RESEARCH ARTICLE
MYCORRHIZAL STATUS OF JUNCUS MARITIMUS, RIPARIAN SPECIES OF SIDI BOUGHABA RESERVE (NORTHWEST OF MOROCCO)
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INTRODUCTION
In Morocco, Sidi Boughaba Reserve is located on the territories of the rural town of Sidi Taibi and the urban commune of Mehdia, Suburbs of Kénitra province. It is a natural site of special scientific interest (Bathaoui, 2001). This site is characterized by the existence of a permanent body of water (Merja Sidi Boughaba) and marshy meadows.

Along the Merja, alternately in the tip area and immersed depending on the season, there is vegetation on variable width formed of large Cyperceae and Juncaceae, that surrounds the shores almost continuously. It is composed of more or less spaced tufts of Juncus acutus L., Juncus maritimus L., Cyperus longus L., Scirpus holoschoenus L., Scirpus maritimus L. (Thévenot, 1976). The mycorrhizal status of trees and shrubs is little known in Morocco. In particular, the riparian species who suffer of various pressures (drying out of the lake, anarchic exploitation of the reserve resources ….). Donmergues and Mangenot (1970) reported that among the taxa which do not form any mycorrhizal association, we found Juncaceae and Cyperaceae. These two families were considered for a long time as families that don’t form any mycorrhizal association (Rouquerol, 1962; Gerdeman, 1968) and this lack of mycorrhiza was also cited in other works (Selivanov and Yelesenova, 1974; Trappe, 1981; Mousain, 1988, Harley and Smith, 1983; Silveria, 1992). According to Mason (1928), Juncus maritimus is a non-mycorrhizal species. Koh and Stasovski (1990); Bledsoe et al. (1990) studied the mycorrhizal status of a large number of plant species in a Canadian Arctic area and noted that the roots of Juncaceae species do not form any mycorrhizal associations.

In some other works (Blaszkowski et al., 1998; Hoefnagels et al., 1993; Pratt-Zossoungbo and Biber, 2009; Harley et Harley, 1987; Silva, 2001; Chafi et al., 2012) have demonstrated that the roots of Juncaceae family species were mycorrhizal (J. balticus, J. articulatus, J. roemerianus, J. microcephalus and J. maritimus).

The objective of this investigation was to determine the mycorrhizal status of Juncus maritimus. In fact, limited information is known about the mycorrhizae of this species in Morocco. The parameters those were taken into account were the mycorrhizal frequency and intensity, arbuscular and vesicular contents and spore spectrum diversity.

MATERIALS AND METHODS
Choice of sites
The study area was the reserve of Sidi Bouhaha: a portion of the Atlantic coastal area in south of Gharb, bounded on the north by the estuary of Oued Sebou, South by the marabout of Sidi Bouhaha which gives its name to the reserve, to the east by cultures land and to the west by the resort city of Mehdia. It is located at 1 km from the Atlantic coast in the north of Rabat near to Mehdia (Idrissi, 1982; Nattah et al., 2013).

Samplings
Soil samples were collected from the riparian of the reserve of Sidi Bouhaha. The samples were collected in the month of April 2013 in the rhizosphere of Juncus maritimus at a depth of 25 to 50 cm and a composite soil sample was carried out by Site. Very fine roots, more likely to be mycorrhized and more easily microscopically observable.

Mycorrhizal rate inside the roots
The roots were prepared according to the method of Koske and Gemma (1989). They were first washed with water; the finest roots were then cut into a length of 1 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 H₂O₂ (hydrogen peroxide) for 20 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 (Phillips and Hayman, 1970), at 90°C for 15 min.

Roots were examined with a compound microscope for the presence of structures characteristic of AM such as arbuscules and vesicles. The AM percent root colonization was estimated by a grid intersect method using a dissecting microscope (Giovannetti and Mosse 1980). The mycorrhizal frequency and intensity were quantified using the technique of Phillips and Hayman (1970), as modified by Koske and Gemma (1989). The frequency and the intensity of arbuscules and vesicles of AMF inside the root bark were measured by assigning an index of mycorrhization from 0 to 5 (Trouvelot et al., 1986; Derkowska et al., 2008).

**Spores extraction**

Five 1-kg samples were collected at random from each site in April 2013. Spores were extracted from the substrate by wet sieving and decanting (Gerdemann and Nicolson 1963). The AM fungi were identified based on their morphological characters.

**Spore’s appearance frequency**

The appearance frequency of the species corresponds to the percentage of sites where each species is detected.

**Statistical Analysis**

The statistical treatment of the obtained results focused on the analysis of variance with a single classification criterion (ANOVA1).

**RESULTS**

The observation of *Juncus maritimus* root fragments, prepared by the method of Hayman and Philips (1970) and colored by cresyl blue, helped to demonstrate the presence of different mycorrhizal structures. At the root fragments of this species, internal and external fungal hyphae are essentially linear (Fig. 2. A, B, C, D), vesicles had a regular forms, sometimes irregular (Fig. 2. C, D), and arbuscular were present in the root cells (Fig. 2B, C). Endophytes were equally observed in the roots of *J. maritimus* (Fig. 2. E).

The frequency of root colonization of *J. maritimus* is total (F = 100%), with a low mycorrhizal intensity, in the order of 28%. Arbuscular and vesicular contents were respectively 13% and 2.1% (Fig. 3).

Concerning the estimation of the spore’s density in the rhizosphere of *Juncus maritimus* in the riparian of Sidi Boughaba (Fig. 4), the average was 72 spores/100 g of soil. A first preliminary identification, based only on the morphological criteria of the spores, has noted 11 species of AM fungi. Five species belonging to the genera of *Glomus*, two...
to the Acaulospora genera, two to Scutellospora genera and one for both of Gigaspora and Entrophospora genera. The isolates Glomus species were G. aggregatum, G. etunicatum, G. geosporum, G. macrocarpum, and G. verruculosum; those belonging to the Scutellospora genera were S. gilmorei and S. nigra; those belonging to Acaulospora genera were A. laevis, and A. gedanensis. The species belonging to Gigaspora and Entrophospora genera were respectively Gigaspora decipiens and Entrophospora infrequens. Scutellospora nigra was the most dominate species; its appearance frequency attained 28%.

DISCUSSION AND CONCLUSION

All the roots samples of Juncus maritimus have demonstrate the presence of the endomycorrhizal structures (vesicles, arbuscules, hyphae). Root arbuscular contents, only indication of functional mycorrhizal fungi, are relatively greater than those of the vesicles. Juncus maritimus is therefore mycotrophic, as other species of the same genera. In fact, roots of Juncus microcephalus (Silva et al., 2001), J. effuses (Oliveira et al., 2001) and J. roemerianus (Pratt-Zossoungbo and Biber, 2009) are also mycorrhized with a colonization rates respectively of 37, 50 and 38%. Other species of the same genera (J. articulatus, J. novazeelaniadie, J. planifolius and J. squarrosus) are considered as mycotrophic.

Working on the mycorrhizal status of the Angiosperms in Hawaii, Koske et al. (1992) noted the root colonization of J. plataniolius by the endomycorrhizal fungi. Harley and Harley (1987) also reported that the roots of 63% of Juncaceae species in the Britanic islands are mycorrhizal. Mycorrhizal intensity (28%), arbuscular (13%) and vesicular (2,1%), content are low at J. maritimus in the riparian of Mehdia. In Algeria, Chafi et al. (2012) reported that the roots mycorrhization of this species is low or null during the season of autumn and winter, but becomes important in the spring and summer (May and June). According to Otto (1962) and Bolan and Abott (1983), mycorrhizal colonization of plant roots is important in the hot weather. Other studies have demonstrated that the degree of the mycorrhizal colonization in salt marshes varies with the season (Cooke et Lefor, 1990; Brown and Bledsoe, 1996). Carvalho et al. (2001) reported that the degree of colonization is more marked during the summer season. The authors (Donnemgues and Mangenot, 1970; Selivanov and Yeleusenova, 1974; Trappe, 1981; Mousain, 1988; Harley and Smith, 1983; Silveria, 1992; Rouquerol, 1962; Gerderman, 1968) who reported the absence of mycorrhiza in the roots of Juncus species have probably taken the roots samples during unfavorable seasons. The analysis communities of the endomycorrhizal spores isolated from the rhizosphere of J. maritimus shows that an average does not exceed the number of 72 spores / 100g of soil. On the other side of the riparian shore, studies performed on Lycium europeum have shown that the number of spores is equally 75 and 50 spores / 100g of soil respectively in the mobile and the immobile dunes (Touati et al., 2013). This number also does not exceed 100 spores / 100 g of soil in the coastal dunes in the southern of Morocco (Hatimi and Tahrouch, 2007). Blaszowski and Tadych (1998), have reported that the number of spores in the rhizosphere of J. balticus was approximately 6 spores/100g of soil and 10 to 204 spores/100g of soil in the rhizosphere of J. articulatus.

Soil was collected from the rhizosphere of Juncus maritimus, 11 endomycorrhizal species were isolated. A preliminary identification, based only on the morphological criteria, have revealed that these species belong to four genera: Glomus (5 species), Acaulospora (2 species), Scutellospora (2 species), Gigaspora (1 species) and Entrophospora (1 species). Scutellospora nigra was the most dominate species, its appearance frequency is approximately 28%. Indeed, this species is the most frequently isolated from the rhizosphere of Lycium europeum developing in mobile and immobile dunes (Touati et al., 2013). The appearance frequency of this endomycorrhizal species in both types of dunes reaches 80%.

References

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