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Glomales species associated with surface and deep rhizosphere of *Faidherbia albida* in Senegal

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Abstract Five arbuscular mycorrhizal (AM) fungal species were isolated and propagated from surface and deep rhizospheres of *Faidherbia albida* trees growing in two ecoclimatic zones of West Africa: the semi-arid Sahelian and the more humid Sudano-Guinean areas. Of these species, *Glomus aggregatum*, *Glomus caledonium*, and *Glomus mosseae* were trapped by *F. albida* roots when cultivated with either surface or deep soils. *Glomus fasciculatum* was found exclusively at the semi-arid Sahelian sites of Louga and Diokoul and *Gigaspora margarita* was isolated only from 16.5-m and 34-m-deep samples. Comparable glomalean fungal species richness was identified in deep (1.5–34 m) and surface (0.15 m) samples. The isolation and the propagation of glomalean fungi from *F. albida* rhizospheres confirmed the presence of viable AM fungal propagules, down to the water table, as deep as 34 m.

Keywords Glomales · Soil depth · *Faidherbia albida*

Introduction

The leguminous tree, *Faidherbia albida* Del. A. Chev., when grown on sandy soils, develops an impressive tap root system that can reach up to 50 m depth (Alexandre and Ouédraogo 1992). This adapted root morphology improves the plant water uptake capacity from the lower soil horizons and takes advantage of deep soil moisture to differentiate larger and more abundant lateral and secondary roots (Osonubi et al. 1992). The growth-promoting ability of arbuscular mycorrhizal (AM) fungi on *Faidherbia* species has been demonstrated on both seedlings (Ducousso et al. 1992; Osonubi et al. 1992; Bâ and Guissou 1996) and transplanted trees (Cornet et al. 1982). AM fungal root colonization levels were found to either increase with (Virginia et al. 1986) or be unaffected by (Ingleby et al. 1997) soil depth. The AM fungal spore populations of the *F. albida* mycorrhizosphere were found to rapidly decrease with depth lower than 1.5 m, but spores were still found present 4–5 m deep in soil (Diop et al. 1994a). Whether deep roots of *F. albida* maintain functional symbiotic associations with AM fungi has not yet been clearly demonstrated.

The objectives of the present study were to evaluate the AM fungal species richness of *F. albida* surface and deep rhizosphere, to isolate and identify the *F. albida* root-colonizing AM fungal species and consequently to demonstrate the viability of the deep rhizosphere propagules. Practically, the isolation and the subsequent in vitro monoxenic cultivation of native glomalean species (Diop et al. 1998) adapted to Sahelian and Sudano-Guinean climates provides a basic AM fungus inoculum for the production of *F. albida* mycorrhizal seedlings dedicated to land rehabilitation.

Materials and methods

Methods of sampling

Indigenous AM fungi associated with *F. albida* rhizospheres were assessed in two distinct ecoclimatic zones of Senegal; in the northern semi-arid Sahelian region, Louga and Diokoul sites, and in the southern more humid Sudano-Guinean zone, Djinaki and Kabrousse sites. Data on geography, and soil properties of these

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sites were previously published by Diop et al. (1994a). Samples were recovered from the rhizosphere of single-species stands of 15- to 20-m *F. albida* trees using lightweight drilling equipment (Dormer's Engineering Equipment, South Murwillumbah, Australia) (Dupuy and Dreyfus 1992). Holes were dug 10 m from the tree trunk, and cores were drilled with a shell-type auger (50 mm diameter) to the water table which fluctuated between 1.50 and 4.5 m in the Sudano-Guinean zone and from 16.5 to 34 m in the Sahelian one. Fifteen surface (0–15 cm) and one deep (4.5–34 m), 20-cm-long cores were collected from each locality. Samples were placed in separate pouches, immediately stored at 4 C, brought back to the laboratory and maintained at 4 C for a maximum of 3 months until use.

Fungus propagation

Soil samples from each sampling site and depth were mixed together and used as a source of inoculum with *F. albida* seedlings as the trapping plant host. Soil material was distributed in ten pot cultures of 2.5 l each containing 1 kg Terragreen, a calcined clay (Plenchette and Perrin 1992). Pots were placed in a growth chamber set at 27/24 C (day/night) with 65% relative humidity and a 12-h photoperiod, watered daily to field capacity and fertilized weekly with 1/10 strength Long Ashton nutrient solution (Hewitt 1966). After 4 months' growth, roots of *F. albida* trapping plants were extracted, washed in distilled water, and mixed together. Root aliquots were stained according to the method of Phillips and Hayman (1970). Root segments that contained either intraradical hyphae, vesicles or arbuscules were considered colonized. The remaining roots were used as source of inoculum for the isolation of AM fungi that actively colonize *F. albida* roots. Roots were distributed amongst three pot cultures per sampling site and depth. Pot cultures were sown with *Allium porrum* L. and grown under the same cultural conditions as above. After 6 months' growth, AM fungal spores were extracted from pots by wet sieving (Gerdemann and Nicolson 1963) followed by density gradient centrifugation (Furlan et al. 1980) and isolated manually under a dissecting microscope. Spores were mounted on microscope slides in PVLG mounting media (Omar et al. 1979), and identified using light and differential interference contrast microscopy combined with UV autofluorescence, and Melzer's and Cotton Blue histochemical stains. The morphology of isolated spores was compared to type-specimens, herbarium specimens, and, when available, live cultures. Voucher specimens were deposited in DAOM, National Mycological Herbarium, Ottawa, Canada.

Single spore cultures were prepared in plastic multipots (Somapo-Sopirec, Diemeringer, France) containing 15 g sterilized calcined clay and sowed with *A. porrum* under the same cultural conditions as above. After 6 months' growth, root colonization was evaluated and AM fungal cultures were kept as pure in vivo reference cultures. For each of the five identified species, root segments, 0.5 cm long, originating from the root system of the respective single spore cultures were surface sterilized, in vitro cultured on transformed carrot roots according to Diop et al. (1994b) protocol, and maintained in the collection of the Université Cheihh Anta Diop, Dakar, Senegal (Diop et al. 1998).

Results

A total of five species of Glomales were trapped by *F. albida* plants when cultivated in the presence of indigenous surface- and deep-soil samples (Table 1). Four of the trapped species belonged to *Glomus* and one to *Gigaspora*: *Glomus aggregatum* Schenck and Smith emend. Koske, (DAOM 227128) *G. caledonium* (Nicol. and Gerd.) Trappe and Gerd. (DAOM 227129), *G. fasciculatum* (Thaxter) Gerd. and Trappe emend. Walker and Koske (DAOM 227130), *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe (DAOM 227131), and *Gigaspora margarita* Becker and Hall (DAOM 227127). Species richness did not differ substantially between the semi-arid Sahelian and the more humid Sudano-Guinean harvesting sites. *Gigaspora margarita* species appeared to be restricted to deep roots at the Sahelian sites while *Glomus fasciculatum* species occurred only at the Louga Sahelian site. Neither of these species were recovered from the Sudano-Guinean sites. All the other species were recovered from either surface or deep roots of all four harvesting sites except *G. aggregatum* that was absent from the Diokoul Sahelian site. In all trapping cultures, *Allium* and *Faidherbia* roots inoculated with either surface- or deep-soil roots, were colonized at levels >50%.

The five isolated species showed quite typical spore morphology based on published descriptions and type-specimen observations (Figs. 1, 2, 3, 4, 5, 6). Spores of *G. aggregatum* (Fig. 1) were usually spherical, occasionally pyriform to tuberculate and always attached to a network of ramified hyphae. Spores of *G. caledonium* were usually found singly in soil, sometimes grouped in clusters and in sporocarps (Fig. 2). Sporocarps, 2–4 mm in diameter, contained four to eight (or ten) spores surrounded by a pale, almost whitish peridium organized in a tight hyphal network, similar to the ones described

Table 1 Arbuscular mycorrhizal fungal (AMF) species trapped from *F. albida* roots with soil depth in the four harvesting sites

Type of climate	Sahelian				Sudano-Guinean			
Harvesting sites Mean annual rainfall (mm)	Louga 200–300		Diokoul 400–600		Djinaki 800–1200		Kabrousse 1000–1500	
Depth (m)	0.15	34	0.15	16.5	0.15	4.5	0.15	1.5
AMF species								
Gigaspora margarita	−	+	−	+	−	−	−	−
Glomus aggregatum	+	−	−	−	−	+	+	−
Glomus caledonium	+	−	−	+	+	+	+	+
Glomus fasciculatum	+	+	−	−	−	−	−	−
Glomus mosseae	+	−	+	+	−	+	−	+
Total species site^{-1} depth^{-1}	4	2	1	3	1	3	2	2
Total species site^{-1}	5		3		3		3	

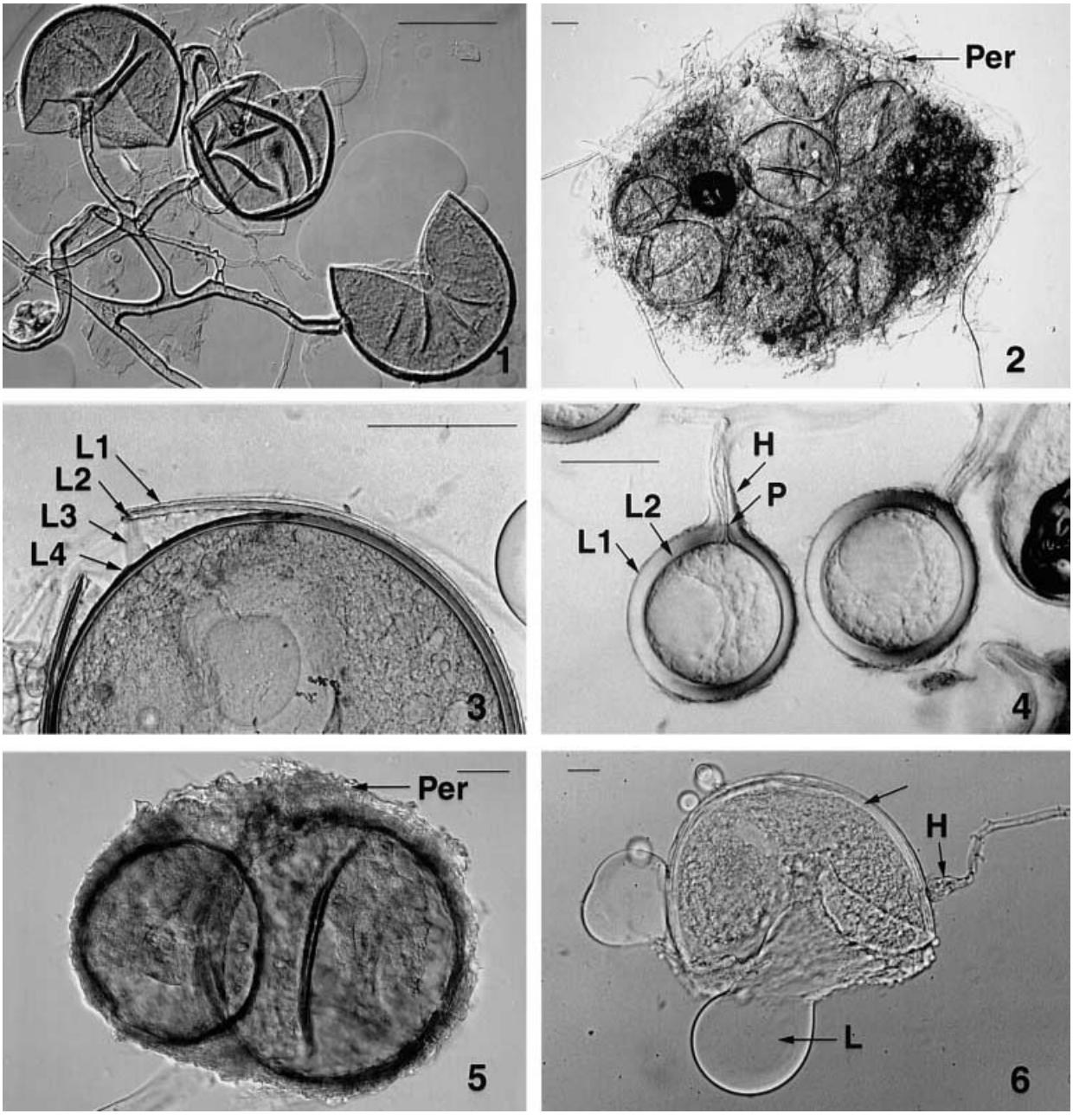

Fig. 1 Spores of *Glomus aggregatum* attached to ramified hyphae; *bars* = 50 μm

Fig. 2 Sporocarp of *Glomus caledonium* surrounded by peridium (*Per*); *bars* = 50 μm

Fig. 3 Crushed spore of *G. caledonium* showing the four wall layers (*L1, L2, L3, L4*); *bars* = 50 μm

Fig. 4 Spores of *Glomus fasciculatum* with open pore (*P*) and subtending hyphae (*H*); *bars* = 50 μm

Fig. 5 Sporocarp of *Glomus mosseae* surrounded by peridium (*Per*); *bars* = 50 μm

Fig. 6 Crushed spore of *Gigaspora margarita* with bulbous hyphae (*H*) and lipid content (*L*); *bars* = 50 μm

by McGee (1986). Spore and spore wall morphologies (Fig. 3) corresponded to that of the INVAM UK 301 live specimen used by Morton (1996) in the species redescription. *G. fasciculatum* spores occurred mainly in clusters of two to five spores (Fig. 4) with 6 to 7.5-μm-thick walls made of a thin evanescent outer layer, sometimes absent, a thick laminated middle layer, and a membranous inner one difficult to detect in juvenile spores. Typical *G. mosseae* spores were found singly in soil or in sporocarps (Fig. 5) containing usually two to three, sometimes up to ten spores of small size such as described by Dodd et al. (1996). *Gigaspora margarita* spore morphology corresponded in size, shape, and spore wall organization to the original description (Fig. 6).

Discussion

The present investigation is the first to report the identity of AM fungal symbionts trapped from the rhizosphere of *F. albida*. Of all the previous studies on AM fungal populations in tropical soils (Redhead 1977; Mathur and Vyas 1995; Udaiyan et al. 1996; Requeña et al. 1996; Roldan et al. 1997; Mohan and Singh 1997), only Bâ et al. (1996) reported on the AM fungal species identities in *Acacia holosericea* A. Cunn. ex G. Don and *A. mangium* Willd rhizospheres. None of the ten species isolated by Bâ et al.(1996) from Burkina Faso were found in the Senegal sites. Moreover, the *Acaulospora* sp. and *Sclerocystis* sp. spores detected in the indigenous soils collected from *F. albida* mycorrhizosphere (Diop et al. 1994a) have not been isolated from the present trapped cultures. The *Glomus deserticola* Trappe, Bloss & Menge species commonly occurring in arid and semi-arid regions of India (Borges and Chaney 1988) has not been isolated from Senegalese soils. However, except for *G. caledonium*, strains of the four other species trapped by *F. albida* plants have all been previously tested as artificial inocula on various Acaciaceae species (Borges and Chaney 1988; Miyasaka et al. 1993; Udaiyan et al. 1996). *Gigaspora margarita* and *Glomus mosseae* have been found to be the most efficient ones for *Acacia scleroxyla* Tuss. seedling development (Borges and Chaney 1988).

The AM fungal species trapping procedure described by Stutz and Morton (1996) is usually performed with soil material, thus permitting the detection of non-sporulating species at the time of harvesting, and consequently permits a more complete picture of the species richness of Glomales in soil. Due to the small amount of root material available in the cores taken from *F. albida* deep-soil samples, a two-step trapping procedure using original soil as the inoculum for *F. albida* seedlings and then the roots of those *F. albida* seedling as the inoculum for *A. porrum* L. was performed. This allowed, in a few cultivation steps, the simultaneous isolation and propagation of glomalean root-inhabiting species associated with *F. albida* trees. Unfortunately, as the root samples containing trapped AM fungal species were mixed together, it was only possible to evaluate the presence or absence of the species. Whether the symbiosis stays active and functional with soil depth still remains to be demonstrated. However, the present results clearly confirmed the presence of viable AM fungal propagules in *F. albida* rhizosphere, up to a depth of 34 m, and the potential of these AM fungal strains to colonize *F. albida* seedlings.

AM fungal root colonization levels usually remain high even at considerable soil depth (Nappi et al. 1985; Virginia et al. 1986; Diop et al. 1994a) although spore populations tend to decrease with depth (Diop et al. 1994a; Ingleby et al. 1997). In the present study, one to four AM fungal species were simultaneously trapped either from the surface or deep roots (Table 1) showing at least one AM fungal species distributed at both depths for each harvesting site. The simultaneous presence of AM fungal species at the surface and at soil depth suggests that roots of young *Faidherbia* seedlings, naturally colonized by indigenous AM fungal species, may carry down, during their tap root elongation, part of the mycorrhizal flora caught from the surface soil layers. An alternative scenario is that spores may be washed down through the soil profile during heavy rains, germinate and colonize roots deep in the soil when encountered. Whatever their propagation scenarios are, the presence of viable AM fungi deep in soil and their demonstrated capacity to colonize *F. albida* seedlings means that the artificial inoculation of tree seedlings may benefit plants throughout their life cycle. The selection of high-performance AM fungal strains among the isolated indigenous species, and their propagation and inoculation of *F. albida* seedlings, could be an efficient research and development avenue to follow in order to improve both the quality and yield of leguminous trees under tropical climates.

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