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Research Article

Diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Plantago coronopus* in Northwestern Algerian coast

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Abstract

Mycorrhizal fungi play a major role in the functioning of ecosystems. However, their identification has remained a challenge for scientific research. This study presents the first identification report of species of arbuscular mycorrhizal fungi in the rhizosphere of the halophyte *Plantago coronopus* L. in Algeria. Samples of rhizospheric soil were collected in spring 2018 at three sites in the Bomo-plage dunes west of Oran, Algeria. The spores were isolated by wet sieving, morphologically identified, and quantified. The mean spore density was 107.94 spores 100 g^{-1} dry soil, which is high compared to other dune ecosystems. Endomycorrhizal spore morphotypes were involved in the following Genus: *Glomus, Septoglomus, Rhizophagus, Diversispora, Funneliformis, Dentiscutata, Claroideoglomus, Scutellospora,* and *Entrophospora,* to the following Family: *Glomeraceae, Gigasporaceae, Diversisporaceae, Claroideoglomeraceae,* and *Acaulosporaceae.* The *Glomeraceae* was the most dominant identified family. The identification of indigenous arbuscular mycorrhizal fungi has been shown to be essential for future programs to restore disturbed dune ecosystems.

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Introduction

Coastal dunes represent special habitats along a sea-land environmental gradient which creates a typical zonation of vegetation (Acosta et al., 2007). They are characterized by specific abiotic conditions such as salt spray, mobile or semi-mobile substrates, edaphic drought and mineral deficiency, pollution and human activities such as urbanization and recreation (Hesp et al., 1991; Maun, 2009). Coastal plant species have a particular adaptive quality to these constraints (Hesp et al., 1991; Calvão et al., 2013) which is due, in large part, to the beneficial interaction with symbiotic microorganisms, such as mycorrhizal fungi.

Arbuscular mycorrhizal fungi (AMF) are an integral component of natural ecosystems (Becerra et al., 2019). They constitute a group of obligate root biotrophs that exchange mutual benefits with about 80% of plants (Berruti et al., 2016). They ingest sugars and lipids to complete their life cycle (Bago et al., 2000; Jiang et al., 2017), and in return, they influence plant community development, nutrient cycling, and soil formation and aggregation. They also act as bio-protectors against biotic and abiotic stresses (Jeffries et al., 2003). They have been grouped into a single phylum *Glomeromycota* (Schüβler et al., 2001). Recently, they have been classified in the phylum *Mucoromycota* as subphylum *Glomeromycotina* (Spatafora et al., 2016). Despite their ancient origin, 400 million years (Selosse et al., 2015), only 334 species have been described (www.amf-phylogeny.com/amphylospecies.html, 2020). However, there are still concerted efforts to make more discoveries of new species in different ecosystems and by various means, both classical and modern.

Plantago coronopus of the *Plantaginaceae* family is an annual herbaceous to perennial flowering plant with the following botanical characteristics: it is

3 to 30 cm high, with spread out or ascending stems, exceeding the leaves, and with taproots. The leaves are linear to lanceolate, toothed, fleshy, and arranged in a basal rosette. The flowers are anemophilous and arranged in cylindrical or oblong spikes, and the fruit is a capsule with three to four brown seeds (Milcent, 2011; Schmidt et al., 2016). Regarding its distribution and use, it grows on dunes and shingles close to the sea, cliffs and dikes, on meadows and heaths. It is native to Eurasia and North Africa but is widely naturalized outside its native range. It is a characteristic Mediterranean halophile plant and it is used in saline agriculture and pharmaceutical industries (Gonçalves and Romano, 2016; Bueno and Cordovilla, 2021).

Mutualistic interactions between halophytes and AMF are the subjects of particular attention from scientists. They influenced the way plants to perceive and respond to their abiotic environments (Maherali, 2020). It has been reported that *Plantago coronopus* is considered an endomycorrhizal plant (Oliveira et al., 2005); however, the identification of mycorrhizal fungi in its rhizosphere remains poorly understood. In this respect, the objective of this study was to morphologically characterize and quantify the AMF isolated from soil samples collected under *P. coronopus*, growing on the coastal dunes of Bomo beach of Oran (Northwestern Algeria).

Materials and Methods

Study area

The study area is located at the coast of Oran in the region of Cap Falcon, which is characterized by a succession of beaches. The region is characterized by a semi-arid Mediterranean climate, psammophilous and heterogeneous vegetation, substrate light, permeable and therefore deficient in water and mineral elements. The study was carried out exactly at the foredune of Bomo beach (35°45'03" North Latitude, 0°49'46" West Longitude, with altitude 4 meters above sea level (masl) in the Commune of Bousfer, Daira of Ain El Turk, Wilaya of Oran (North-West Algeria) (Figure 1), where *Plantago coronopus* was abundant. Rhizospheric soil samples of *P. coronopus* were collected in spring 2018 at a depth of 20 cm from three sites (S1, S2, and S3). Morphological analyses of the spores were performed after air-drying the soils and sieving them through a 2 mm diameter mesh sieve.

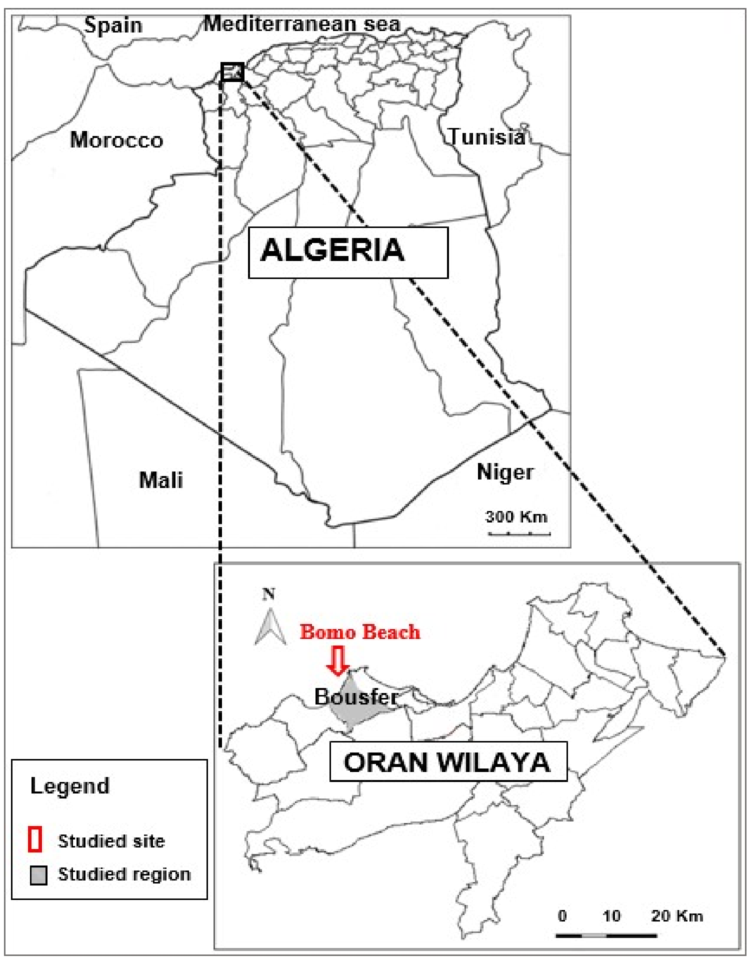

Figure 1. Location of the study sites in the coastal dune of Bomo beach in Oran, Northwest Algeria.

Extraction, enumeration and morphological identification of spores

Mycorrhizal spores were extracted from 100 g of each sample of *P. coronopus* rhizosphere soil by wet sieving (Gerdemann and Nicolson, 1963) using different sieves with mesh sizes ranging from 700 to 32 µm and then centrifuged (ROTINA 380, Germany) with sucrose solution (Jenkins, 1964). Healthy and intact spores were examined, counted and grouped into morphotypes using a binocular loupe (ZEISS, Germany) at 40X. Microscopic examination (AXIO SCOPE A1, ZEISS, Germany) was carried out in the presence of polyvinyl-lacto-glycerol reagent (PVLG) and PVLG mixed with Melzer's reagent (1:1, v/v). Morphotyping and identification of spores and their recognition into genera and species were carried out by referring to the online databases related to the work of the INVAM (2020) and Blaszkowski (2020).

Calculation of spore densities and relative abundances

Based on the spore count, the spore density (SD), the family relative abundance (FRA), and the species relative abundance (SRA) were determined. The spore density (SD) is defined as the number of spores in 100 g of dry soil. The relative spore abundances of families and species (Johnson et al., 1991) were obtained according to the following formula:

$$RA = \frac{n}{N} \times 100$$

N: Total number of spores identified, n: Number of spores for each species (or genus).

Statistical analysis

One-way analysis of variance (One-Way ANOVA) was used to test the difference in AMF spore densities between the three selected sites. Fisher's least significant difference (LSD) test was used to compare the means between the different sites.

Results and Discussion

In the Mediterranean, coastal dunes are fragile ecosystems vulnerable to climatic variations that limit plant survival and contribute to soil degradation. The use of mycorrhizae as an approach remains necessary to restore semi-arid and arid ecosystems (Barea et al., 2011), improving the survival, performance, and adaptation of plants under adverse conditions. The present study aimed to determine the density and the different morphotypes of AMF spores in the rhizosphere of *P. coronopus* in its natural distribution range.

Diversity of AMF spores

The study of mycorrhizal diversity revealed the existence of fourteen AMF species belonging to nine genera (*Glomus, Septoglomus, Rhizophagus, Diversispora, Funneliformis, Dentiscutata, Claroideoglomus, Scutellospora*, and *Entrophospora*) and five families (*Glomeraceae, Gigasporaceae, Diversisporaceae, Claroideoglomeraceae*, and *Acaulosporaceae*). Our results agree with those of Koske et al. (2004), who reported that 1 to 14 AMF species could thrive in the roots of dune plants. Eleven (11) AMF species of *Lotus creticus* were identified in the coastal dunes of the Wilaya of Ain Temouchent (West of Oran, about 90 km) in Algeria (Nehila et al., 2015). Furthermore, 10 AMF species of five psammophytes were found in the dunes of the Gulf of Valencia (Spain) (Guillén et al., 2019). When dunes are unstable and disturbed, spore numbers decrease, and conversely (Koske et al.,2004).

The results showed an increased presence of *Glomeraceae* was characterized by seven species (*Rhizophagus* sp., *Septoglomus constrictum*, *Septoglomus deserticola*, *Glomus* sp.1, *Glomus* sp.2, *Funneliformis verruculosum*, and *Funneliformis geosporum*). *Gigasporaceae* were represented by three species (*Dentiscutata* sp.1, *Dentiscutata* sp.2, and *Scutellospora* sp.), followed by the *Diversisporaceae* with two species (*Diversispora epigaea* and *Diversispora spurca*), the *Claroideoglomeraceae* (*Claroideoglomus claroideum*) and the *Acaulosporaceae* (*Entrophospora* sp.) with one species each (Table 1). The results indicate a high mycorrhizal richness in the three sites studied (Figure 2). In contrast, low levels of richness and dominance of the species *Funneliformis geosporum* have been reported among halophytes in European salt marshes (Carvalho et al., 2001; Hildebrandt et al., 2001; Landwehr et al., 2002). Some research indicates that a community with high diversity is characterized by low dominance (Castillo et al., 2016). Mycorrhizal diversity is important in the functioning of the mycorrhizae themselves and their plant partners, namely the origin, evolution, distribution, and development of plants (Liu and Wang, 2003). The composition and richness of AMF species were similar in the three sites in the present study, which could be explained by the proximity of the chosen sites.

AMF spore density

AMF spore density in rhizospheric soil of *P. coronopus* was highly and significantly different between the study sites ($F = 6.92$, $p < 0.01$). Abiotic factors such as soil properties could contribute to these differences (Melo et al., 2019). The results of the count showed that spores were present with a high average density of 107.94 spores/100 g^{-1} of dry soil (Table 2). This is close to that reported by Wang et al. (2021) (109.9/100 g of dry soil) for the species *Cynanchum chinense* on a coastal beach in North Jiangsu, China. In addition, the spore density found was important and higher than that recorded in the rhizosphere of some coastal psammophytes in Algeria and Morocco (Touati et al., 2013; Nehila et al., 2015). However, it is low compared to that estimated in *Plantago maritima* in salt marshes in Hungary (Landwehr et al., 2002) and *Asteriscus maritimus* from dunes in Spain (Estrada et al., 2013). It is also low compared to other species in other arid and semi-arid ecosystems in Algeria, such as *Tamarix articulata* in steppes and *Arthrocnemum macrostachyum* in saline sites (Bencherif et al., 2015; Nehila et al., 2015).

Table 1. Morphotypic identification of AMF spores.

No	Color	Size (µm)	Species	Genus	Family
1	Hyaline	40 - 81	*Rhizophagus* sp.	*Rhizophagus*	*Glomeraceae*
2	Red brown-Black	198 - 240	*Septoglomus constrictum*	*Septoglomus*	
3	Orange-Brown	86 - 102	*Septoglomus deserticola*		
4	Pale yellow	223 - 235	*Glomus* sp.1	*Glomus*	
5	Orange	97 - 117	*Glomus* sp.2		
6	Yellowish-Orange	220 - 250	*Funneliformis verruculosum*	*Funneliformis*	
7	Yellow-Orange	182 - 224	*Funneliformis geosporum*		
8	Orange	148 - 168	*Diversispora epigaea*	*Diversispora*	*Diversisporaceae*
9	Sub-Hyaline	104 - 122	*Diversispora spurca*		
10	Cream-Light yellow	110 - 117	*Claroideoglomus claroideum*	*Claroideoglomus*	*Claroideoglomeraceae*
11	Red brown	298 - 343	*Dentiscutata* sp.1	*Dentiscutata*	*Gigasporaceae*
12	Dark brwn	245 - 282	*Dentiscutata* sp.2		
13	Red	134 - 146	*Scutellospora* sp.	*Scutellospora*	
14	Cream	35 - 71	*Entrophospora* sp.	*Entrophospora*	*Acaulosporaceae*

Note: Spores size data are represented from minimum to maximum value.

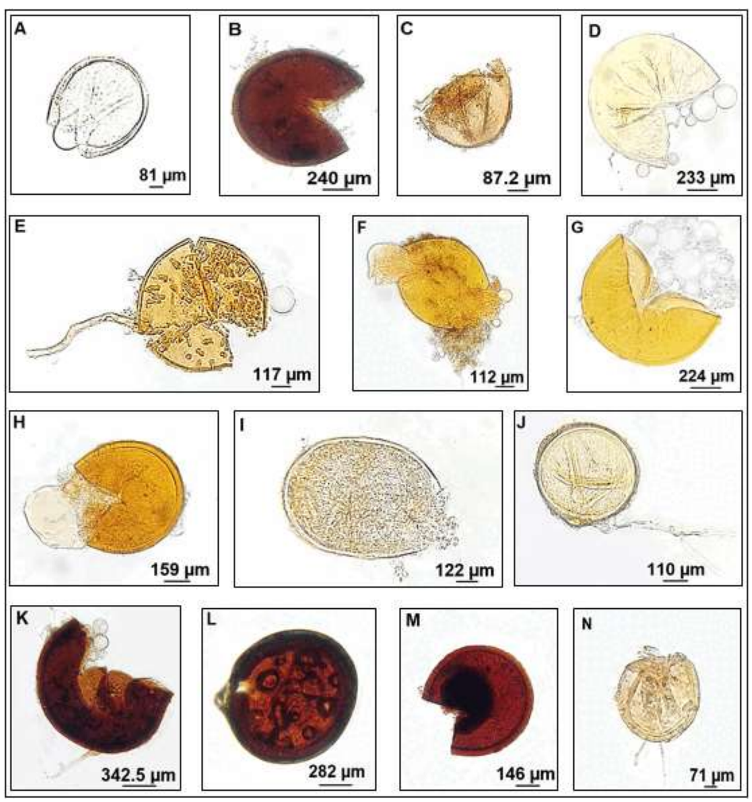

Figure 2. AMF spores isolated from the trap crop and rhizosphere soils of *P. coronopus.*
A: *Rhizophagus* sp.; B: *Septoglomus constrictum*; C: *Septoglomus deserticola*; D: *Glomus* sp.1; E: *Glomus* sp.2; F: *Funneliformis verruculosum*; G: *Funneliformis geosporum*; H: *Diversispora epigaea*; I: *Diversispora spurca*; J: *Claroideoglomus claroideum*; K: *Dentiscutata* sp.1 (germ walls with warts, and a darker orange-brown oblong germ shield); L: *Dentiscutata* sp.2 (dark, with multilayers, and a sporogenous cell); M: *Scutellospora* sp. (ornamented, and a dark reddish-brown reaction of germ wall in Melzer's reagent); N: *Entrophospora* sp. (with two scars)

Table 2. AMF spores density (per 100 g dry soil) for each species or genus identified in the rhizospheric soil samples of *P. coronopus.*

Species	Sites		
	S1	S2	S3
Rhizophagus sp.	22.8 ± 3.16^{a}	14 ± 4.3^{b}	17.8 ± 3.9^{c}
Septoglomus constrictum	17.2 ± 3.46^{a}	9.8 ± 3.16^{b}	12 ± 1.8^{c}
Septoglomus deserticola	3.6 ± 2.24^{a}	5.8 ± 2.34^{b}	6.8 ± 1.4^{b}
Glomus sp.1	12.8 ± 3.39^{a}	8.8 ± 4.3^{b}	8 ± 2.9^{ab}
Glomus sp.2	12 ± 2^{a}	8 ± 2.3^{b}	14 ± 2.5^{c}
Funneliformis verruculosum	6 ± 1^{a}	3.8 ± 2.3^{b}	9.8 ± 2.2^{c}
Funneliformis geosporum	4 ± 1.8^{a}	2 ± 2.7^{b}	7 ± 2.2^{c}
Diversispora epigaea	7 ± 2.3^{a}	3 ± 2.4^{b}	8.4 ± 3.8^{c}
Diversispora spurca	7.8 ± 1^{a}	4 ± 3^{b}	9 ± 1^{c}
Claroideoglomus claroideum	10 ± 2^{a}	3 ± 2.7^{b}	8 ± 1.5^{a}
Dentiscutata sp.1	2 ± 1.5^{a}	10 ± 3.6^{b}	5 ± 2.5^{c}
Dentiscutata sp.2	4 ± 2.9^{a}	2 ± 2.34^{b}	10 ± 2.5^{c}
Scutellospora sp.	3 ± 2.23^{a}	4 ± 2.3^{a}	6.6 ± 1.8^{b}
Entrophospora sp.	3 ± 2.3^{a}	5 ± 2.7^{b}	2 ± 2.1^{a}
Total	115.2^{a}	83.2^{b}	125.4^{c}

Note: Spores density results are presented as mean ± standard deviation of five replicates per sample. Mean values in columns, followed by the same letter, do not differ significantly ($p < 0.05$) as determined by the LSD test.

Relative abundance of AMF families

The *Glomeraceae* is the most abundant family in our samples (Figure 3). This profile of the AMF group characterizes Mediterranean coastal dunes (Campubri et al., 2010; Stürmer et al., 2018). In addition, members of *Glomeraceae* are regenerated mainly from hyphal fragments that colonize roots more rapidly than members of *Gigasporaceae* that are regenerated from spores (Hart and Reader, 2002). The dominance of *Glomeraceae* shows that they are also better adapted to stressful conditions (aridity and drought) (Panwar and Tarafdar, 2006).

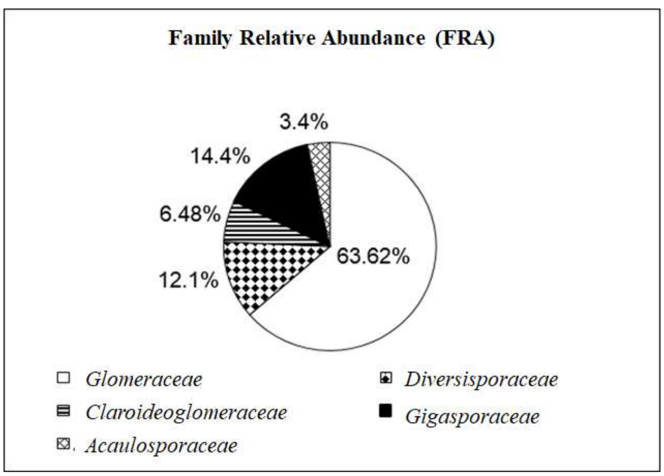

Figure 3. Relative abundance (%) of AMF families in rhizospheric soils of *P. coronopus* from the three sites considered.

Relative abundance of AMF species

The relative abundances of *Rhizophagus* sp. and *Septoglomus constrictum* is higher than other species with an average rate of 16.94% and 12.08%, respectively, in the three sites (Figure 4). The presence of *Rhizophagus* sp., *Septoglomus constrictum*, *Glomus* sp.1, and *Claroideoglomus claroideum* is high in site S1 with 19.79%, 14.93%, 11.12%, and 8.68%. The relative abundances of *Glomus* sp.2*, Dentiscutata* sp.2, *Funneliformis verruculosum*, *Diversispora spurca*, *Diversispora epigaea*, *Funneliformis geosporum* and *Scutellospora* sp. are high at site S3 with values of 11.16%, 7.97%, 7.82%, 7.17%, 6.7%, 5.58%, and 5.26%, respectively. *Dentiscutata* sp.1, *Septoglomus deserticola*, and *Entrophospora* sp., are represented in site S2 with values of 12%, 6.97%, and 6%, respectively, rather than in the other sites. Estrada et al. (2013) showed that *Septoglomus constrictum* was the most abundant species in the rhizosphere of *Asteriscus maritimus* growing on coastal dunes in Spain. The results of the present research show the existence of *Septoglomus constrictum* in all sampling sites, but it is not the most abundant. *Rhizophagus* sp., which is transparent, is characterized by its small size and is frequently detected in the sites. This proliferation may be due to its high dispersal capacity.

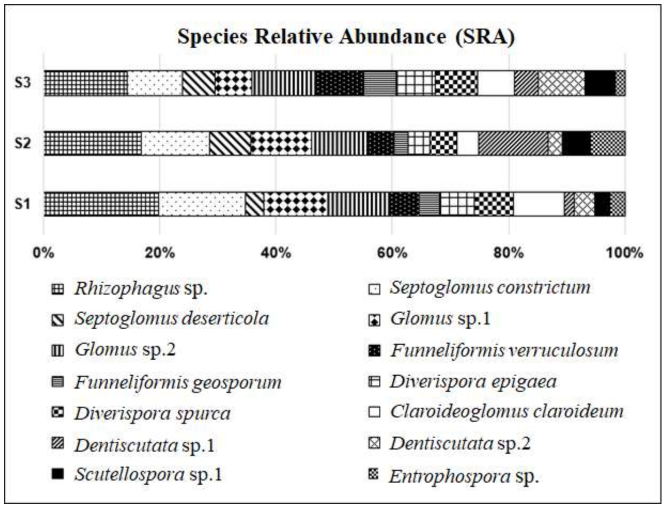

Figure 4. Relative abundance of AMF species identified in *P. coronopus* rhizospheric soil from the three sites considered. The number of AMF species (species richness) for each site is 14.

Conclusion

This study is the first inventory report concerning the mycorrhizal fungi existing in the rhizosphere of *P. coronopus*. It showed that this plant has high spore densities and a range of fourteen AMF morphotypes in this fragile ecosystem. These AMF species can be used in the production of inoculums to ensure the functional balance of coastal dune ecosystems. This research needs to be expanded by multiplying the sites, applying a more complete spatio-temporal sampling across the Algerian and Mediterranean coastal dunes to study in detail the mycorrhizal and fungal flora of the Mediterranean region.

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