

ARBUSCULAR MYCORRHIZAL FUNGI (GLOMALES) IN EGYPT. II. AN ECOLOGICAL VIEW OF SOME SALINE AFFECTED PLANTS IN THE DELTAIC MEDITERRANEAN COASTAL LAND

H. E. AGWA¹ and G. M. ABDEL-FATTAH²

¹*Dept. Biol. & Geol. Sci., Faculty of Education, Tanta University, Kafr El-Sheikh, Egypt*

²*Dept. Botany, Faculty of Science, Mansoura University, Egypt*

E-mail: Sinfac@mum.mans.eun.eg

(Received 25 February 2001)

A field survey was conducted to investigate the importance of saline soil factors on the occurrence of arbuscular mycorrhizal (AM) fungi in three localities (Baltim, New Damietta and Gamasa). Out of the 45 species of native and cultivated plants collected from three sites, 36 species including all agronomic were infected with arbuscular mycorrhizal fungi. However, few plant species belonging to the families Chenopodiaceae, Cruciferae and Cyperaceae were found to be non-mycorrhizal. In general, soil pH and CaCO₃ content have no direct effect on the number of mycorrhizal spores in the rhizosphere of all plants tested. A positive correlation between densities of Glomales and the organic matter in the rhizosphere of cultivated salt tolerant plants were observed. Generally, spore densities and subsequent level of mycorrhizal infection were negatively correlated with the degree of salinity in the soil. However, number of mycorrhizal spores in the rhizosphere of wild halophytes were not significantly correlated to infectivity. Arbuscular mycorrhizal spores isolated from the collected soils were identified as two genera, *Glomus* and *Acaulospora*. Spores belonging to the genus *Glomus* were abundant in this survey, many of these were identified as *Glomus mosseae* and *Glomus etunicatum*. *G. mosseae* was the most frequent species reported in the salt rhizosphere. Thus, the results suggest that soil salinity and organic matter might play an important role in regulating the distribution of AM fungal communities in the saline soil.

Key words: arbuscular mycorrhiza, edaphic factors, Glomales spores, halophytic plants, mycorrhizal colonization, saline soils

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are the most widespread symbiotic microorganisms associated with higher plants. Their ubiquitous distribution is related to their capacity for active and passive dispersal by many vectors (Hetrick 1984, Diop *et al.* 1994, Miller and Jackson 1998). In natural ecosystems, the occurrence of AM fungi can be related to soil disturbance (Jasper *et al.* 1991, Johnson-Green *et al.* 1995, McGonigle and Miller 2000), to agricultural prac-

tices (Black and Tinker 1979, Singh and Tiagi 1989), to chemical properties of the soil (Johnson *et al.* 1992, Mankarios and Abdel-Fattah 1994), to the seasons (Hayman 1970, Diop *et al.* 1994) and to the activities of other microorganisms (Azcon-Aguilar and Barea 1985).

In Egypt, AM fungi are known to colonize a wide range of natural and cultivated plants (Ishac *et al.* 1986, Agwa 1990, Mankarios and Abdel-Fattah 1994). Little is known about the ecology of AM fungi in saline habitats. Most studies have concentrated on the effects of inoculation with arbuscular mycorrhizal fungi on the growth of plants in saline soil, and information on the effects of salinity on growth of the fungi is scarce and is often circumstantial (Levy *et al.* 1983, Juniper and Abbott 1993, Mankarios *et al.* 1995, Bhaskaran and Selvaraj 1997). There is clear evidence that germination of spores and subsequent hyphal growth of some endomycorrhizal fungi are reduced by increasing salt concentration (Ho 1987, Copeman *et al.* 1996).

The interaction of AM fungi with salts such as NaCl is not well understood. However, AM colonization of native halophytic vegetation growing in saline environments has been observed (Khan 1974, Pond *et al.* 1984, Kim and Weber 1985, Pfeiffer and Bloss 1988, Agwa 1990, Juniper and Abbott 1993, Mankarios and Abdel-Fattah 1994, Bhaskaran and Selvaraj 1997). Reports of AM fungi in salt marsh plants are rather conflicting (Mohan Kumar and Mahadevan 1986, Sengupta and Chaudhuri 1990, Cooke *et al.* 1993). AM fungi have increased and suppressed herbaceous plant growth in saline conditions compared with non-mycorrhizal control (Hirrel and Gerdemann 1980, Poss *et al.* 1985). These differential effects might be due to the origin of the AM fungi since mycorrhizal fungi adapt to edaphic conditions (Copeman *et al.* 1996).

AM colonization has been reported to increase plant growth under saline conditions by overcoming the detrimental growth effects of NaCl (Rinaldelli and Mancuso 1998). This may be due to increased uptake of phosphorus leading to increased growth and subsequent dilution of toxic ion effects, to an ameliorative effect of mycorrhizas on water stress of plants (Pond *et al.* 1984).

In this study, we present an ecological survey of AM fungi in three saline areas of deltaic Mediterranean coastal land. The study intended to quantitatively assess AM fungi associated within plant roots, physicochemical properties of the soil, counting and identification of dominant AM spores in the rhizosphere soil.

MATERIAL AND METHODS

Sampling procedures

Forty-five rhizosphere soils and root samples of wild halophytes and cultivated salt tolerant plant (at least three replicates for each plant species) were collected from saline localities (Baltim, New Damietta and Gamasa) (Fig. 1). Soil sampling for each plant species was carried out at three different times during the flowering stages to give 415 soil specimens. Soil samples were taken by core (2.2 cm diameter \times 15 cm deep) from the center of each replicate, then placed in presterilised bags, labelled in the field, transported to the laboratory and stored at 4 °C until they were processed. Root system from each species, freed from adhering soil, was carefully removed and immediately fixed in FAA (formalin-acetic acid-alcohol, 90 : 5 : 5 v/v). A representative of each wild plant species was pressed, dried, identified and deposited in the Botany Department Herbarium, Mansoura University, Egypt.

Soil characteristics

Soil texture analysis was carried out using the sieving method (Ball 1986). Total organic matter was determined according to Jackson (1960). The soil pH was determined in water by electric pH meter model WHPYE M29 (USA). Total soluble salt was measured using a conductivity meter (Type D-K-102

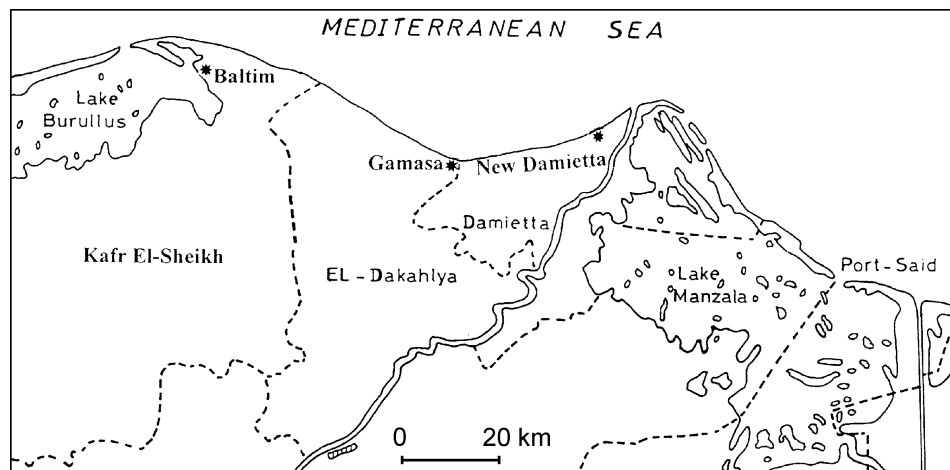


Fig. 1. Location map of the studied area [*]

Radelkis) according to the procedure of Jackson (1962). The concentrations of Na, K and Ca in soil extracts were estimated by a Perkin Elmer (Germany) flame photometer (model 2100) (Allen *et al.* 1974).

Extraction and estimation of mycorrhizal spores

From each rhizosphere soil sample, spores were extracted from 100 g of wet-soil using a modification technique of Gerdemann and Nicolson (1963). We used a smaller mesh (25 μm) instead of that recommended by Gerdemann and Nicolson (50 μm) to minimise loss of the smallest spore. This process was repeated twice again to retain most of the spores in the rhizosphere soil. In order to remove colloidal large pieces of soil organic material, the debris retained on the sieve was either thoroughly washed under a steam of water or resuspended and decanted again through the sieve. The spore suspension and small amounts of the remaining organic matter were blended and subjected to 20%, 40% and 60% sucrose density gradient centrifugation (Daniels and Skipper 1982) to separate spores from heavy organic matter. The supernatant (with spores) were pour onto a 25-mesh sieve and rinsed with water to remove the sugar. Collected spores were spread onto a gridded membrane filter and then examined using a dissecting microscope (WILD M8, Switzerland) to determine the quantity of spores present in each rhizosphere according to the technique of Mckenney and Lindsey (1987). Soil samples were oven-dried at 105–110 °C to determine moisture content and then spore numbers per 100 g dry weight soil were then calculated.

Staining and estimation of mycorrhizal root infection

Fixed roots in FAA were rinsed repeatedly in tap water, cut into small segments (0.8 \pm 0.3 cm) and cleared at appr. 90 °C in 10% KOH for 1 h; darker roots were bathed in alkaline hydrogen peroxide for 20 min (Kormanik and McGraw 1982). Thereafter, the roots were washed with tap water and stained with trypan blue (SIGMA) in lactophenol (Phillips and Hayman 1970). Fifty randomly selected stained root pieces of each species were mounted on slides in lactoglycerol and examined microscopically (Leitz Wetzlar, Germany) for estimation of the mycorrhizal root colonization (Trouvelot *et al.* 1986). Fungal hyphae, vesicles and arbuscules were recorded.

Trap pot cultures of mycorrhizal fungi

Mixture of the infected plant roots and rhizosphere soil from each location were used as inocula for the preliminary trap cultures with autoclaved

sand (1 : 1 v/v) as the substrate and *Linum usitatissimum* L. as a host plant. Plants were grown in a glasshouse (26 °C day / 18 °C night) under natural light conditions. The soil surface of each pot was covered with gravel to reduce air borne contamination and the plants were watered with tap water to maintain soil moisture. After four months (life period) of plant growth, spores were isolated from the culture soils and used for identification or reinoculation to the host plant to get pure spores for each arbuscular mycorrhizal species.

Spore isolation and identification

Fungal spores were recovered from soil by wet sieving and sucrose gradient centrifugation (Daniels and Skipper 1982). Identification of AM species was based on spore colour, size, surface ornamentation, and wall structure using mostly reference cultures or slides from the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi, INVAM (Morton *et al.* 1993), and species descriptions (Schenck and Perez 1990). Colour of whole spores was characterised using a printed chart (available from INVAM, West Virginia University, USA). Spores were mounted in polyvinyl-alcohol-lactic-acid-glycerol (PVLG) mounting medium (Koske and Tiessier 1983), or PVLG mixed with Melzer's reagent (1 : 1 v/v) to make permanent slides. Slides were examined using a compound microscope (100–1000×). Voucher specimens of each of the identified fungal species were retained and deposited in the Plant Pathology Herbarium, Mansoura University, Egypt.

Correlation coefficients between total number of spores and some physicochemical properties of the soil were calculated by using SAS (Statistical Analysis System).

RESULTS AND DISCUSSION

Survey of AM fungi in Egyptian saline affected soils

Table 1 lists the pioneer salt marsh species of wild and cultivated plants examined, sites of collection, mycorrhizal status, percentage of root colonization and spore densities of each rhizosphere soil. Most plant families examined in this study were reported to be mycorrhizal. Of the 45 plant species examined, 36 had mycorrhizal infection and 9 plants were non-mycorrhizal. Some cultivated plants were heavily infected with these fungi, e.g. *Trifolium alexandrinum*, *Vicia faba*, *Lactuca sativa* and *Allium porrum*. Low levels of AM infection were found in native halophytic plants, like *Zygophyllum aegyptium*, *Sporobolus spicatus*, *Tamarix nilotica* and *Inula crithmoides*. These results are in

Table 1
Spore densities, percentage of arbuscular mycorrhizal (AM) infection and mycorrhizal status in roots of pioneer species collected from three sites of Egyptian salt affected soils. Each value is the mean of 9 samples \pm SE

Locality	Plant species	Family	Spore density/ 100 g soil	M*%	Fungal struct.		
					H	A	V
Saline area of Baltim	<i>Arthrocnemum macrostachyum</i> Del.	Chenopodiaceae	0.0 \pm 0.0	0.0 \pm 0.0	-	-	-
	<i>Atriplex portulacoides</i> L.	Chenopodiaceae	13 \pm 1.2	0.0 \pm 0.0	-	-	-
	<i>Beta vulgaris</i> var. <i>rapa</i> L.	Chenopodiaceae	198 \pm 13.7	45.3 \pm 5.8	+	+	-
	<i>Beta vulgaris</i> var. <i>cicla</i> L.	Chenopodiaceae	75 \pm 16.0	35.1 \pm 7.1	+	+	-
	<i>Suaeda vera</i> Forsk.	Chenopodiaceae	40 \pm 13.4	29.5 \pm 3.5	-	+	-
	<i>Trifolium alexandrinum</i> L.	Leguminosae	412 \pm 29.3	55.8 \pm 8.2	+	+	+
	<i>Vicia faba</i> L.	Leguminosae	326 \pm 25.2	52.0 \pm 6.9	+	+	-
	<i>Melilotus indica</i> L.	Leguminosae	305 \pm 18.3	48.2 \pm 6.3	+	+	-
	<i>Inula crithmoides</i> L.	Compositae	25 \pm 2.5	15.9 \pm 2.1	+	-	+
	<i>Lactuca sativa</i> L.	Compositae	288 \pm 18.3	45.0 \pm 3.2	+	+	-
	<i>Asparagus africanus</i> Lam.	Liliaceae	180 \pm 11.3	33.0 \pm 4.1	-	+	-
	<i>Allium porrum</i> L.	Liliaceae	290 \pm 14.2	60.1 \pm 6.3	+	+	-
	<i>Allium cepa</i> L.	Liliaceae	311 \pm 16.1	68.3 \pm 5.4	+	+	-
	<i>Triticum vulgare</i> L.	Gramineae	220 \pm 13.1	42.0 \pm 9.1	+	-	-
	<i>Solanum tuberosum</i> L.	Solanaceae	180 \pm 9.3	23.0 \pm 3.5	+	-	+
	<i>Malva parviflora</i> L.	Malvaceae	218 \pm 10.4	53.0 \pm 6.1	+	-	+
	<i>Arthrocnemum macrostachyum</i> Del.	Chenopodiaceae	0.0 \pm 0.0	0.0 \pm 0.0	-	-	-
	<i>Suaeda vera</i> Forsk.	Chenopodiaceae	19 \pm 2.1	0.0 \pm 0.0	-	-	-
	Saline area of New Damietta	<i>Melilotus indicus</i> L.	Leguminosae	255 \pm 18.9	49.0 \pm 3.5	+	+
<i>Vicia faba</i> L.		Leguminosae	280 \pm 19.1	45.0 \pm 4.2	+	+	-
<i>Medicago sativa</i> L.		Leguminosae	307 \pm 16.5	43.0 \pm 3.5	+	+	-
<i>Lotus halophilus</i> Boiss.		Leguminosae	225 \pm 13.1	37.0 \pm 4.6	+	+	+
<i>Inula crithmoides</i> L.		Compositae	129 \pm 9.3	18.0 \pm 1.2	+	-	-
<i>Senecio glaucus</i> L.		Compositae	13 \pm 2.3	22.0 \pm 2.3	+	-	-
<i>Lactuca sativa</i> L.		Compositae	239 \pm 15.6	48.0 \pm 6.3	+	+	-

Table 1 (continued)

Locality	Plant species	Family	Spore density/ 100 g soil	M*%	Fungal struct.
					H A V
Saline area of New Damietta	<i>Allium cepa</i> L.	Liliaceae	290±11.3	51.9±3.2	+ - -
	<i>Lolium temulentum</i> L.	Gramineae	211±14.2	33.2±2.1	+ - -
	<i>Phragmites australis</i> Cav.	Gramineae	153±11.1	28.1±3.1	+ - +
	<i>Sporobolus spicatus</i> Kunth	Gramineae	112±10.3	22.5±2.5	+ - +
	<i>Triticum vulgare</i> L.	Gramineae	293±15.3	44.2±3.5	+ - -
	<i>Lycopersicon esculentus</i> L.	Solanaceae	35±3.5	28.6±1.8	+ + -
	<i>Zygophyllum aegyptium</i> L.	Zygophyllaceae	18±2.3	13.3±3.1	+ - -
	<i>Spergularia marina</i> L.	Caryophyllaceae	211±8.2	26.2±5.1	+ + -
	<i>Cyperus laevigatus</i> L.	Cyperaceae	0.0±0.0	0.0±0.0	- - -
	<i>Tamarix nilotica</i> Ehrenb.	Tamaricaceae	24±4.1	16.3±2.1	+ - +
	<i>Cakile maritima</i> Scop.	Cruciferae	0.0±0.0	0.0±0.0	- - -
	<i>Arthrocnemum macrostachyum</i> Del.	Chenopodiaceae	0.0±0.0	0.0±0.0	- - -
	<i>Atriplex portulacoides</i> L.	Chenopodiaceae	0.0±0.0	0.0±0.0	- - -
	<i>Melilotus indica</i> L.	Leguminosae	513±13	53.3±5.2	+ - -
	<i>Lolium perenne</i> L.	Gramineae	211±9.3	38.2±3.1	+ + -
	<i>Parapholis incurva</i> L.	Gramineae	63±5.2	33.2±1.6	+ + -
	<i>Bromus rigidus</i> Roth	Gramineae	113±10.9	33.9±2.8	+ + -
<i>Zygophyllum aegyptium</i> L.	Zygophyllaceae	123±8.3	13.3±1.6	+ - -	
<i>Inula crithmoides</i> L.	Compositae	0.0±0.0	0.0±0.0	- - -	
<i>Solanum lycopersicum</i> L.	Solanaceae	139±9.4	21.0±2.1	+ - +	

*M = intensity of mycorrhizal root infection, H = internal hyphae, A = arbuscules, V = vesicles
+ = present, - = absent

Table 2
Edaphic characteristics of rhizosphere soil samples of different plant species collected from three sites of Egyptian salt affected soils.
Each value is the mean of 9 samples \pm SE

Locality	Plant species	Soil texture	Salinity (jumhos)	pH	O. M.* (%)	CaCO ₃ (%)	
Saline area of Baltim	<i>Arthrocnemum macrostachyum</i>	Sandy	6750 \pm 37.3	7.66 \pm 0.5	0.50 \pm 0.21	10.0 \pm 2.4	
	<i>Atriplex portulacoides</i>	Sandy	6780 \pm 34.9	7.72 \pm 0.4	0.50 \pm 0.11	7.0 \pm 2.1	
	<i>Beta vulgaris</i> var. <i>rapa</i>	Sandy clay loam	870 \pm 18.6	8.43 \pm 0.5	0.81 \pm 0.3	19.8 \pm 3.3	
	<i>Beta vulgaris</i> var. <i>cicla</i>	Sandy clay loam	1150 \pm 21.6	8.21 \pm 0.6	0.93 \pm 0.41	20.0 \pm 4.8	
	<i>Suaeda vera</i>	Sandy clay loam	3000 \pm 29.2	7.83 \pm 0.4	0.71 \pm 0.32	11.5 \pm 2.5	
	<i>Trifolium alexandrinum</i>	Sandy	800 \pm 10.5	8.12 \pm 0.4	1.20 \pm 0.56	21.0 \pm 1.9	
	<i>Vicia faba</i>	Sandy clay loam	1100 \pm 22.6	8.15 \pm 0.6	1.30 \pm 0.68	15.0 \pm 2.3	
	<i>Melilotus indica</i>	Sandy clay loam	1680 \pm 28.0	8.04 \pm 0.3	1.00 \pm 0.4	22.0 \pm 4.2	
	<i>Inula crithmoides</i>	Sandy	4650 \pm 32.0	7.91 \pm 0.3	0.75 \pm 0.42	18.0 \pm 2.1	
	<i>Lactuca sativa</i>	Sandy clay loam	700 \pm 11.0	8.14 \pm 0.5	1.20 \pm 0.61	14.0 \pm 3.0	
	<i>Asparagus africanus</i>	Sandy clay loam	840 \pm 13.0	8.20 \pm 0.6	0.85 \pm 0.54	17.0 \pm 3.8	
	<i>Allium porrum</i>	Sandy clay loam	1000 \pm 22.0	8.12 \pm 0.6	1.50 \pm 0.74	15.0 \pm 1.3	
	<i>Allium cepa</i>	Sandy clay loam	800 \pm 17.3	7.95 \pm 0.5	1.60 \pm 0.65	16.0 \pm 2.2	
	<i>Triticum vulgare</i>	Sandy clay loam	1050 \pm 21.0	7.88 \pm 0.5	0.72 \pm 0.41	11.3 \pm 2.5	
	<i>Solanum tuberosum</i>	Sandy clay loam	350 \pm 15.0	8.19 \pm 0.7	0.82 \pm 0.23	14.5 \pm 3.5	
	<i>Malva parviflora</i>	Sandy clay loam	940 \pm 21.3	8.23 \pm 0.7	0.76 \pm 0.43	18.6 \pm 5.4	
	<i>Arthrocnemum macrostachyum</i>	Sandy	6520 \pm 34.1	7.96 \pm 0.4	0.32 \pm 0.11	8.6 \pm 1.3	
	Saline area of New Damietta	<i>Suaeda vera</i>	Sandy	2900 \pm 29.0	7.67 \pm 0.5	0.24 \pm 0.10	7.9 \pm 1.6
		<i>Melilotus indicus</i>	Sandy clay loam	500 \pm 13.0	8.12 \pm 0.6	1.10 \pm 0.53	14.0 \pm 3.8
<i>Vicia faba</i>		Sandy clay loam	490 \pm 09.0	8.10 \pm 0.7	0.93 \pm 0.60	13.0 \pm 2.0	
<i>Medicago sativa</i>		Sandy clay loam	190 \pm 13.5	7.95 \pm 0.5	0.91 \pm 0.60	19.3 \pm 5.8	
<i>Lotus halophilus</i>		Sandy	190 \pm 11.0	7.92 \pm 0.5	0.40 \pm 0.20	13.0 \pm 2.3	
<i>Inula crithmoides</i>		Sandy	3950 \pm 19.5	7.67 \pm 0.3	0.30 \pm 0.12	7.8 \pm 1.1	
<i>Senecio glaucus</i>		Sandy	320 \pm 11.0	7.50 \pm 0.4	0.63 \pm 0.41	9.0 \pm 1.8	
<i>Lactuca sativa</i>		Sandy clay loam	460 \pm 12.0	8.10 \pm 0.6	0.81 \pm 0.43	13.0 \pm 1.4	
<i>Allium cepa</i>		Sandy clay loam	400 \pm 11.0	7.97 \pm 0.3	0.65 \pm 0.32	19.8 \pm 3.8	

Table 2 (continued)

Locality	Plant species	Soil texture	Salinity (µmhos)	pH	O. M.* (%)	CaCO ₃ (%)
	<i>Lolium temulentum</i>	Sandy	240±08.0	7.87±0.2	0.22±0.09	14.0±2.3
	<i>Phragmites australis</i>	Sandy	820±16.1	7.93±0.3	0.43±0.21	12.0±1.5
	<i>Sporobolus spicatus</i>	Sandy	2500±25.1	7.38±0.4	0.91±0.41	16.0±3.5
	<i>Triticum vulgare</i>	Sandy clay loam	315±10.2	7.93±0.3	0.90±0.43	14.0±2.9
	<i>Lycopersicon esculentus</i>	Sandy clay loam	370±08.0	7.90±0.6	0.80±0.36	9.0±1.9
	<i>Zygophyllum aegyptium</i>	Sandy	2500±27.1	7.58±0.5	0.82±0.33	7.9±2.3
	<i>Spergularia marina</i>	Sandy	1090±18.9	7.56±0.4	0.56±0.15	13.0±2.4
	<i>Cyperus laevigatus</i>	Sandy	950±15.1	7.68±0.5	0.56±0.11	18.0±2.9
	<i>Tamarix nilotica</i>	Sandy	315±09.3	7.93±0.4	0.51±0.23	15.3±3.2
	<i>Cakile maritima</i>	Sandy	250±10.2	7.80±0.3	0.62±0.31	11.3±3.5
Saline area of Gamasa	<i>Arthrocnemum macrostachyum</i>	Sandy	5520±32.4	7.35±0.3	0.32±0.09	8.0±2.3
	<i>Atriplex portulacoides</i>	Sandy	1200±19.4	7.53±0.30	0.71±0.39	8.5±1.95
	<i>Melilotus indica</i>	Sandy clay loam	950±18.3	8.15±0.35	0.91±0.52	13.0±3.4
	<i>Lolium perenne</i>	Sandy	192±08.1	7.30±0.39	0.55±0.22	10.2±2.5
	<i>Parapholis incurva</i>	Sandy	1120±17.3	7.75±0.12	0.35±0.14	2.5±0.9
	<i>Bromus rigidus</i>	Sandy	320±11.2	7.53±0.20	0.32±0.11	9.3±1.2
	<i>Zygophyllum aegyptium</i>	Sandy	2500±26.1	7.94±0.20	0.35±0.12	5.0±0.96
	<i>Inula crithmoides</i>	Sandy	2390±24.1	7.78±0.30	0.46±0.06	10.0±2.0
	<i>Solanum lycopersicum</i>	Sandy clay loam	238±09.3	8.10±0.40	0.86±0.37	14.0±2.71

*O. M. = organic matter

agreement with various reports (Khan 1974, Pond *et al.* 1984, Mohan Kumar and Mahadevan 1986, Sengupta and Chaudhuri 1990, Johnson-Green *et al.* 1995, Bhaskaran and Selvaraj 1997) who concluded that the occurrence of mycorrhizas on halophytes were highly affected by soil salinity in saline environments.

In our survey, a number of plant species that were found to be non-mycorrhizal, are in agreement with previous literatures (Berch *et al.* 1988, Mankarios and Abdel-Fattah 1994). These include *Arthrocnemum macrostachyum*, *Atriplex portulacoides* and *Suaeda vera* (Chenopodiaceae), *Cyperus laevigatus* (Cyperaceae), *Cakile maritima* (Cruciferae) and *Inula crithmoides* (Compositae). On the other hand, under natural saline conditions, few species of Chenopodiaceae and Compositae were colonized by AM fungi (Schwab *et al.* 1982, Harley and Harley 1987). In this connection, Sengupta and Chaudhuri (1990) and Bhaskaran and Selvaraj (1997) suggested that stress factors in the saline habitat might have made the chenopodiaceous plants susceptible to colonization by AM fungi.

Observations of stained root pieces taken from the infected plant species showed the presence of typical vesicles and/or arbuscules in the root cortex. Extent of colonization and characteristics of AM in the roots are given in Table 1.

In this study, total spore densities in the rhizosphere soil cultivated salt tolerant plants were positively correlated ($r = 0.581, 0.635$ and 0.583 in Baltim, Damietta and Gamasa sites, respectively) with the percentage of mycorrhizal colonization (Table 3). However, these two parameters were not correlated in the rhizosphere soil of wild halophytic plants. In this connection, number of spores were absent or present in very low amounts in soils where non-mycorrhizal plants were found. Furthermore, we observed large variation in the percentage of colonization in some plants growing close to one another and collected at the same time. These results are supported by Smith and Newton (1986). This variation might be explained in a number of ways: it may result from local variation in edaphic factors (Antibus and Lesica 1985, Johnson *et al.* 1992, Diop *et al.* 1994, McGonigle and Miller 2000), soil nutrients (Sparling and Tinker 1975, Miller and Jackson 1998) or differences in plant genotype (Johnson-Green *et al.* 1995) and stage of development (Hayman *et al.* 1975, Copeman *et al.* 1996, Bhaskaran and Selvaraj 1997).

Our results demonstrated that the formation of mycorrhizal fungi, particularly in wild halophytic plants, may either decreased or disappear by increasing soil salinity (Table 2). Salt in the soil may have had a direct effect of the mycorrhizal fungi (caused turgor loss and desiccation of fine hyphae) or it may have reduced colonization indirectly via its deleterious effect on root growth in surface soil (Poss *et al.* 1985). In contrast to our data, Hartmond *et al.* (1987) reported that the formation of mycorrhizal colonization of citrus was

Table 3

Correlation coefficient between spore densities in rhizosphere soils of wild halophyte plants (WHP) and cultivated salt tolerant plants (CSTP), and each mycorrhizal root colonization (M%) and some edaphic properties of soil collected from three saline sites

Spore density	M%	Edaphic factors			
		Salinity	pH	O.M.	CaCO ₃
Baltim					
WHP	0.419	-0.675*	0.454	0.465	0.498
CSTP	0.581*	-0.509*	0.244	0.862**	0.192
Damietta					
WHP	0.335	-0.980**	0.411	0.208	0.446
CSTP	0.635*	-0.595*	0.598*	0.838**	0.452
Gamasa					
WHP	0.368	-0.936**	0.275	0.324	0.250
CSTP	0.583*	-0.588*	0.492	0.618*	0.366

* and ** significant at $p = 0.05\%$ and $p = 0.01\%$, respectively

not affected by increasing the level of salinity in the soil up to 0.15 mol l^{-1} . More research is needed which emphasises the growth and physiology of the fungal symbiont in saline conditions.

Physicochemical properties of the studied saline soils and their spore density

Data concerning the physicochemical properties of the soil and the number of AM spores are listed in Table 2. All the soils collected during this study were slightly alkaline. No significant correlations were found between the number of spores and the level of soil pH (except in Damietta rhizosphere soil of cultivated salt tolerant plants, $r = 0.598$, Table 3). Our findings did not support the results of Green *et al.* (1976) who concluded that the soil factors like pH is a limiting agent in the distribution of AM spores. In the meantime, Sheikh *et al.* (1975) reported that soil at pH 6.2 contains the greatest number of AM spores and that the number decreases with an increase of pH.

The present investigation shows that spore densities of AM fungi in the rhizosphere of cultivated plants were positively correlated with the organic matter content ($r = 0.862, 838$ and 0.618 in Baltim, New Damietta and Gamasa sites, respectively, Table 3). However, this relation was not present in the wild halophytic plants. An increase in number of AM spores with increasing organic matter content has been documented by other investigators (Diop *et al.* 1994, Mankarios and Abdel-Fattah 1994). We suggested that the organic matter contains substances which stimulate the growth of both fungi and their host, and also establishment of the symbiosis (Miller and Jackson 1998). In ad-

Table 4

Average means of physicochemical nature of rhizosphere soil, percentage of infection and associated mycorrhizal fungi identified from three sites of Egyptian affected soils

Parameters	Locality		
	Saline area of Baltim	Saline area of New Damietta	Saline area of Gamasa
pH (H ₂ O)	8.01	7.81	7.71
E. C. (μ mhos)	2081	1248	1596
Extractable cations (mg/100 g soil)	Ca ⁺⁺	471	360
	Na ⁺	965	752
	K ⁺	223	169
Intensity of mycorrhizal infection (%)	40.1	28.3	24.5
Mycorrhizal fungi	<i>Glomus mosseae</i> , <i>G. etunicatum</i>	<i>Glomus mosseae</i> , <i>G. etunicatum</i> , <i>Acaulospora</i> sp.	<i>Glomus mosseae</i>

dition, organic matter may play an important role in providing the substrate for limited saprophytic existence of AM fungi (Brechtel 1990).

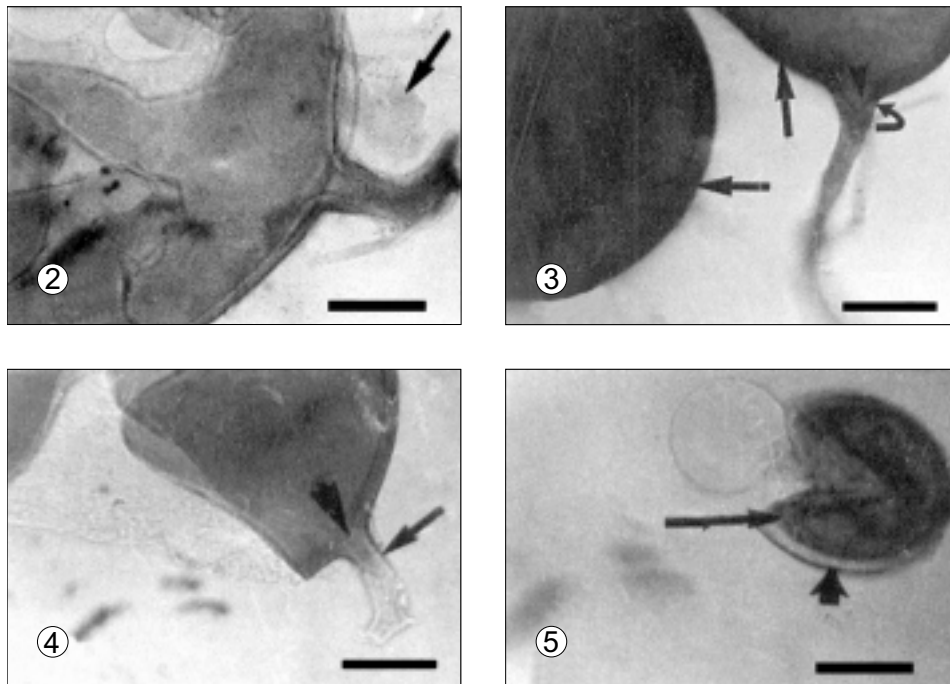
During this study, soil salinity appeared to be a limiting factor on the occurrence of AM spores in the rhizosphere soils (Table 3). In this connection, spore densities of mycorrhizal fungi in rhizosphere soil of plant species, particularly in wild halophytes, were negatively correlated (at $p = 0.01$) with the degree of soil salinity. These observations are in good agreement with other investigators (Estaun 1990, Juniper and Abbott 1993) who reported that the rate of germination of spores of mycorrhizal fungi in soil reduced with increasing concentration of NaCl in the soil solution up to 0.14 mol l^{-1} . Furthermore, NaCl in the growth medium may induce changes not only in the hyphal length but also in other morphological properties of hyphae (Hirrel 1981). Moreover, Juniper and Abbott (1993) suggested that the primary effect of salinity on spore germination and their hyphae is likely due to osmotic forces rather than the toxicity of sodium or chloride ions. More work is needed to investigate the effects of salinity on different stages of germination and to compare the sensitivities of different species and isolates of fungi to salt stress.

Morphological characteristics and identification of AM species isolated from Egyptian saline soils

The isolated and identified AM fungal spores found in saline soils are listed in Table 4 and illustrated in Figures 2–5. Spores were identified as: *Glomus mosseae* (Nicolson et Gerdemann) Gerdemann et Trappe (Figs 2, 3), *Glomus etunicatum* Becker et Gerdemann (Fig. 4) and *Acaulospora* sp. (Fig. 5). *G. mosseae* was the most frequent species reported in the rhizosphere soil samples

during this survey. Next was *G. etunicatum* and insignificant numbers of spores of *Acaulospora*.

Physicochemical properties of soil samples from three saline locations in Egypt indicated that all the soils examined were slightly alkaline (pH 7.30 to 8.43), with high soluble salinity (1263 to 2081 μmhos) and mycorrhizal infection ranged from 24 to 40% (Table 4). The effect of edaphic factors like pH and CaCO_3 on the distribution of AM fungi and abundance of particular species was difficult to explain (Walker *et al.* 1982). During our investigations no relation was observed between soil pH and the abundance of *G. mosseae*. However, the mycelia of *G. mosseae* prefer alkaline soil (Khaliel 1989), whereas *G. fasciculatum* (Thaxter sensu Gerdemann) Gerdemann et Trappe prefers acidic soils (Hayman 1983). The predominance of *G. mosseae* in the saline areas supports the observation of Khan (1974) who recorded this fungus in the rhizosphere



Figs 2–5. A crushed spore of *Glomus mosseae* mounted in Melzer's reagent. 2 = Note the outer sloughing layer (arrow) stained red, bar = 50 μm ; 3 = showing globose (left) and elliptical (right) spores. Note the thickness of the laminate layer of the wall (straight arrows), an open pore (arrowhead) and the characteristic funnel shaped subtending hypha (recurved arrow), bar = 50 μm ; 4 = showing occluded hyphal attachment by the spore wall. Note the hyaline cylindrical hyphal attachment (arrow), bar = 50 μm ; 5 = A crushed spore of *Acaulospora* sp. mounted in Melzer's reagent. The outer wall (short arrow) does not stained, but the inner wall stained dark red-purple (long arrow), bar = 50 μm

soil of halophytic plants in California. These results concluded that soil salinity and organic matter content (rather than other estimated soil factors) play an important role in the occurrence of AM fungi in saline environments. In a future study, we will attempt to determine whether the occurrence of AM fungal spores and their colonization in halophytic plants can be directly related to one or more of physicochemical properties of soil, plant genotype, stage of plant development and proximity to other plant species.

*

Acknowledgements – The authors would like to thank Dr I. Mashaly (Ecology Department, Mansoura University, Egypt) for his help during the field trips and sample collection. Special thanks to Prof. Dr Zahran who kindly confirmed the identification of the collected wild plants.

REFERENCES

- Agwa, H. E. (1990): *Vesicular-arbuscular mycorrhiza and nodulation in some Egyptian plants*. – Ph. D. Thesis, Tanta University, Egypt, 320 pp.
- Allen, S., Griwshay, H. M., Parakinson, J. A. and Quarmbly, C. (1974): *Chemical analysis of ecological materials*. – Blackwell, Oxford, 565 pp.
- Antibus, R. K. and Lesica, P. C. (1985): Effects of soil parent material on mycorrhizal infection of alpine plants. – *Jpn. J. Bot.* **12**: 359–408.
- Azcon-Aguilar, C. and Barea, J. M. (1985): Effect of soil microorganisms on formation of vesicular-arbuscular mycorrhizas. – *Trans. Br. Mycol. Soc.* **84**: 536–537.
- Ball, D. F. (1986): *Site and soils*. – In: Moor, P. D. and Chapman, S. B. (eds): *Methods in plant ecology*. Blackwell, Oxford, pp. 215–284.
- Berch, S. M., Gamiet, S. and Deom, E. (1988): Mycorrhizal status of some plants of southwestern British Columbia. – *Can. J. Bot.* **66**: 1924–1928.
- Bhaskaran, C. and Selvaraj, T. (1997): Seasonal incidence and distribution of VA-mycorrhizal fungi in native saline soils. – *J. Environ. Biol.* **18**: 209–212.
- Black, R. and Tinker, P. B. (1979): The development of endomycorrhizal root systems. II. Effects of agronomic factors and soil conditions on the development of vesicular-arbuscular mycorrhizal infection in barley and on the endophyte spore density. – *New Phytol.* **83**: 401–413.
- Brechelt, T. (1990): Effect of different organic manures on the efficiency of VA mycorrhiza. – *Agri. Ecosy. Environ.* **29**: 323–327.
- Cooke, J. C., Butler, R. H. and Madole, G. (1993): Some observations on the vertical distribution of vesicular arbuscular mycorrhizae in roots of salt marsh grasses growing in saturated soils. – *Mycologia* **85**: 547–550.
- Copeman, R. H., Martin, C. A. and Stutz, J. C. (1996): Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soils. – *Hort. Sci.* **31**: 341–344.
- Daniels, B. A. and Skipper, H. D. (1982): *Methods for the recovery and quantitative estimation of propagules from soil*. – In: Schenck, N. C. (ed.): *Methods and principles of mycorrhizal research*. Ammr. Phytopathol. Soc. Press., St. Paul, Minnesota, pp. 29–35.

- Diop, T. A., Gueye, M., Dreyfus, B. L., Plenchette, C. and Strullu, D. G. (1994): Indigenous arbuscular mycorrhizal fungi associated with *Acacia albida* Del. in different areas of Senegal. – *Appl. Env. Microbiol.* **60**: 3433–3436.
- Estaun, M. V. (1990): Effect of sodium chloride and mannitol on germination and hyphal growth of the vesicular-arbuscular fungus *Glomus mosseae*. – *Agric. Ecosyst. Env.* **29**: 123–129.
- Gerdemann, J. W. and Nicolson, T. H. (1963): Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. – *Trans. Brit. Myc. Soc.* **46**: 235–244.
- Green, N. E., Graham, S. O. and Schenck, N. C. (1976): The influence of pH on the germination of vesicular-arbuscular mycorrhizal spores. – *Mycologia* **68**: 929–934.
- Harley, J. L. and Harley, E. L. (1987): A check-list of mycorrhiza in the British Flora. – *New Phytol.* **105**: 1–102.
- Hartmond, U., Schaesberg, N. V., Graham, J. H. and Syvertsen, J. P. (1987): Salinity and flooding stress effects on mycorrhizal and non-mycorrhizal citrus rootstock seedlings. – *Plant Soil* **104**: 37–43.
- Hayman, D. S. (1970): *Endogone* spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. – *Trans. Brit. Mycol. Soc.* **54**: 53–63.
- Hayman, D. S. (1983): The physiology of vesicular-arbuscular endomycorrhizal symbiosis. – *Can. J. Bot.* **61**: 944–963.
- Hayman, D. S., Johnson, A. M. and Ruddlesdin, I. (1975): The influence of phosphate and crop species on *Endogone* spores and vesicular-arbuscular mycorrhiza under field conditions. – *Plant Soil* **43**: 489–495.
- Hetrick, B. A. D. (1984): *Ecology of VA mycorrhizal fungi*. – In: Bagyaraj, D. J. (ed.): *VA-mycorrhiza*. CRC Press, Boca Raton, Fla., pp. 35–36.
- Hirrel, M. C. (1981): The effect of sodium and chloride salts on the germination of *Gigaspora margarita*. – *Mycologia* **73**: 610–617.
- Hirrel, M. C. and Gerdemann, J. W. (1980): Improved growth of onion and bell pepper in saline soils by two vesicular-arbuscular mycorrhizal fungi. – *Soil Sci. Soc. Amer. J.* **44**: 654–655.
- Ho, I. (1987): Vesicular-arbuscular mycorrhizae of halophytic grasses in the Alvord desert of Oregon. – *Northwest Sci.* **61**: 148–151.
- Ishac, Y. Z., El-Haddad, M. E., Daft, M. J., Ramadan, E. M. and El-Demardeshi, M. E. (1986): Effect of seed inoculation, mycorrhizal infection and organic amendment on wheat growth. – *Plant Soil* **90**: 373.
- Jackson, M. L. (1960): *Soil chemical analysis*. – Prentice Hall, Inc. Ingle-Wood, 498 pp.
- Jackson, M. L. (1962): *Soil chemical analysis*. – Prentice Hall of India, New Delhi.
- Jasper, D. A., Abott, L. K. and Robson, A. D. (1991): The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soils from different vegetation types. – *New Phytol.* **118**: 471–476.
- Johnson-Green, P. C., Kenkel, N. C. and Booth, T. (1995): The distribution and phenology of arbuscular mycorrhizae along an inland salinity gradient. – *Can. J. Bot.* **73**: 1318–1327.
- Johnson, N. C., Tilman, D. and Wedin, D. (1992): Plant and soil controls on mycorrhizal fungal communities. – *Ecology* **73**: 2034–2042.
- Juniper, S. and Abbott, L. (1993): Vesicular-arbuscular mycorrhizas and soil salinity. – *Mycorrhiza* **4**: 45–57.
- Khaliel, A. S. (1989): Mycorrhizal status of some desert plants and correlation with edaphic factors. – *Trans. Mycol. Soc. Japan* **30**: 231–237.

- Khan, A. G. (1974): The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes and of Endogone spores in adjacent soils. – *J. Gen. Microbiol.* **81**: 7–14.
- Kim, C. and Weber, D. J. (1985): Distribution of VA mycorrhiza on halophytes on inland salt plains. – *Plant Soil* **83**: 207.
- Kormanik, P. P. and McGraw, A. C. (1982): *Quantification of vesicular-arbuscular mycorrhizae in plant roots*. – In: Schenck, N. C. (ed.): *Methods and principles of mycorrhizal research*. American Phytopathological Society, St. Paul, MN, pp. 37–45.
- Koske, R. E. and Tiessier, B. (1983): A convenient, permanent slide mounting medium. *Mycol. Soc. Amer. Newslett.* **34**: 59–63.
- Levy, Y., Dodd, J. and Krikun, J. (1983): Effect of irrigation, water salinity and rootstock on the vertical distribution of vesicular-arbuscular mycorrhiza in citrus roots. – *New Phytol.* **95**: 397–403.
- Mankarios, A. T. and Abdel-Fattah, G. M. (1994): Ecology of VA-mycorrhiza in some Egyptian soils. – *Egypt. J. Bot.* **34**: 135–152.
- Mankarios, A. T., Gianinazzi, S. and Abdel-Fattah, G. M. (1995): Improved growth and nutrition of soybean (*Glycine max* L.) in a saline soil associated with colonization by vesicular-arbuscular mycorrhizal fungi. – *Egypt. J. Bot.* **35**: 101–117.
- Mckenney, M. C. and Lindsey, D. L. (1987): Improved method for quantifying endomycorrhizal fungi spores from soil. – *Mycologia* **79**: 779–782.
- McGonigle, T. P. and Miller, M. H. (2000): The inconsistent effect of soil disturbance on colonization of roots by arbuscular mycorrhizal fungi: a test of the inoculum density hypothesis. – *Applied Soil Ecology* **14**: 147–155.
- Miller, R. L. and Jackson, L. E. (1998): Survey of vesicular-arbuscular mycorrhizae in lettuce production in relation to management and soil factors. – *J. Agric. Sci.* **130**: 173–182.
- Mohan Kumar, V. and Mahadevan, A. (1986): Survey of vesicular-arbuscular mycorrhizae in mangrove vegetation. – *Curr. Sci.* **55**: 936.
- Morton, J. B., Bentivenga S. P. and Wheeler, W. W. (1993): Germ plasm in the international collection of arbuscular and vesicular-arbuscular mycorrhizal fungi (INVAM) and procedures for culture development, documentation and storage. – *Mycotaxon* **48**: 491–528.
- Pfeiffer, C. M. and Bloss, H. E. (1988): Growth and nutrition of guayule (*Parthenium argentatum*) in saline soil as influenced by vesicular-arbuscular mycorrhiza and phosphorus fertilization. – *New Phytol.* **108**: 315–321.
- Phillips, J. M. and Hayman, D. S. (1970): Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. – *Trans. Br. Mycol. Soc.* **55**: 158–161.
- Pond, E. C., Menge, J. A. and Jarrell, W. M. (1984): Improved growth of tomato in salinized soil by vesicular-arbuscular mycorrhizal fungi collected from saline soils. – *Mycologia* **76**: 74–84.
- Poss, J. A., Pond, E. C., Menge, J. A. and Jarrell, W. M. (1985): Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. – *Plant Soil* **88**: 307–319.
- Rinaldelli, E. and Mancuso, S. (1998): Short-term and long-term response of mycorrhizal and non-mycorrhizal olive plants (*Olea europaea* L.) to saline conditions. – *Olivae* **74**: 45–49.
- Schenck, N. C. and Perez, Y. (1990): *Manual for the identification of VA mycorrhizal fungi*. (3rd edition). – Synergistic Publications, Gainesville, Florida, USA.

- Schwab, S. M., Johnson, E. L. V. and Menge, J. A. (1982): Influence of simazina on formation of vesicular-arbuscular mycorrhizae in *Chenopodium quinoa*. – *Plant Soil* **64**: 283–288.
- Sengupta, A. and Chaudhuri, S. (1990): Vesicular-arbuscular mycorrhiza (VAM) in pioneer salt marsh plants of the Ganges river delta in West Bengal (India). – *Plant Soil* **122**: 111–113.
- Sheikh, N. A., Saif, S. R. and Khan, A. G. (1975): Ecology of Endogone. II. Relationships of Endogone spore population with chemical soil factors. – *Islamabad J. Sci.* **2**: 6–9.
- Singh, C. S. and Tiagi, S. P. (1989): Study on the occurrence of vesicular-arbuscular mycorrhizal (VAM) fungi in the root of *Aeschynomene indica* under the influence of various ecological factors. – *Z. f. Mikrobiol.* **144**: 241–248.
- Smith, V. R. and Newton, I. P. (1986): Vesicular-arbuscular mycorrhizas at a subarctic island. – *Soil Biol. Biochem.* **18**: 547–549.
- Sparling, G. P. and Tinker, P. B. (1975): *Mycorrhizas in pennine grassland*. – In: Sanders, F. E., Mosse, B. and Tinker, P. B. (eds): *Endomycorrhizas*. Academic Press, London, pp. 546–560.
- Trouvelot, A., Kough, J. L. and Gianinazzi-Pearson, V. (1986): Mesure du taux de mycorrhization VA d'un système racinaire recherche de méthodes d'estimation ayant une signification fonctionnelle. – In: Gianinazzi-Pearson, V. and Gianinazzi, S. (eds): *Physiological and genetical aspects of mycorrhizae*. INRA Publications, Paris, pp. 217–221.
- Walker, C., Mize, C. W. and McNabb, H. S. (1982): Populations of edogonaceous fungi at two locations in Iowa. – *Can. J. Bot.* **60**: 2518–2529.