both structurally and physiologically compatible. This compatibility depends on the host plant, mycorrhizal species, and environmental factors (Koïde and Scheiner 1992, Plenchette et al. 1983). AMF associations can also contribute to the maintenance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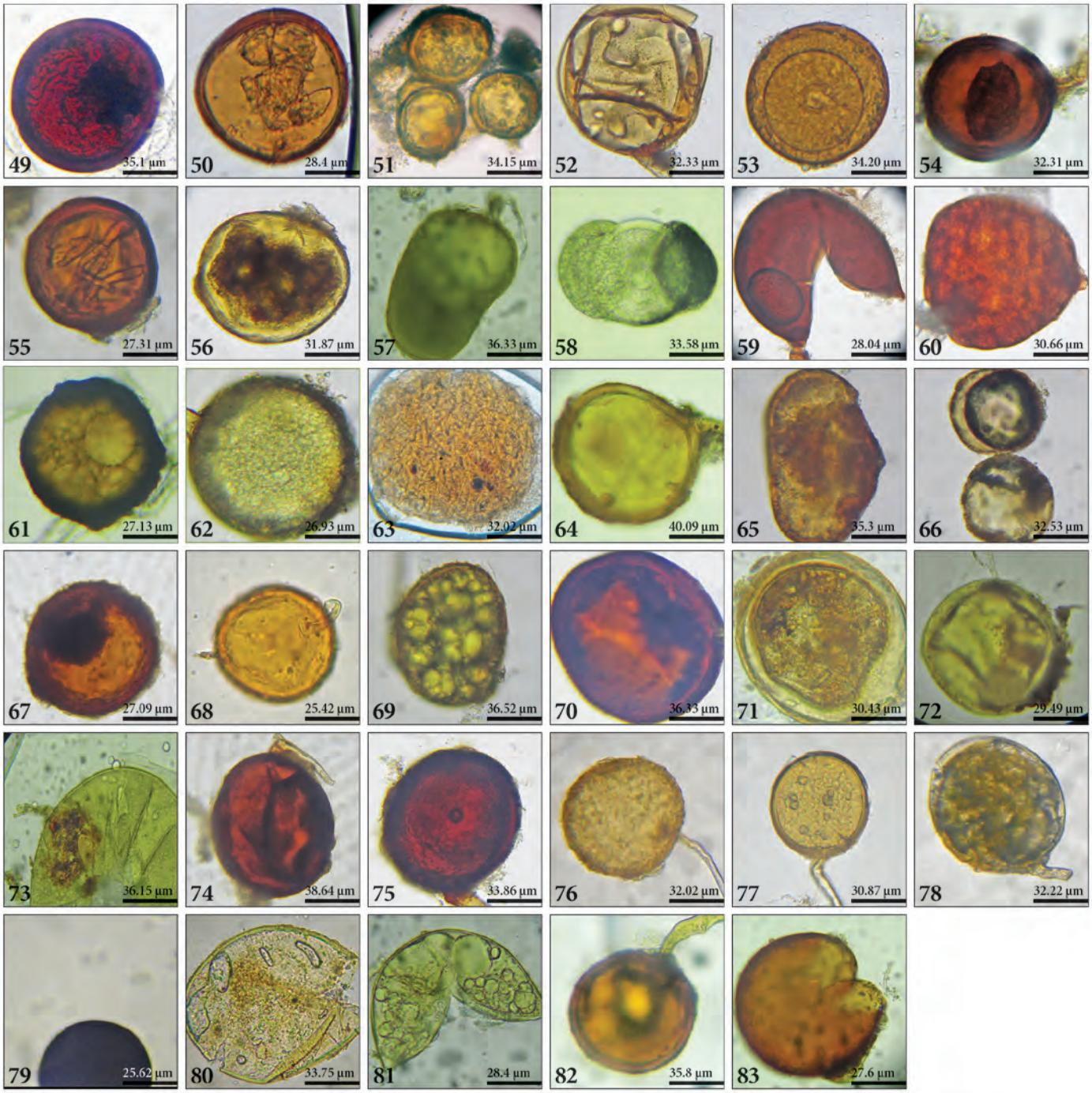

Figure 6. A total of 83 morphotypes of endomycorrhizal fungi were isolated from the rhizosphere of *Casuarina*. See table 1 for additional details. (Photos by N. Hibilik, 2015)

of plant biodiversity and thus have a positive impact on terrestrial ecosystems (Duponnois et al. 2013).

Conclusion

This study demonstrates that *Casuarina* sp. is highly mycotrophic with a high diversity of AMF. This diversity enhances the capacity of trees to thrive in difficult environments by improving mineral nutrition, increasing tolerance to drought, floods, salt stress, and diseases. Thus, AMF inoculation has great potential for use in reforestation and restoration programs including growing *Casuarina* and other plants in the nursery and outplanting them to degraded ecosystems.

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