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Arbuscular-mycorrhizal fungi (Glomales) in Egypt. III: Distribution and ecology in some plants in El-Omayed Biosphere Reserve

Hamdy E. Agwa* and Yassin M. Al-Sodany

Biological and Geological Sciences Department, Faculty of Education, Kafr El-Sheikh, Tanta University, Egypt. Emails: dragwa2002@yahoo.com or yalsodany@yahoo.com

ABSTRACT

Roots and rhizospheric soils of 26 plant species belonging to 18 families representing five different habitats at El-Omayed Biosphere Reserve were collected and examined for arbuscular-mycorrhizal fungal (AMF) associations. Plant species recorded in the habitat of coastal sand dunes had the highest percentage of infection and spore number, followed by those of non-saline depressions, while those of saline depressions showed the lowest percentage of infection and spore numbers. There was a significant negative correlation between the percentage of infection and both salinity and phosphorus concentration in the study area. Cluster analysis in correlation matrices indicated that spore numbers, $CaCO_3$, Fe, percentage of infection and K were closely correlated.

KEYWORDS: Arbuscular mycorrhiza, habitats, sand dunes, salt marshes, *Glomus*

INTRODUCTION

Plant roots provide an ecological niche for many microorganisms that are limited to soil. In natural ecosystems much of the root system can be colonized by mycorrhizal fungi. Colonization is restricted to the root cortex and does not enter the vascular cylinder. Arbuscular-mycorrhizal fungi (AMF) occupy the roots of the majority of plants in natural ecosystems throughout the world (Mukerji & Sharma 1996). The nature and abundance of propagules of these fungi determine their resistance during periods of inactivity, response to disturbance, resistance to predation by other soil organisms, and their capacity for dispersal to new locations (Brundrett & Abbott 1994). Propagules of AMF are thought to include spores, dead root fragments and other colonized materials as well as the network of hyphae in soil.

In semi-arid Mediterranean ecosystems, the scarce and irregular rainfall, long dry and hot summer, and man-mediated degradative activities may synergistically act as driving-forces for the promotion of the desertification process (Azcón-Aguilar *et al*. 2003). Degradation of natural plant communities, in terms of population structure, successional patterns or species diversity, is known to occur concommitantly with the degradation of physio-chemical and biological soil properties (Requena *et al.* 2001). Variation in the populations of the fungi and their symbiosis with plant roots is related to both soil properties and host plants (Hayman 1982). In addition, species and isolates of AMF differ in their tolerance to adverse physical and chemical conditions in soil (Juniper & Abbott 1993). Destruction of forests and damage by grazing livestock in the subsequent century would permit the formerly stabilized dunes to become mobile again and lead to the problem of sand drifting (McCaffrey & Leatherman 1979).

The distribution of AMF in different ecological regions and their relations to soil properties and native plants have been investigated by several researchers. In Egypt, knowledge of AMF spores in Biosphere Reserves is limited. This study is carried out as a

* Address for Correspondence

part of an ecological survey assessing AMF associated with common plants in the protectorate areas in Egypt. No previous assessment has been made of El-Omayed Biosphere Reserve.

MATERIALS AND METHODS

The El-Omayed Biosphere Reserve is located on the western Mediterranean coastal region of Egypt, 80 km west of Alexandria. Its landscape is differentiated into a northern coastal plain and a southern inland plateau. The coastal plain is characterized by alternating ridges and depressions running parallel to the coast in the E-W direction. This physiographic variation leads to the recognition of five types of habitats. These habitats are more or less arranged in the same sequence from north to south as follows: a coastal ridge composed mainly of snow-white oolitic calcareous rocks, and overlain by dunes; saline depressions with brackish water and saline calcareous deposits (i.e. salt marshes); non-saline depressions with a mixture of calcareous and siliceous deposits of deep loess; inland ridges formed of limestone with a hard crystallized crust, and less calcareous than the coastal ridge; inland plateau that characterized by an extensive flat rocky surface and shallow soil; and inland siliceous deposits, sporadically distributed on the inland plateau and occasionally forming dunes, specially in more inland sites.

The climate of this region belongs to the warm coastal desert climate (Meigs 1973): the warmest summer month (August) has a mean temperature less than 30 °C, and the coldest winter month (January) has a mean temperature above 10 °C. Occasional short rainstorms occur mainly in winter. The ratio of annual precipitation to annual evaporation is between 0.03 and 0.2. A comparison of metereological records of two stations, one near the Mediterranean coast at Burg El-Arab, and the other about 40 km to the south at Dammanhur, demonstrates the N-S climatic gradient in this region. These records indicate an increase in the environmental aridity and thermal continentality from north to south.

Samples were collected from the rhizospheres of different plants during spring 2003 after the maturity of plants. Fine roots in each sample were removed, rinsed with tap water and fixed (in formalin, acetic acid, alcohol) for the determination of root infection. Soil samples were then air-dried in the shade at laboratory temperature for spore counting.

Spores were extracted from 50 gm soil in triplicate for each sample by wet-sieving followed by floating-centrifugation in 50% sucrose (Dalpe 1993). The spores were collected on grid-pattern filter paper and counted using a dissecting microscope at x30 magnification.

For character observation and identification, spores were mounted on glass slides in PVLG and PVLG + Melzer's reagent (Schenck & Perez 1988). The number of spores was expressed as the mean of three replicates. Roots were rinsed with distilled water, cleared by 10% KOH for 15 minutes at 90 ºC, bleached in alkaline hydrogen peroxide for 20 minutes, acidified in 1% HCl, stained using trypan blue (Phillips & Hayman 1970) and quantified for AMF infection according to Giovannetti & Mosse (1980).

Three soil samples were collected from profiles (0-50 cm) of each sampled stand for estimating their physical and chemical characteristics. Total organic matter was determined by weight-loss-on-ignition at 450°C, and total $CaCO_3$ was estimated by Collin's calcimeter. Soil-water extracts (1:5) were prepared for the estimation of electrical conductivity (EC in mS/cm) using an electric conductivity meter, soil reaction (pH) using a pH-meter. and chlorides by direct titration against silver nitrate using 5% potassium chromate as indicator. Soil extracts of 5-gm soil samples were prepared using 2.5% v/v glacial acetic acid for estimating P, K, Na, Mn and Fe. Potassium, and Na were estimated

by flame photometry, and Mn and Fe by atomic absorption spectrophotometry. Molybdenum blue and indophenol methods were used for estimation of P and N, respectively, using calorimetric spectrophotometer. All these procedures followed Allen *et al*. (1974).

Correlation analysis was performed to evaluate the relationships between different soil properties, the number of spores and the percentage root infection. The variation in soil variables in relation to habitats was assessed using one-way analysis of variance (ANOVA). To assess the complex multivariate relationships among the variables, agglomerative hierarchical cluster analysis was performed on the correlation matrices and the results expressed as a dendrogram (Romesburg 1984).

RESULTS

The total number of sampled species was 26 species belonging to 18 families distributed in five habitats (Table 1). The family Compositae and Chenopodiaceae showed the highest number of species (4 species each). *Adonis dentata* which inhabits coastal sand dunes had the highest percentage of infection, followed by *Lygum spartum*: *Limoniastrum monopetalum* and *Suaeda vera* along saline depressions and *Salsola kali* along non-saline depressions had no infection. Similarly, while the soil of *Ammophila arenaria* along the coastal sand dunes had the highest total number of spores, that of *Suaeda vera* along the saline depressions showed the lowest number.

The most dominant genera of AMF in the rhizosphere soils of the study area were *Glomus* followed by *Gigaspora* and *Scutellospora* but *Acaulospora* and *Entrophospora* were scanty or absent.

The soil of coastal sand dunes had the highest percentage of infection and number of spores, while that of saline depressions had no infection and the lowest number of spores (Table 2). Regarding the soil variables, the soil of coastal sand dunes had the highest values of $CaCO_3$ and potassium, and the lowest values of pH, organic matter, phosphorus, nitrogen and iron. Soil of saline depressions had the highest values of salinity and phosphorus, while that of inland ridges had the highest values of pH, nitrogen and iron. The soil of the inland plateau had the the highest organic matter. Soils of non-saline depressions had the lowest values of salinity, $CaCO_3$ and potassium.

Table 1: Means of (a) percentage of infection and (b) spore number in soil distributed in different habitats in El-Omayed Biosphere Reserve. The habitats are: CD: coastal sand dunes, SD: saline depressions, ND: non-saline depressions, IR: inland ridges and IP: inland plateau .

(a)

Species	Family	Habitats					Total
		CD	SD	ND	IR	IP	mean
		Percentage of infection					
Adonis dentate L.	Ranunculaceae	71.0					71.0
Ammophila arenaria (L.) Link	Graminaea	35.3					35.3
Anabasis articulata (Frossk.) Moq.	Chenopodiaceae			4.0	12.0	0.0	5.3
Artemisia herba-alba Asso	Compositae					68.7	68.7
Artemisia monosperma Delile	Compositae			55.7			55.7
Asphodelus ramosus L.	Liliaceae			0.0		3.3	1.7
Citrullus colocynthis (L.) Schrad.	Cucurbitaceae				3.3		3.3
Crucianella maritima	Rubiaceae	20.7					20.7
Deverra tortuosa (Desf.) DC.	Umbelliferae	14.7			7.0		10.9
Echinops hussonii Boiss.	Compositae	37.3					37.3
Echinops spinosissimus Turra	Compositae	69.3		38.0			53.7
Helianthemum lippii (L.) Dum. Cours.	Cistaceae	9.0	0.0				9.0
Limoniastrum monopetalum (L)Bioss	Plumbaginaceae						0.0
Lycium europaeum L.	Solanaceae	6.0					6.0

Lygum spartum Loefl. ex L.	Gramineae			69.7			69.7
Muscaria racemosum (L.) Mill.	Liliaceae	55.7					55.7
Noaea mucronata (Forssk.) Asch. & Schweinf.	Chenopodiaceae			11.7			11.7
Ononis vaginalis Vahl.	Leguminosae	23.3					23.3
Phlomis floccosa D. Don	Labiatae				37.0		37.0
Plantago albicans L.	Plantaginaceae					43.7	43.7
Retama raetum (Forssk) Webb & Berthel	Leguminosae	45.3					45.3
Salsola kali L.	Chenopodiaceae			0.0			0.0
Salvia lanigra Poir.	Labiatae	60.7	0.0				60.7
Suaeda vera Forssk. ex J.F. Gmel.	Chenopodiaceae						0.0
Thymelaea hirsuta (L.) Endl.	Thymelaeaceae	4.7	0.0	14.7		5.0	8.1
Zygophyllum album L.	Zygophyllaceae	4.7					2.4

(b)

		Spore number					
Adonis dentate L.	Ranunculaceae	131.0					131.0
Ammophila arenaria (L.) Link	Graminaea	160.7					160.7
Anabasis articulata (Frossk.) Moq.	Chenopodiaceae			126.0	129.3	88.7	114.7
Artemisia herba-alba Asso	Compositae					128.3	128.3
Artemisia monosperma Delile	Compositae			140.7			140.7
Asphodelus ramosus L.	Liliaceae			116.0		91.0	103.5
Citrullus colocynthis (L.) Schrad.	Cucurbitaceae				126.3		126.3
Crucianella maritima	Rubiaceae	132.7					132.7
Deverra tortuosa (Desf.) DC.	Umbelliferae	110.0			115.3		112.7
Echinops hussonii Boiss.	Compositae	158.7					158.7
Echinops spinosissimus Turra	Compositae	149.0		114.7			131.9
Helianthemum lippii (L.) Dum. Cours.	Cistaceae	97.0					97.0
Limoniastrum monopetalum (L)Bioss	Plumbaginaceae		104.3				104.3
Lycium europaeum L.	Solanaceae	136.7					136.7
Lygum spartum Loefl. ex L.	Gramineae			119.0			119.0
Muscaria racemosum (L.) Mill.	Liliaceae	135.7					135.7
Noaea mucronata (Forssk)Asch & Sch.	Chenopodiaceae			97.0			97.0
Ononis vaginalis Vahl.	Leguminosae	128.7					128.7
Phlomis floccosa D. Don	Labiatae				129.3		129.3
Plantago albicans L.	Plantaginaceae					105.0	105.0
Retama raetum (Forssk) Webb & Berthel	Leguminosae	142.3					142.3
Salsola kali L.	Chenopodiaceae			109.3			109.3
Salvia lanigra Poir.	Labiatae	131.3					131.3
Suaeda vera Forssk. ex J.F. Gmel.	Chenopodiaceae		85.3				85.3
Thymelaea hirsuta (L.) Endl.	Thymelaeaceae	88.3		106.3		109.3	101.3
Zygophyllum album L.	Zygophyllaceae	121.7	106.7				114.2

Table 2: Means of percentage of infection, spore number of arbuscular mycorrhizal fungi and some soil characters collected from different habitats of El-Omayed Biosphere Reserve. The habitats are: CD: coastal sand dunes, SD: saline depressions, ND: non-saline depressions, IR: inland ridges and IP: inland plateau. F-values are indicated. *: $P \leq 0.05$, **: $P \leq 0.01$ and ***: $P \leq 0.001$ according to Anova (all with 4,10 df).

Variable	CD	SD	ND	IR	IP	Mean	F-value
Percentage of infection	32.7	0.0	24.2	14.8	24.1	24.5	4.01**
Spore number	130.3	98.8	116.1	125.1	104.5	119.8	5.06***
pH	8.3	8.8	9.4	9.6	9.6	6.4	0.21
EC (µmhos/cm)	120	2600	76.5	260.0	140.0	864.1	21.7**
O.M. %	1.5	15.1	4.7	10.6	12.3	9.2	2.19
CaCO3	98.4	38.5	19.4	21.8	22.4	21.2	0.43
P	40.0	1076	218.9	282.2	92.0	352.7	0.08
N mg/100g	31.0	320.1	347.7	468.3	184.6	346.6	2.01
K	40.0	17.5	3.8	5.2	10.0	7.6	3.08
Fe	11.0	36.3	20.2	47.8	17.3	24.3	200.8***

As shown in Table 3, the percentage of infection had a significant negative correlation with both salinity and phosphorus. On the other hand, pH had a significant negative correlation with both $CaCO_3$ and potassium; salinity had a significant positive correlation with phoshorus; and $CaCO_3$ had positive correlation with potassium.

Table 3: Correlation coefficients between percentage of infection, spore number and some soil variables in El-Omayed Biosphere Reserve. %: percentage of infection, No.: number of spores.

	pH	EC	OM	$CaCO_3$	P	N	K	Fe	%	No.
pH	---	-0.310	0.418	-0.918*	-0.176	0.093	-0.931*	0.393	-0.115	-0.237
EC		---	0.652	-0.041	0.979**	0.003	0.068	0.411	-0.883*	-0.665
OM			---	-0.595	0.673	-0.109	-0.471	0.611	-0.829	-0.794
$CaCO_3$				---	-0.190	-0.109	0.985**	-0.502	0.425	0.503
P					---	0.144	-0.100	-0.536	-0.941*	-0.651
N						---	-0.224	0.716	-0.261	0.518
K							---	-0.508	0.342	0.359
Fe								---	-0.754	-0.113
%									---	0.644
No.										---

Cluster analysis of the correlation matrix indicated that the spore numbers, $CaCO_3$, Fe, percentage of infection and K were closely correlated, while other soil variables such as salinity, phosphorus and pH vary independently to both percentage of root infection and spore number (Figure 1).

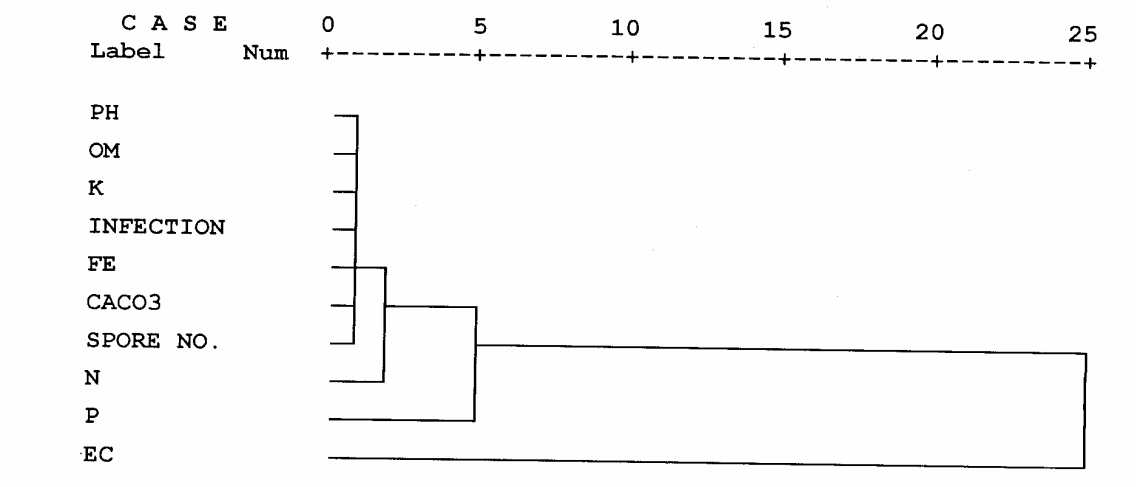

Figure1: Dendrogram based on the Ward method to represent cluster analysis of the correlation matrix of percentage of infection, spore number and soil variables.

DISCUSSION

This study shows that arbuscular mycorrhizas are present in the El-Omayed Biosphere Reserve and that colonization and AMF spores occur in different habitats. However, not all plant species were colonized and species belonging to Ranunculaceae, Gramineae, Compositae, Labiatae and Liliaceae display high levels of colonization. The presence of very high levels of colonization in these families has previously been reported (Agwa 1990; Agwa & Abdel-Fattah 2002).

The absence of AMF infection in *Limoniastrum monopetalum* in this investigation was also noted by Agwa (1990). Although *Suaeda vera* and *Salsola kali* (Chenopodiaceae) showed no infection in the present study, other investigations showed that some plant species of this family, which is usually referred to as non-mycorrhizal (Harley & Smith 1983), show mycorrhizal infection (Hirrel *et al*. 1978; Agwa 1990; Hildebrandt *et al*. 2001; Agwa & Abdel-Fattah 2002) or become mycorrhizal when salt stressed (Khan 1974; Hirrel 1981; Katembe *et al*. 1998). Other results show that the salt tolerance of some plants increases under saline conditions when they are mycorrhizal with certain AMF (Jindal *et al*. 1993; Aboul-Khair & El-Sokkary 1994).

The number of AMF spores in rhizosphere soils differed among plant species of the same habitat. The number of spores in *Ammophila arenaria* rhizosphere soil was about twice that of *Thymelaea hirsuta* in coastal sand dunes. This suggests that AMF distribution does not coincide with the zonation pattern of vegetation. These differences may be related to the different behaviour of each AMF species, even in similar ecosystem (Kilronomos *et al*. 1993). A good number of spores was recorded in the rhizosphere soil of non-colonized plant species in saline depression habitat. Tressner & Hayes (1971) and Hirrel (1981) suggested that AMF sporulation is stimulated under salt stress conditions, but McMillen *et al.* (1998) reported that spore germination and hyphal growth of AMF were inhibited in severely saline soils. This may again cause accumulation of spores in saline soils. The results also revealed that the genus *Glomus* was found to be dominant. This dominance might be due to the earlier sporulation of *Glomus* than *Gigaspora* and *Scutellospora*. Moreover, *Acaulospora* colonizes in acidic pH (Green *et al*. 1976). Further study is needed through a series of trap and pure cultures to allow non-sporulated AMF species to appear and be identified (Morton J.B.,West Virginia University, personal communication).

In the present study, the coastal sand dunes had the highest number of spores and percentage of root infection, which gives this habitat an important value because the presence of mycorrhizae lead to stabilization of these sand dunes (Koske & Gemma 1997). Moreover, this habitat in the study area is characterized by a high diversity of plant species: it has 127 plant species, of which 35 are unique to this habitat in the Egyptian Mediterranean region (Shaltout & Al-Sodany 2002).

The present study indicated that the percentage of root infection had a significant negative correlation with phosphorus. This result agrees with that of Hetrick *et al* (1994) who showed that mycorrhizal dependence decreases while root fibrousness increases as phosphorus level increases.

The ability of spores to germinate and hyphae to grow from those spores is affected by pH (Porter *et al*. 1987), and increasing soil pH beyond 5 decreases the germination of spores (Hepper 1984). The present study indicated that the percentage of root infection had a significant negative correlation with pH. This result agrees with that of Hepper's study who indicated that the differences between soils in the germination of spores of mycorrhiza apperead to be negatively correlated with the differences in soil pH. It shows that no conclusions can be drawn about the mechanism by which raising the soil pH restricts spore germination or hyphal growth. However, the effects of limiting pH soil in decreasing the

germination of spores and hyphal growth may also be related to effects on hydrogen ion concentration, although this requires further research.

The results of this study raise questions important to our understanding of the role of mycorrhizas in the ecology of Bioreserves and protectorate areas.

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الملخص العربى

الفطريات الجذرية الشجيرية فى مصر
3- دراسة بيئية على بعض النباتات فى محمية العميد الطبيعية

حمدى الرفاعى عجوة و يس محمد السودانى

قسم العلوم البيولوجية والجيولوجية – كلية التربية بكفر الشيخ – جامعة طنطا – مصر

فى هذا البحث تم دراسة ارتباط جذور 26 نوع من النباتات مع الفطريات الجذرية الشجيرية فى كل من التربة الملاصقة للجذور وداخل الجذور ذاتها. وكانت هذه النباتات تنتمى الى 18 فصيلة نباتية مختلفة وتمثل 5 مواطن بيئية فى محمية العميد الطبيعية.

وقد أثبتت الدراسة أن الأنواع النباتية فى الكثبان الساحلية الرملية كانت تحتوى فى جذورها على أعلى اصابة بهذه الفطريات، واحتوت أيضاً فى التربة الملاصقة لجذورها على أعلى عدد من جراثيم هذه الفطريات، وقد تلاها فى هذا الصدد الأنواع النباتية فى موطن المنخفضات غير الملحية، بينما أظهرت الأنواع النباتية فى موطن المنخفضات الملحية أقل نسبة من الإصابة وأقل عدد من جراثيم هذه الفطريات.

وقد أظهرت الدراسة أن هناك علاقة ارتباطية دالة سالبة بين نسبة الإصابة ودرجة الملوحة وكمية الفوسفور فى التربة قيد البحث. وقد أظهرت نتائج التحليل التقابلى على جداول الإرتباط أن العلاقة الإرتباطية بين كل من أعداد الجراثيم فى التربة وكمية كربونات الكالسيوم والحديد فى التربة ونسبة الإصابة فى الجذور وأيضاً كمية البوتاسيوم فى التربة كانت متقاربة.