

**MYCORRHIZAL STATUS OF *POPULUS ALBA* AND ACCOMPANYING SPECIES OF RIPARIAN FOREST IN THE RESERVE OF SIDI BOUGHABA (NORTHWEST OF MOROCCO)**

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ABSTRACT. This study describes the mycorrhizal status of *Populus alba*, the main species of the riparian reserve of Sidi Boughaba (northwest of Morocco) and accompanying species: *Smyrniium olusatrum*, *Urtica membranacea*, *Ononis natrix*, *Pteridium aquilinum*. Microscopic examinations of these species roots have revealed the presence of endomycorrhizal and endophytic structures in all samples. Mycorrhizal frequency and intensity have respectively reached 100% and 75%. The spore's number of the endomycorrhizal species those were isolated from the rhizosphere of these plant species reached 78 spores/100g of soil. Spores belonging to the genera of *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Kuklospora*. *Scutellospora nigra* and *Scutellospora* sp1 are the two most abundant in the sites of the Sidi Boughaba riparian.

Key words. Morocco, reserve of Sidi Boughaba, riparian forest, mycorrhizal status.

INTRODUCTION

The riparian, vegetation along rivers and permanent or temporary water, is composed of trees formation, shrubs, and herbaceous [26]. This is an ecotone, the interface between aquatic and terrestrial environments, effect with edge, done side of aquatic and terrestrial environments across, biologically very rich, and of which the trees structure promotes the connection between the different habitats [5]. Riparian forest plays a structuring role for biodiversity and it is a key element of the ecosystems functioning [33]. Among the many functions of these formations, we include the regulation of flow dynamics, stream bank stability, water quality, ecosystem functioning [4]. In Morocco, the reserve of Sidi Boughaba is located on the territories of the rural town of Sidi Taibi and the urban commune of Mehdiya, outskirts of the province of Kenitra, is a natural site of special scientific interest [3]. It is characterized by the existence of a permanent body of water (Merja of Sidi Boughaba) and marshy meadows, protected by a dense afforestation and not very penetrable [1]. The border (Southeast) of the lake is occupied by a hygrophilic matorral of *Populus alba* L. which combines a *Tamarix*. This riverine forest features undergrowth bracken fern (*Pteridium aquilinum* L.) and found there *Rubus ulmifolius* Schott [20]. At the west side, we encounter a dense Junipers that is difficult to penetrate in, most often degraded to the east and making room for a scrub to *Retama monosperma* L. or even to *Chamaerops humilis* L. [16]. The role of mycorrhizal fungi in the growth and nutrition of plants is well established [22]. In general, mycorrhizal status of trees and shrubs is little known, especially, riparian species which undergo large diverse pressures (drying up of the lake, clearing, anarchic exploitation of resources in the reserve ...). Yet, this is in part due to symbiotic microorganisms (mycorrhizal fungi, Rhizobium) that the majority of ligneous are able to be installed and to maintain on soils of different biotopes [12]. Mycorrhizae is a result of a mutual benefit association between a soil fungus and the roots of a host plant.

The objective of this investigation is to determine the mycorrhizal status of *Populus alba*, ligneous essence of riparian reserve of Sidi Boughaba, and of some accompanying species: *Smyrniium olusatrum*, *Urtica membranacea*, *Ononis natrix*, *Pteridium aquilinum*. Indeed, little information is known about these mycorrhizal species associated to these plants in Morocco. Riparian forests, in general, remain unstudied areas. The parameters taken into account are: Mycorrhizal frequency and intensity, arbuscular content and the diversity of spore spectrum in symbiosis with these ligneous.

MATERIALS AND METHODS

Choice of Sites

The study area was the reserve of Sidi Boughaba: 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 Boughaba which gives its name to the reserve, to the east by cultures land and to the west by the resort city of Mehdiya. It is located at 1 km from the Atlantic coast in the north of Rabat near to Mehdiya. [15, 21].

Sample Collection

Soil samples were collected from the riparian of the reserve of Sidi Boughaba. The samples were collected in the month of April 2013, in the rhizosphere of *Populus alba*, *Smyrniium olusatrum*, *Urtica membranacea*, *Ononis natrix*, *Pteridium aquilinum*, 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.

Rate of the root mycorrhization

The roots were prepared according to the method of Koske and Gemma [19]. 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 [25], at 90°C for 15 min.

After the final rinse, thirty pieces of dyed roots of 1 cm length were randomly selected and mounted, in groups of 10 to 15 segments, in glycerine between slide and coverslip [18]. The remaining roots were kept in glycerol acid. The slides were examined under a microscope, each fragment being thoroughly checked over its entire length, at magnifications of 100 x and 400 x to observe and to note the mycorrhizal structures: arbuscules, hyphae, vesicles, external hyphae, intra and intercellular hyphae and even the endophytes structures. Vesicular and arbuscular frequencies and content of the endomycorrhizal fungi inside the roots were measured assigning a mycorrhization index ranging from 0 to 5 [6], 0: absent, 1: traces, 2: less than 10%, 3: 11 to 50%, 4: 51 to 90%, 5: more than 91%.

Mycorrhizal Frequency

Mycorrhizal Frequency (F %), it reflects the infection importance of the host plant root system by the endomycorrhizal fungi:

$$F\% = 100 (N - N_0) / N$$

N: number of the observed fragments and N₀: number of non-mycorrhized fragments.

Mycorrhizal intensity:

Mycorrhizal intensity (M %) expresses the portion of the cortex colonized compared to the entire root system:

$$(M\%) = (95 n_5 + 70 n_4 + 30 n_3 + 5 n_2 + n_1) / N$$

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

Arbuscular content (A %) of the mycorrhized part :

$$A\% = (100 m_{A3} + 50 m_{A2} + 10 m_{A1}) / 100$$

Where;

m_{A3}, m_{A2}, m_{A1} are the percentages (%) respectively assigned to the notes A₃, A₂, A₁, with, m_{A3} = (95 n_{5A3} + 70 n_{4A3} + 30 n_{3A3} + 5 n_{2A3} + n_{1A3}) / N. The same for A₁ and A₂, n_{5A3} represents the number of fragments marked 5 with A₃; n_{4A3} 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 %) :

$$(V\%) = (100 m_{V3} + 50 m_{V2} + 10 m_{V1}) / 100$$

Where m_{V3}, m_{V2}, m_{V1} are the percentages (%) respectively assigned notes V₃, V₂, V₁, with V₃; m_{V3} = (95 n_{5V3} + 70 n_{4V3} + 30 n_{3V3} + 5 n_{2V3} + n_{1V3}) / N. The same for V₁ and V₂. n_{5V3} represents the number of fragments marked 5 with V₃; n_{4V3} marked the number of fragments 4 with V₃; V₀: no vesicles; V₁: some vesicles 10% V₂: 50% moderately abundant vesicles; V₃ abundant vesicles: 100%.

Spores extraction:

The spores were extracted by the method of wet sieving described by Gerdemann and Nicolson [10]. 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 μm). 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 solution of 40% sucrose in each centrifuge tube [32]. 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.

Frequency of spores' occurrence

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

Statistical analysis: The statistical treatment of results focused on the analysis of variance to a single criterion of classification (ANOVA).

RESULTS

The observation of the roots fragments of *Populus alba* and other accompanying species (*Smyrniium olusatrum*, *Urtica membranacea*, *Ononis natrix*, *Pteridium aquilinum*), prepared by the method of Philips et Hayman [24] and colored by cresyl blue, allowed to demonstrate the presence of mycorrhizal structures. At the root fragments of those species, internal and external fungal hyphae are essentially linear (fig.2 A, B, H), the vesicles are regularly shaped, sometimes irregular, arbuscular are present in the root cells (fig. 2 C, D). Mycorrhizal helper, such as those reported by Druva-Lusite (2010), was observed in the roots of *Pteridium aquilinum* (fig. 2 F). Endophytes were also observed in the roots of all study plant species (fig. 2 C, G, H). Root colonization rate of the study plant species varies from one species to another (figure 3). This colonization is total in *Smyrniium olusatrum* and *Ononis natrix* (F = 100%). A high rate of colonization was equally observed in *Urtico membranacea* and *Populus alba* (F = 93%). The roots of *Pteridium aquilinum* are more weakly mycorrhized (F = 53%). The mycorrhizal intensity is high among *Smyrniium olusatrum* (75.33%) and so weak in *Urtico membranacea* and *Pteridium aquilinum*, respectively 8.06%, 1.9% (fig. 3).



Fig. 1. *Populus alba* (Pa): Principal species in the riparian reserve of Sidi Boughaba

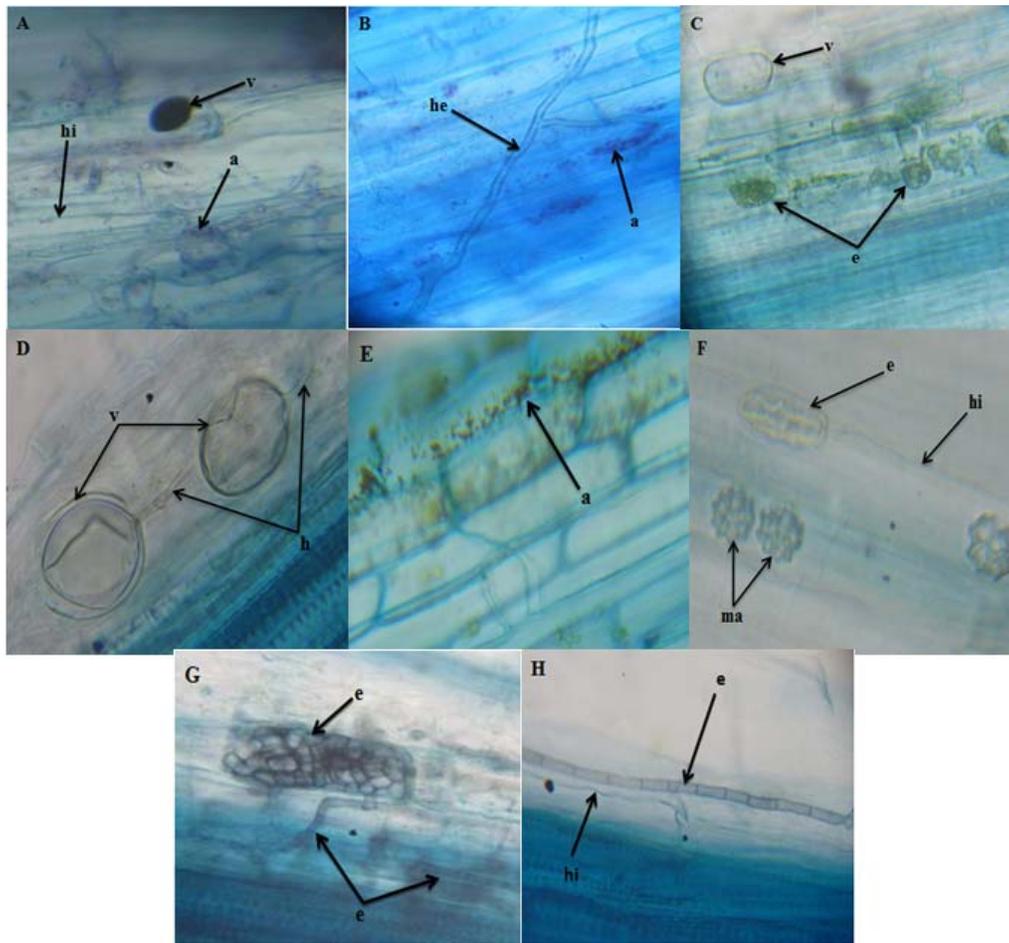


Fig. 2. Endomycorrhizal and endophytic fungal structures in the roots of *Urtica membranacea* (A, H) ; *Populus alba* (B) ; *Pteridium aquilinum* (C, E, F) ; *Ononis natrix* (D, H) : a, arbuscule ; e, endophytes ; hi, internal hyphae; he, external hyphae; v, vesicule ; h, hyphe ; ma, mycorrhizal helper (G. ×400).

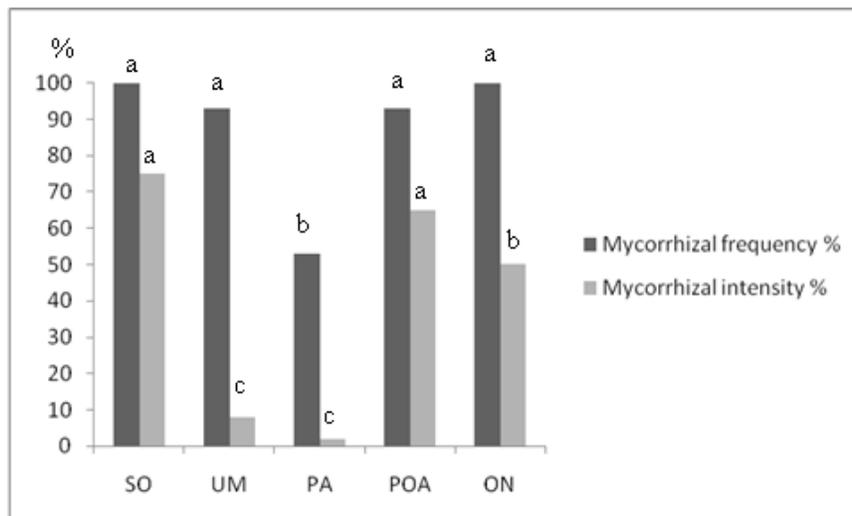


Fig-3: Mycorrhizal frequency and intensity of the endomycorrhizae inside the roots of the study species. Two results affected by the same letter were not significantly different at 5%

SO: *Smyrniolum olusatrum*, UM: *Urtica membranacea*, PA: *Pteridium aquilinum*, POA: *Populus alba*, ON: *Ononis natrix*

Furthermore, the arbuscular contents vary between 65% and 60% respectively at the roots of *Smyrniun olusatrum* and *Populus alba* (Figure 4). In the other hand, these levels are low at the roots of *Urtico membranacea* and *Pteridium aquilinum*. The vesicular contents did not exceed 17% at the roots of all the study plant species (Figure 4). It is 16.22% in *Smyrniun olusatrum* and 0.4% and 0.62% respectively in *Urtico membranacea* and *Pteridium aquilinum*. Concerning the estimation of the spore's density in the rhizosphere of *Populus alba*, developing in the study site (Fig. 5), the unregistered mean was 78 spores/100 g of soil. Some collected soil in the rhizosphere of poplar tree, 20 species of endomycorrhizal were isolated.

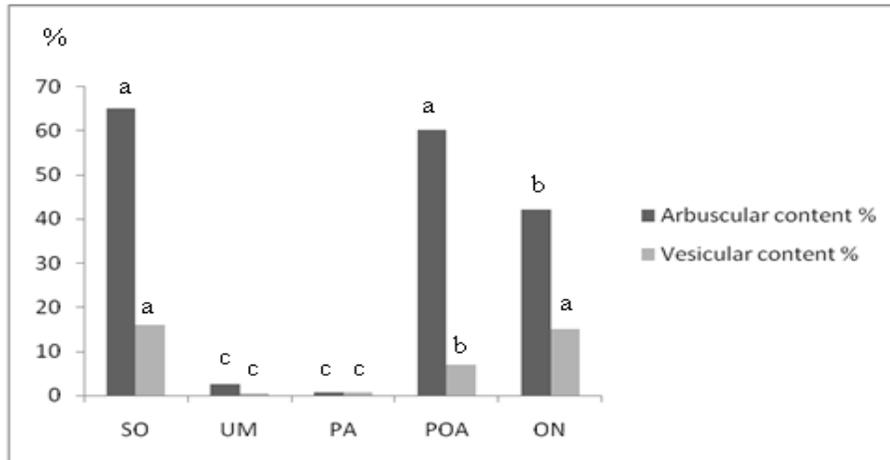


Fig.4. Arbuscular and vesicular content of the endomycorrhizae inside the roots of the study species. Two results affected by the same letter were not significantly different at 5%.

SO: *Smyrniun olusatrum*, UM: *Urtico membranacea*, PA: *Pteridium aquilinum*, POA: *Populus alba*, ON: *Ononis natrix*.

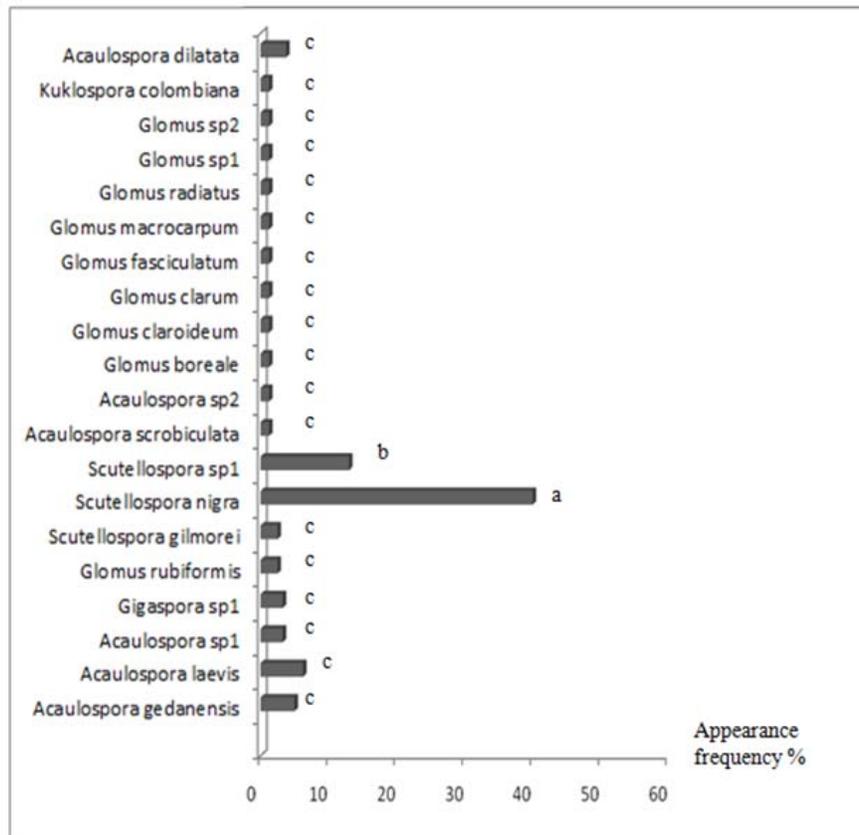


Fig. 5. Appearance frequency of the endomycorrhizal species isolated from the rhizosphere of *Populus alba*. Two results affected by the same letter were not significantly different at 5%.

Nine species belonging to the *Glomus* genera (*Glomus* sp1, *Glomus* sp2, *G. radiatus*, *G. macrocarpum*, *G. fasciculatum*, *G. clarum*, *G. claroideum*, *G. boreale* and *G. rubiformis*), six to the *Acaulospora* genera (*A. scrobiculata*, *Acaulospora* sp1, *Acaulospora* sp2, *A. laevis*, *A. gedanensis* and *A. dilatata*), three to the *Scutellospora* genera (*Scutellospora* sp1, *S. nigra* and *S. gilmorei*) and one to the genera *Gigaspora* (*Gigaspora* sp1) and *Kuklospora* (*Kuklospora colombiana*). *Scutellospora nigra* and *Scutellospora* sp1 are the two most abundant species, their appearance frequency attained 40 % and 20 %.

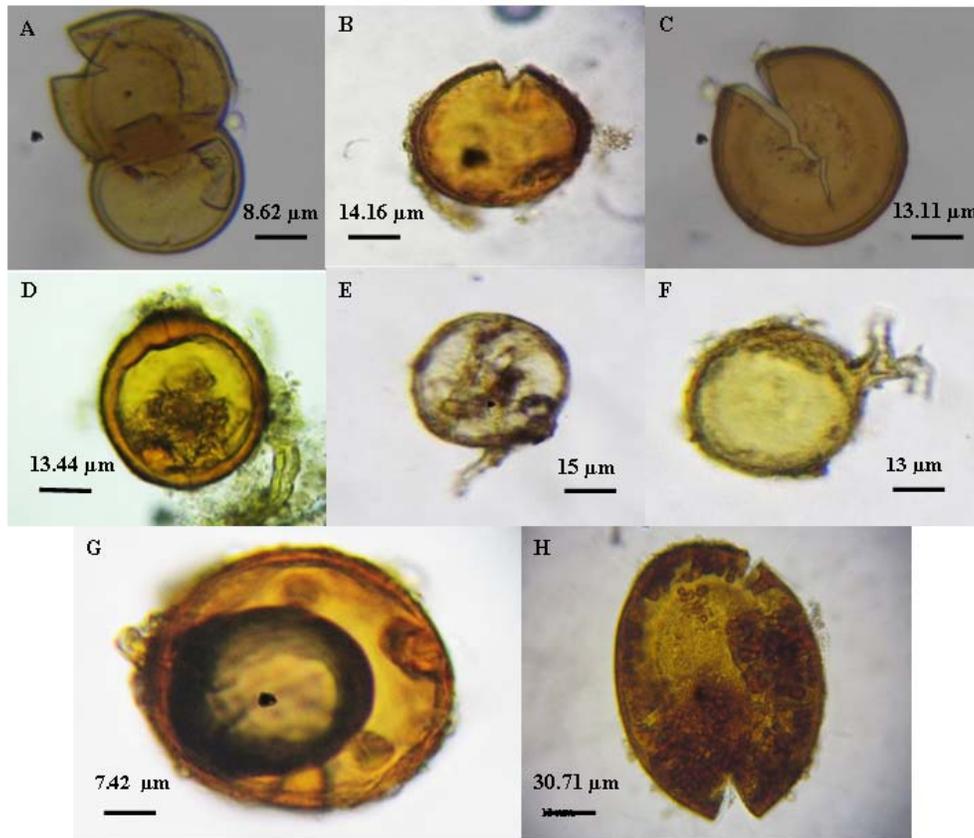


Fig. 6: Some endomycorrhizal species isolated from the rhizosphere of *Populus Alba*: *Acaulospora laevis* (A) and (C); *Kuklospora colombiana* (B); *Glomus macrocarpum* (D); *Scutellospora* sp1. (E) ; *Acaulospora lacunosa* (F) ; *Glomus rubiformis* (G) ; *Gigaspora* sp1. (H) (G. ×400).

DISCUSSION AND CONCLUSION

Populus alba, pioneer species of riparian vegetation, and the vegetal species associated with (*Smyrniolum olusatrum*, *Urtica membranacea*, *Ononis natrix*, *Pteridium aquilinum*) have shown the presence of all mycorrhizal structures inside the roots samples (vesicles, arbuscules, hyphae), suggesting a functionally active state of symbiosis. Arbuscular contents of roots, only indication of the mycorrhizae performance, are relatively greater than those of the vesicles. The obtained results showed a high mycorrhizal intensity in *Populus alba*, that it might be considered as a source of fungal inoculum for other plant species. Gardes *et al.* [9] reported that poplar tree is a vector of diversity for riparian forests: fungal assemblages that are directly associated are extremely varied at different levels of organization. It constitutes a fungal inoculum reservoir for all mycotrophic plant species that may develop in this environment. Sometimes the first plant species that settle are those little dependent on the mycorrhizal symbiosis [8]. Thereafter, the enrichment of soil with the mycorrhizal structures, the most mycotrophic species takes over with a strong positive correlation between fungal and plant biodiversities [13, 17, 27, 30]. Some species are highly mycotrophic settled in early successional vegetation on the degraded soils and subsequently promote the development of other plant species [2]. The low observed rate of mycorrhization in *Urtico membranacea* and *Pteridium aquilinum* could be due to their very low requirements to minerals and also by adverse environmental conditions in the growing season. Druva-Lusite and Ievinsh (2010) reported that environmental stress can affect the morphology of the symbiosis.

Analysis of the endomycorrhizal fungi spore's communities that were isolated from the rhizosphere of *Populus alba* showed that on their average number did not exceed 78 spores / 100 g of soil. On the other side of the riparian shore, studies performed on *Lycium europaeum* have shown that the number of spores was also 75 and 50 spores / 100g of soil respectively in the mobile and fixed dunes [29]. This number also did not exceed 100 spores / 100 g of soil in the coastal dunes in the Moroccan south [14]. The collected soil from the rhizosphere of poplar and associated species, 20 endomycorrhizal species were isolated. A preliminary identification based solely on morphological criteria, revealed that these species belong to five genera: *Glomus* (9 species), *Acaulospora* (6 species), *Scutellospora* (3 species), *Gigaspora* (1 species) and *Kullospora* (1 species). *Scutellospora nigra* was the most abundant species; its appearance frequency was about 40%. These species abound probably in the study site. In deed, at the level of the riparian west shore, *Scutellospora nigra* was the most isolated species in the rhizosphere of *Lycium europaeum* developing in the mobiles and fixed dunes in the reserve of Sidi Boughaba [29]. The appearance frequency of this endomycorrhizal species in the two types of dunes attained 80 %. Poplar, woody essence with a pioneer vocation, often dominant in the early stages of the riparian evolution, is considered as an interesting vegetal model for the comprehension of the mycorrhizal symbiosis [9, 11]. According to these authors, the poplar tree is known as a rare vegetal species with a host power on two mycorrhizal species simultaneously: ectomycorrhizae and endomycorrhizae [31].

This double root colonization was able to increase the ecological amplitude of the vegetal host with improving its mineral alimentation [9]. Endomycorrhizae facilitates the phosphate nutrition of the plant [23] and the nitrogen nutrition [28]. The introduction of the poplar tree in the conservation programs and the riparian forests restoration has allowed the maintenance of the plant and fungal diversity in this ecosystem. This species is able to adjust to the environment variations through its fungal partners.

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