

Arbuscular Mycorrhizal Symbiosis in Two Cannabis Varieties (Khardala and Critical) in Morocco

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ABSTRACT

The richness of the soil in beneficial and growth-stimulating microorganisms and their symbiotic interactions with plants is a factor to be discovered. This kind of relationships is still poorly studied in cannabis (*Cannabis sativa* L.). The endomycorrhizal status of cannabis was assessed in two cannabis varieties (Khardala and Critical) cultivated in the Taounate region located in northern Morocco. Soil samples collected from the rhizospheric soil of two cannabis parcels of khardala and critical were studied using the wet sieving method. Rhizospheric soil of khardala presents a spore density of 200 spores/100 g of soil and a combination of 12 different AMF species divided morphologically into 6 genera predominated by *Glomus* and *Funneliformis*. In the other hand, 243 spores per 100 g of soil were noted in association with critical variety, the endomycorrhizal fungi community was represented by 15 species of 6 genera predominated by *Glomus*. Contrary to expectations, cannabis is found to be mycorrhizal, this symbiotic association can be exploited in the soon future to develop profitability and cope with different types of stress.

Keywords: cannabis, critical, khardala, Morocco, mycorrhizal symbiosis.

INTRODUCTION

Cannabis (*Cannabis sativa* L) is a medicinal plant historically and widely used, because of the cannabinoids, as a medicine and even as an illicit drug. (Karas et al., 2020). It is used to reduce anxiety and insomnia, stimulates the appetite, relieves pain, and is also utilized as an anti-bacterial agent (Bourassa, 1972). The cultivation of cannabis covers a large area in Morocco where it was used for centuries. Among the northern regions, the isolated Rif mountains, characterized by poor soils at risk of severe erosion, are the main cultivation area (Afsahi, 2015). In recent years, cannabis plantations have spread south from Ketama towards Taounate and

the Fez region, and even further west, notably towards Ksar El Kebir and around Larache. This barrier zone, quite densely populated, is characterized by its own local varieties that were replaced by new hybrid varieties such as Khardala and Critical. This rapid trend towards hybrid varieties (water-intensive) is depleting the region's water resources and worsening soil conditions (Labrousse & Romero, 2001).

Different regulating biological processes can assist in the maintenance of water resources and restoration of soil structure and its fertility under interaction activation with organisms of the ecosystems (Bever et al., 1997). Among soil microorganisms communities, Arbuscular Mycorrhizae Fungi (AMF) represent a "key" component in

plant-soil systems. Known for its multiple advantages and benefits on plant production and health, these growth-stimulating microorganisms can form symbiotic relationships with plants (Paliwoda & Mikiciuk, 2020). They are the most common plant symbiosis worldwide optimizing the development of various host plants through different pathways (Van der Heijden et al., 1998). AMF association could be the best solution for plants with limited root systems such as cannabis, a plant of great economic and pharmaceutical interest. To date, due to the restrictions imposed on this type of cultivation, few studies are interested in the identification of AMF in association with cannabis.

The objective of this work is to evaluate the diversity of Arbuscular Mycorrhizae Fungi population in new hybrid cannabis varieties cultivated in the traditional fields and under environmental conditions of the Taounate region.

MATERIAL AND METHODS

Surveys and sampling

Surveys were conducted in Taounate region, mostly located at mountainous areas in the north of Morocco. The area is situated in a Mediterranean climate at an Altitude of 188m, Latitude: 34.55, Longitude: -4.93333 (34° 33' 0" Nord, 4° 55' 60" Ouest). Sampling was carried out in June (dry season) by random sampling of the soil at a depth of 20 cm.

Spore extraction

Using the wet sieving method described by Gerdemann and Nicolson (1963) for the extraction of mycorrhizal spores, 100 g of each cannabis soil composite sample was studied by immersion in 0.5 L of tap water and agitated for one minute. After rapid decantation, the supernatant is passed twice through four sieves of decreasing mesh size from 500 to 50 microns. The contents of the small-mesh sieves were recovered using sterile distilled water and centrifuged in tubes for 4 min at 9000 rpm. After removing the supernatant, 20 ml of a 40% sucrose solution was added to each centrifuge tube (Walker, 1983; Walker, 1992; Walker & Vestberg, 1998). The tubes are shaken manually and centrifuged at 9000 rpm for 1 min. Finally, the supernatant (spores suspended

in the sucrose solution) was collected in a 50-micron mesh sieve. Then rinsed with distilled water to remove sucrose, disinfected with streptomycin solution and recovered in flasks. The spores recovered were observed using a binocular magnifying glass and identified according to the species descriptions provided by The International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), the classification of Schenck and Smith (1982) and the criteria provided by Redecker et al. (2013).

RESULTS

The study of the Arbuscular Mycorrhizae Fungi related the cannabis rhizosphere cultivated in fields of the Taounate region revealed a fairly significant diversity of endomycorrhizal fungi which includes several species belonging to several genera. The number of endomycorrhizal fungal spores isolated from the rhizosphere of cannabis varied over the cannabis varieties.

The morphological study of soil spores of the khardala variety indicated the presence of 12 morphotypes: represented by *Dentiscutata heterogama*, *Glomus microcarpum*, *Funneliformis geosporum*, *Glomus mosseae*, *Glomus deserticola*, *Funneliformis verruculosum*, *Scutellospora nigra*, *Glomus constrictum*, *Funneliformis constrictum*, *Rhizophagus fasciculatus*, *Acaulospora sp.*, *Acaulospora bireticulata*. These species belonged to 6 genera (*Dentiscutata*, *Glomus*, *Funneliformis*, *Acaulospora*, *Rhizophagus*, and *Scutellospora*), 3 Families (Glomaceae, Acaulosporaceae, and Gigasporaceae), and 2 orders (Glomerales, and Diversisporales) (Figure 1). A spore density of 200 spores/100 g of soil was noted in the rhizospheric soil of Khardala variety.

In the other hand, the Critical variety soil showed 15 different morphotypes represented by the following species: *Acaulospora sp.*, *Glomus macrocarpum*, *Funneliformis geosporum*, *Glomus macrocarpum*, *Rhizophagus intraradices*, *Glomus deserticola*, *Acaulospora morrowiae*, *Scutellospora fulgida*, *Acaulospora excavata*, *Glomus pellucidum*, *Glomus versiforme*, *Glomus fasciculatum*, *Gigaspora candida*, *Glomus ambisporum*, *Glomus claroideum*. These species are representatives of 3 families (Glomaceae, Acaulosporaceae, and Gigasporaceae), and 2 orders (Glomerales, and Diversisporales) (Figure 2). with a spore density of 243 spores/100 g of soil.

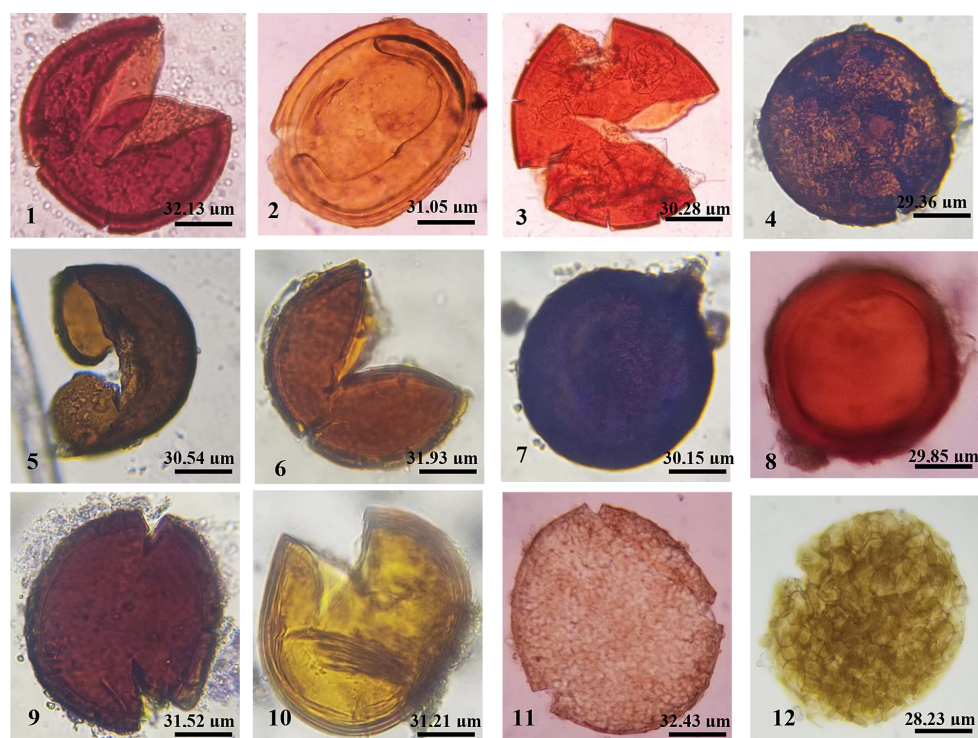


Figure 1. Endomycorrhizal species isolated from the khardala rhizosphere: 1 – *Dentiscutata heterogama*, 2 – *Glomus microcarpum*, 3 – *Funneliformis geosporum*, 4 – *Glomus mosseae*, 5 – *Glomus deserticola*, 6 – *Funneliformis verruculosum*, 7 – *Scutellospora nigra*, 8 – *Glomus constrictum*, 9 – *Funneliformis constrictum*, 10 – *Rhizophagus fasciculatus*, 11 – *Acaulospora* sp., 12 – *Acaulospora bireticulata*

DISCUSSION

Due to the restrictions imposed on cannabis production in many countries, researches on this plant are very scarce. This study is one of the few studies focused on investigating the interactions of cannabis with soil biological components. The result highlighted a significant richness of the soil of two cannabis varieties with mycorrhizal structures (spores) belonging to different genera and families.

According to Aubin and his collaborators (2016), the identification of cannabis-related mycorrhizal strains would allow a more in-depth analysis of the behavior of different species. The identification of spores would also make it possible to characterize the AMF community composition in association with this species of particular benefits and to study the complementarity or competitiveness between strains for resources or any other significant interaction. Plant species have different mycorrhizal dependencies, therefore different colonization levels and adaptation strategies to the environment. In addition, the biological mechanisms by which the specific richness of plants and their composition are regulated are still poorly known (Bationo et al.,

1986). According to the descriptions of Beaulieu and Doucet in 2013, cannabis has a taproot 15 to 30 cm in length with a tightly limited development capacity and linked to the structure of the soil. These criteria influence the development and productivity of cannabis in biomass and seeds. In an effort to reduce stress related to root physiology, resorting to the use of mycorrhizae in cannabis is not only a more environmentally friendly method, but it also optimizes root efficiency and the uptake of nutrients and water (Kakabouki et al., 2021).

One of the few studies, Citterio and his co-workers, in 2005, noted changes in growth and heavy metal accumulation in the roots of *Cannabis sativa* in presence of mycorrhizal fungus. The results showed that *Glomus mosseae* induced a reduction in biomass yield correlated with an increase in root colonization by mycorrhizae. The same study showed considerable improvement in metal translocation from root to shoot in *Cannabis sativa* L. The possibility of increasing the accumulation of metals in the shoots is very interesting for phytoextraction purposes, it helps to better exploit soil components, specifically for plants with high biomass production and low

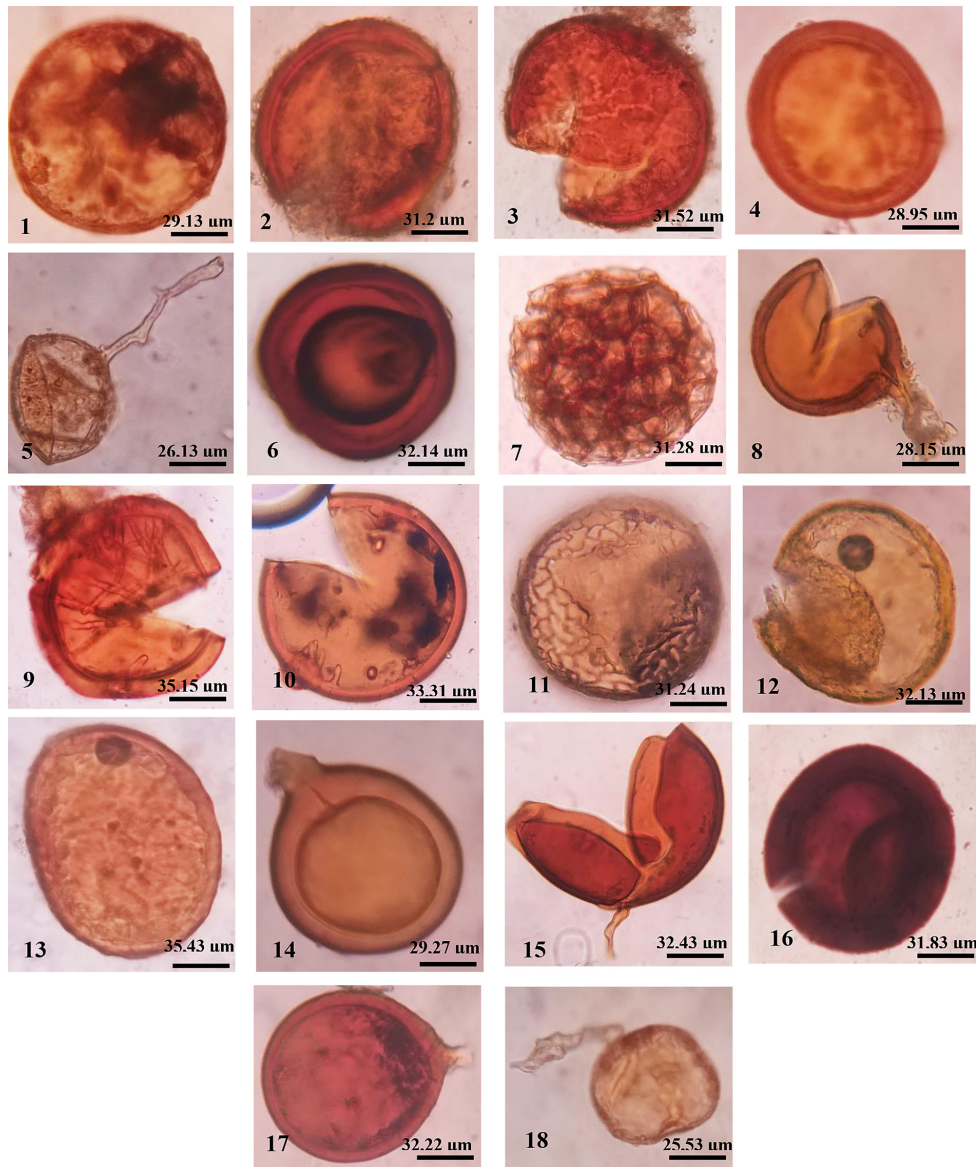


Figure 2. Endomycorrhizal species isolated from the Critical rhizosphere: 1 – *Acaulospora* sp., 2 – *Glomus macrocarpum*, 3 – *Funneliformis geosporum*, 4 – *Glomus macrocarpum*, 5 – *Rhizophagus intraradices*, 6 – *Glomus deserticola*, 7 – *Acaulospora morrowiae*, 8 – *Funneliformis geosporum*, 9 – *Glomus macrocarpum*, 10 – *Scutellospora fulgida*, 11 – *Acaulospora excavata*, 12 – *Glomus pellucidum*, 13 – *Glomus versiforme* 14 – *Glomus fasciculatum* 15 – *Gigaspora candida*, 16 – *Glomus deserticola*, 17 – *Glomus ambisporum*, 18 – *Glomus claroideum*

metal translocation as cannabis. In another crop, *Glomus* plays an important role in the overall nutrient cycle of ecosystems (Schüßler, 2001) and (Sharma & Yadav., 2013).

Aubin demonstrated in 2016 that mycorrhizal application resulted in significant effect on the yield and development of industrial hemp. Moreover, although the effect was not statistically significant, the average grain yield of the plots inoculated with mycorrhizae was higher than the yields of the control plots.

Besides cannabis, most plants are able to form a cross-relationship with mycorrhizal fungi. This

symbiotic relationship has a beneficial effect on plant growth and performance and on soil health. Mycorrhizae contribute through their networks of filaments to increase the absorption of mineral nutrients (available phosphorus) and water and also to strengthen the vigor of the plant and its resistance to biotic and abiotic stresses (Gianinazzi et al., 2010). The orientation towards the positive exploitation of beneficial fungi is a very useful tool to prevent the appearance of pests and diseases that can affect the cultivation of cannabis and to improve the health of the plant and its yield. (Msairi et al., 2021).

CONCLUSION

Mycorrhizae is a widely accepted beneficial relationship between plant roots and certain beneficial soil fungi. The analysis of cannabis soil in the Taounate region has revealed a significant diversity of mycorrhizal spores. These structures are identified and related to different genera and families. The presence of this type of fungus at the rhizospheric soil level can be considered as a good sign that can be exploited in more advanced research aiming to improve the performance of the cannabis root system.

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