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Arbuscular mycorrhizal fungi and the plant chemistry of *Sulla aculeolata* spp. in Mediterranean semi-arid environments

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Abstract

This study is the first to investigate the mycorrhizal associations of Sulla (*Sulla aculeolata*) growing wild in the semi-arid region of northeastern Morocco. The physicochemical properties of the soil and the chemical composition of the plants were analyzed at two different sampling sites (Ras El-ma and Bni Chiker). Spores were extracted from the rhizosphere, counted, and identified. The most probable number (MPN) approach was used to assess the indigenous soil mycorrhizal potential. Field surveys revealed that the plant biomass was 6512.5 kg DM/hectare at Ras El-ma and 2000 kg DM/hectare at Bni Chiker. *Sulla aculeolata* from Ras El-ma had a significantly higher organic matter content (86.89%) and a lower ash content (13.11%) compared to the Bni Chiker (73.15% and 26.85%, respectively). Chemical composition analysis showed no significant difference ($P > 0.05$) in crude protein content between the two sites. Similarly, the assessment of macronutrients revealed no differences in N, P, Mg, and S between the sites. Conversely, *S. aculeolata* from Ras El-ma had a higher K content (1.75%) and a lower Na concentration (0.22%), whereas plants from Bni Chiker exhibited the highest levels of Ca (4.67%) and Na (0.6%). The fungal spore density ranged between 1123 and 2300 spores per 100 g of soil, with spores predominantly belonging to the genus *Glomus*. The indigenous mycorrhizal potential of the soil was high, reaching 6400 propagules per kg. Root analysis revealed endomycorrhizal structures, including arbuscules, vesicles, and hyphae. The mycorrhizal frequency ranged between 88.89% and 92.22%, while mycorrhizal intensity and arbuscular intensity reached 26.2% and 39.52%, respectively. Selecting and utilizing arbuscular mycorrhizal fungi (AMF) present in the rhizosphere could enhance the production of vigorous Sulla plants, supporting their preservation and domestication in Mediterranean semi-arid pastures while improving forage productivity.

Keywords – *Sulla aculeolata* – endomycorrhizae – biomass forage – chemical composition, semi--arid climate.

Introduction

Introducing new legume species with high forage yield and nutritive value can be beneficial in increasing low-cost meat production. In 2020, the demand for forage in Morocco had reached nearly three billion units of feed. The decline in soil fertility has been a significant constraint to developing the forage sector, particularly in semi-arid ecosystems. The excessive use of chemical fertilizers is potentially hazardous to the environment and the proliferation of soil microbial populations.

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According to Luan et al. (2020), chemical fertilizers not only increase production costs but also reduce overall yield.

Sulla (*Sulla aculeolata*) is native to temperate Europe and the Mediterranean basin. This legume has been cultivated for centuries due to its high nutritional value and ability to enrich soils. The most beneficial characteristic is its ability to fix atmospheric nitrogen through a symbiosis with rhizospheric rhizobacteria. Such a process enriches the soil with nitrogen, benefiting other plants. The flowers attract pollinators like bees, which is crucial for the reproduction of many plant species in their habitats. The species participates in the valuation of the fallows by adding organic nitrogen and fixing soil. Sulla is a legume characterized by high protein content and its palatability for ruminants. Forage contains moderate levels of condensed tannins. De Koning et al. (2003) reported that in Australia, the agricultural productivity of Sulla reached 26 t/ha in the second year. This Fabaceae is also used as an ornamental and medicinal plant to treat a variety of ailments such as fever, cough, and cold. Studies on Sulla focus on its ecological roles and applications in sustainable agriculture due to its nitrogen-fixing abilities. *Sulla aculeolata* is rare and even endangered in northwestern Morocco, this genetic erosion led to symbiosis changes, specifically, a reduction in mycorrhizal symbiosis (Duponnois et al. 2013).

Arbuscular mycorrhizal fungi (AMF) are the most ancient and widespread mycorrhizal type, formed by > 80% of plants (Harrison et al. 2012) and involving 240 fungal species (Spatafora et al. 2016). AMF belong to the Glomeromycota phylum (Brundrett & Tedersoo 2018). The positive effects of this symbiosis on plant development and quality are well demonstrated (Begum et al. 2019). In fact, the fungi have been found to increase soil nutrient content, the phosphorus (P) uptake (Püschel et al. 2021) and other nutrients such as N, S, Cu and Zn (Bhandari & Garg 2017).

The use of AMF can reduce the need for chemical fertilizers, promoting more sustainable agricultural practices. Plant development is attributed to the favorable effects of AMF that invade plant roots or reside in the rhizosphere, these fungi assist the host plants by beneficially modifying the rhizospheric soil properties. Additionally, AMF enhance the host plant's capacity to adapt to severe ecological conditions, such as drought, heavy metals, microplastics, pathogens, and herbivorous insects (Li et al. 2023). Although AMF are frequently found in natural soils and contribute to agricultural sustainability, cultural methods most significantly disrupt their communities and diversity (Jansa et al. 2003). Based on these circumstances, numerous studies have shown that using AMF spores or propagules for soil biofertilization results in improved plant growth and enhanced quality. Furthermore, M'saouar et al. (2024) revealed that the co-inoculation with *Rhizobium sullae* and AMF enhanced the biomass and the growth parameters of *Sulla flexuosa* compared with non-inoculated treatments.

Since the systematic analysis of the mycorrhizal status of *S. aculeolata* has not yet been conducted, the purpose of this study was to (i) assess the soil mycorrhizal potential, (ii) determine the natural mycorrhization rate of Sulla plants, (iii) measure the AMF spore density, and (iv) evaluate the mineral content of this forage legume. In furnishing essential background information, the paper provides the basis for any Sulla development program in the semi-arid ecosystems through the use of AMF, as they are most beneficial in this context.

Materials and methods

Plant sampling and soil analysis

Plant samples (stems and leaves) of *S. aculeolata* were collected from three-square plots of 1m^2 set up at Bni Chiker and Ras El-ma sites across the northeastern of Morocco. Samples were harvested at flowering stage, oven-dried at 60°C, and finally crushed using POLYMIX® PX-MFC 90 D Hammer mill. Climatic parameters (annual rainfall, minimum and maximum temperatures) of the study areas were retrieved from the National Oceanic and Atmospheric Administration (NOAA) available at https://www.ncei.noaa.gov. Additionally, three topsoil samples (0–20 cm) per site were collected to evaluate the physicochemical soil characteristics. A homogeneous sample of 1kg was prepared from a mixture of collected soils and kept in a clean bag until analysis. The main physical and chemical characteristics of the soil were determined by conventional analyses performed by the National Institute of Agronomic Research (INRA), Rabat, Morocco. The densimetry method was used to determine the soil granulometry (Bouyoucous 1962). The total $CaCO_3$ determination was conducted using Bernard's calcimeter. Soil pH was measured by a Mettler Toledo SevenEasy 728 Metrohm pH meter. Organic matter was assessed using the Walkley-Black method (Walkley & Black 1934). The Kjeldahl method (ISO 11261, 1995) was used to determine total nitrogen (N), and the Olsen method to assess the available phosphorus (Olsen, Cole & Watanabe 1954). Potassium was extracted by CH_3COONH_4 at pH 7 (Bower et al. 1952) and measured using a flame photometer, model CL-378.

Shoot chemistry analysis

Major mineral elements (N, Ca, K, Mg, P, S, and Na) were determined using the wavelength-dispersive X-ray fluorescence (WDXRF) method. The samples were milled and compressed into a pellet under high pressure. Mineral content analysis was performed using eight analyzer crystals and three types of detectors: scintillation, gas-flow, and sealed xenon. Analyses were conducted at the Technical Support Unit for Scientific Research (UATRS) of the National Center for Scientific and Technical Research (CNRST) in Rabat, Morocco. Furthermore, plant samples were examined for organic matter (OM) by incineration at 550 °C for 12 hours (AOAC, 1997). Crude protein content was determined using the Kjeldahl method, involving sample digestion with sulfuric acid, ammonia distillation and absorption, followed by titration to calculate protein content using a 6.25 conversion factor.

Isolation and quantification of AMF spores

The spore extraction from the rhizosphere was achieved through wet sieving, followed by sucrose centrifugation for two minutes at 1000 revolutions per minute (Gerdemann & Nicolson 1963). After being enumerated, the spores were analyzed for their morphological characteristics and classified at the genus level. The density of AMF spores was determined by calculating the richness per 100g of dry soil. The original classification of the AMF species published on the home page of the International Collection of Vesicular Arbuscular Mycorrhizal fungi (INVAM) was used as a reference for the characterization test.

Soil mycorrhizogenic potential

The dilution method was applied to evaluate the indigenous soil inoculum potential by the well-known "Most Probable Number" method (MPN) according to the statistical outlined in the Cochran table (Cochran et al. 1950). Six dilution levels were obtained by carefully mixing the original soil at 1/10 quantity with an autoclaved sandy soil (120 °C, 40 min, two times). Five replicates were prepared for each degree of dilution. The seeds of *Sorghum vulgare* (trap plant) were pre-germinated for two days on humid filter paper (El Gabardi et al. 2019a). One germinated seed was then transferred into each of the small plastic pots filled with 100g of different soil dilutions. The complete root system was mounted on a microscope slide and analyzed at a 400x magnification under the microscope to verify the presence of arbuscular mycorrhizal structures. Data were presented as the number of AM fungal propagules in 100 g of dry soil.

Determination of AMF root colonization

To assess mycorrhizal rate and visualize the fungi structures in the plants, thirty root pieces (1cm/plant) were collected, cleared with KOH, stained in Trypan blue, and mounted on slides (Phillips & Haymann 1970). A systematic analysis was carried out to establish the abundance of AMF hyphae, arbuscules, and vesicles in the root bark. The frequency of AM colonization (F%), intensity of AM colonization (M%) and arbuscule abundance (A%) were evaluated with MYCOCALC software, available at: Mycorrhizal Manual (Trouvelot et al. 1986).

Statistical analysis

Significant differences between treatments were determined using Duncan's multiple range tests as part of SAS (version 9.1.3, SAS Institute Inc., Cary, NC, USA). The results were statistically compared by ANOVA test (P value < 0.05).

Results

Research sites and soil analyses

The study area is located in the semi-arid northeastern Morocco. From 2011 to 2022, the average annual rainfall was 353.33mm at Bni Chiker and 412.2 mm at Ras El-ma (Figs. 1 and 2). The mean minimum temperature was 10.33 °C and 2.56 °C respectively, at Bni Chiker and Ras El-ma. At Ras El-ma, the maximum temperature was 39 °C while it was 30.14 °C at Bni Chiker.

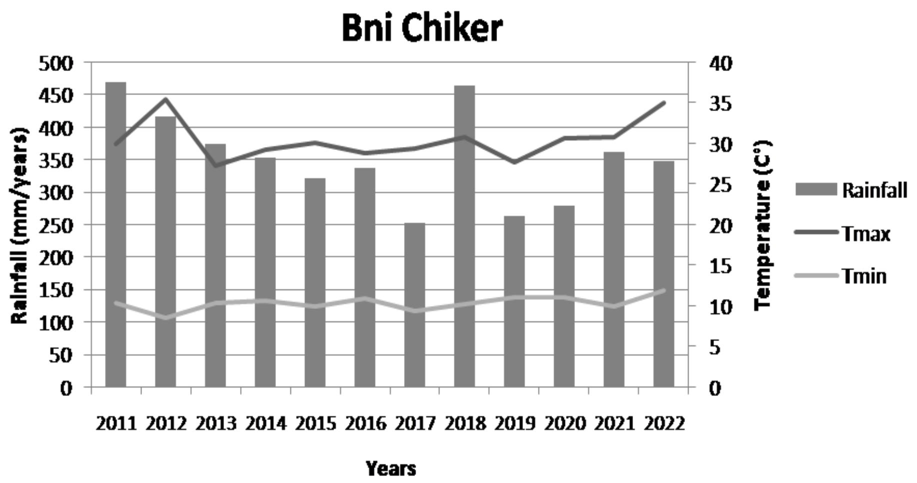


Fig. 1 – Mean annual rainfall and temperature at Bni Chiker.

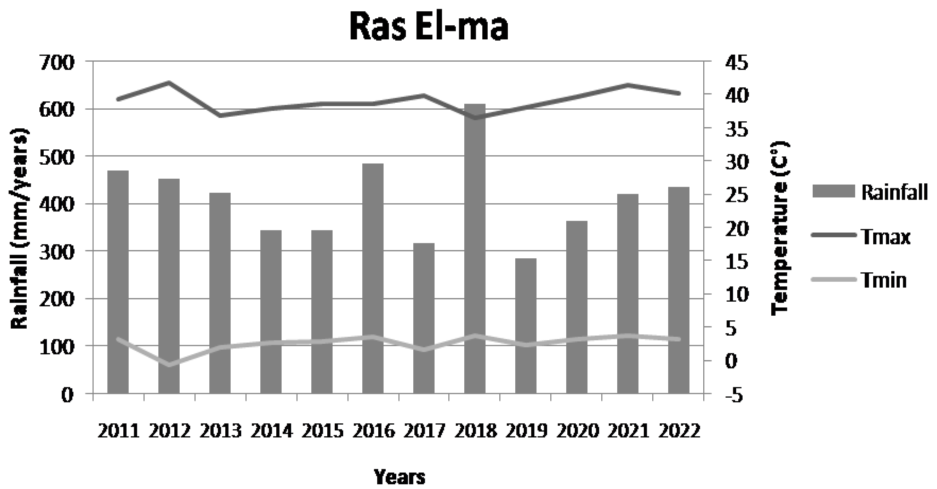


Fig. 2 – Mean annual rainfall and temperature at Ras El-ma.

The physicochemical analysis of the soil beneath *S. aculeolata* (Table 1) showed a sandy texture at Bni Chiker and loamy sand at Ras El-ma. The pH is alkaline for all the sites. The soil is generally poor in P_2O_5 and nitrogen. Organic matter content was high at Bni Chiker and poor in Ras El-ma. The highest level of K_2O was recorded in Ras El-ma, but the lowest was at Bni Chiker. The soils are enriched in calcium carbonate, particularly at Bni Chiker.

Table 1. Soil characteristics of the sampling sites.

Sites	Texture %					pH	OM	P_2O_5	K_2O	N	$CaCO_3$
	C	FSi	CSi	FSa	CSa	(H_2O)	(%)	(ppm)	(ppm)	(%)	(%)
Bni Chiker	10.2	10.2	4.1	27.4	14.4	8.8	2	5.1	111.5	0.09	33.6
Ras El-ma	5.1	20.5	17.3	35.2	0.8	8.8	0.6	6.1	250	0.08	21

C: Clay; **FSi**: Fine silt; C**Si**: Coarse silt; **FSa**: Fine sand; **CSa**: Coarse sand

Plant biomass

The results presented in Fig. 3 show that the *S. aculeolata* biomass was higher at Ras El-ma; the recorded value was 6512.5 Kg/hectare with a statistically significant difference from Bni Chiker (2000 Kg/hectare).

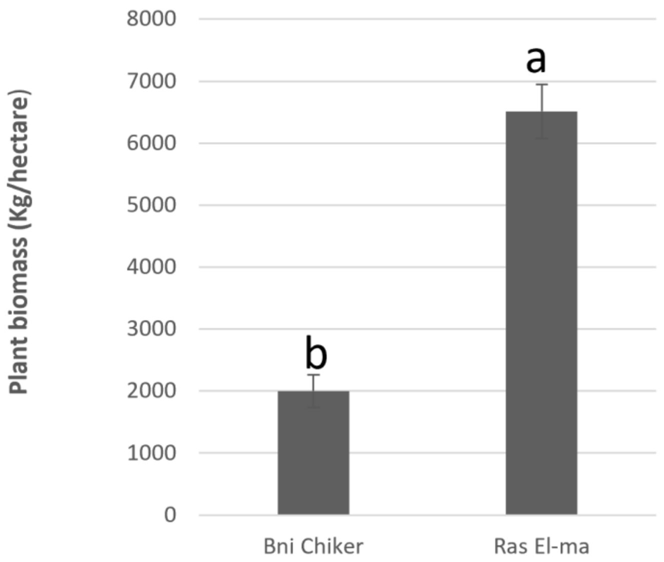


Fig. 3 – Mean plant biomass of *S. aculeolata.* Data followed by the different letters are significantly different ($P < 0.05$)

Aboveground tissue chemistry

The chemical analysis results of *S. aculeolata* are shown in Table 2. The highest organic matter concentration and the lowest ash content were obtained at Ras El-ma. The survey revealed no statistically significant (P> 0.05) difference between the sites in the rate of crude proteins.

Table 2. Mean chemical composition of *S. aculeolata.*

Sites	Bni Chiker	Ras El-ma
OM	73.15^b	86.89^a
Ash	26.85^a	13.11^b
CP	13.76^a	13.97^a

OM: Organic matter; **CP**: Crude proteins.
Data followed by the different letters are significantly different ($P < 0.05$)

Shoot mineral analysis

The study showed that sites had no significant effect (P <0.05) on the nitrogen (N), phosphorus (P), magnesium (Mg), and sulfur (S) content (Table 3). However, the above-ground tissue potassium

(K) increased significantly at Ras El-ma. The calcium (Ca) and sodium (Na) concentrations of S. *aculeolata* from Bni Chiker were statistically higher.

Table 3. Macro mineral elements present in *S. aculeolata.*

Sites	Bni Chiker	Ras El-ma
Nitrogen	2.2[a]	2.23[a]
Phosphorus	0.14[a]	0.14[a]
Potassium	0.95[b]	1.75[a]
Calcium	4.67[a]	1.11[b]
Magnesium	0.27[a]	0.24[a]
Sulfur	0.55[a]	0.62[a]
Sodium	0.6[a]	0.22[b]

Data followed by the different letters are significantly different ($P < 0.05$)

AMF spore abundance

A very abundant and diversified population of mycorrhizal fungi was revealed by assessing the spore density in the rhizosphere of *S. aculeolata* (Fig. 4). Statistically, the total number of spores at Bni Chiker was significantly higher compared to Ras El-ma.

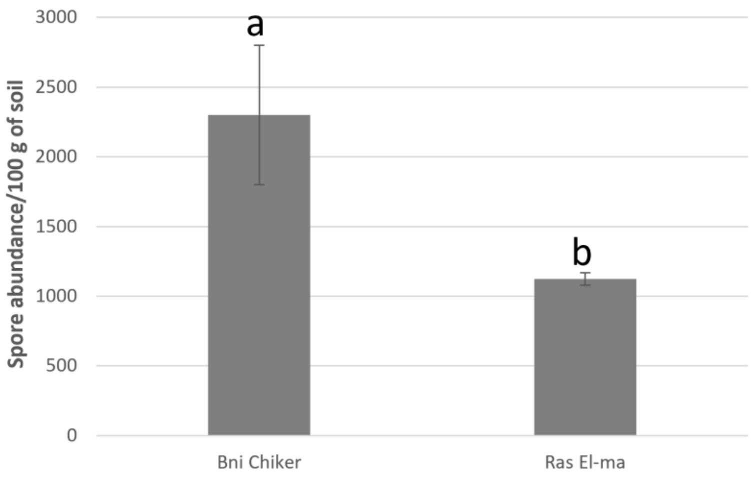
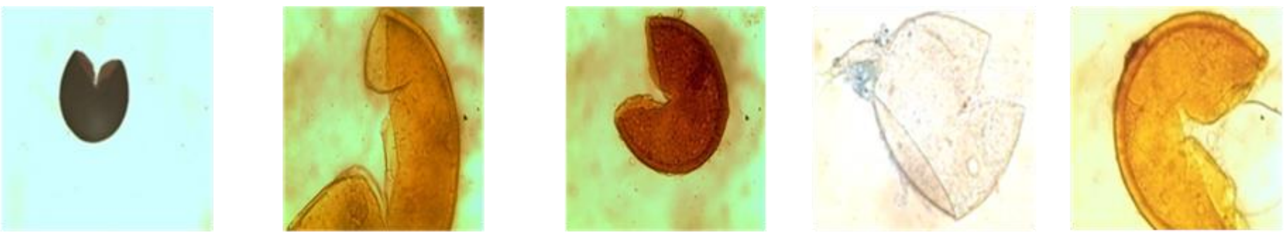


Fig. 4 – Spore abundance of the soil beneath *S. aculeolata.* Data followed by the different letters are significantly different ($P < 0.05$)

Within the Glomales order (Fig. 5), the spores were classified into three genera: *Septoglomus, Scutellospora* and *Glomus* (the most abundant). The spores were sub-spherical, with two or three walls, and varied in color. Their sizes ranged from 110 µm to 240 µm.



Septoglomus sp *Glomus sp* *Glomus* sp *Scutellospora* sp *Scutellospora sp*

Fig. 5 – AMF spores isolated from the rhizosphere beneath *S. aculeolata.*

Soil mycorrhizogenic potential

Mycorrhizogenic potential was high in both sites (Fig. 6). Mycorrhizal propagules in 1Kg of soil were abundant; they reached 3500 at Ras El-ma and 6400 at Bni Chiker. AMF propagules exist as spores, colonized root fragments, and vegetative hyphae.

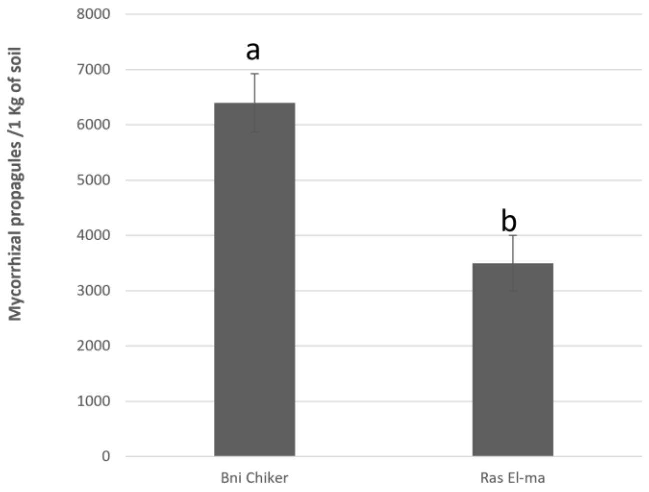


Fig. 6 – Mycorrhizal potential of soil beneath *S. aculeolata*. Data followed by the different letters are significantly different ($P < 0.05$)

Mycorrhizal rate of Sulla

Based on the root examination, the plants were mycorrhized and densely colonized, with varying mycorrhizal rates depending on the site (Fig. 7). The mycorrhization frequency of *Sulla aculeolata* was 92.22% at Bni Chiker and 88.89% at Ras El-ma. At Bni Chiker compared to Ras El-ma, there was a higher mycorrhizal intensity in the plant roots. The mycorrhizal intensity and frequency did not vary significantly between the sites. Finally, the arbuscular intensity was significantly higher at Ras El-ma.

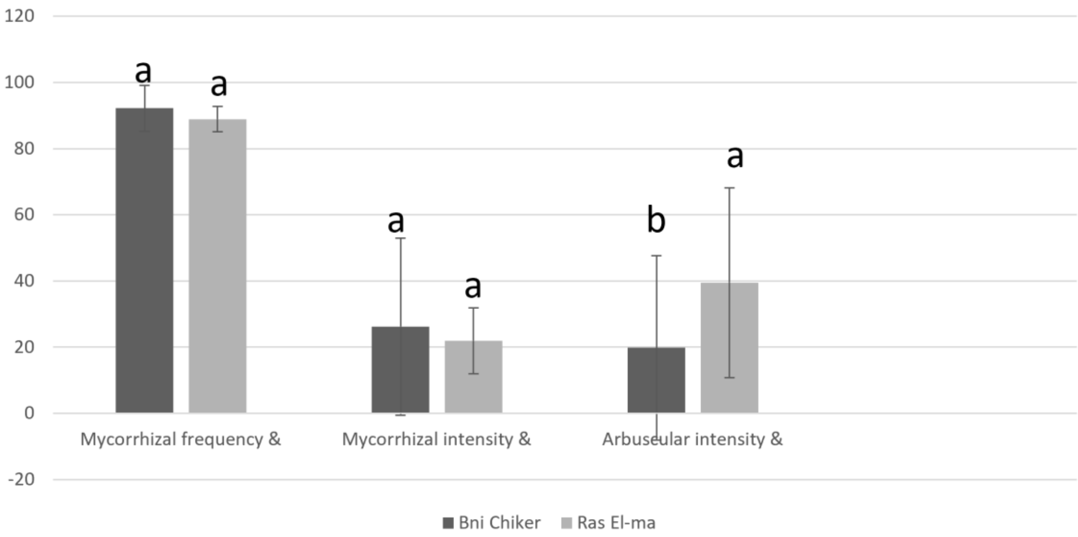


Fig. 7 – Mycorrhizal parameters of *S. aculeolata* roots. Data followed by different letters are significantly different ($P < 0.05$)

The root observation revealed the presence of endomycorrhizal structures in the two sites, such as coils, arbuscules, hyphae, and intraradical spores (Fig. 8). The non-mycorrhized roots were extremely rare. The majority of the hyphae were coenocytic, while septate forms were infrequently observed.

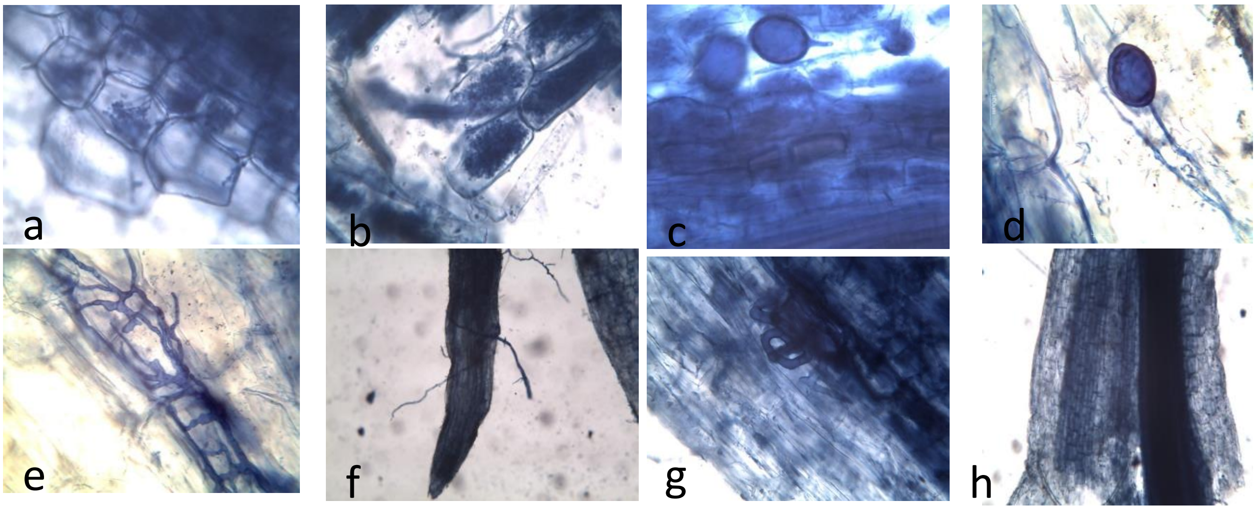


Fig. 8 – Mycorrhizal infection of *S. aculeolata*. (G: 400). a–b arbuscules. c–d spores at the root cortex. e intraradical hyphae. f extraradical hyphae. g coils. h Non-mycorrhized root

Discussion

Our study highlighted a previously unknown AMF related to *S. aculeolata* in two semi-arid regions of northeastern Morocco. It emphasized the need to understand the link between these ubiquitous plant root symbionts and the shoot chemical composition. Soil analyses are thought to be crucial for understanding the diversity and abundance of AMF. The soils beneath this legume tend to be deficient in phosphorus and nitrogen, while potassium is typically abundant. Compared to Bni Chiker, the soil of Ras El-ma had less organic matter.

There is a lack of data on the chemical composition of this species, thus, it is crucial to screen this species in order to assess its potential use in ruminant diets. The species had high crude protein levels compared to other Sulla species, such as *Sulla pallida*, which had only 12.5%, but less compared to *Sulla spinosissima* (22.7%) (El Yemlahi et al. 2024). *Sulla aculeolata* can establish symbiosis with rhizobia, affecting crude protein content (Kishinevsky et al. 2003). The bacteria provide most of the nitrogen needed by the host plant, with some efficient species providing more than 90% (Sulas et al. 2009, Fitouri et al. 2012). Compared with some forage species currently used as fodder in Mediterranean semi-arid pastures, this forage legume showed a higher organic matter content (Molina et al. 1997, Aboukhalid et al. 2002, Haddi et al. 2009, Louhaichi et al. 2021, Nunes et al. 2022). Likewise, the aboveground parts contained a higher ash rate than other Sulla species. El Yemlahi et al. (2024) found less ash in the shoots of *Sulla spinosissima* (20%) and *Sulla pallida* (14%). Aboveground biomass is a key ecosystem component and an essential indicator of plant development (Fu et al. 2013). S*ulla aculeolata* shoot biomass reached 6512.5 kg/hectare; the increased biomass content, especially at Ras El-ma, may be linked to high mycorrhizal rates, particularly to its arbuscular mycorrhizal intensity. In relation to the recommended range of mineral elements for ruminants, *S. aculeolata* has significant levels of Ca and Mg and is moderately below the mineral requirements for other nutrients (McDowell 1997).

Analysis of AMF spores and propagules isolated from the soil revealed that they were generally abundant, showing an important mycorrhizal potential. These results are in agreement with those of M'saouar et al. (2019) beneath *Sulla flexuosa* (5840 spores/100g of soil). *Sulla aculeolata* has the ability to nourish and biofertilize the soil through the production of mycorrhizal propagules. This biofertilization is crucial for reducing ecological stress effects and improving sustainable ecosystem management (Elkoca et al. 2008).

According to Rivaton (2016), the high spore abundance may be attributed to the soil sandy texture. Moreover, a positive and statistically significant correlation was demonstrated between the spore density and soil organic matter exceeding 0.5% (Mohammad et al. 2003). The soils beneath *S.*

aculeolata were very poor in P_2O_5 (< 15 ppm) (El Oumlouki et al. 2014). The phosphorus depletion may be explained by the degraded ecosystem, nonetheless, it has been shown that plant tissues absorb high phosphorus amounts (Azcon & Barea 1997). Phosphorus is essential to the germination and development of AMF spores. Soils with decreased phosphorus availability may have higher spore concentrations (Pyoabalo et aal. 2021). Sulla grows on alkaline and calcareous soil (Gagnard et al. 1988), due to high pH (8.8) and $CaCO_3$ levels. Calcareous soils often have more than 15% $CaCO_3$; the majority of soil nutrients, including N, P, K, and S, commonly remain less accessible to plants (FAO 2020, Pasricha et al. 2000, Taalab et al. 2019). To overcome the soil's mineral depletion, *S. aculeolata* develops a beneficial symbiosis with the rhizosphere endomycorrhizae. These symbionts produce compounds that modify the biochemistry of plant-fungal interaction and promote the dissolution of minerals, thereby releasing nutrients for plant uptake (Hinsinger et al. 2011).

Morphological identification of AMF is essential to confirm, with some certainty, the identity of the species. The spores are important and convenient for identifying mycorrhizae faster than other approaches (Fall et al. 2022). AMF biodiversity showed the presence of three genera based on detailed morphological analysis: *Glomus*, *Acaulospora*, and *Septoglomus*. Other authors have also noted similar results. Kachkouch et al. (2012) isolated five genera from the soil beneath olive trees; Meddich et al. (2017) found three genera in Marrakech and Tafilalet palm tree soils. Most of the species belong to the genus *Glomus*, which generally dominates AMF communities in Moroccan soils (El Hazzat et al. 2017, El Khaddari et al. 2019, el Aymani et al. 2019, Artib et al. 2019, El Gabardi et al. 2019b, Maazouzi et al. 2021, 2023, Sellal et al. 2021). Additionally, this genus is widespread globally, from the dry forests of Ethiopia (Tesfaye et al. 2004) to the tropical rainforests of Mexico (Haas et al. 1990). *Glomus* dominates due to its high reproductive capacity (Bever et al. 1996, Ourras et al. 2021, Sellal et al. 2024) and its competition with indigenous AMF (Moreira et al. 2003). Significant mycorrhizogenic potential reflects a well-functioning soil (Chantelot 2003); this parameter is considered adequate above 1500 propagules per Kg of soil. Thus, at Bni Chiker, the assessment showed the highest mycorrhizogenic potential. In this site, *S. aculeolata* enriches the soil with the mycorrhizal propagules. Likewise, *S. aculeolata* has a dense branching and taproot system, which enables it to multiply and effectively trap mycorrhizal fungi.

If the spore analysis reveals diverse AMF, the differences in mycelium and mycorrhizal structures provide clear confirmation of this fact. Inside the roots, mycorrhizal fungi create vesicles, arbuscules, and hyphae, which are storage, transfer, and transport structures, respectively. *Sulla aculeolata* exhibited a greater root colonization rate. Currently, there is no information available regarding its mycorrhizal status. However, significant mycorrhizal colonization suggests a high dependence on mycorrhizae. Mycorrhizal frequency and intensity in Sulla roots reflect high soil propagule pressure. Considering the abundant arbuscules, it appears that there is high exchange intensity between the fungi and *S. aculeolata*. The extent of fungi's spread within the roots can be clearly indicated by these parameters. Sulla appeared to be more receptive to the establishment of mycorrhizal structures. The sampling period may have contributed to the high frequency of root mycorrhization; indeed, Birhane et al. (2012) proved that spring is the most frequent colonization season. Also, the high mycorrhizal intensity is negatively correlated to the available soil phosphorus concentration, as found by Dai et al. (2014). In addition, Bowen (1987) found that the root colonization increases up to 35 °C, which could be confirmed for *S. aculeolata* based on the temperature of the sampling site. According to Diagne & Ingleby (2003), beyond 12% mycorrhizal intensity, the benefits obtained by the plant symbiont are significant. Hartmann et al. (2008) found that the plant genotype influences both the quantity and quality of rhizo-deposits, thereby; the change in root exudates necessary for AMF proliferation may lead to an increase in mycorrhizal values of *Sulla aculeolata*.

The diversity of endomycorrhizal structures at the roots, such as the thickness and appearance of the mycelium, as well as the shapes of the arbuscles and vesicles, suggests a morphogenetic richness of the mycorrhizal community living in symbiosis with this legume. Our study revealed variation in hyphal thickness, which is consistent with the results of Zubek et al. (2008) and Giovanetti et al. (2010), who mentioned that the thickness of fungal hyphae varies between 2 and 20 μm. According to Dodd et al. (2000) and Blaszkowski (2003), fine mycelia correspond to the genus *Glomus*. *Scutellospora* mycelia has a thick and coil-like structure. Arbuscules are tree-like structures growing inside the roots to improve the interaction between fungi and plant cells and facilitate the exchange of nutrients (Hause & Fester 2005). The hyphal networks enhance soil aggregation, aeration, and water retention, creating a favorable environment for Sulla. The growth of *S. aculeolata* in this semi-arid ecosystem may be the result of a wide network of hyphae in the soil, as demonstrated in earlier research (Zhong-Qun et al. 2007, Tahat et al. 2008). The abundance of vesicles in the roots of this legume is a valuable source with high inoculation potential (Strullu & Romand 1986, Mosse 1988). Strullu et al. (1997) hypothesized that substances accumulated in vesicles within the roots enable the plant to grow independently.

Conclusions

The current study is the first report on an in-depth description of the mycorrhizal associations of *Sulla aculeolata*. The survey was conducted at Bni Chiker and Ras El-ma, two sites in semi-arid northeastern Morocco. This forage legume grows in these environments where soils are nutrient-poor; however, its aboveground tissues contain significant amounts of chemical compounds. The results demonstrated that the plant is a mycotrophic species, known for its high mycorrhizal rate. It establishes a beneficial symbiosis with arbuscular mycorrhizal fungi, thereby enhancing its chemical composition and facilitating growth in the semi-arid ecosystems of northeastern Morocco.

Future studies are necessary to further select and identify AMF species with high infectivity and excellent adaptability to various types of environments. Fungal bio-inoculation can be used as a promising approach for the integrated conservation and sustainable management of degraded semi-arid ecosystems. Additionally, this applied method can also generate high-quality livestock fodder for optimal nutrition while avoiding chemical fertilizers.

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