Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2196

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **4 (1):** 35-44 (2016)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Mycorrhizal status of *Eryngium maritimum* in the mobile dunes of Mehdia (Northwest of Morocco)

Nawal HIBILIK, Karima SELMAOUI, Jihane TOUATI, Mohamed CHLIYEH, Amina OUAZZANI TOUHAMI, Rachid BENKIRANE and Allal DOUIRA*

Laboratoire de Botanique, Biotechnologie et de Protection des Plantes, Université Ibn Tofaïl

Faculté des Sciences, B.P. 133, Kénitra, Maroc (Morocco)

*Corresponding Author E-mail: douiraallal@hotmail.com

Received: 20.01.2016 | Revised: 28.01.2016 | Accepted: 08.02.2016

ABSTRACT

The objective of this work was to study the mycorrhizal status of Eryngium maritimum in the mobile dunes of Mehdia coastal area depending on the depth level reached by the roots. The obtained results showed that all the roots were mycorrhizal, different fungal structures were observed (arbuscules, vesicles, hyphae, spores and endophytic). However, the identified mycorrhizal parameters varied from the surface to the depth reached by the roots. The mycorrhizal frequency reached 88% by the depth. The highest mycorrhizal intensity (26.4%) was recorded at a depth of 53.05 to 73.05 cm. The highest arbuscular contents (22.72%) and vesicular content (11.80%) were recorded respectively at depths of 53.05 to 73.05 cm and 33.05 to 53.05 cm. The spores density in the rhizosphere of E. maritimum varied between 10 to 20 spores / 100 g of soil. Similarly, 19 spore's morphotypes were isolated from five different genera: Gigaspora, Acaulospora, Entrophospora, Scutellospora and Glomus.

Among these morphotypes, ten species of endomycorrhizal fungi were determined: Glomus badium, Glomus aggregatum, Glomus multiforum, Gigaspora sp., Glomus invermaium, Glomus geosporum, Glomus glomerulatum, Glomus albidum, Glomus enticatum, *Scutellospora verrucosa, Scutellospora calospora, Scutellospora heterogama, Acaulospora colaussica, Entrophospora infrequens. Mycorrhization of E.maritimum roots in depth, with development of exchange structures (arbuscular) probably facilitated the installation of this species, which grows well in the mobile dunes.*

Keywords: *Morocco, Mehdia, mobile dunes, Eryngium maritimum, roots, depth, mycorrhizal status.*

INTRODUCTION

The *Eryngium* genus, Umbelliferae family, includes more than 220 species distributed in temperate and warm areas of both hemispheres, with the exception of southern and tropical Africa[11]. According to the characters of plants and pollens, Cerceau-Larrival[12] defined within that genus three series: the American series, the Eurasian series, including *E. maritimum*, and the intermediate series.

Cite this article: Hibilik, N., Selmaoui, K., Touati, J., Chliyeh, M.,Touhami, A.O., Benkirane, R. and Douira, A., Mycorrhizal status of *Eryngium maritimum* in the mobile dune of Mehdia (Northwest of Morocco), *Int. J. Pure App. Biosci.* **4(1):** 35-44 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2196

In Morocco, *E. maritimum* was cited at the embryonic dunes of Morocco[3,5,28]. The dunes are characterized by mobility of the soil, wind and drought in the harsh environment characterized by sandy and moving substrate, lack of soil in the pedological meaning, a salted humidification and strafing by sand grains[44]. To adapt to these conditions, some plants grow superficial fibrous root system to collect rainwater, but others, develop deep roots to absorb water, case of *E. maritimum*. This species also presents a major environmental interest as it contributes to the maintenance of sand dunes.

The role of microflora in the dune dynamics is known[26,27]. Endomycorrhizal fungi form symbiotic associations with many plant species growing on maritime soils[21,33,42] but their behavior is a function of the dune system. Distribution, for example, of endomycorrhizae in the sand dunes is depending on the plant species, the dunes stability degree, the amount of organic matter and microbiological activity[43].

The aim of this investigation was to determine the mycorrhizal status of *Eryngium maritimum*, plant species developing in the mobile dunes. Indeed, little informations are known about mycorrhizae of this species. Anderson [2] has studied only seasonal variations in the mycorrhizal intensity and frequency of infection in roots of *E. maritimum*. In this study, the considered parameters, depending on the depth reached by the roots, are the mycorrhizal frequency and intensity, the arbuscular and vesicular content, and diversity of spores.

MATERIALS AND METHODS

1. Site selection

The studied area, coastal dunes of Mehdia is located at 35 km from Rabat and 13 km from Kenitra. This part of the Atlantic coast also includes in Sidi Boughaba reserve a lake of 6 km a long and 100-350 meters a wide, it presents a depression which separates two sand dunes, mobile in the west and consolidated in the east. The sand of mobile dunes is directly attached to the beach of Mehdia.

In this study, soil samples were collected from mobile dunes of the littoral zone of Mehdia.

2. Root Mycorrhization

Two plants of *E.maritimum*, were randomly selected from mobile dunes of Mehdia coastal zone. The length of the roots of each plant was measured starting from the onset of the finest roots. The size of the root indicates its depth at the sand.

After the roots were cut from the top to the bottom into fragments of 20 cm. Each fragment refers to a given depth. Roots were first washed with water; the finest roots were then cut into a length of 1 to 2 cm then immersed in a solution of 10% KOH (Potassium hydroxide) and placed in the water bath at 90 °C for one hour to eliminate cytoplasmic contents. At the end of this period, roots were rinsed and transferred in a solution of H_2O_2 (Hydrogen peroxide) for 15 min at 90°C in the water bath until the roots became white. Roots were then rinsed, after this; they were dyed with Cresyl blue at 90°C for 15 min.

The evaluation of mycorrhizal parameters was performed by observing thirty root fragments with 1 cm length, randomly selected to quantify mycorrhizae. These fragments were mounted, in groups of 10 to 15 segments, in glycerine between slide and cover slip[32]. The slides were examined under a microscope, each fragment being thoroughly checked over its entire length, at magnifications of 100 X and 400 X.

Vesicular and arbuscular frequencies and content of the endomycorrhizal fungi inside the roots were measured assigning a mycorrhization index ranging from 0 to 5 [15]:

0 : absent ; 1 : traces ; 2 : less than 10% ; 3 : from 11 to 50% ; 4 : from 51 to 90% ; 5 : more than 91%.

Mycorrhizal Frequency:

F% = 100 (N-No)/N

N: number of the observed fragments and No: number of non-mycorrhized fragments.

Mycorrhizal intensity:

(M %) = (95 n5 + 70 n4 + 30 n3 + 5 n2 + n1) / N

Where,

n = number of fragments assigned with the index 0, 1, 2, 3, 4 or 5.

Arbuscular content (A %)

A% = (100 mA3 + 50 mA2 + 10 mA1) / 100

Where,

MA3, MA2, MA1 are the percentages (%) respectively assigned to the notes A3, A2, A1, with, MA3 = (95n5 A3 + 70 n4 A3 + 30 n3 A3 + 5 n2 A3 + n1A3) / N.

The same for A1 and A2, n5A3 represents the number of fragments marked 5 with A3; n4A3 marked the number of fragments 4 with A3; etc...

A0: no arbuscules, A1: some arbuscules 10%, A2: moderately abundant arbuscular 50%, A3: very abundant arbuscular: 100%.

Vesicular content (V %)

(V %) = (100 mV3 + 50 mV2 + 10 mV1) / 100

Where,

MV3, MV2, MV1 are the percentages (%) respectively assigned notes V3, V2, V1, with V3; MV3 = (95V3n5 + 70n4V3 + 30 n3V3 + 5 n2 V3 + n1V3) / N.

The same for V1 and V2.n5V3 represents the number of fragments marked 5 with V3; n4V3 marked the number of fragments 4 with V3; V0: no vesicles; V1: some vesicles 10% V2: 50% moderately abundant vesicles; V3 abundant vesicles: 100%.

3. Spores extraction

The spores were extracted by the method of wet sieving[20]. In a beaker of 1L, 100g of each composite soil sample was submerged in 0.5 L of tap water and it was stirred with a spatula for 1 minute.

After 10 to 30 seconds of settling, the supernatant was passed through four superimposed sieves with decreasing meshes (500, 200, 80 and 50 Mm). This operation was repeated two times. The selected content by the screen 200, 80 and 50 microns was divided into two tubes and centrifuged for 4 min at 9000 RPM. The supernatant was discarded and a viscosity gradient was created by adding 20 mL of a of 40% sucrose in each centrifuge tube[59]. The mixture was quickly stirred and the tube was handed back into the centrifuge for 1 min at 9000 RPM. Unlike the first centrifugation process, the supernatant was poured into the sieve mesh of 50 microns; the substrate was rinsed with distilled water to remove the sucrose, and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with distilled water in an Erlenmeyer flask.

Estimation of spore's number in the soil was made by counting the spores contained in one mL of the supernatant and by extrapolation on the total volume (100 mL).If no spores are observed, all the supernatant is reduced to one ml and observed again.

The spore's characteristics (color, shape, size and number of separation membranes ...) are highlighted by mounting between slide and cover slip 0.1 mL of the supernatant.

A preliminary identification of the spores genera was performed based on the criteria proposed by several authors [6,14,18,25,38,39,47,48,59], and the available information in different databases.

RESULTS

Microscopic observations have shown that the roots of *E. maritimum* were colonized by endomycorrhizal fungi. Indeed, various endomycorrhizal structures were observed: arbuscular, vesicles often with varying shapes (oval, round, rectangular,...), external and internal hyphae and endophytes:

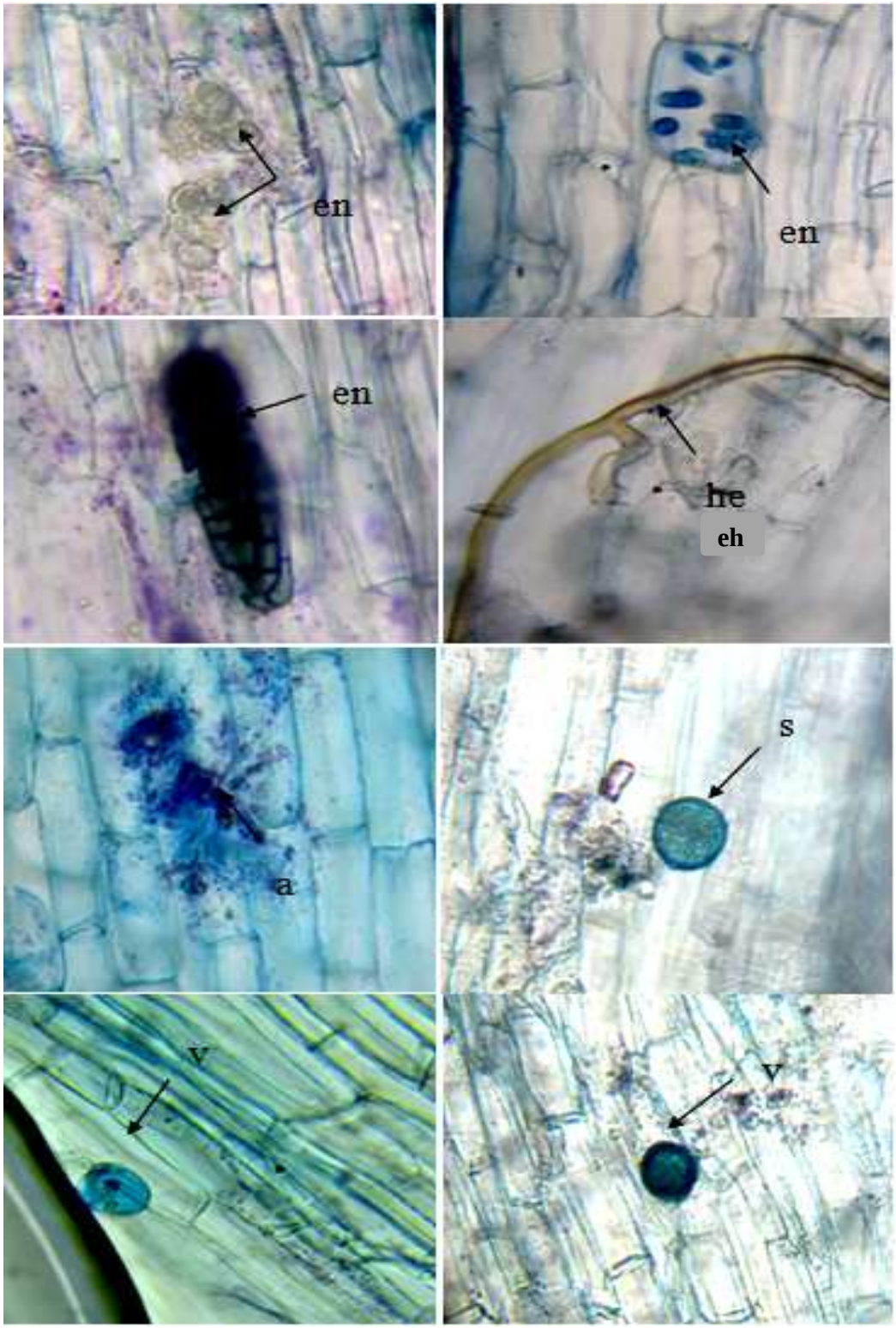

Fig. 1: Roots of *E.maritimum* presenting arbuscular mycorrhizal fungi (a); external hyphae (eh) and spores (s); vesicles (v) and entophytes (en). (M. × 400)

The roots mycorrhizal frequencies of *E. maritimum* identified based on depth levels reached by the roots are almost identical (88%) but decrease (about 77.49%) at 13.05 to 33.05 cm deep. These intensities reach 22.18% and 26.4% respectively at depths of 33.05 to 53.05 cm, and 53.05 to 73.05 cm

Roots arbuscular content of *E. maritimum* increases with the root's depths. It east of 6.94% to a depth of 13.05 to 33.05 cm and 22.72% to 53.05 at 73.05 cm in contrast, the vesicular content decreases in depth, until it reached 2.36%.

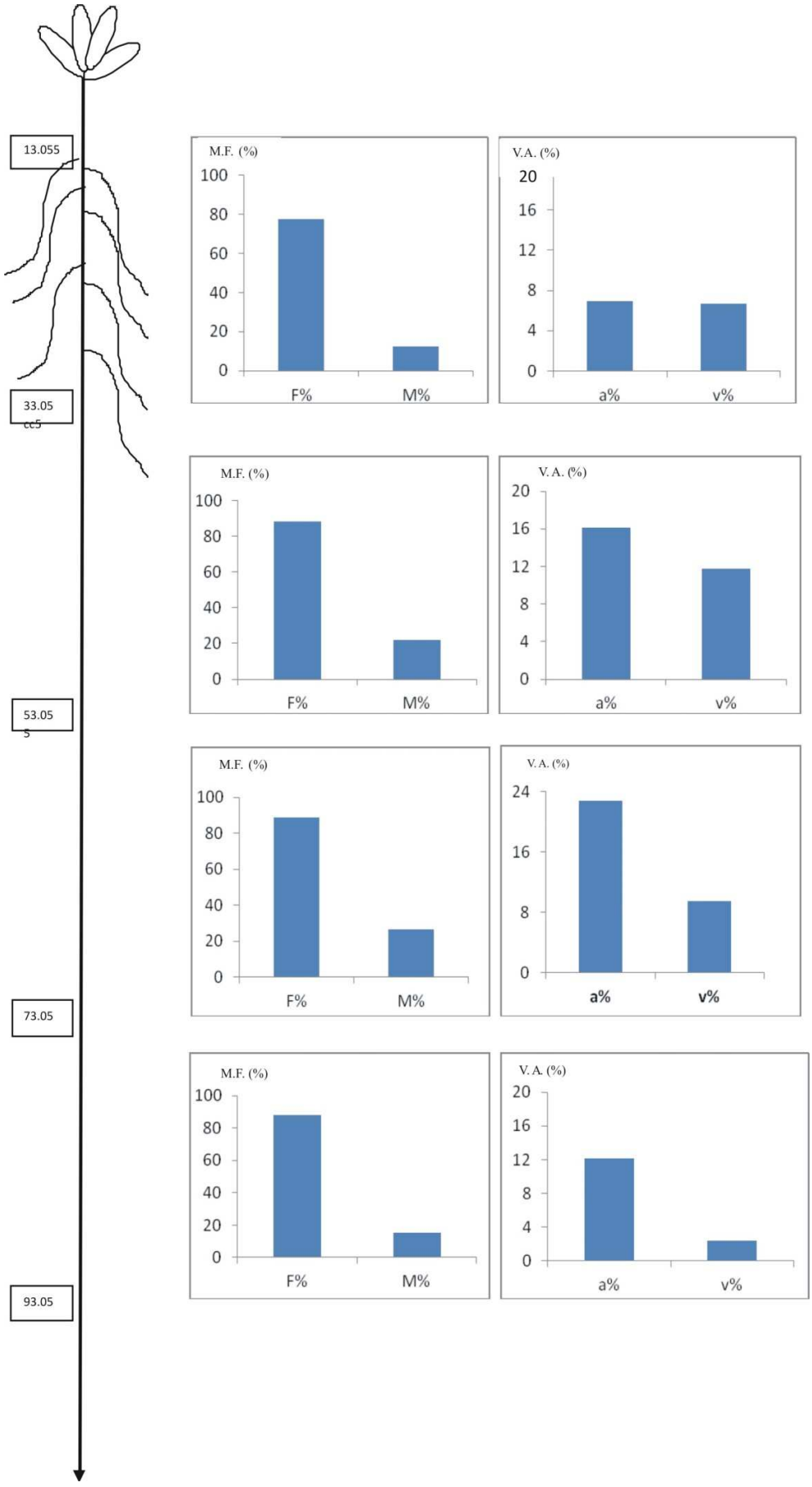

Fig. 2: Mycorrhizal parameters based on different depths reached by the plant roots. M: Mycorrhizal intensity; F: Mycorrhizal frequency; V: Vesicular content; A: Arbuscular content

Preliminary identifications showed that spores encountered in the rhizosphere of *E.maritimum* roots belong to five genera: *Gigaspora* (*Gigaspora* sp.), *Acaulospora* (*Acaulospora colaussica*), *Entrophospora* (*Entrophospora infrequens*), *Scutellospora* (*Scutellospora verrucosa, Scutellospora calospora, Scutellospora heterogama,*) and *Glomus* (*Glomus badium, Glomus aggregatum, Glomus multiforum, Glomus invermaium, Glomus geosporum, Glomus glomerulatum, Glomus albidum, Glomus enticatum,*).

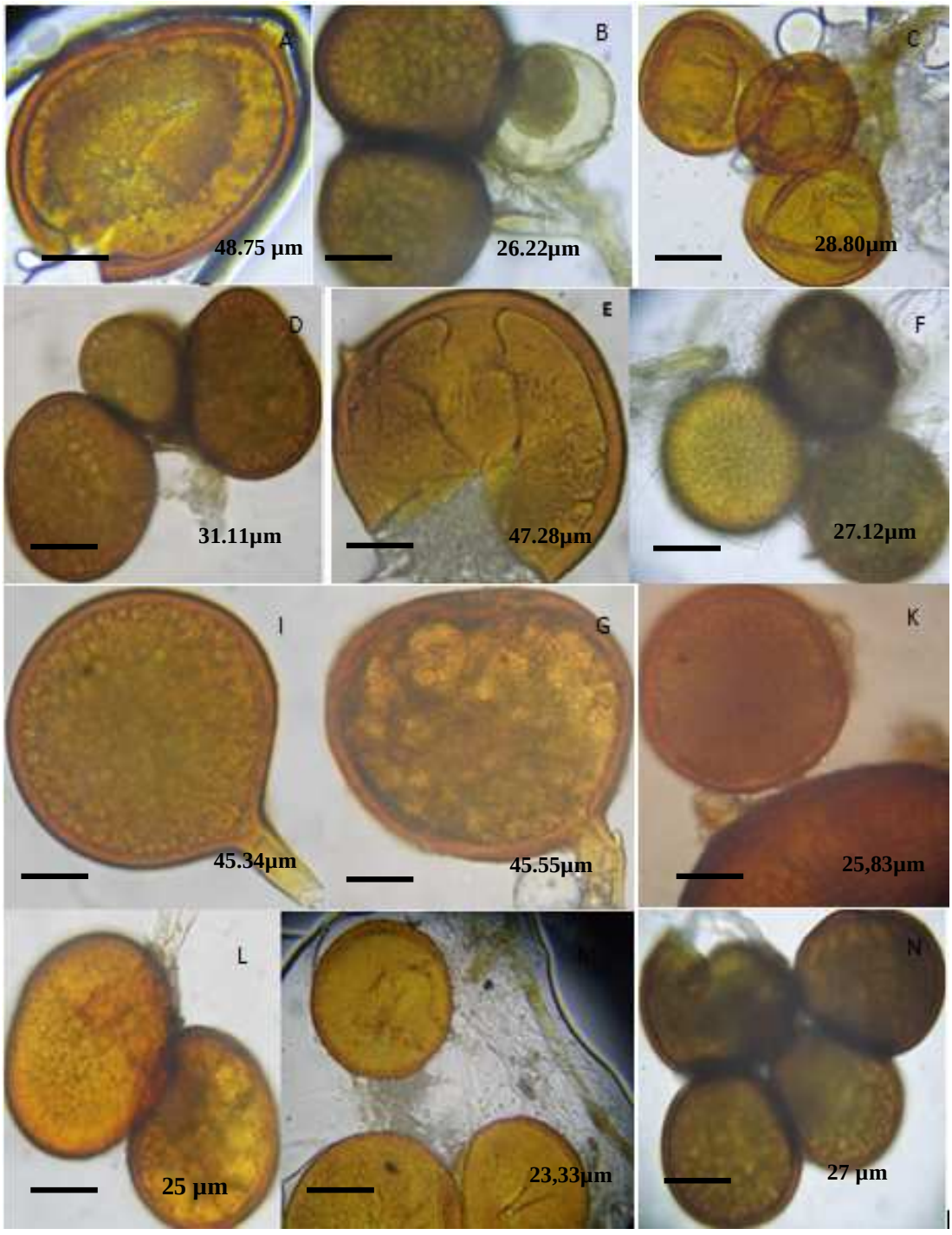

Fig. 3: Endomycorrhizal species isolated from the rhizosphere of *E.maritimum*: Spore of *Glomus badium* (A), *Scutellospora calospora* (B), *Glomus aggretatum* (C), *Glomus geosporum* (D), *Glomus glomerulatum* (E), *Entrophospora infrequens* (F), *Gigaspora* sp. (I), *Glomus enticatum* (G), *Scutellospora verrucosa (*K), *Acaulospora colaussica* (L), *Glomus albidum* (M), *Glomus multiforum* (N) (x400).

DISCUSSION AND CONCLUSION

The coastal dune habitats are relatively poor in nutrients, grown plant species have strategies for the conservation of nutrients and water as well as adaptations favoring the absorption of nutrients, mycorrhizal symbiosis is one of them[35]. Dune vegetation contributes to the formation and stabilization of sand dunes[58].

E. maritimum, is a mycotrophic mobile dune plant species. Observed mycorrhizal structures in the roots (vesicles arbuscules internal and external hyphae) clearly confirm this symbiotic association. The roots mycorrhizal frequencies and intensities of *E. maritimum* vary with depth levels reached by the roots. Mycorrhizal frequency is about 77.49% at 13.05 to 33.05 cm. Mycorhization intensity is 26.4% at depths of 53.05 to 73.05 cm.

According to Anderson[2], the mycorrhizal intensities and frequencies remain relatively high in *E. maritimum* roots throughout the growing season and are not influenced by local environmental factors. However, mycorrhizal intensity decreases during the month of August and increased significantly in September. Anderson[2] reported a linear negative correlation between the leaves chlorophyll content and roots mycorrhizal intensity.

The arbuscular content is very variable; it is a function of the depth reached by the roots. Indeed, more the roots of *E. maritimum* penetrate deeply, more the arbuscular content increases. The highest arbuscular content was recorded at a depth of 53.05 to 73.05 cm. In contrast, the vesicular content decreases with increasing depth, it is relatively lower than arbuscular one. The highest vesicular content was noted at the depth of 13.05 to 33.05 cm, which indicate that there is a storing of resources[10].

Arbuscules are considered as the preferred site of nutrient exchange between symbionts. The vesicles also are present, and the internal and external hyphae in the roots reflect the presence of a very active mycorrhizal symbiosis[10] in the mobile dunes. According to Branderett [10], the vesicles are swellings of hyphae that act as storage organs and allow accumulation of AM fungi lipid. The presence of sporocarps and spores on *E. maritimum* extra-roots hyphae also reflect the abundance of organic carbon in study site, which facilitates the reproduction of endomycorrhizae[10].

The number of the isolated spores in the *E. maritimum* rhizosphere varied from 10 to 20 spores / 100 g of soil. Conducted studies in the same area on *Lycium europaeum* showed that the number of spores is 75 to 50 spores / 100 g of soil respectively in the mobile dunes and consolidated dunes [56]. Hatimi and Tahrouch[26] reported in the coastal dunes of southern Morocco, a number of 42 spores / 100 g soil. This number is nearly identical to that reported by Ambouta[1] in the dunes of Gouré Department (Niger). Strümer and Belli[51] reported 298 spores / 100 g of soil in the dunes of Brazil.

Based on the morphological criteria of spores, ten species belonging to five different genera were identified: *Glomus badium, G. aggregatum, G. multiforum, G. invermaium, G. geosporum, G. glomerulatum, G. albidum, G.enticatum, Scutellospora verrucosa, S. calospora, S. heterogama, Acaulospora colaussica, Entrophospora infrequens et Gigaspora* sp. The dominance of the genus *Glomus* was also demonstrated in Morocco in the rhizosphere of the olive tree[30,31], the oleaster [50], date palm [9,49], Carob tree[17,54], poplar[53], Juncus[52] and *Lycium europaeum*[56]. *Glomus* is well adapted to African soils[23,34], particularly in semi-arid areas of Senegal[16] and Burkina Faso[23], in. rainforest of Cameroon[41] or South Africa[19], the southwest of Ethiopia[29,40], African dry mountain forests of Ethiopia[55]. Although there are very few studies on the diversity of Glomales populations in Africa, particularly in West Africa[23].

This domination of species of the genus *Glomus* was also reported in several studies conducted in Latin America [13,22,36,60] in arid and semi-arid northern Jordan[37] and coastal dunes[7,21,26,42,45,46].

Various authors have linked the domination of *Glomus* with its ability to produce more spores in a shorter time than other genera such as *Gigaspora* and *Scutellospora* and also for its ability to adapt to drought and to soil salinity[8,24].

The intertidal zone, devoid zone of vegetation, followed toward the interior with a zone composed of pioneer species, case of *E. maritimum*. The roots of this plant species tend to develop in depth to partially fix the sand[3] and mycorrhizal roots deep well to facilitate this development. *E. maritimum* is itself followed by a belt of *Ammophilia arenaria* and *Lycium europaeum*. Both species are also a mobile dunes mycotrophic species[56].

REFERENCES

1. Ambouta, K. J-M., Ibrahim, D., Bara, S., Statut mycorhizien de dix espèces ligneuses prélevées des dunes menaçant d'ensablement des cuvettes dans le département de Gouré (Niger). Geo-Eco-Trop., 33, NS: 107-114 (2009).
2. Anderson, U., Druva-Lūsīte, I., Ieviņa, B., Karlsons, A., Ņečajeva, J., Samsone, I. and Ievinsh, G., The use of nondestructive methods to assess a physiological status and conservation perspectives of *Eryngium maritimum* L. *J Coast Conserv,* **15:** 509–522 (2011).
3. Atbib, M., Etude phytoécologique de la Réserve biologique de Mehdia (littoral atlantique du Maroc). 2- La végétation du milieu dunaire, suppl. 7, 112 p.5 tabl. h.t. Bulletin de l'Institut Scientifique, Rabat, **4:** 99-188 (1983).
4. Atbib, M., La végétation du littoral du Maroc septentrional. Thèse de Doctorat d'Etat. Université Mohammed V, Rabat, 273p (1988).
5. Bammi, J., Mouhiddine, M., Fassi, D., et Douira, A., Contribution à la connaissance de la végétation des Doukkala-Abda (Maroc Atlantique): Approche éco-géomorphologique. *Journal of Animal & Plant Sciences,* **20(3):** 3202-3211 (2014).
6. Berch, S., Endogonaceae: taxonomy, specificity, fossil record, phylogeny. Front Appl Microbiol, **2:** 161-188 (1986).
7. Bergen, M., Koske R.E., Vesiculararbuscular mycorrhizal fungi from sand dunes of Cape Cod, Massachusetts. Transactions of the British Mycological Society, **83:** 157-158 (1984).
8. Blaszkowski, J., Tadych, M., Madej, T., Arbuscular mycorrhizal fungi (Glomales, Zygomycota) of the bledowska desert, Poland. Societastis Botanicorum Poloniae, **71(1):** 71-85 (2002).
9. Bouamri, B., Dalpé, Y., Serrhini, M.N. and Bennani, A., Arbuscular Mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera* l. in Morocco. African Journal of Biotechnology, **5(6):** 510-516 (2006).
10. Brundrett, M.C., Mycorrhizas in natural ecosystems. In A. MacFayden, M. Begon and A.H. Fitter (eds). Advances in Ecological Research. Academic Press, London. p. 171-133 (1991).
11. Cerceau-Larrival, M.T., Corrélations de caractères pour une étude biologique du genre *Eryngium* L. In: Anonymous Comptes rendus du 96 Congre`s National des Sociétés Savantes, Section des Sciences, Toulouse, 1971, Bibliothèque Nationale, Paris, **5:** pp. 451–477 (1973).
12. Cerceau-Larrival, M.T., Plantules et pollens dans le genre *Eryngium* L. Relations avec biogéographie. C.R. Somm. Séances Soc. *Biogéogr.* **424:** 103–112 (1971).
13. Cruz, S.J.C., Estudio de la simbiosis micorrızica vesicular arbuscular en el cultivo de *Coffea arabica* var. *caturra.* Fitopatol Colomb. **13:** 56 – 64 (1989).
14. Dalpé, Y., *Gigaspora margarita*. Fungi Canadenses No. 331. Can.J.Pl. Pathol., **16:** 229-230 (1995).
15. Derkowska E., Sas Paszt L., Sumorok B., Szwonek E., Głuszek S., The influence of mycorrhization and organic mulches on mycorrhizal frequency in apple and strawberry roots. J. fruit Ornam. Plant Res., **16:** 227-242 (2008).
16. Diallo, A.T., Samba, P.I. and Ducousso, M., Arbuscular mycorrhizal fungi in the semi-arid areas of Senegal. *European Journal of Soil Biology,* **35(2):** 65-75 (1999).
17. El Asri, A., Talbi, Z., Ait Aguil, F., Chliyeh, M., Sghir, F., Touati, J., Ouazzani Touhami, A., Benkirane, R. and Douira, A., Arbuscular Mycorrhizal Fungi Associated with Rhizosphere of Carob Tree (*Ceratonia siliqua* L.) in Morocco. *Int. J. Pure App. Biosci.* **2(3):** 286-297 (2014).
18. Ferrer, R.L., Herrera, R.A., El género Gigaspora Gerdemann et Trappe (Endogonaceae) en Cuba. Rev. Jardin. Bot. Nacional, Habana, **1:** 43–66 (1981).
19. Gaur, A., Van Greuning, J.V., Sinclair, R.C. and Eicker, A., Arbuscular mycorrhizas of *Vangueria infausta* Burch. sub sp. Infausta (Rubiaceae) from South Africa. *S. Afr. J. Bot.,* **65(5):** 1-3 (1999).
20. Gerdemann J.W., Nicholson T.H., Spores for mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc, **46:** 235-244 (1963).
21. Giovannetti, M., Nicolson, T.H., Vesicular arbuscular mycorrhiza in Italian sand dunes. *Trans. Br. Mycol. Soc.,* **80(3):** 552-556 (1983).

22. Guadarrama, P. and Alvarez-Sanches, F.J., Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico. Mycorrhiza, 8: 267- 270 (1999).
23. Guissou, T., La symbiose mycorhizienne à arbuscules chez des espèces ligneuses: diversité des Glomales, dépendance mycorhizienne, utilisation des phosphates naturels et tolérance à un stress hydrique, Thèse de Doctorat, Université de Ouagadougou, Burkina Faso, 124 pp (2001).
24. Haas, J.H., Menge J.A., VA mycorrhizal fungi and soil characteristics in avocado (*Persea americana* Mill.) orchard soil. Plant and Soil, The Hague, *127(2):* 207-212 (1990).
25. Hall, I. R., A review of VA mycorrhizal growth responses in pastures. Angew. Bot. 61, 127-134 (1987).
26. Hatimi, A. and Tahrouch, S., Caractérisations chimique, botanique et microbiologique du sol des dunes littorales du Souss- Massa. *Biomatec Echo*, **2(5):** pp 85-97 (2007).
27. Hatimi, A., Symbiotes racinaires de trois légumineuses arborescentes de dunes littorales de Souss-Massa. Ed. INRA, Paris, Les Collogues, **77:** 183-190 (1995).
28. Ionesco, T., Considérations sur la méthode floristico-écologique appliquée à l'étude des milieux dans les Doukkala. Bull. *Soc. Sc. Nat., Maroc*, T. 36 (1956).
29. Jefwa, J.M., Mung'atu, J., Okoth, P., Muya, E., Roimen, H. and Njuguini, S., Influence of Land use types on occurrence of arbuscular mycorrhizal fungi in the high altitude regions of Mt. Kenya. Trop. Subtrop. Agroecosystems, 11: 277–290 (2009).
30. Kachkouch, W., Touati, J., Ouazzani Touhami, A., Filali-Maltouf, A., El Modafar, C., Moukhli, A., Oukabli A., Benkirane R. and Douira A., Diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Olea europaea* in three regions of Morocco (Tafilalt, Zagora and Taounate). *Int. J. Pure App. Biosci.* **2(5):** 178-195 (2014).
31. Kachkouch, W., Ouazzani Touhami A., Filali-Maltouf A., El Modafar C., Moukhli A., Oukabli A., Benkirane R. and Douira A., Arbuscular mycorrhizal fungi species associated with rhizosphere of *Olea europaea* L. in Morocco. *Journal of Animal & Plant Sciences*, **15(3):** 2275-2287 (2012).
32. Kormanik P.P., McGraw A.C., Quantification of Vesicular-arbuscular Mycorrhizae in Plant Roots. In Methods and Principles of Mycorrhizal Research. Ed. N.C. Schenck. The American Phytopathological Society, 37-36 (1982).
33. Koske R.E., Gemma J.N., Mycorrhizae and succession in plantings of beachgrass in sand dunes. *Amer. J. Bot.*, **84(1):** 118-130 (1997).
34. Laminou Manzo O., Fixation des dunes dans le sud-est du Niger: évaluation de l'efficacité de la barrière mécanique, espèces ligneuses adaptées et potentialités d'inoculation mycorhizienne. Thèse de Doctorat, communauté française de bélgique académie universitaire Wallonie-Europe Université de Liège (ulg) Gembloux Agro Bio tech (gxabt), 142p (2009).
35. Logan, V.S., Clarke, P.J., Allaway, W.G., Mycorrhizas and root attributes of plants of coastal sand-dunes of New South Wales. Australian Journal of Plant Physiology **16:** 141–146 (1989).
36. Lopes, E.S., Oliveira, E., De Dias, R.A. and Schenck, N.C., Occurrence and distribution of vesicular arbuscular mycorrhizal fungi in coffee (*Coffea arabica* L.) plantations in central Sao Paulo State, Brazil. Turrialba, **33:** 417–422 (1983).
37. Mohammad, J.M., Rushdi Hamadt, S. and Issa Malkawit, H., Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. *Journal of Arid Environments*, **53:** 409- 417 (2003).
38. Morton, J.B., Benny, G.L., Revised classification of arcuscular mycorrhizal fungi (Zygomycetes): A new order, Glomales, two new suborders, Glomineae and Gigasporineae, with an emendation of Glomzceae. *Mycotaxon*, **37:** 471-491 (1990).
39. Mukerji, K.G., Taxonomy of endomycorrhizal fungi. In Mukerji KG, Mathur B., Chamola B.P., Chitralekha P. (eds.) Advances in Botany, 213-221. APH Publishing Corporation, New Delhi, pp. 211-221 (1996).
40. Muleta, D., Assefa, F., Nemomissa, S., Granhall, U., Distribution of arbuscular mycorrhizal fungi spores in soils of systems in; southwestern Ethiopia. *Biology and fertility of soils.* **44(4):**653-659 (2008).

41. Musoko, M., Last, F.T., and Mason, P.A., Populations of spores of vesicular arbuscular mycorrhizal fungi in undisturbed soils of secondary semideciduous moist tropical forest in Cameroon. *Forest Ecology and management*, **63:** 359-377 (1994).
42. Nicolson, T.H., Johnston, C., Mycorrhiza in the Gramineae. III. *Glomus fasciculatum* as the endophyte of pioner grasses in a maritime sand dune. *Trans. BR. Mycol. Soc.*, **72:** 261-268 (1979).
43. Nicolson, T.H., Mycorrhiza in the Gramineae. II. Developpement in different habitats, particularly sand dunes. *Trans. Br. Mycol. Soc.*, **43(1):** 132-145 (1960).
44. Paskoff, R., Caractérisation et gestion d'un type de dune littorale : les avant-dunes. Sécheresse, **16(4):** 247-53 (2005).
45. Ragupathy, S., Nagarjan G., Mahadevan A., Mycorrhizae in coastal sand dunes of Tuticorin, Tamil Nadu. *Jour. Envir. Biol.*, **19(3):** 281-284 (1998).
46. Schenck, N.C., Kinloch, R.A., Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. *Mycologia*, **72:** 445-456 (1980).
47. Schenck, N.C., Perez, Y., Manual for the Identification of VA Mycorrhizal Fungi. Second Edition. International Culture Collection of VA Mycorrhizal Fungi (INVAM), University of Florida, Gainesville, Florida. (1987).
48. Schenck, N.C., Smith, G., Additional new unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia*, **74:** 77- 92 (1982).
49. Sghir, F., Touati, J., Chliyeh, M., Ouazzani Touhami, A., Filali Maltouf, A., El Modafar, C., Moukhli, A., Oukabli, A., Benkirane, R., and Douira, A., Diversity of arbuscular mycorrhizal fungi in the rhizosphere of date palm tree (*Phoenix dactylifera*) in Tafilalt and Zagora regions (Morocco). *Int. J. Pure App. Biosci.* **2(6):** 1-11 (2014).
50. Sghir, F., Chliyeh, M., Kachkouch, W., Khouader, M., Ouazzani Touhami, A., Benkirane, R. and Douira, A., Mycorrhizal status of *Olea europaea* spp. *oleaster* in Morocco. *Journal of Applied Biosciences*, **61:** 4478 – 4489 (2013).
51. Stürmer, S.L., Bellei, M.M., Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. *Can. J. Bot.*, **72:** 359-363 (1994).
52. Talbi, Z., Chliyeh, M., Selmaoui, K., Ouazzani Touhami, A., Benkirane, R. and Douira, A., Mycorrhizal Status *Juncus maritimus*, Riparian species of Sidi Boghaba Reserve (Northwest of Morocco. *International Journal of Recent Scientific Research*, **5(4):** 792-795 (2014b).
53. Talbi, Z., Chliyeh, M., Selmaoui, K., Ouazzani Touhami, A., Benkirane, R. and Douira, A., mycorrhizal status of *Populus alba* and accompanying species of riparian forest in the Reserve of Sidi Boughaba (Northwest of Morocco). *International Journal of Plant, Animal and Environmental Sciences*, **4(2):** 126-133 (2014a).
54. Talbi, Z., El Asri, A., Touati, J., Chliyeh, M., Ait Aguil, F., Selmaoui, K., Sghir, F., Ouazzani Touhami, A., Benkirane, R. and Douira, A., Morphological characterization and diversity of endomycorrhizae in the rhizosphere of Carob tree (*Ceratonia siliqua*) in Morocco. *Biolife*, **3(1):** 196-211 (2015).
55. Tesfaye, G., Teketay, D., Assefa, Y. and Fetene, M., The influence of fire on soil seed bank composition and density and regeneration in a humid tropical forest, southeast Ethiopia. *Mount. Res. Dev.* **24:** 354 – 361 (2004).
56. Touati, J., Chliyeh, M., Ouazzani Touhami, A., Benkirane, R. and Douira, A., Mycorrhizal status of *Lycium europaeum* in the coastal dunes of Mehdia (Northwest of Morocco). *Journal of Applied Biosciences*, **71:** 5728– 5741 (2013).
57. Varsha, J. and Rodrigues, Occurrence and distribution of arbuscular mycorrhizal fungi in coastal sand dune vegetation of Goa. *Current Science*, **80:** 1101-1115 (2001).
58. Walker, C., Mize, C.W., Mc Nabb, H.S., Populations of endogonaceous fungi at two localities in central Iowa. *Can. J. Bot.*, **60:** 2518-2529 (1982).
59. Walker, C., Systematics and taxonomy of the arbuscular mycorrhizal fungi. *Agronomie*, **12:** 887-897 (1992).
60. Zhao, Z.W., Xia, Y.M., Qin, X.Z., Li, X.W., Cheng, L.Z., Sha, T. and Wang, G.H., Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. *Mycorrhiza*, **11:** 159-162 (2001).