

Arbuscular mycorrhizal and dark septate endophyte fungal associations in *Asparagus*

Muthukumar THANGAVELU*, Muthuraja RAJI

Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India

Received: 06.02.2016 • Accepted/Published Online: 24.05.2016 • Final Version: 06.12.2016

Abstract: We studied the arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal associations in five species of pot-grown *Asparagus* (*A. aethiopicus*, *A. densiflorus*, *A. setaceus*, *A. racemosus*, and *A. umbellatus*). Root colonization by AM and DSE fungi and AM spore numbers in the soil were assessed. AM fungal diversity indices like species richness, Shannon–Weiner index, Simpson's index, evenness, and Jaccard's index were determined. Relationships among the fungal and soil variables were also examined. All the species of *Asparagus* examined were colonized by both AM and DSE fungi, and these associations have been reported for the first time in four species. Root colonization by both the fungi varied significantly among *Asparagus* species and was related to each other and soil factors. The AM colonization patterns reported in four *Asparagus* species for the first time were characterized by either intermediate or *Arum–Paris* type. The AM fungal community was dominated by members belonging to the Glomerales and was significantly influenced by *Asparagus* species and soil factors. We conclude that *Asparagus* species could support a diverse AM fungal community even under pot conditions; however, certain AM fungal species are sensitive to changes in soil factors and host species.

Key words: Arbusculate coils, arbuscules, diversity, *Glomus*, morphology, species richness, soil factors

1. Introduction

Plant roots in natural and cultivated soils by default are colonized by a wide range of soil fungi that may be either beneficial or detrimental to plant growth. Among the soil fungi that associate with plant roots, the most common and widespread type is the arbuscular mycorrhizal (AM) fungi belonging to the phylum Glomeromycota. These fungi associate with members of more than 80% of plant families (Smith and Read, 2008). Arbuscular mycorrhizal symbiosis helps plants in the acquisition of nutrients, especially phosphorus (P) and other nutrients beyond the nutrient depletion zones surrounding the roots from deficient soils. The AM fungi also impart other benefits to the host plants like protection against various abiotic and biotic stresses (Smith and Read, 2008). In addition, AM fungi may also modify soil structure through the production of a glycoprotein, glomalin. In turn, the AM fungi obtain photosynthates from the associated host plant. Many studies have investigated the occurrence of AM fungal association with members of several plant families (Wang and Qiu, 2006; Brundrett, 2009). In spite of this, roots of only <5% of plant species have been actually examined for their mycorrhizal status (Brundrett, 2009).

In addition to AM fungi, Ascomycetous fungi that are primarily conidial or sterile forms also colonize living plant roots without causing any negative effects (Rodriguez

et al., 2009). These fungi possess melanized or hyaline structures such as inter- and/or intracellular hyphae and microsclerotia within plant roots. This association is reported in around 600 plant species growing in a wide range of terrestrial ecosystems (Jumpponen and Trappe, 1998). A recent meta-analysis on the influence of DSE fungi on plant growth suggests that these fungi improve plant performance under controlled conditions, especially when nitrogen (N) is available in organic forms (Newsham, 2011).

Asparagus, belonging to the family Asparagaceae, includes about 217 species and two subspecies distributed around the world (The Plant List, 2013). Roots of *Asparagus* like *Asparagus curillus* Buch.-Ham. ex Roxb., *Asparagus racemosus* Willd., and *Asparagus filicinus* Buch.-Ham. ex D.Don are used in traditional herbal preparations to cure various ailments (Negi et al., 2010). Furthermore, young shoots of garden asparagus (*Asparagus officinalis* L.) are eaten as a vegetable for its unique taste and nutritional value in many countries (Wang et al., 2010). Although the role of AM fungi in promoting plant growth (Xu et al., 2014) and control of soil-borne diseases in *A. officinalis* (Nahiyan and Matsubara, 2012) has been extensively studied, information on the occurrence of root fungal associations in most *Asparagus* species is largely lacking. So far, only roots of *Asparagus acutifolius*, *A. officinalis*,

* Correspondence: tmkum@yahoo.com

and *A. racemosus* (Wang and Qiu, 2006; Gaur and Kaushik, 2011) have been examined for the presence of AM fungal association.

A wide variation in the intra- or intercellular distribution of AM fungal structures within roots exists for plants growing in natural and cultivated ecosystems. Based on the inter- and/or intracellular presence of AM fungal structures, colonization patterns within plant roots are distinguished into *Arum*, *Paris*, and intermediate types (Dickson, 2004). There is a presumption that the majority of the cultivated plants growing in open sunlight tend to form *Arum*-type AM, while those growing under shaded conditions in natural ecosystems form *Paris*-type AM (Dickson et al., 2007). Recent studies have shown that typical *Arum*- or *Paris*-type AM is not very common in most plant families, and AM type may vary with host and fungal species (e.g., Dickson, 2004). Observations from earlier studies have shown that both *A. acutifolius* and *A. racemosus* (Dickson et al., 2007) formed typical *Arum*-type morphology.

Soil conditions and management practices are important factors that determine AM fungal diversity and AM formation and function in both natural and agroecosystems (Smith and Read, 2008). Wacker et al. (1990) found a decline in root colonization by AM fungi and spore density in the soil with increasing number of years cropped with asparagus. Furthermore, Cuenca et al. (2003) suggested that the proliferation of AM fungi in pot cultures is restricted to species that are able to tolerate those conditions. In a recent study, Trejo-Aguilar et al. (2013) showed that several cycles of trap culture result in the loss of AM fungal diversity. These suggest that AM fungal diversity should be less in container-grown plants. The first step in exploiting endophytic fungal benefits in plant production systems is to understand their mycorrhizal status. As many of the *Asparagus* species are grown as ornamentals or as vegetables, it is primarily essential to understand their AM and DSE fungal status. Therefore, the present study was conducted to test the following objectives. First, we examined the AM and DSE fungal associations and AM morphology in species of *Asparagus*. Second, we determined the diversity of AM fungi associated with species of *Asparagus* and assessed the influence of soil factors on fungal colonization of roots and AM fungal species diversity.

2. Materials and methods

2.1. Sample collection

We collected root and soil samples from five *Asparagus* species [*Asparagus aethiopicus* L., *Asparagus densiflorus* (Kunth) Jessop, *Asparagus setaceus* (Kunth) Jessop, *A. racemosus*, and *Asparagus umbellatus* Link] growing in 30-cm-diameter pots from home gardens in Coimbatore (11°1'6"N 76°58'21"E, 411.2 m a.s.l.), Tamilnadu, India. All the plants were grown in an Alfisol soil originating from the same locality for more than 14 months and regularly watered and fertilized at monthly intervals with 5–7–4 NPK

fertilizer and 200 g of farmyard manure every 4 months. For each *Asparagus* species five plants were sampled. Samples collected beneath each plant were composited (hereafter referred to as sample). The root systems were excavated and roots were washed free of soil and stored in formalin/acetic acid/alcohol mixture (FAA; 90 mL:5 mL:5 mL) for the assessment of fungal colonization. Two hundred grams of soil were collected at four points around each plant using a soil corer (5-cm diameter) for chemical analysis and enumeration and isolation of AM fungal spores. Roots present in soil cores was also washed and added to root samples for determining fungal colonization. Approximately 2 g of fine feeder roots (fresh weight) were collected for each plant. Soils collected from a plant were bulked, air-dried, and stored at 4 °C until analysis. In all, there were 25 root and soil samples included in the study. The nomenclature for *Asparagus* species follows *The Plant List* (2013).

2.2. Soil chemical analysis

The soil samples were sieved to 2.0 mm for chemical analysis. Soil pH (in water) and electrical conductivity were measured using respective digital meters. The total N was determined by Kjeldahl digestion and available P was measured in the triple acid digest by molybdenum blue method (Jackson, 1973). Exchangeable potassium (K) in soil was extracted using ammonium acetate solution (pH 7) and measured with a digital flame photometer (Chapman, 1965).

2.3. Root processing and assessment of fungal colonization

The roots stored in FAA were washed, cut into 1-cm-long pieces, cleared in 2.5% KOH at 90 °C for 30–60 min, and rinsed in several changes of water (Koske and Gemma, 1989). The root pieces were then acidified in 5 N HCl for 8 h and stained with 0.05% trypan blue in lactoglycerol overnight. Thirty randomly chosen stained root pieces were mounted on microscopic slides in clear lactoglycerol, covered with cover glasses, squashed, and observed under a compound microscope (Olympus BX51). The images were captured using a ProgRes 3 digital camera. The percentage of root length with AM and DSE fungal structures and total root length colonization was assessed according to McGonigle et al. (1990). One hundred and fifty intersections were observed for each root sample at 400×. AM colonization patterns were determined according to Dickson (2004).

2.4. Isolation of AM fungal spores and diversity assessment

Spores of AM fungi from the soil samples were extracted and enumerated using modified wet sieving and decanting technique (Muthukumar et al., 1996). The morphological characters of the isolated spores and subcellular characters were examined in material mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG) and PVLG/Melzer's reagent (Omar et al., 1979) mixture using an Olympus BX51 microscope. Only intact AM fungal spores that were free from parasitism or any signs of deterioration were counted. The nomenclature of AM fungal species is after Schüßler and Walker (2010), Oehl et al. (2011), and Sieverding et al. (2011).

The structure and diversity of the AM fungal community associated with *Asparagus* species were evaluated by ecological parameters like frequency of isolation, relative abundance, species richness, Shannon–Wiener index, evenness, Simpson’s index, and Jaccard’s index (Magurran, 1988).

The abundance of each AM fungal spore morphotype was assessed using the formula

Abundance (%) = No. of spores of a particular morphotype in a sample/total number of spores in the sample × 100

Similarly, the frequency of each spore morphotype was determined as Frequency (%) = No. of soil samples in which a particular spore morphotype was present/total number of soil samples examined × 100.

Species richness (S) is defined by: $S = \sum n$, where n is number of AM fungal species associated with an *Asparagus* species.

Shannon–Wiener index (H') was calculated using the formula $H' = -\sum P_i \ln P_i$, where $P_i = ni/N$, ni is the spore numbers of a species, and N is the total number of identified spores in a sample.

Simpson’s index (D) was determined as $D = S [ni(ni - 1)/N(N - 1)]$, where ni is the spore numbers of an AM fungal species and N is the total number of identified AM fungal spores in a sample.

Species evenness (E) was calculated as $E = H'/H'_{max}$, where H' is the Shannon–Wiener index and H'_{max} is $\ln S$

We calculated Jaccard’s index (Krebs, 1989) to assess the similarity in AM fungal communities between *Asparagus* species. Jaccard’s index (S_{ij}) between two *Asparagus* species is defined by $S_{ij} = a/a + b + c$, where a = number of AM fungal species common for two *Asparagus* species, b = number of AM fungal species present for the i th species and absent for the j th species, and c = number of AM fungal species absent for the i th species and present for the j th species.

2.5. Data analysis

Data on soil chemical analysis and AM and DSE fungal parameters were subjected to one-way analysis of variance

(ANOVA) to analyze the influence of *Asparagus* species on AM and DSE fungal colonization as well as AM spore numbers. Pearson’s correlation analysis was used to assess the relationships between various fungal and soil variables. Data on fungal colonization were arcsine transformed and spore numbers were log transformed to achieve normalization prior to statistical analysis. The statistical analysis was performed using SPSS for Windows, version 9.

To better understand the relationship between AM fungal communities and soil factors associated with *Asparagus* species we conducted a principal component analysis (PCA) using XLSTAT for Windows, version 2016, using the spore abundance data of individual AM fungal species and soil factors.

3. Results

3.1. Soil characteristics

All the soil chemical properties (pH, EC, N, P, and K) examined showed significant variation with *Asparagus* species (Table 1). Soil pH and EC varied from 7.3 (*A. aethiopicus*) to 8.5 (*A. umbellatus*), and 0.15 (*A. aethiopicus*) to 0.29 (*A. racemosus*), respectively, for different *Asparagus* species. Total N was higher (23.56 mg/g) in soil under *A. racemosus* and lower (18.35 mg/g) under *A. umbellatus*. Available P varied between 0.87 mg/g (*A. umbellatus*) to 1.72 mg/g (*A. setaceus*). Maximum and minimum exchangeable K was recorded respectively for soils under *A. racemosus* (35.48 mg/g) and *A. umbellatus* (23.58 mg/g) (Table 1).

3.2. AM and DSE fungal association

All the *Asparagus* species examined in the present study had dual association of both AM and DSE fungi. AM fungal colonization was characterized by an appressorium at the point of fungal entry into roots (Figures 1a and 1b). The AM fungal hyphae were in the form of coils (Figure 1c) or linear inter- or intracellular hyphae (Figures 1d and 1e) bearing arbuscules (Figure 1d–1h). Vesicles were either inter- or intracellular (Figure 1i). DSE fungal colonization was characterized by dark or hyaline regularly

Table 1. Soil characteristics of different *Asparagus* species investigated.

Species	Soil characters ^a				
	pH	EC (dSm ⁻¹)	Nitrogen (mg g ⁻¹)	Phosphorus (mg g ⁻¹)	Potassium (mg g ⁻¹)
<i>A. aethiopicus</i>	7.3 ± 0.06a	0.15 ± 0.01a	19.27 ± 0.70a	1.25 ± 0.01b	27.28 ± 0.65b
<i>A. densiflorus</i>	7.5 ± 0.07a	0.24 ± 0.01c	21.52 ± 0.58b	1.32 ± 0.02c	29.52 ± 1.08bc
<i>A. racemosus</i>	8.2 ± 0.09c	0.29 ± 0.01d	23.56 ± 1.05b	1.56 ± 0.02d	35.48 ± 1.27d
<i>A. setaceus</i>	7.9 ± 0.07b	0.26 ± 0.01c	22.31 ± 0.86b	1.72 ± 0.02e	31.47 ± 0.95c
<i>A. umbellatus</i>	8.5 ± 0.10d	0.19 ± 0.01b	18.35 ± 0.39a	0.87 ± 0.02a	23.58 ± 0.67a
F value (df = 4,20)	37.813***	40.128***	8.182***	310.382***	21.972***

^aMean ± SE. Means in a column followed by same letter(s) are not significantly different ($P > 0.05$) according to DMRT

***Significant at $P < 0.001$

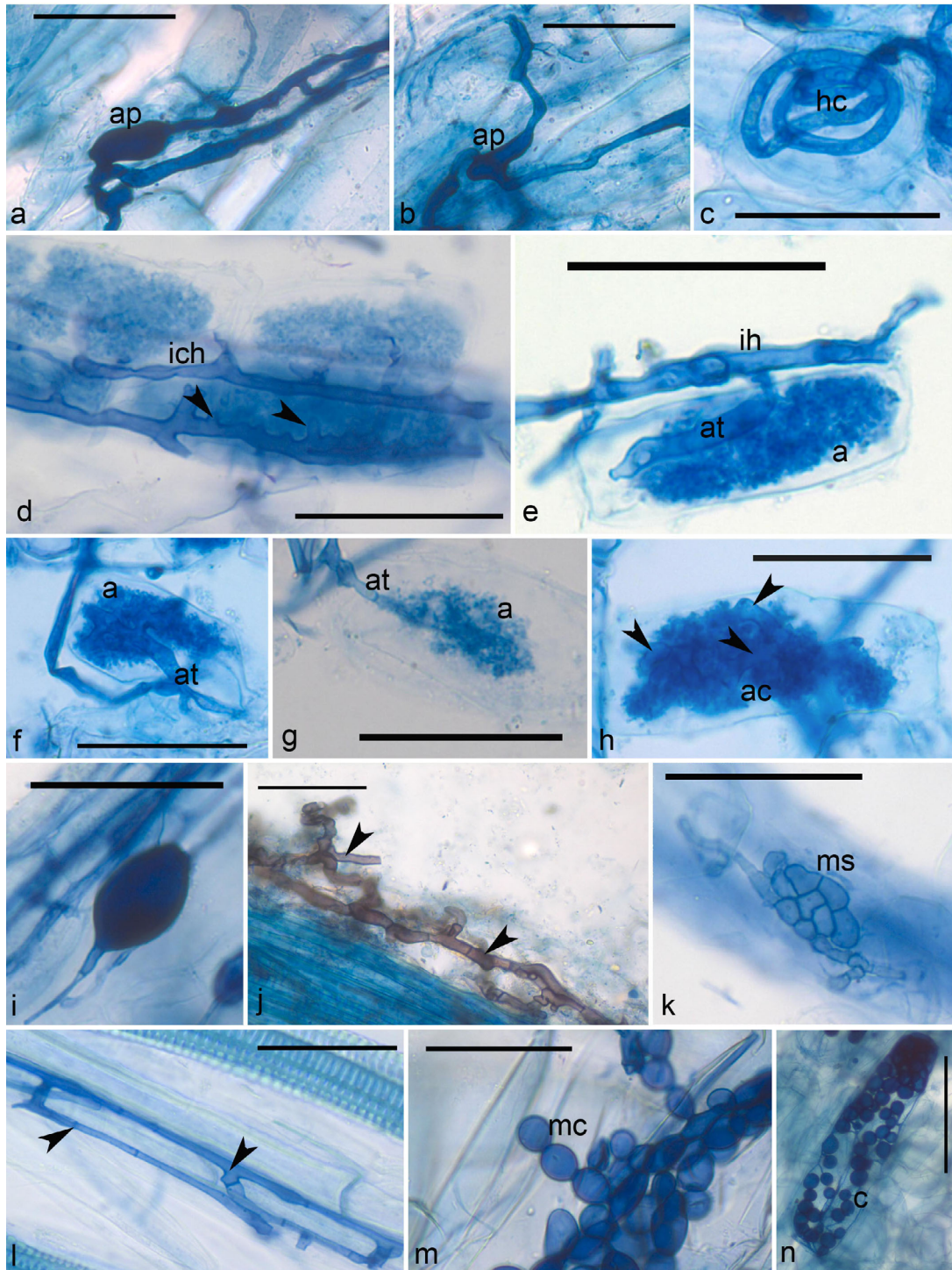


Figure 1. Arbuscular mycorrhizal (AM) (a-i) and dark septate endophyte (DSE) fungal (j-n) association in *Asparagus*. (a, b) appressorium (ap) on root surface of *A. racemosus* (a) and *A. umbellatus* (b), (c) hyphal coil (hc) in *A. setaceus*, (d) Intracellular hyphae (ich) and arbuscules in *A. racemosus*. Note the knobby outgrowth on one side of the hyphae (arrow heads), (e) Intercellular hyphae (ih), arbuscular trunk (at) and arbuscule (a) in *A. setaceus*, (f, g) arbuscular trunk (at) and arbuscule (a) in *A. aethiopicus* (f) and *A. umbellatus* (g), (h) Arbusculate coil (ac) and hyphae (arrow heads) in *A. setaceus*, (i) Vesicle in *A. racemosus*, (j) Melanized DSE fungal hyphae (arrow heads) in inner cortex of *A. racemosus*, (k) Microsclerotia (ms) in *A. setaceus*, (l) Intercellular septate hyphae (arrow heads) with 'H' connections in *A. aethiopicus*, (m) Moniliform cells (mc) in *A. umbellatus*, (n) Chlamydospore-like structures (c) in root cortical cell of *A. racemosus*. Scale bars = 50 µm.

septate hyphae that formed microsclerotia or moniliform cells within root cells (Figure 1j–1m). Aggregates of chlamyospore-like structures were also seen in cortical cells (Figure 1n). We did not find any AM or DSE fungal structures in the storage roots of any of the *Asparagus* species.

3.3. AM morphology

Intermediate AM morphology was present in four of the five *Asparagus* species (Table 2). Of the four *Asparagus* species that formed intermediate type AM morphology, *A. aethiopicus* and *A. densiflorus* had intermediate type 1 morphology, whereas *A. racemosus* and *A. umbellatus* had intermediate type 3 AM morphology. *A. setaceus* had Arum–Paris-type morphology (Table 2; Figure 1).

3.4. Extent of AM and DSE fungal colonization

Root length containing different AM and DSE fungal structures as well as total root length colonization of these two fungi differed significantly among the *Asparagus* species (Table 3). The percentage of root length containing linear hyphae/hyphal coils (RLH%) ranged between 15.14 (*A. aethiopicus*) and 25.64 (*A. densiflorus*). The percentage

of root length containing arbuscules/arbusculate coils (RLA/RLAC%) and vesicles (RLV%) varied from 29.17 (*A. densiflorus*) to 42.65 (*A. racemosus*) and 10.38 (*A. aethiopicus*) to 23.54 (*A. racemosus*). The percentage total root length (RLTC%) colonized by AM fungi varied between 61.36 (*A. aethiopicus*) and 84.55 (*A. racemosus*) (Table 3).

The percentage root length colonized by DSE fungal hyphae (RLDSH%) ranged from 5.89 (*A. aethiopicus*) to 10.32 (*A. racemosus*) (Table 3). Similarly, the percentage root length containing moniliform hyphae (RLDMH%) and microsclerotia (RLDMS%) varied from 3.12 (*A. aethiopicus*) to 9.71 (*A. umbellatus*) and 4.15 (*A. aethiopicus*, *A. umbellatus*) to 7.81 (*A. racemosus*), respectively. The percentage total root length colonized by DSE fungi (RLDTC%) ranged between 13.16 (*A. aethiopicus*) and 23.37 (*A. racemosus*) (Table 3).

Pearson’s correlation indicated the existence of a significant positive correlation between RLH% and RLDMS% (Table 4). Similarly, RLDMS% and RLDTC% were significantly and positively correlated to RLV% and RLTC%.

Table 2. Distribution of various arbuscular mycorrhizal fungal structures in different *Asparagus* species.

Species	Linear hyphae		Hyphal coils	Arbusculate coils	Vesicles		AM type**
	Inter [#]	Intra [#]			Inter [#]	Intra [#]	
	Ar*	Ar*					
<i>A. aethiopicus</i>	-	+	-	-	+	-	Intermediate 1
<i>A. densiflorus</i>	-	+	-	-	+	-	Intermediate 1
<i>A. racemosus</i>	-	+	-	-	+	+	Intermediate 3
<i>A. setaceus</i>	+	-	+	+	-	+	Arum & Paris
<i>A. umbellatus</i>	-	+	-	-	+	+	Intermediate 3

[#] Inter, Intercellular; Intra, Intracellular; *Ar, Arum-type arbuscules; ** According to Dickson (2004)

Table 3. Arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal colonization and AM fungal spore numbers in different *Asparagus* species.

Species	AM fungi ⁱ				SN (per 10 g soil)	DSE fungi ⁱⁱ			
	Colonization (%)					Colonization (%)			
	RLH	RLA/RLAC	RLV	RLTC		RLDSH	RLDMH	RLDMS	RLDTC
<i>A. aethiopicus</i>	15.14 ± 0.79 a ⁱ	35.84 ± 1.02 b	10.38 ± 0.40 a	61.36 ± 0.68 a	39 ± 2.63 a	5.89 ± 0.19 a	3.12 ± 0.35 a	4.15 ± 0.30 a	13.16 ± 0.81 a
<i>A. densiflorus</i>	25.64 ± 0.73 c	29.17 ± 0.68 a	18.74 ± 0.67 c	73.55 ± 1.22 c	58 ± 1.87 b	6.54 ± 0.43 a	8.47 ± 0.34 c	5.02 ± 0.29 a	20.03 ± 0.76 b
<i>A. racemosus</i>	18.36 ± 1.08 ab	42.65 ± 1.22 c	23.54 ± 0.98 d	84.55 ± 2.01 d	63 ± 4.21 b	10.32 ± 0.60 b	5.24 ± 0.36 b	7.81 ± 0.38 b	23.37 ± 1.15 d
<i>A. setaceus</i>	15.86 ± 1.89 ab	35.89 ± 0.92 b	16.32 ± 0.70 b	68.07 ± 2.89 b	56 ± 2.60 b	10.16 ± 0.65 b	5.94 ± 0.38 b	6.84 ± 0.33 b	22.94 ± 0.82 cd
<i>A. umbellatus</i>	18.97 ± 0.69 b	36.87 ± 0.84 b	11.48 ± 0.56 a	67.32 ± 1.38 b	55 ± 2.01 b	6.78 ± 0.37 a	9.71 ± 0.47 d	4.15 ± 0.42 a	20.64 ± 0.77 bc
F value (df = 4,24)	13.630***	25.158***	61.486***	23.474***	10.710***	19.836***	46.566***	22.790***	21.923***

ⁱRLH, RLA/RLAC, RLV, RLTC, SN: Root length with AM fungal hyphae/hyphal coils, arbuscules/arbusculate coils, vesicles, total colonization, and spore numbers, respectively

ⁱⁱRLDSH, RLDMS, RLDTC: Root length with DSE fungal hyphae, moniliform cells, microsclerotia, and total colonization, respectively

ⁱMean ± SE. Means in a column followed by same letter(s) are not significantly different (P > 0.05) according to DMRT

***Significant at P < 0.001

Table 4. Pearson's correlation coefficient for arbuscular mycorrhizal (AM), dark septate endophyte (DSE) fungal and soil variables (n = 25).

Variables		AM fungi [†]					DSE fungi ^{††}			
		RLH	RLA/RLAC	RLV	RLTC	SN	RLDSH	RLDMH	RLDMS	RLDTC
Soil ^{†††}	pH	-0.471	-0.092	-0.709***	-0.701***	0.403*	-0.270	-0.367	-0.409*	-0.529**
	EC	0.139	-0.421*	-0.349	-0.363	0.697***	-0.050	0.544**	-0.167	0.238
	N	0.231	-0.257	-0.047	-0.051	0.517**	0.287	0.536**	0.159	0.540**
	P	0.176	0.004	0.032	0.110	0.261	0.276	0.776***	0.106	0.659***
	K	0.180	-0.070	0.080	0.100	0.299	0.398	0.566**	0.280	0.665***
AM fungi	RLH		-0.443*	0.346	0.465*	-0.092	-0.186	0.590**	-0.074	0.231
	RLA/RLAC			0.233	0.457*	-0.598**	0.441*	-0.266	0.450	0.250
	RLV				0.885***	-0.485**	0.597	-0.010	0.717***	0.596**
	RLTC					-0.655***	0.495**	0.147	0.626***	0.601**
	SN						-0.096	0.199	-0.266	-0.036
DSE fungi	RLDSH							-0.140	0.860***	0.785***
	RLDMH								-0.161	0.469*
	RLDMS									0.755***

[†]RLH, RLA/RLAC, RLV, RLTC, SN: Root length with AM fungal hyphae/hyphal coils, arbuscules/arbusculate coils, vesicles, total colonization, and spore numbers, respectively

^{††}RLDSH, RLDMH, RLDMS, RLDTC: Root length with DSE fungal hyphae, moniliform cells, microsclerotia, and total colonization, respectively

^{†††}Soil pH, electrical conductivity, nitrogen (N), phosphorus (P) and potassium (K) respectively

*, **, *** Significant at P < 0.05, P < 0.01, and P < 0.001, respectively

3.5. Relationship of AM and DSE fungal variables to soil factors

Among soil factors, pH was significantly and negatively correlated to RLV% and RLTC% (Table 4). A significant negative correlation also existed between soil EC and RLA/RLAC%. Unlike AM, DSE fungal variables were significantly influenced by soil factors. The %RLDMH was significantly and positively correlated to all the soil factors except pH. In contrast, RLDMS% and RLDTC% had a significant negative correlation with soil pH. The RLDTC% was also significantly and positively correlated to all the soil nutrients examined (Table 4).

3.6. AM fungal species diversity

Spore morphotypes of 16 AM fungal species belonging to *Acaulospora*, *Scutellospora*, *Claroideoglossum*, *Viscospora*, *Funneliformis*, *Glomus*, *Rhizoglossum*, and *Septoglossum* in four families and two orders of Glomeromycota were isolated from the soils of the five *Asparagus* species (Figure 2; Table 5). Of these, one *Glomus* spore morphotype could not be identified to species.

3.7. AM fungal spore numbers

The AM fungal spore numbers ranged between 39 (*A. aethiopicus*) and 63 (*A. racemosus*) per 10 g of soil (Table 3) and was significantly and positively correlated to soil pH, EC, and total N (Table 4). However, AM fungal spore

numbers were significantly and negatively correlated to RLA/RLAC%, RLV%, and RLTC% (Table 4)

There were significant variations in the number of individual spore morphotypes in the rhizosphere of *Asparagus* except for spores of *A. scrobiculata*, *G. sinuosa*, and *F. mosseae* (Table 5). Individual spore numbers in the root zones of *A. densiflorus*, *A. umbellatus*, *A. aethiopicus*, *A. setaceus*, and *A. racemosus* ranged from 2.6 (*R. intraradices*) to 7.6 (*F. mosseae*), 2.2 (*R. aggregatum*) to 8.0 (*C. etunicatum*), 2.0 (*R. intraradices*) to 8.4 (*F. mosseae*), 0.8 (*F. geosporus*) to 9 (*R. aggregatum*), and 1.8 (*Glomus* sp.) to 9.8 (*F. mosseae*, *R. intraradices*) spores per 10 g of soil, respectively (Table 5).

3.8. Relationship between AM fungal spore morphotypes and soil variables

Of the 16 spore morphotypes examined for their relationship with soil variables, only *A. spinosa*, *R. aggregatum*, *C. etunicatum*, *F. geosporus*, *R. microaggregatum*, *S. constrictum*, *Glomus* sp., *G. clavisporum*, and *V. viscosum* exhibited a significant correlation with one or more soil factors (Table 6). Spore numbers of *R. aggregatum* were significantly and positively correlated to all soil variables except pH. In contrast, a significant negative correlation existed between spore numbers of *R. microaggregatum* and *V. viscosum* and all soil variables except for EC and

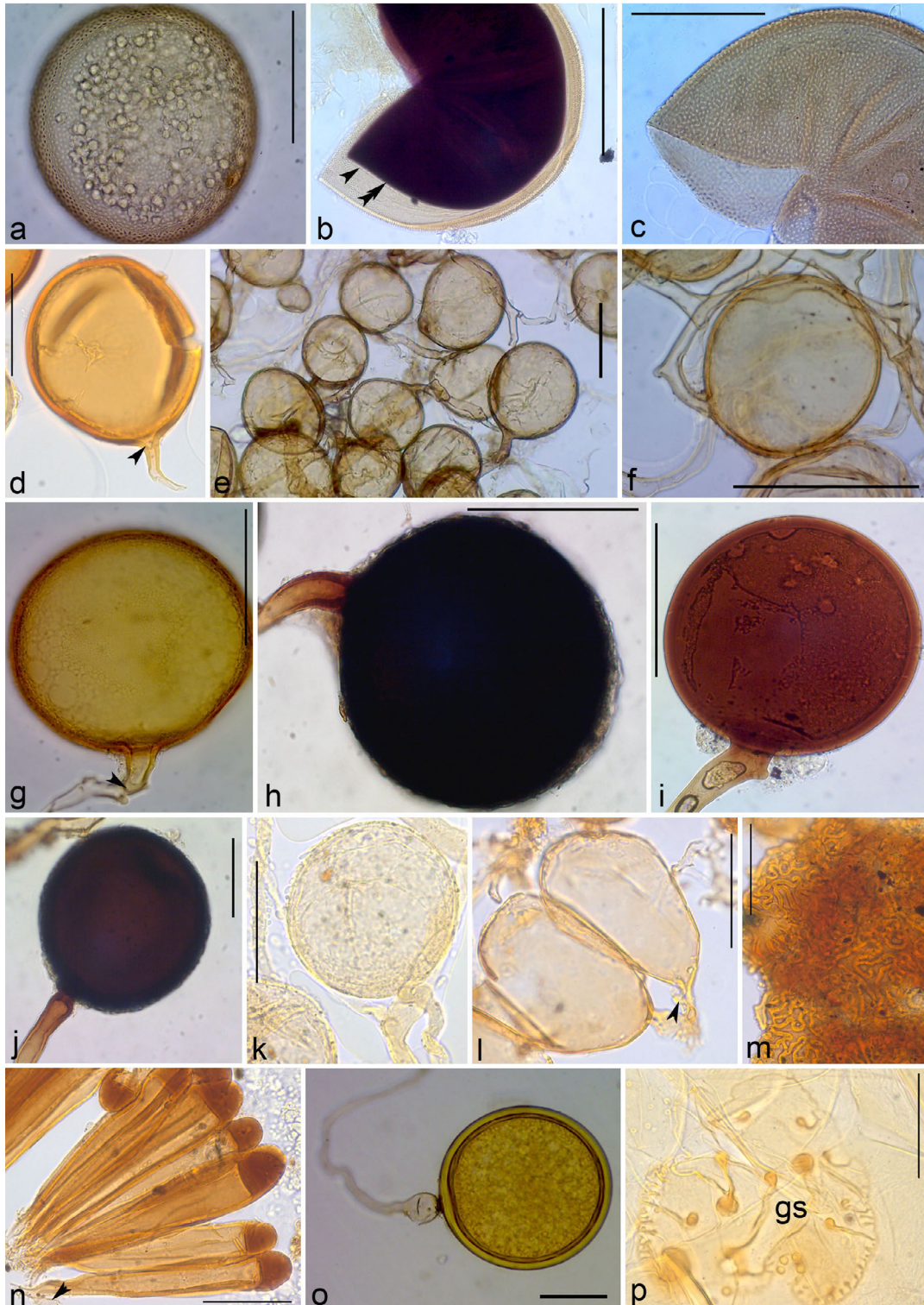


Figure 2. Spores of Glomeromycota isolated from the rhizosphere of *Asparagus*. (a) Spore of *A. scrobiculata*, (b) Fractured spore of *A. scrobiculata* in Melzer's reagent. Note the outer membranous wall (arrow head) and the inner membranous wall stained intense purple (double arrow head), (c) Fractured spore of *A. spinosa*, (d) *C. etunicatum*, (e) *R. aggregatum*, (f) *R. intraradices*, (g) *F. mosseae*. Note the curved septum (arrow head), (h) *F. geosporus*, (i) *Glomus* sp., (j) *S. constrictum*, (k) *V. viscosum*, (l) Spores of *G. sinuosum* with the sporophore branching (arrow head), (m) Peridium of *G. sinuosum*, (n) Spores of *G. clavisporum* with the sporophore branching (arrow head), (o) *S. calospora*, (p) Germination shield (gs) of *S. calospora*. Scale bars = 50 μ m.

Table 5. Spores of Glomeromycota identified and their spore populations in different *Asparagus* species.

Order	Spore numbers per 10 g soil ^a					F value
	<i>A. aethiopicus</i>	<i>A. densiflorus</i>	<i>A. racemosus</i>	<i>A. setaceus</i>	<i>A. umbellatus</i>	
Diversisporales						
Acaulosporaceae						
<i>Acaulospora scrobiculata</i> Trappe	6.4 ± 2.04cd	7.2 ± 1.61d	6.2 ± 1.27e-g	3.2 ± 1.16b	0.0 ± 0.00a	23.740***
<i>Acaulospora spinosa</i> C. Walker & Trappe	0.0 ± 0.00a	0.0 ± 0.00a	4.6 ± 1.38b-e	5.0 ± 1.32bcd	0.0 ± 0.00a	1.857
Gigasporaceae						
<i>Scutellospora calospora</i> (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler	0.0 ± 0.00a	3.6 ± 0.81b	3.2 ± 0.92bcd	4.2 ± 1.27bc	2.8 ± 1.16b	7.438***
Glomerales						
Claroideoglomaceae						
<i>Claroideoglomerum etunicatum</i> (W.N. Becker & Gerd.) C. Walker & A. Schüssler	0.0 ± 0.00a	8.1 ± 2.24d	7.6 ± 2.77fg	8.8 ± 0.92e	6.4 ± 0.81c	11.138***
<i>Viscospora viscosum</i> (T.H. Nicolson) Sieverd., Oehl & G.A. Silva	0.0 ± 0.00a	6.4 ± 1.38cd	5.8 ± 1.69d-g	0.0 ± 0.00a	6.6 ± 2.86c	11.500***
Glomeraceae						
<i>Funneliformis mosseae</i> (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler	7.6 ± 1.07d	7.2 ± 2.89d	8.4 ± 1.78g	6.2 ± 1.16d	9.8 ± 1.36d	1.442
<i>Funneliformis geosporus</i> (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler	2.8 ± 0.92b	3.8 ± 0.59b	5.8 ± 0.92d-g	0.8 ± 0.92a	6.6 ± 1.28c	14.912***
<i>Glomus clavisporum</i> (Trappe) Almeida & Schenck	0.0 ± 0.00a	3.2 ± 1.05b	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	23.273***
<i>Glomus microcarpum</i> Tul. & Tul.	5.4 ± 1.07c	4.2 ± 0.77bc	0.0 ± 0.00a	0.0 ± 0.00a	2.4 ± 0.81b	30.938***
<i>Glomus sinuosum</i> (Gerd. & B.K. Bakshi) R.T. Almeida & N.C. Schenck	3.2 ± 1.16b	4.2 ± 0.77bc	4.6 ± 2.04b-e	3.6 ± 0.81b	2.6 ± 0.81b	1.061
<i>Glomus</i> sp.	0.0 ± 0.00a	3.6 ± 1.07b	0.0 ± 0.00a	0.0 ± 0.00a	1.8 ± 0.59ab	21.600***
<i>Rhizoglomerum aggregatum</i> (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	11.0 ± 1.00e	2.2 ± 0.59ab	4.4 ± 0.81b-e	9.0 ± 1.12e	6.4 ± 0.81c	39.551***
<i>Rhizoglomerum clarum</i> (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl	0.0 ± 0.00a	0.0 ± 0.00a	2.1 ± 0.71a	5.8 ± 1.05cd	0.0 ± 0.00a	49.750***
<i>Rhizoglomerum intraradices</i> (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	2.6 ± 0.81b	4.4 ± 1.07bc	5.2 ± 1.83c-f	6.0 ± 1.12d	9.8 ± 1.53d	10.143***
<i>Rhizoglomerum microaggregatum</i> (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl	0.0 ± 0.00a	0.0 ± 0.00a	3.2 ± 1.36bcd	0.0 ± 0.00a	0.0 ± 0.00a	13.838***
<i>Septoglomerum constrictum</i> (Trappe) Sieverd., G.A. Silva & Oehl	0.0 ± 0.00a	0.0 ± 0.00a	2.4 ± 0.81a	3.2 ± 1.16b	0.0 ± 0.00a	15.200***

^a Mean ± SE. Means in a column followed by same letter(s) are not significantly different (P > 0.05) according to DMRT
 ***Significant at P < 0.001

pH, respectively. Similarly, spore populations of *Glomus* sp. and *G. clavisporum* were significantly and negatively correlated to soil pH and spore numbers of *G. clavisporum* exhibited a similar correlation with soil EC (Table 6). Spore numbers of *A. spinosa* was significantly and positively correlated to soil pH. While soil K was significantly and negatively correlated to spore numbers of *C. etunicatum* and *S. calospora*, it was positively correlated to spore numbers of *G. microcarpum* (Table 6). The PCA on AM fungal abundance and soil factors for different *Asparagus* species also suggested that some AM fungal species could be substantially influenced by soil conditions (Figure 3). The eigenvalues of the first and second axes were 8.83 and 5.44, respectively. The cumulative percentage variance of AM fungal species data showed that the first two PCA axes explain 67.94% of the variability in species data.

3.9. Relative abundance and frequency of AM fungi

Spores of *R. aggregatum*, *C. etunicatum*, and *F. mosseae* were the most abundant in *A. aethiopicus*, *A. densiflorus*, and *A. racemosus* (Table 7). In contrast, the relative abundance was shared by *C. etunicatum* and *R. aggregatum* in *A. setaceus* and *F. mosseae* and *R. intraradices* in *A. umbellatus* (Table 7). Spores of *F. mosseae*, *G. sinuosum*, *R. aggregatum*, and *R. intraradices* were the most frequent, occurring in all the soil samples examined. Nevertheless, spores of *G. clavisporum* were infrequent, occurring only in five of the 25 soil samples examined (Table 7).

3.10. Diversity indices and their correlation with soil variables

Calculated diversity indices varied significantly among *Asparagus* species. Species richness was highest (13) in *A. racemosus* and lowest in *A. aethiopicus* (7) (Figure 4a). The H' index varied from 0.906 (*A. umbellatus*) to 1.052 (*A.*

Table 6. Pearson's correlation coefficient for arbuscular mycorrhizal (AM) fungal species spore numbers and soil factors (n = 25).

AM fungal species	Soil factors				
	pH	EC	Nitrogen	Phosphorus	Potassium
<i>A. spinosa</i>	0.513**	-0.002	-0.152	-0.082	-0.289
<i>A. scrobiculata</i>	0.272	-0.231	-0.241	-0.214	-0.045
<i>C. etunicatum</i>	-0.251	-0.442*	-0.296	-0.220	-0.504**
<i>F. geosporus</i>	0.045	-0.342	-0.368	-0.660***	-0.368
<i>F. mosseae</i>	0.014	-0.043	0.123	-0.235	-0.163
<i>G. clavisporum</i>	-0.550**	-0.628***	-0.287	-0.151	-0.224
<i>G. microcarpum</i>	-0.280	0.087	0.373	0.211	0.394*
<i>G. sinuosum</i>	0.172	-0.176	-0.269	-0.250	-0.123
<i>Glomus</i> sp.	-0.757***	-0.539**	-0.317	-0.166	-0.239
<i>R. aggregatum</i>	0.351	0.814***	0.633***	0.675***	0.721***
<i>R. clarum</i>	0.266	0.161	0.018	0.345	-0.027
<i>R. intraradices</i>	-0.367	0.072	-0.045	-0.061	-0.197
<i>R. microaggregatum</i>	0.493**	-0.296	-0.521**	-0.696***	-0.570**
<i>S. calospora</i>	-0.310	-0.290	-0.344	-0.121	-0.479*
<i>S. constrictum</i>	0.449*	0.057	-0.040	0.048	-0.226
<i>V. viscosum</i>	-0.308	-0.611***	-0.508**	-0.641***	-0.532**

*, **, *** Significant at P < 0.05, P < 0.01, and P < 0.001, respectively

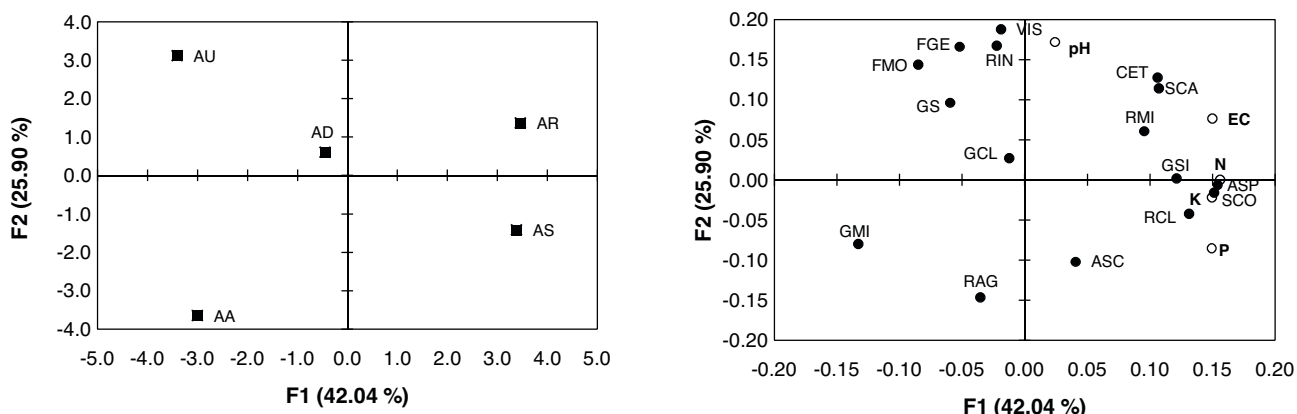


Figure 3. Principle component analysis of the relationship between soil factors (open circles) and arbuscular mycorrhizal (AM) fungal spore abundance (solid circles). The eigenvalues shown on the diagram axes refer to the percentage variation of the respective axis. AA, *A. aethiopicus*; AD, *A. densiflorus*; AR, *A. racemosus*; AS, *A. setaceus*; AU, *A. umbellatus*; ASC, *A. scrobiculata*; ASP, *A. spinosa*; CET, *C. etunicatum*; FGE, *F. geosporus*; FMO, *F. mosseae*; GCL, *G. clavisporum*; GMI, *G. microcarpum*; GS, *G. sp.*; GSI, *G. sinuosum*; RAG, *R. aggregatum*; RCL, *R. clarum*; RIN, *R. intraradices*; RMI, *R. microaggregatum*; SCA, *S. calospora*; SCO, *S. constrictum*; VIS, *V. viscosum*; pH, soil pH; EC, soil electrical conductivity; N, soil nitrogen; P, soil phosphorus; K, soil potassium.

racemosus) (Figure 4b). The D index varied from 0.084 (*A. racemosus*) to 0.168 (*A. aethiopicus*) (Figure 4c). Species evenness varied from 0.913 (*A. aethiopicus*) to 0.984 (*A. racemosus*) (Figure 4d). Jaccard's index was highest for *A. densiflorus*–*A. umbellatus* (0.833) and lowest for *A. aethiopicus*–*A. racemosus* (0.429) (Table 8).

As H' and species richness indices were significantly and negatively correlated to soil factors except pH, a significant positive correlation existed between D and all soil factors except pH (Table 9). No significant correlation was noted between E and soil factors.

Table 7. Relative abundance and frequency of Glomeromycota spores associated with different *Asparagus* species.

AM fungal species	Relative abundance (%) [*]					Frequency (%)
	<i>A. aethiopicus</i>	<i>A. densiflorus</i>	<i>A. racemosus</i>	<i>A. setaceus</i>	<i>A. umbellatus</i>	
<i>A. scrobiculata</i>	15.78 ± 2.53c	12.39 ± 1.71d	10.00 ± 1.56efg	5.63 ± 1.26b	0.00 ± 0.00a	80
<i>A. spinosa</i>	0.00 ± 0.00a	0.00 ± 0.00a	7.59 ± 1.82c-f	8.96 ± 1.43cde	0.00 ± 0.00a	40
<i>C. etunicatum</i>	0.00 ± 0.00a	13.80 ± 2.55d	11.44 ± 2.08fg	16.02 ± 1.64f	11.57 ± 0.73c	80
<i>F. geosporus</i>	6.92 ± 1.19b	6.59 ± 0.74b	9.33 ± 1.14d-g	1.43 ± 1.06a	12.03 ± 1.48c	92
<i>F. mosseae</i>	19.75 ± 1.85d	12.24 ± 2.99d	13.13 ± 1.52g	10.98 ± 0.91e	17.72 ± 1.35d	100
<i>G. clavisporum</i>	0.00 ± 0.00a	5.44 ± 1.06b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	20
<i>G. microcarpum</i>	14.29 ± 2.19c	7.24 ± 0.76bc	0.00 ± 0.00a	0.00 ± 0.00a	4.45 ± 1.07b	60
<i>G. sinuosum</i>	7.98 ± 1.65b	7.22 ± 0.76bc	6.90 ± 1.70b-e	6.37 ± 0.74bc	4.72 ± 0.89b	100
<i>Glomus</i> sp.	0.00 ± 0.00a	6.25 ± 1.23b	0.00 ± 0.00a	0.00 ± 0.00a	3.25 ± 0.64ab	40
<i>R. aggregatum</i>	28.45 ± 1.49e	3.84 ± 0.69ab	7.29 ± 1.34b-f	16.09 ± 0.84f	11.79 ± 1.39c	100
<i>R. clarum</i>	0.00 ± 0.00a	0.00 ± 0.00a	3.22 ± 0.76ab	10.47 ± 1.24d	0.00 ± 0.00a	40
<i>R. intraradices</i>	6.82 ± 1.36b	7.71 ± 1.35bc	8.12 ± 1.81def	10.71 ± 1.03de	17.73 ± 1.55d	100
<i>R. microaggregatum</i>	0.00 ± 0.00a	0.00 ± 0.00a	5.32 ± 1.71bcd	0.00 ± 0.00a	0.00 ± 0.00a	24
<i>S. calospora</i>	0.00 ± 0.00a	6.23 ± 0.96b	5.14 ± 0.99bcd	7.73 ± 1.71bcd	4.95 ± 1.18b	80
<i>S. constrictum</i>	0.00 ± 0.00a	0.00 ± 0.00a	3.69 ± 0.66abc	5.61 ± 1.21b	0.00 ± 0.00a	40
<i>V. viscosum</i>	0.00 ± 0.00a	11.03 ± 1.46cd	8.86 ± 1.12def	0.00 ± 0.00a	11.80 ± 3.24c	60

* Mean ± SE. Means in a column followed by same letter(s) are not significantly different ($P > 0.05$) according to DMRT. Highest values are indicated in bold.

4. Discussion

All the *Asparagus* species examined in the present study had dual colonization of AM and DSE fungi. This is in accordance with studies where species of *Asparagus* have been reported to possess these associations (Wang and Qiu, 2006). However, AM fungal structures were not evident in any of the storage roots examined. This is similar to the observations by Wacker et al. (1990), where AM fungal structures were absent in storage roots of *Asparagus*. Of the five *Asparagus* species examined in the present study, AM and DSE fungal status is known only for *A. racemosus* (Ragupathy and Mahadevan, 1993; Babu and Manoharachary, 2003; Gaur and Kaushik, 2011) and to the best of our knowledge these associations have been reported for the first time in the other *Asparagus* species. Further, the extent of colonization varied with species. Because all the *Asparagus* species grew under fairly homogeneous conditions, the potential confounding factors like soil type, climate, and cultural conditions that are known to influence AM fungal association were negligible. It has been shown that phenolic allelochemicals (e.g., cinnamic acids, methylenedioxycinnamic acids) produced by *Asparagus* species at biologically active concentrations could affect mycorrhization (Pederson et al., 1991). Allelochemicals produced by different

species in a plant genus may vary both quantitatively and qualitatively (Souza et al., 2011), and the varied types and levels of allelochemicals produced by different *Asparagus* species could have influenced AM fungal colonization as observed in the present study. In addition, root morphology, which determines mycorrhizal dependency, can also influence the extent of AM colonization in plant roots (Brundrett, 2009).

In the present study, *A. racemosus* had around 85% of its root length colonized by AM fungi compared to the 64%–70% reported for this species growing under natural conditions (Ragupathy and Mahadevan, 1993; Babu and Manoharachary, 2003). These high AM colonization levels in *A. racemosus* could be attributed to the high root density as plants growing in containers usually have increased rooting density per given volume of soil (Yang et al., 2010), and high root density is known to favor AM formation (Abbott and Robson, 1984). In general, the proportion of RLA/RLAC% in *Asparagus* species was higher compared to other AM fungal structures. This suggests the existence of a mycorrhizal benefit, as nutrient demand of the host induces formation of these structures for the transfer of nutrients from the fungus to the host (Smith and Read, 2008). This clearly indicates that *Asparagus* species are dependent on AM association for their nutrient uptake (Xu et al., 2014).

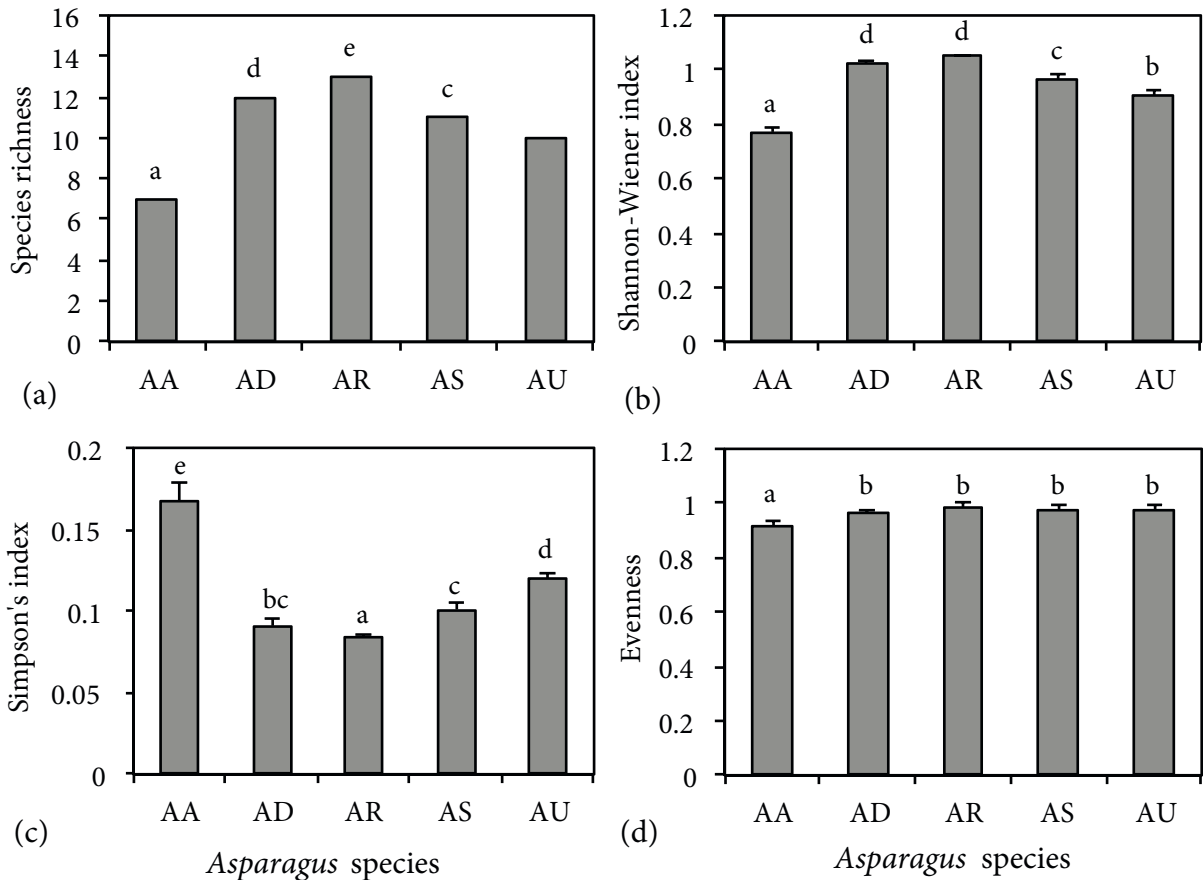


Figure 4. Arbuscular mycorrhizal fungal species richness (a), Shannon–Wiener index (H') (b), Simpson's index (D) (c), and evenness (E) (d). AA, *A. aethiopicus*, AD, *A. densiflorus*, AR, *A. racemosus*, AS, *A. setaceus*, AU, *A. umbellatus*. Error bars indicate ± 1 SE. Bars topped by same letter(s) are not significantly different according to DMRT ($P > 0.05$).

Table 8. Jaccard's index values for arbuscular mycorrhizal fungal communities associated with *Asparagus* species.

<i>Asparagus</i> species	<i>Asparagus</i> species			
	<i>A. densiflorus</i>	<i>A. racemosus</i>	<i>A. setaceus</i>	<i>A. umbellatus</i>
<i>A. aethiopicus</i>	0.636	0.429	0.500	0.545
<i>A. densiflorus</i>		0.563	0.533	0.833
<i>A. racemosus</i>			0.611	0.533
<i>A. setaceus</i>				0.500

Table 9. Pearson's correlation coefficient for arbuscular mycorrhizal fungal diversity indices and soil factors (n = 25)

Diversity indices	Soil factors				
	pH	Electrical conductivity	Nitrogen	Phosphorus	Potassium
Shannon–Wiener index (H')	-0.070	-0.710***	-0.690***	-0.566**	-0.787***
Simpson's index (D)	0.087	0.631***	0.611***	0.486**	0.707***
Evenness (E)	0.069	-0.284	-0.209	-0.249	-0.271
Richness	-0.050	-0.765***	-0.707***	-0.624***	-0.836***

, *Significant at $P < 0.01$ and $P < 0.001$, respectively

To date only *Arum*-type AM morphology has been reported in *Asparagus* (Dickson et al., 2007) and for the first time we report the intermediate and *Arum-Paris*-type AM morphologies in *Asparagus*. The factors that determine AM morphology within plant roots after initial colonization are not fully understood. However, the results of experimental studies do suggest that the AM colonization patterns within roots are the result of an interaction of host and fungal factors (Dickson et al., 2007). Functional studies on AM fungal morphologies revealed transport of P in *Paris*-type coils and arbusculate coils, as well as in intermediate AM morphology as in *Arum*-type symbiosis (Dickson et al., 2007).

In the present study, only soil pH and EC significantly influenced AM colonization and intraradical structures among the different soil factors studied. The negative correlation of RLV% and RLTC% to soil pH contradicts earlier reports where a positive or lack of correlation has been reported for these variables (Lingfei et al., 2005; García and Mendoza, 2008). Soil pH could affect various AM fungal processes like the quantity of inoculum in the soil, spore germination, hyphal growth, and root colonization (Smith and Read, 2008). Further, it is difficult to interpret the effect of soil pH on AM fungal colonization or structures, because many chemical properties of the soil and plant physiology tend to vary with changes in soil pH (Brundrett, 2009). The influence of EC on RLA/RLAC% can vary with host species. For example, García and Mendoza (2008) showed that soil EC had a positive correlation to root length with arbuscules in *Lotus tenuis*, but a negative correlation existed for these indexes in the roots of grasses. As soil salinity could alter plants nutrient demand, it could alter the formation of arbuscules. The existence of a negative correlation between %RLH/RLHC and %RLA/RLAC might be an expression of the symbionts' physiological condition as suggested by Lugo et al. (2003).

The AM fungal species richness (7–13) in the present study is similar or higher to those reported for species of *Asparagus* growing under field conditions (Babu and Manoharachary, 2003; Gaur and Kaushik, 2011; Kowalczyk and Blaszkowski, 2011). Therefore, our study does not support the view that continuous pot culture (trap culture) results in a loss of AM fungal diversity (Trejo-Aguilar et al., 2013). One possible reason for the high diversity of AM fungi in the present study could be the perennial nature of the host. The high diversity of Glomerales compared to Diversisporales suggests that taxa in the former order are well adapted to conditions of pot culture than those in the latter are (Kennedy et al., 2002). Lovera and Cuenca (2007) indicated that taxa in Diversisporales are more influenced by environmental factors than those in Glomerales are. In addition, species in Glomerales are considered to be generalists and their competitive ability enables their presence in most plant communities (Lekberg et al., 2007).

The frequent occurrence and high abundance of *F. mosseae*, *R. aggregatum*, and *R. intraradices* indicate good adaptation of these fungi to different host and soil conditions. This is evidenced by a lack of correlation or presence of a positive correlation of spore populations of these species to soil factors. The exclusive occurrence of *G. clavisporum* (= *Sclerocystis clavispora*) with *A. densiflorus* indicates that certain AM fungal taxa can exhibit some degree of host preference as shown elsewhere (Su et al., 2011).

In the present study, the nature of the correlation between soil factors and spore numbers varied with total and individual spore numbers. For example, spore populations of *C. etunicatum*, *G. clavisporum*, *Glomus* sp., and *V. viscosum* were negatively correlated to soil EC, whereas the total spore counts had a positive correlation with soil EC. A similar trend was also observed for correlations between spore numbers and soil N. This resembles the variation in the nature of correlation for spore numbers, observed by Del Val et al. (1999), where total AM fungal spore numbers on a long-term sewage sludge field experiment site located at the Federal Research Center for Agriculture in Braunschweig, Germany, was significantly and negatively correlated to soil total and available P. In contrast, spore populations of *Glomus* species III were not correlated to soil total and available P and *Glomus* species V had a significant negative correlation with the same soil variables (Del Val et al., 1999). This clearly shows that different AM fungal species in a community may respond variedly to changes in a soil variable.

The current study supports the view that host species significantly influences the AM fungal diversity and community structure (e.g., Lovelock and Ewel, 2005). For example, *A. densiflorus* and *A. racemosus* supported greater diversity of AM fungal species than other *Asparagus* species. The calculated Jaccard index of similarity indicated that all the *Asparagus* species except *A. aethiopicus* and *A. racemosus* had more than 50% of AM fungal species in common. Nevertheless, there were significant differences in the abundance of AM fungal species associated with an *Asparagus* species. Though AM fungi are considered broad generalists, a certain degree of host preference and influence has been demonstrated (Kernaghan, 2005). The influence of host species on AM fungal diversity could be attributed to the differential resource allocation of the plant host to its fungal symbionts as well as the ability of the fungus to compete for scarce resources (Hart et al., 2013). Therefore, sporulation of one AM fungal species at times could happen at the expense of others, and this could also be regulated by interspecific competition of resources, spatial restriction, and/or soil factors (Gemma and Koske, 1989).

The colonization morphology of DSE fungi within *Asparagus* roots is similar to that described by Yu et al. (2001) for the DSE fungus *Phialocephala fortinii* colonizing roots of *A. officinalis*. In the present study, DSE fungal colonization was always three- to five-fold lower than that of AM fungal colonization. This clearly suggests that the conditions prevailing under *Asparagus* cultivation are more suitable for AM than for DSE fungi. This is in accordance with the results of other studies where a higher proportion of AM fungal colonization than DSE fungal colonization has been reported (Lingfei et al., 2005). Further, the positive correlation between RLTC% and RLDTTC% suggests that these two fungal types do not compete within roots. Lingfei et al. (2005) also reported a positive correlation between %RLDTTC and %RLH in grassland plants in southwest China. This is in contrast to the observations reported by Wu et al. (2009) where RLDTTC% was negatively correlated to RLH%, while RLDTM% was positively correlated with RLV% and RLA%. However, in contrast to the results published by Lingfei et al. (2005), all the soil factors except EC were correlated to RLDTTC%. Li et al. (2015) also reported the existence of a positive correlation between DSE fungal

structures and available soil P similar to the present study. Although experimental evidence on the role of soil factors on DSE fungal colonization and function is not well resolved, available evidence does indicate that DSE fungal processes could be influenced by soil factors like for AM fungi.

In conclusion, the results of the present study clearly indicate that *Asparagus* could associate with both AM and DSE fungi. However, the benefit from this dual association has to be elucidated experimentally. The high AM fungal diversity suggests that *Asparagus* could sustain diverse AM fungal taxa even under pot conditions. This would enable maintenance of AM fungal trap cultures for long periods without much loss in diversity. The influence of soil factors on the two fungal types showed that DSE fungi were more sensitive to changes in soil factors than AM fungi were. The present study also indicated that soil factors could influence different AM fungi variedly and ultimately the AM fungal community. Considering the plant growth potentials of both AM and DSE fungi in horticulture, further evaluation of these fungi for their various plant growth promoting ability would enable their use in sustainable plant production systems.

References

- Abbott LK, Robson AD (1984). The effect of root density, inoculum placement and infectivity of inoculum on the development of vesicular-arbuscular mycorrhizas. *New Phytol* 97: 285-299.
- Babu KS, Manoharachary C (2003). Occurrence of arbuscular mycorrhizal fungi in rhizosphere soils of some medicinal plants. *Indian Phytopathol* 56: 223-227.
- Brundrett MC (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320: 37-77.
- Chapman HD (1965). Cation exchange capacity. In: Black CA, editor. *Methods of Soil Analysis. Part 2. Agronomy Monograph No. 9.* Madison, WI, USA: American Society of Agronomy, pp. 891-901.
- Cuenca G, de Andrade Z, Lovera M, Fajardo L, Meneses E, Márquez M, Machuca R (2003). Pre-selección de plantas nativas y producción de inóculos de hongos micorrízicos arbusculares (HMA) de relevancia en la rehabilitación de áreas degradadas de La Gran Sabana, estado Bolívar, Venezuela. *Ecotrópicos* 16: 27-40.
- Del Val C, Barea JM, Azcón-Aguilar C (1999). Diversity of arbuscular mycorrhizal fungus populations in heavy metal contaminated soils. *Appl Environ Microb* 65: 718-723.
- Dickson S (2004). The *Arum-Paris* continuum of mycorrhizal symbioses. *New Phytol* 163: 187-200.
- Dickson S, Smith FA, Smith SE (2007). Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza* 17: 375-393.
- García I, Mendoza R (2008). Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients. *FEMS Microbiol Ecol* 63: 359-371.
- Gaur S, Kaushik P (2011). Biodiversity of vesicular arbuscular mycorrhiza associated with *Catharanthus roseus*, *Ocimum* spp. and *Asparagus racemosus* in Uttarakhand State of Indian Central Himalaya. *Int J Bot* 7: 31-41.
- Gemma JN, Koske RE (1989). Field inoculation of American beachgrass (*Ammophila breviligulata*) with VA mycorrhizal fungi. *J Environ Manage* 29: 173-182.
- Hart MM, Forsythe J, Oshowski B, Bucking H, Jansa J, Kiers ET (2013). Hiding in a crowd - does diversity facilitate persistence of a low-quality fungal partner in the mycorrhizal symbiosis? *Symbiosis* 59: 47-56.
- Jackson ML (1973). *Soil Chemical Analysis*. New Delhi, India: Prentice-Hall.
- Jumpponen A, Trappe JM (1998). Dark septate endophytes: a review of facultative biotrophic root colonizing fungi. *New Phytol* 140: 295-310.
- Kennedy LJ, Tiller RL, Stutz JC (2002). Associations between arbuscular mycorrhizal fungi and *Sporobolus wrightii* in riparian habitats in arid southwestern North America. *J Arid Environ* 50: 459-475.
- Kernaghan G (2005). Mycorrhizal diversity: Cause and effect? *Pedobiologia* 49: 511-520.

- Koske RE, Gemma JN (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycol Res* 92: 486-488.
- Kowalczyk S, Blaszkowski J (2011). Arbuscular mycorrhizal fungi (Glomeromycota) associated with roots of plants of the Lubuskie province. *Acta Mycol* 46: 3-18.
- Krebs CJ (1989). *Ecological Methodology*. New York, NY, USA: Harper and Row.
- Lekberg Y, Koide RT, Rohr JR, Ahrlich-Wolfe L, Morton JB (2007). Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *J Ecol* 95: 95-105.
- Li B, He X, He C, Chen Y, Wang X (2015). Spatial dynamics of dark septate endophytes and soil factors in the rhizosphere of *Ammopiptanthus mongolicus* in Inner Mongolia, China. *Symbiosis* 65: 75-84.
- Lingfei L, Anna Y, Zhiwei Z (2005). Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiol Ecol* 54: 367-373.
- Lovelock CE, Ewel JJ (2005). Links between tree species, symbiotic fungal diversity and ecosystem functioning in simplified tropical ecosystems. *New Phytol* 167: 219-228.
- Lovera M, Cuenca G (2007). Diversidad de los hongos micorrízico arbusculares (HMA) y potencial micorrízico del suelo de una sabana natural y una sabana perturbada, Venezuela. *Interciencia* 32: 108-114.
- Lugo MA, González Maza ME, Cabello MN (2003). Arbuscular mycorrhizal fungi in a mountain grassland II: seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia* 95: 407-415.
- Magurran AE (1988). *Ecological Diversity and Its Measurement*. Princeton, NJ, USA: Princeton University Press.
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990). A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 115: 495-501.
- Muthukumar T, Udaiyan K, Manian S (1996). Vesicular-arbuscular mycorrhizae in tropical sedges of Southern India. *Biol Fertil Soils* 22: 96-100.
- Nahiyani ASM, Matsubara Y (2012). Tolerance to *Fusarium* root rot and changes in antioxidative ability in mycorrhizal asparagus plants. *Hortscience* 47: 356-360.
- Newsham KK (2011). A meta-analysis of plant responses to dark septate root endophytes. *New Phytol* 190: 783-793.
- Negi JS, Singh P, Joshi GP, Rawat MS, Bisht VK (2010). Chemical constituents of *Asparagus*. *Pharmacogn Rev* 4: 215-220.
- Oehl F, Sieverding E, Palenzuela J, Ineichen K, da Silva GA (2011). Advances in Glomeromycota taxonomy and classification. *IMA Fungus* 2: 191-199.
- Omar MB, Bolland L, Heather WA (1979). PVA (polyvinyl alcohol). A permanent mounting medium for fungi. *Bull Br Mycol Soc* 13: 31-32.
- Pederson CT, Safir GR, Siqueira JO, Parent S (1991). Effect of phenolic compounds on asparagus mycorrhiza. *Soil Biol Biochem* 23: 491-494.
- Ragupathy S, Mahadevan A (1993). Distribution of vesicular arbuscular mycorrhizae in the plants and rhizosphere soils of the tropical plains, Tamil Nadu, India. *Mycorrhiza* 3: 123-126.
- Rodriguez RJ, White FA Jr, Arnold AE, Redman RS (2009). Fungal endophytes: diversity and functional roles. *New Phytol* 182: 314-330.
- Schüßler A, Walker C (2010). Glomeromycota Glomeromycota species list [online]. Website http://www.arbuscular-mycorrhiza.net/amphylo_species.html [accessed 04 August 2015].
- Sieverding E, Silva GA, Berndt R, Oehl F (2011). *Rhizoglossum*, a new genus of the Glomeraceae. *Mycotaxon* 129: 373-386.
- Smith SE, Read DJ (2008). *Mycorrhizal Symbiosis*, 3rd ed. San Diego, CA, USA: Academic Press Inc.
- Souza MC, Carvalho LB, Alves PLCA, Giancotti PRF (2011). Allelopathy in pigweed (a review). *Commun Plant Sci* 1: 5-12.
- Su YY, Sun X, Guo LD (2011). Seasonality and host preference of arbuscular mycorrhizal fungi of five plant species in the inner Mongolia steppe, China. *Braz J Microbiol* 42: 57-65.
- The Plant List (2013). Version 1.1. Published on the Internet; <http://www.theplantlist.org/> (accessed 3 July, 2015).
- Trejo-Aguilar D, Lara-Capistrán L, Maldonado-Mendoza IE, Zulueta-Rodríguez R, Sangabriel-Conde W, Mancera-López ME, Barois I (2013). Loss of arbuscular mycorrhizal fungal diversity in trap cultures during long-term subculturing. *IMA Fungus* 4: 161-167.
- Wacker TL, Safir GR, Stephenson SN (1990). Evidence for succession of mycorrhizal fungi in Michigan *Asparagus* fields. *Acta Horti (ISHS)* 271: 273-278.
- Wang B, Qiu YL (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 96: 299-363.
- Wang JS, Wang XP, Zhou Y, Du LC, Wang QM (2010). Fumonisin detection and analysis of potential fumonisin-producing *Fusarium* spp. in asparagus (*Asparagus officinalis* L.) in Zhejiang Province of China. *J Sci Food Agr* 90: 836-842.
- Wu YQ, Liu TT, He XL (2009). Mycorrhizal and dark septate endophytic fungi under the canopies of desert plants in Mu Us Sandy Land of China. *Front Agric China* 3: 164-170.
- Xu P, Liang LZ, Dong XY, Xu J, Jiang PK, Shen RF (2014). Response of soil phosphorus required for maximum growth of *Asparagus officinalis* L. to inoculation of arbuscular mycorrhizal fungi. *Pedosphere* 24: 776-782.
- Yang Z, Hammer G, van Oosterom E, Rochais D, Deifel K (2010). Effects of pot size on growth of maize and sorghum plants. In: George-Jaeggli B, Jordan DJ, editors. Proceedings of the 1st Australian summer grains conference, Gold Coast, Australia, 21-24 June 2010. Canberra, Australia: Grains Research and Development Corporation, pp.1-7.
- Yu T, Nassuth A, Peterson RL (2001). Characterization of the interaction between the dark septate fungus *Phialocephala fortinii* and *Asparagus officinalis* roots. *Can J Microbiol* 47: 741-753.