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Evaluation of the mycorrhizal potential of *Hedysarum flexuosum* L. in relation with the soil chemical characteristics in the northwest of Morocco

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Abstract

This study was set up to evaluate the mycorrhization of *Hedysarum flexuosum* L., in relation with the soil chemical characteristics in the northwest of Morocco. A detailed description of the mycorrhizal associations in this specie soil and roots is reported for the first time in this study. Rhizospheric soils were randomly collected for spores' enumeration and morpho-anatomic identification, composite soil sample was taken for physicochemical analysis, from each site; three plants were sampled to evaluate roots mycorrhization. Spores number was between 1230 and 5840/100g of soil. The encountered genera were: *Scutellospora, Glomus, Acaulospora,* and *Septoglomus*. Mycorrhizal frequency and intensity have respectively reached 97.78% and 45.01%. The roots had typical endomycorrhizal structures: coils, arbuscules, hyphae, and vesicles. The rate of K_2O, Nitrogen and P_2O_5 in the soils was respectively between 51.2 ppm and 218.7 ppm, 0.061% and 0.15%, 3.5 ppm and 14.9 ppm. Principal component analysis (PCA) showed positive and negative correlation between the mycorrhizal parameters and the minerals concentration of the soils. *H. flexuosum* is a mycotrophic legume establishing a close symbiosis between arbuscular mycorrhizal fungi. The endomycorrhizae can be a tool for the introduction of this forage in the degraded areas.

Keywords: *Hedysarum flexuosum*, soils, mycorrhizal rate, PCA.

Introduction

In Morocco, there is a need to identify suitable forages that will be incorporated in cattle diets and support low-cost meat production. Under such circumstances, legume species are important for facilitating nutrient cycling and increasing plant diversity by transferring significant amounts of symbiotically fixed nitrogen (N) to neighboring plants (Selosse *et al.*, 2006). Sulla (*Hedysarum flexuosum* L.,) is one of these interesting forage resources (Issolah *et al.*, 2011), this legume is known as an ecotype of the northwest of Morocco (Errassi *et al.*, 2018). The species are growing on marly and marly-limestone substrates where the annual rainfall exceeds 500 mm (Abdelguerfi-Berrakia *et al.*, 1991). It participates in the valuation of the fallows and their enrichment in organic (N) as well as in the soil fixation. The agronomical features of this legume are: remarkable productivity; palatability for ruminants and a high forage quality (Errassi *et al.*, 2018). However in the northwest of Morocco its populations are predisposed to an increasing anthropological pressure, overgrazing, soil degradation and climate changes (Ben Fadhel *et al.*, 2006).

The increasing demand for inexpensive and environmentally friendly agricultural practice has warranted the use of microbial fertilizers. In this association, arbuscular mycorrhizal fungus (AMF) receives the photosynthetic products prepared by the plant and in turn facilitates the plant by enhancing the availability of major essential nutrients particularly phosphorus (P) and(N). Many plants form

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symbiotic association with fungus that protects them from diseases and toxicities of heavy metals (Birhane *et al.*, 2012; Liu *et al.*, 2015). Amir et al (2019) and Raklami *et al.* (2019) show the beneficial effect of AMF on plants growth and their role in the improvement of the nutrition. Several forage legumes are mycotrophic plants like *Retama monosperma*, *Spartium Junceum* (Jihane *et al.*, 2014) and *Arachis hypogaea* (Bouhraoua *et al.*, 2015). This investigation aims to evaluate the mycorrhizal rate of Sulla in relation with the soil chemical parameters, in fact limited information is known about the mycorrhization of this species in Morocco.

Materials and methods

Sites of soil and roots sampling

For covering the principal populations of *H. flexuosum* in Morocco, survey was conducted in 6 localities (Figure 1): Boukhalef (**BO**), Pont anassers (**PA**), Achakar (**AC**), Melloussa (**ME**), Khandak Lihoudi (**KH**) and Bni garfet (**BG**).

Physicochemical analyses of the soils

3 sampling soils per site were collected in the first 20 centimeters deep. To determine the physicochemical soils characteristics, a mixture of samples gave rise to a composite sample. The main physical and chemical characteristics of the soil were determined by conventional analyzes performed by the National Center of Scientific and Technical Research (CNRST) in Rabat, Morocco.

AMF spores extraction and identification

The spores were extracted from the rhizospheric soil using the wet sieving technique (Gerdemann & Nicolson, 1963) followed by sucrose centrifugation for 2 min at 1000 rpm. The spores were enumerated, screened according to their morphological characteristics and classified to the genus level. The richness of AMF spores was calculated per 100g of dry soil. The original descriptions of species provided on the website of the INVAM (According to the latest update in July 2014) served as a reference for the identification exercise.

The roots mycorrhization rate

About 30 root pieces (1 cm)/plant were collected, cleared, stained as described by Phillips & Haymann (1970) and finally mounted on slides. Quantification of arbuscular mycorrhizal infection was performed using the notation scale described by Trouvelot *et al.* (1986). Parameters of mycorrhization were calculated with MYCOCALC software, available at http://www.dijon.inra.fr/mychintec/Myc13ocalc-prg/download.html (2016) and expressed as mycorrhizal frequency (**F**), mycorrhizal intensity (**M**) and arbuscular abundance (**A**).

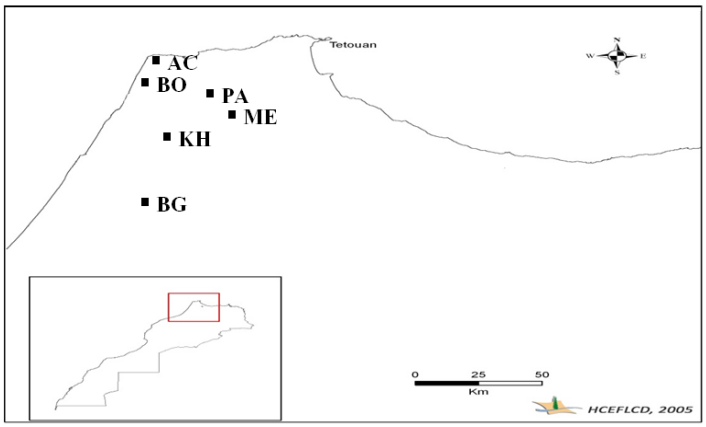

Figure 1. Location of study sites: BO, Boukhalef; AC, Achakar; PA, Pont anassers; ME, Melloussa; KH, Khandak Lihoudi; BG, Bni Garfet.

Statistical analysis

The results were statistically compared by ANOVA test. A p-value ≤0.05 was considered to be significantly different, the data were then subjected to statistical analyzes on **Xlstat** for the principal components analysis (PCA).

Results

Properties of soil

The physico-chemical analysis of the soil, summarized in (Table 1), show a sandy clay texture in (AC) and clayey in others sites, pH is alkaline for all the studied sites (pH from 7.5 in (PA) and 8.5 in (AC)). The highest concentration of organic matter (2.3%) is recorded in (PA) but only 0.2% in (BG).The contents of mineral (N) range from 0.061% in (AC) to 0.15% in (PA). The level of K_2O and P_2O_5 vary respectively between 51.2 ppm in (KH) and 218.7 ppm in (PA) and 3.5 ppm in (AC) and 17 ppm in (KH).

Richness, diversity of AMF spores

The density of spores in the soil varies between 5840 spores/100g of soil in (PA) and 1230 in (KH). Statistically, the spores' number in (PA) and (ME) is significantly high with respect to the other sites (Figure 2). A detailed analysis of the morphological characteristics revealed the presence of four genera in the order of Glomales: *Glomus*, *Acaulospora* and *Septoglomus* (Figure 3). *Glomus* was dominant in (KH); *Scutellospora* is the most abundant in other sites.

Table 1. Physical and chemical properties of soil samples. BO, Boukhalef; PA, Pont Anassers; ME, Melloussa BG, Bni Garfet; AC, Achakar; KH, Khandak Lihoudi; C, Clay; FS, Fine silt; CS, Coarse silt; FS, Fine sand; CS, Coarse sand; OM, Organic Matter; N, Nitrogen.

Sites	Texture					pH (water)	MO %	P_2O_5 ppm	K_2O ppm	N %
	A%	LF%	LG%	SF%	SG%					
BO	68.83	10.64	2.04	6.49	9.52	8	0.8	4.3	84.3	0.146
PA	58.22	7.04	8.93	5.11	6.33	7.5	2.3	14.9	218.7	0.15
ME	52.63	15.79	10.76	1.84	1.53	7.8	1.4	8.1	138.5	0.147
BG	58.2	31.75	0.39	0.85	4.55	8	0.2	7	108.4	0.113
AC	20.41	5.10	0.91	7.60	40.87	8.5	0.7	3.5	102.3	0.061
KH	47.12	26.18	12.87	2.20	1.88	7.9	0.9	17	51.2	0.097

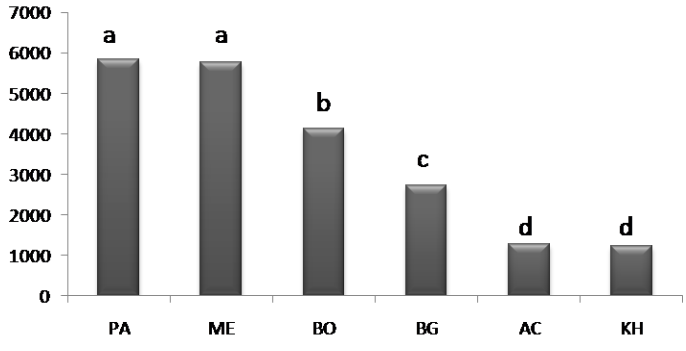

Figure 2. Abundance of the endomycorrhizal genera spores isolated from the rhizosphere of *Hedysarum flexuosum* (Data followed by different letters are significantly different, p≤0.05). BO, Boukhalef; PA, Pont Anassers; ME, Melloussa; BG, Bni Garfet; AC, Achakar; KH, Khandak Lihoudi.

Mycorrhizal rate of Sulla

The microscopic observation of the roots reveals the existence of special fungal structures (coils, arbuscules, hyphae, and vesicles). Two colonization morphologies are detected: *Arum-type* and *Paris-type* (Figure 4). All the roots were highly and densely mycorrhized. The mycorrhizal intensity has been statistically different, reaching 45.01% in (BO) and 0.95% in (KH), the highest arbuscular intensity was recorded in (BO) (28.83%) meanwhile the recorded one in (KH) reached 0.02% (Figure 5).

Principal component analysis (PCA)

The principal component analysis (PCA) allows for

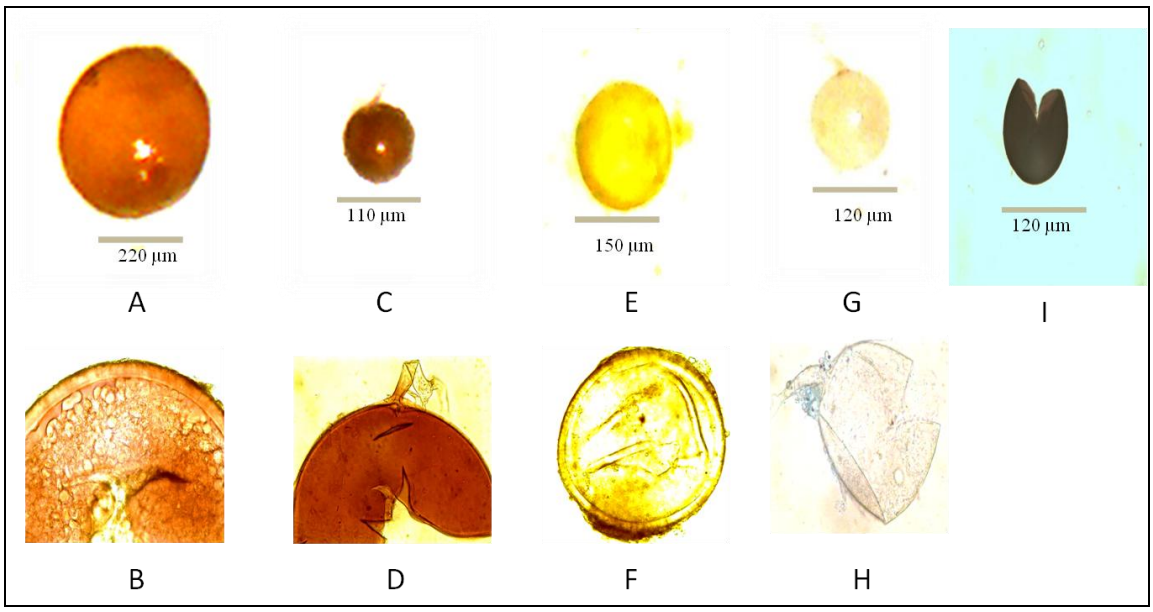

Figure 3 Spores of (AMF) encountered in the sampled soils. *Glomus* (A: Gx50; B: Gx400), *Acaulospora* (C: Gx50; D: Gx400), *Scutellospora* (E-G: Gx50; F-H: Gx400), *Septoglomus* (I: Gx50).

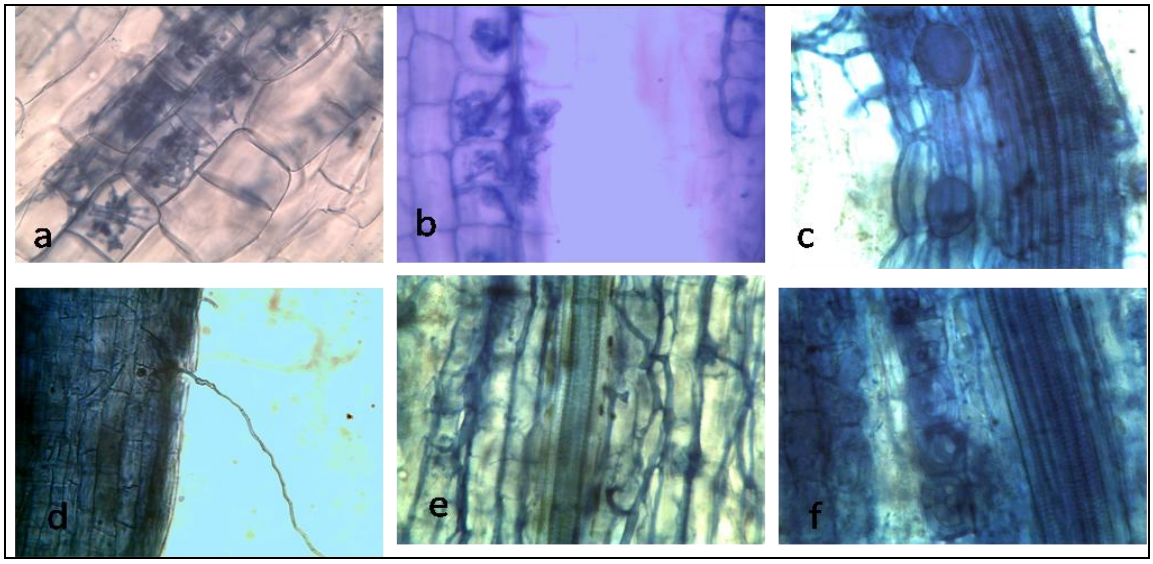

Figure 4. Mycorrhizal infection of Sulla (G: 400). a-b, arbuscules; c, vesicles; d, Extraradical hyphae; e, Intraradical hyphae; f, coils.

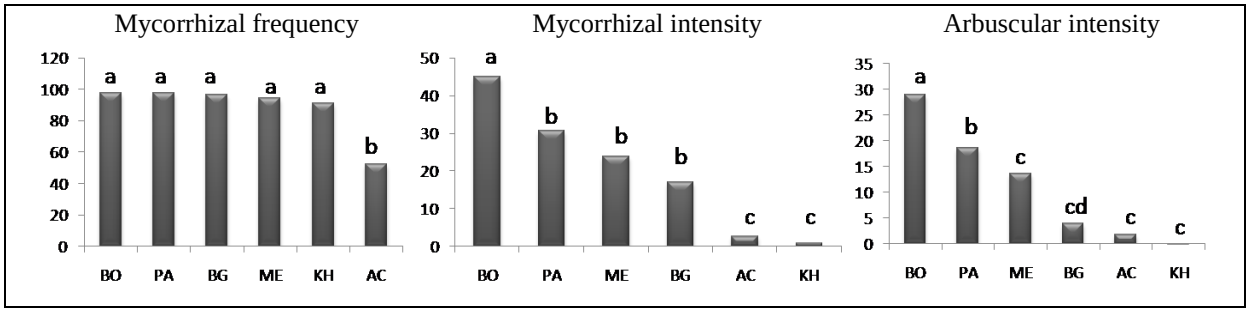

Figure 5. Mycorrrhizal parameters of *Hedysarum flexuosum* roots (Data followed by different letters are significantly different, $p \leq 0.05$).

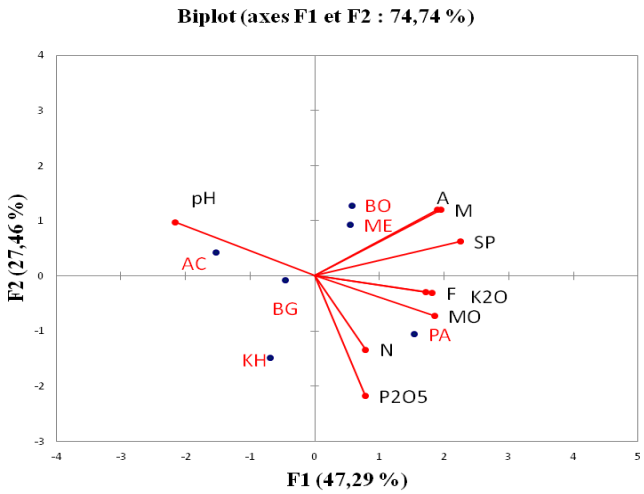

Figure 6. Principal component analysis with traits recorded on mycorrhizal parameters of Sulla plant and the chemical characteristic of soils.

graphic representations of Sulla mycorrhization parameters and the soil chemical characteristics. The two axes describe 74.74% of the total variation (Figure 6). The first axis expresses the highest rate of variation 47.29%. It is positively correlated with the spore number (SP), frequency (F), intensity (M) and arbuscular rate (A) of mycorrhizal fungi and the level of K_2O, organic matter (OM), N and P_2O_5. It is negatively correlated with soil pH. The second axis represents 27.46% of information. It is positively correlated with pH, (A), (M), and (SP). It is negatively correlated with (F), K2O, OM, N and P_2O_5.There is a negative correlation between pH and mycorrhizal properties including (SP), (F), (M) and (A).

Discussion

The pH is alkaline in all the sites, it creates more favorable environment for mineral nutrition and plant growth. It promotes the activity of beneficial microorganisms in the soil. The rate of the soils organic matter is poor or moderately poor; it will increase the soil erosion and decrease its fertility. Also, more the soil is poor in organic matter, more decreases the levels of the naturally bioavailable nitrogen. The number of (AMF) sporesof *H. flexuosum*was high and reached 5480 spores/100 g of the soil of (PA), this abundance appeared high compared with those recorded in Morocco by Abbas *et al.* (2009) and Hatimi *et al.* (2007) respectively *Tetraclinis articulata* (400/100g) and *Retama Monosperma* (63/100g). The density of spores is due to their formation, degradation and germination processes. This high abundance of the spores indicates significant mycorrhizal potential infection and biological fertility of the soil of (PA). Four genera of AMF were isolated from the sampled soils, *Glomus*, *Septoglomus*, *Acaulospora* and *Scutellospora.* According to Le Tacon (1978) AMF are non specific and present in all environment but differ by their mycorrhizal potential, their number in the soil depends on the host plant and competition with other endomycorrhizal species. Previous reports have already described that many plants from the Mediterranean area form arbuscular mycorrhizae association and have been classified as "obligatory mycorrhizal" or as "highly dependent on mycorrhiza" (Ferrol & al. 2004). For this reason, autochthonous plant species are widely used for reclaiming degraded lands in Mediterranean areas (Caravaca *et al.*, 2002). The mycorrhizal rate of *H. flexuosum* observed in this study appeared high compared with those recorded in Morocco by Abbas *et al.* (2006) (*Tetraclinis articulata*: between 27 and 57%) and Hatimi *et al.* (2007) (*Retama monosperma:* 43%). The mycorrhizal frequency and intensity reflect high soil propagules pressure on the roots of Sulla, the high arbuscular intensity means a relatively large abundance of arbuscules in the roots. These two intensities indicate the ability of fungi to spread into the root system of the plant and to establish

exchanges through the fine arbuscular ramifications. Bouamri *et al.* (2006) reported a negative correlation between the mycorrhizal intensity of root cortex and the concentration of available (P) in the soil. Indeed, the highest intensity of root mycorrhization of the plants was observed in (BO) (45.01%) where the soil is deficient on (P) (4.3 ppm), contrary in (KH) the mycorrhizal intensity is 0.95% and the (P) rate is 17 ppm. The analysis of PCA demonstrates that our result is in accordance with the study of Tibbett *et al.* (2008), they indicated that the high diversity of AMF is positively correlated with the frequency of colonization by AMF.

Conclusion

Hedysarum flexuosum L., is regarded as a mycotrophic legume establishing a symbiosis between the endomycorrhizae of the rhizosphere, the diversity of AMF in the roots provides the growth in degraded areas. This forage legume has the ability to promote the development of fungal propagules in the rhizosphere. This type of propagules can be selected and used in the restoration of degraded area of *H. flexuosum*, the production of vigorous Sulla plants in order to mitigate the anthropological pressure and the valuation of the fallows and their enrichment in organic nitrogen.

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