Chapter

Saffron endomycorrhizae: diversity and effect on plant growth and corm formation

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Abstract

Saffron cultivation is an important alternative for marginalized areas. Due to low soil fertility and low water availability, arbuscular mycorrhizal fungi are an essential alternative for maintaining fertility and water economy, stimulating growth and protecting plants against soil diseases. Studies on the diversity of endomycorrhizal fungi in the rhizosphere of *Crocus sativus* in Taliouine (Tinfat), located in Morocco, revealed the impact of age saffron plantations. A greater endomycorrhizal fungi density was recorded in the rhizosphere of saffron plants from plots operated for 2 years (138.66/100 g of soil) over that occupied for 10 years. Seventeen morphotypes of collected spores belong to 5 genera: *Glomus* (seven species), *Acaulospora* (seven species), *Rhizophagus*, Densicitata, and *Funneliformis* (one species). The weak endomycorrhizal species richness can be explained by the accumulation of *C. sativus* residues over time and its allelopathic effect. The beneficial effect of composite endomycorrhizal inocula, originating from Moroccan saffron plantations, was obvious in the growth of saffron plants, mother bulb number, the leaves length, root, and vegetative masses. These inocula mycorrhized over time saffron plants' roots and can sporulate at the level of the rhizosphere of these plants. The use of composite inocula, as biofertilizers, can be one of the solutions for sustainable agriculture.

Keywords: Morocco, saffron, diversity, endomycorrhizal, composite endomycorrhizal inoculums, growth parameters, bulb multiplication

1. Introduction

Saffron (*Crocus sativus* L.) is an autumn flowering plant from the *Iridaceae* family, renowned worldwide for its red stigmas, which represent the most precious spice in the world [1], a kilogram of good quality saffron produced from *C. sativus* can cost over 2000 US dollars. Approximately 150,000 flowers are needed to produce 1 kilogram of dried saffron, and to growth is amount one would typically require some 2000 m^2 under cultivation per kg harvest [2, 3]. It has been cultivated in the Mediterranean area and Near East since the ancient period, used as a condiment for food, as a dye for textiles, and in traditional medicinal preparations [4]. Nowadays, saffron cultivation gaining interest due to its beneficial health effects, including antioxidant, anticancer, anti-inflammatory, and anti-depressive properties [5]. Iran, India, Greece, Morocco, and Spain are the main saffron-producing countries, with respective average annual productions of 180 tons, 9 tons, 5.5 tons, 3 tons, and 1 ton, recorded over the period 2004–2008 [6]. Saffron is also grown in small areas in Azerbaijan, Afghanistan, France, Switzerland, Italy, Turkey, Australia, and China [1, 3]. In recent years the productivity in Iran has increased enormously, Nevertheless, Spain remains the country most associated with this spice, notably thanks to its main production area, this position makes Spain currently control the world market of saffron and more particularly that of the United States, considered to be the main market of Spain [3], packs and re-exports about 40–50% of Iranian saffron. The United Arab Emirates is also an important importer of Iranian saffron [7].

Saffron is a hardy plant that thanks to its morphology and physiology can with stand very severe climatic conditions [8]. In Morocco, the saffron sector represents a major challenge for this local product both economically and socially. This sector is a promising way to reduce poverty and income inequalities in saffron-growing areas.

Indeed, it is one of the pillars of the economy of the Taliouine-Taznakht region, which is characterized by difficult soil and climate conditions [9], high rates of poverty and income inequality, and a high level of the rural exodus [10].

The annual production has increased according to ANDZOA from 3 tons of dry stigmas and an area of 600 ha in 2009 to 6.8 tons and about 1800 ha in 2018. The cultivation of saffron has been extended and intensified in recent years (Green Morocco Plan). The objective is to reach 9 tons in 2020 according to the contract program signed in 2016 between the Moroccan Interprofessional Federation of Saffron and the government. The cultivation area in Morocco is concentrated in the area of Taliouine–Tazenakht (**Figure 1**).

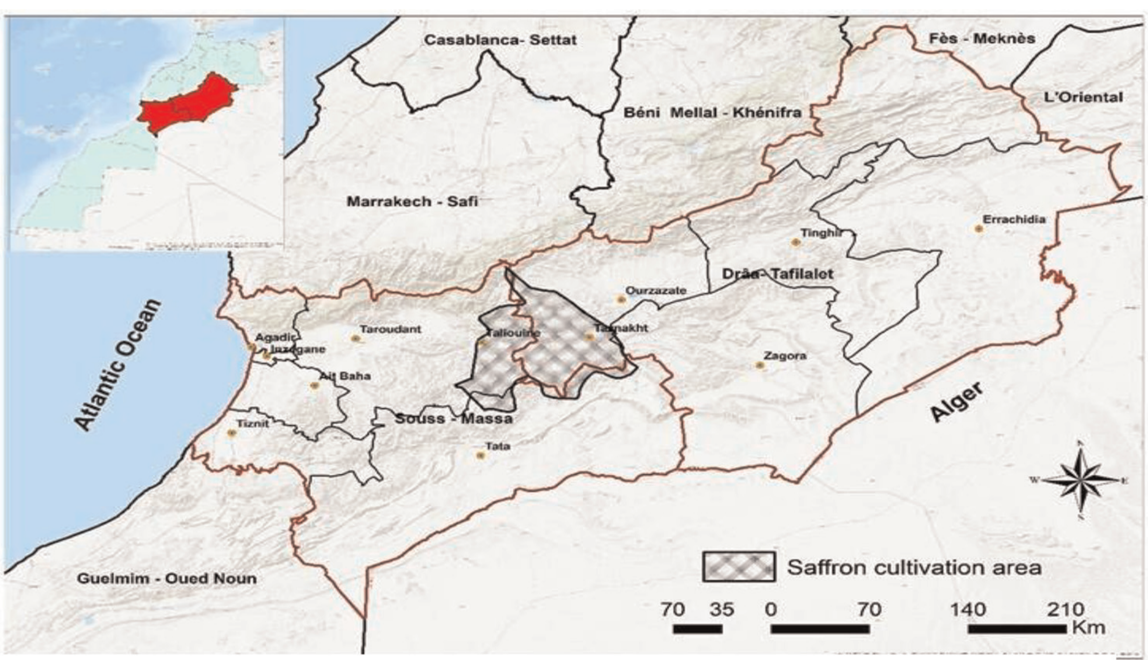

Figure 1.
Geographical distribution of saffron production area in Morocco.

According to the Regional Office of Agricultural Development (ORMVAO), the region of Souss-Massa ensures about 95% of the national production of saffron, which represents an annual turnover of 75 million DH. Around Two-thirds of the production is destined for the international market.

Moroccan saffron has a great reputation at national and international levels [11]. Data from the 'Office des Changes du Maroc' show that the main destination for "Moroccan" saffron is France. These data also show (mass imports registered with the customs services and which come essentially from the Islamic Republic of Iran or Greece. These quantities are redirected to Qatar or other lesser-known destinations. This intruding mass is cheaper, but affects the reputation of Moroccan saffron and competes with local productions.

Saffron cultivation management depends on the development of new technical production practices adapted to the pedoclimatic conditions of traditional areas for this crop. Among, the major constraints that limit its production and productivity is poor management of saffron cultivation, as it involves in adequate plant population, the incidence of corm rot disease [12], nutrient depletion, and lack of irrigation facilities. Moreover, environment and cultivation management affect strongly flower induction in C. sativus [13]. Accordingly, Increasing saffron yield and quality, reducing production costs, and flowering modulation may need new technology implementation as proposed in the Mediterranean environment viz., soilless cultivation systems were proposed but only limited and controversial reports are present in literature. Another option for saffron plant performance is through the use of biostimulants, or microorganisms applied to plants to enhance nutrition efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of the irnutrient content. Soil microorganisms such as arbuscular mycorrhizal fungi (AMF) can form a mutualistic symbiosis with about 80% of land plant species, including several crops [14]. This association provides ecological stability to the environment [15, 16], and enhances water and nutrient uptakes such as phosphorus, nitrogen, and micronutrients, thus improving plant growth and resistance to biotic and abiotic stresses [17, 18].

This chapter presents an overview of saffron cultivation in Morocco and information on the diversity of arbuscular mycorrhizal fungi in the Taliouine region. Inocula, based on these fungi, originating from the saffron rhizosphere, were tested to promote plant growth and bulb production and multiplication.

2. Mycotrophic nature of Saffron plants

As saffron does not grow from seeds, reproduction is only possible by vegetative propagation, using the corms which can withstand a long dry dormant period before sprouting. Thus, corms are indispensable to saffron propagation reproducing vegetatively into corm lets that ultimately develop into new plants (**Figure 2**) [19].

The traditional area of saffron production in Morocco is characterized by very poor soils, however, the rhizosphere of cultivated saffron supports very interesting populations of microorganisms such as endomycorrhizae that are supposed to be essential for plant growth and ecosystem functioning.

Like most geophytes, saffron produces a coarse root system; these root systems have under gone extensive mycorrhization and benefit from this symbiosis [20]. Indeed, field-grown saffron is extensively mycorrhized [21, 22]. The Saffron plant

Figure 2.
A, B: Saffron fields in Talouine region; C: Saffron plant at flowering stage.

shows a high incidence of AMF colonization. It was observed that the corm, despite being the modified stem, shows AMF colonization [23]. Morphological examination of fine roots comprised in rhizospheric soil samples collected in October 2020 from 15 different plots of saffron located in the Taliouine area (Morocco) under light microscope revealed the presence of AMF structures inside the examined roots which showed different colonization levels (**Figure 3**).

Ourras et al. [24] pointed out a high mycorrhization rate of 95.02% which reflects the inoculum pressure or propagules rate infecting the surrounding substrate. Likewise, El Aymani et al. [12] have reported different root mycorrhizal frequencies of 93.33% and 96.67% associated with the rhizosphere of *C. sativus* grown in the Taliouine region. This mycorrhizal colonization demonstrates relevant interaction between *C. sativus* roots and native endomycorrhizal populations under field conditions through which mechanisms such as modulation of water transport [25], nutrient acquisition [26], and stimulating the exudation of root phosphatase [27, 28] under drought conditions, are active in mycorrhizal plants to alleviate environmental stressors.

With the identification of native arbuscular mycorrhizal fungi and their application, it could be possible to expand the saffron cultivated area and increase the performance of arable lands.

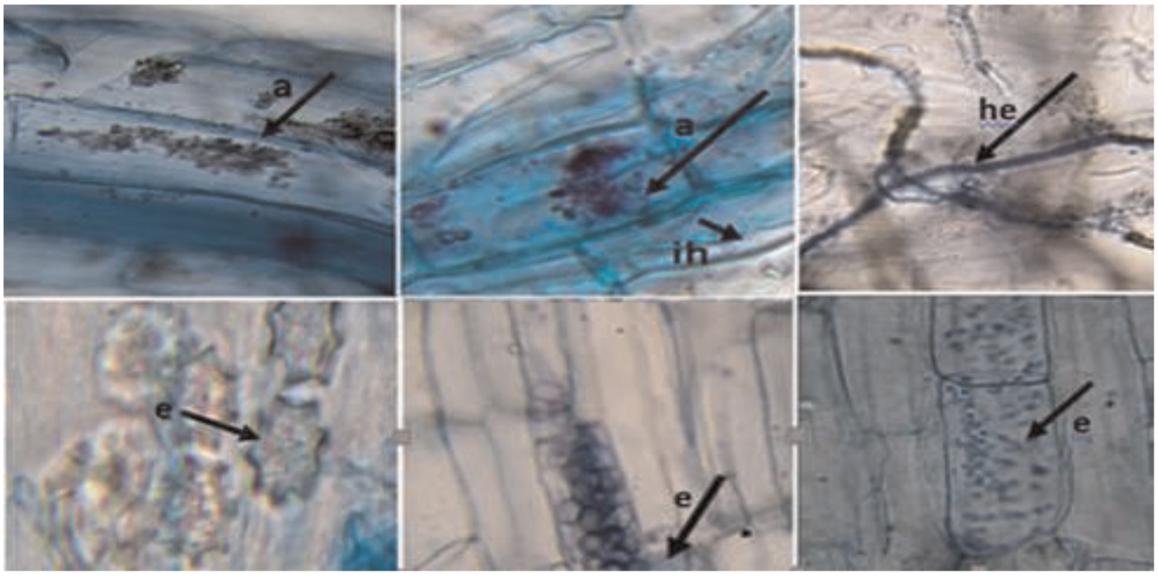

Figure 3.
AMF structures inside the examined roots of saffron plants grown in fields in Taliouine regions.

3. Diversity of arbuscular mycorrhizal fungi from moroccan saffron plantations areas

The knowledge of the current richness of Glomale an AM fungi (Glomerales; Glomeromycota) and biological diversity associated with plants grown in adverse environments could be necessary to expand the cultivated area and increase the performance of arable lands.

AMF diversity has been scarcely studied in the Taliouine-Taznakht region where saffron cultivation is exclusively concentrated [29].

This diversity was unveiled by direct spore isolation and morphological characterization of rhizospheric soil and roots samples from different sites in the main saffron production region. A variety of endomycorrhizal fungi has been noted in the rhizosphere of *C. sativus* grown in the Taliouine region (Tinfat) (**Figure 4**) [30]. These authors have detected 36 morphologically distinctive AMF species that were directly retrieved from field samples. All the encountered species in the different studied sites belong to six genera: *Glomus* (14 species), *Acaulospora* (10 species), *Scutellospora* (6 species), *Gigaspora* (2 species), *Pacispora* (2 species), and *Entrophospora* (1 species). The genus *Glomus* was the most dominant, it occurred in all studied sites with a proportion of distribution that varies between 8.33% and 30.56%.

A low number of AMF species have been reported by Ourras et al. [24]. Chamkhi et al. [31] detected 11 species of AMF spores among which *Funneliformis* and *Rhizoglomus* species were the most abundant (>35%). Yang et al. [32] reported that AMF soil community evolved in the function of environmental conditions. Another factor affects AMF survival and community composition in association with the saffron crop.

3.1 Age of plantation

Age of plantation seemed to be affected the richness of AM fungi, spore density and root colonization rates [12, 24, 31]. Indeed, the greatest richness of AM fungi was registered in the site at 4 years of successive exploitation by saffron (24 species),

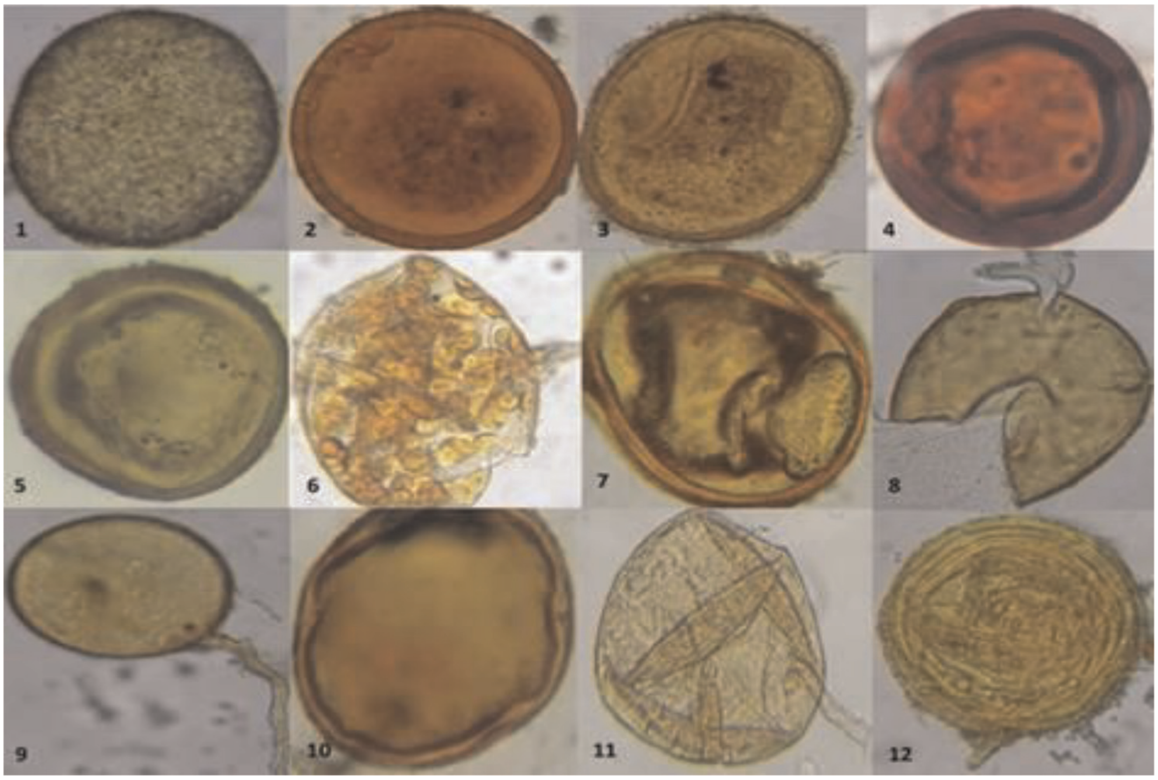

Figure 4.
Some indigenous endomycorrhizal fungals pecies isolated from the rhizosphere of Crocus sativus *grown in Taliouine areas.*

followed by the site at 6 years of occupation by saffron (21 species), while the lowest number of species was recorded at the sites of two, three and 10 years of soil use by saffron [33]. According to Yu et al. [34], stand age significantly changed the structure of the AM fungal community. The increase of spore density with field age has been cited in previous literature reviews [35, 36]. Rengifo-Del Aguila et al. [37] found that intra-radical colonization and AM fungal phylogenetic diversity increased with plantation age, while AM fungal richness was still constant across time but a significant compositional turnover was detected.

Many studies have demonstrated that the shift in the AM fungal community over ecological succession is associated with variations in soil properties induced by stand age, such as soil texture [38], pH [39], and nutrient availability [40]. Krüger et al. [41] pertain that P has become increasingly limited in old soils as the amount and availability of P declines over time. The changes in soil's chemical and physical properties can play an important role in decreasing saffron yields even after 6 years of cultivation [42].

4. AMF as biofertilizers on Saffron

Regarding the biological activity, as biofertilizers, AMF offers a way to replace, at least partially, the use of chemical fertilizers and pesticides seeing their fatal effects on the environment and health. In low soil fertility of saffron cultivation areas [43, 44], they can serve as an AMF inoculum which may be part of the solution for sustainable agriculture. Cases in point showed successful application on saffron production under controlled conditions.

4.1 Corm production

In addition to the spice yield, another economically important attribute of saffron is the number of replacement corms. Corms inoculation by composite AMF inoculum significantly initiated and stimulated the production of new corms (**Figure 5**) [30].

Such promoting effect of AMF on initiating the new underground stem propagules was observed previously too [18, 45–47]. Considering that tuber initiation in potato is hormonally mediated [48], it may be that in the present study too AMF affected hormone balance in the saffron plant, leading to earlier initiation and production of corms. Inoculation of corms with spores has enhanced the growth of corms and the number of spores per 10 g soil and results recorded as 13–52 with the highest occurrence in December–January [49]. Caser et al. [50] approved the fact that AMF symbiosis enhanced the production of replacement corms and reduced the occurrence of fungal diseases.

4.2 Growth response

Although AMF increased the number of corms produced, it increased also shoot fresh weight and root dry weight [51]. A significant increment of the overall growth parameters such as length of aerial and below ground of saffron plants occurred too after AMF colonization [30].

The use of arbuscular mycorrhizal symbionts as biostimulants positively affected saffron cultivation, improving the crop performances and the content of important nutraceutical compounds. In particular, the inoculum composed by *R. intraradices* and *F. mosseae* increased flower production and saffron yield [52]. Such compatibility between AMF fungi and host plant was previously observed in other plants also such as onion [47] and Potato [18] cultivars.

Van der Heijden et al. [53], found that in the grassland ecosystem, inoculation of 14 AM fungal species increased plant diversity by 105% and plant productivity by 42% compared to that in the ecosystem inoculated with only 1 AM fungal species. A study has also revealed that different AM fungi can absorb P at different distances from the

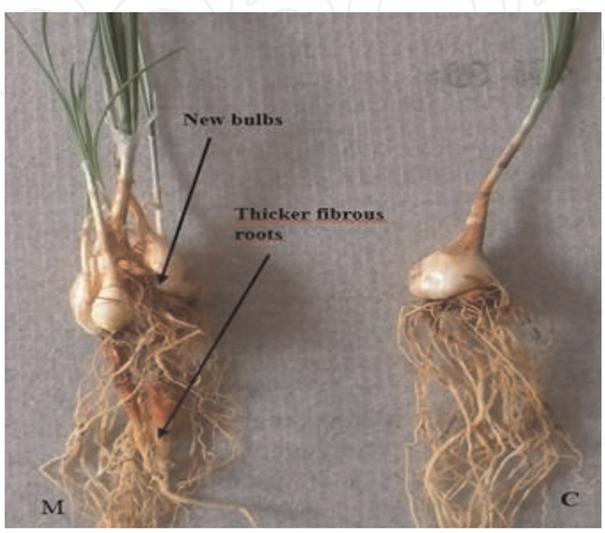

Figure 5.
New bulbs production; ***M:*** *Inoculated plant;* ***C:*** *Control.*

root of the plant, making the host plants inoculated with mixed AM fungal species grow better than those inoculated with AM fungal species.

Variations in the growth of saffron plants are possibly observed as a function of the age of saffron plantation as seen below (**Figure 6**).

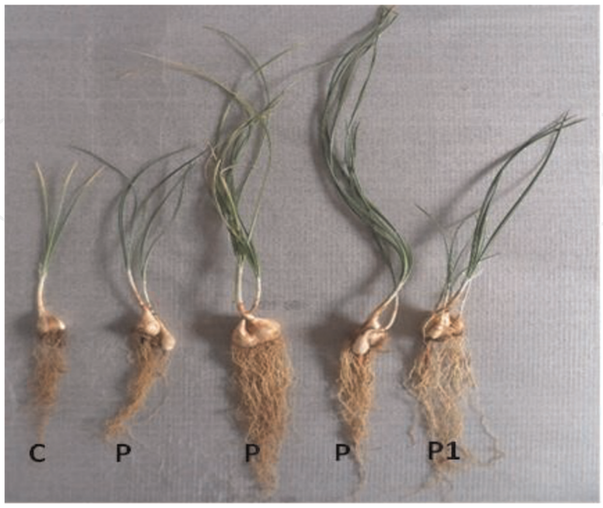

Figure 6.
Performance of Crocus sativus *plants as affected by the presence of AMF and age of saffron plantations.*

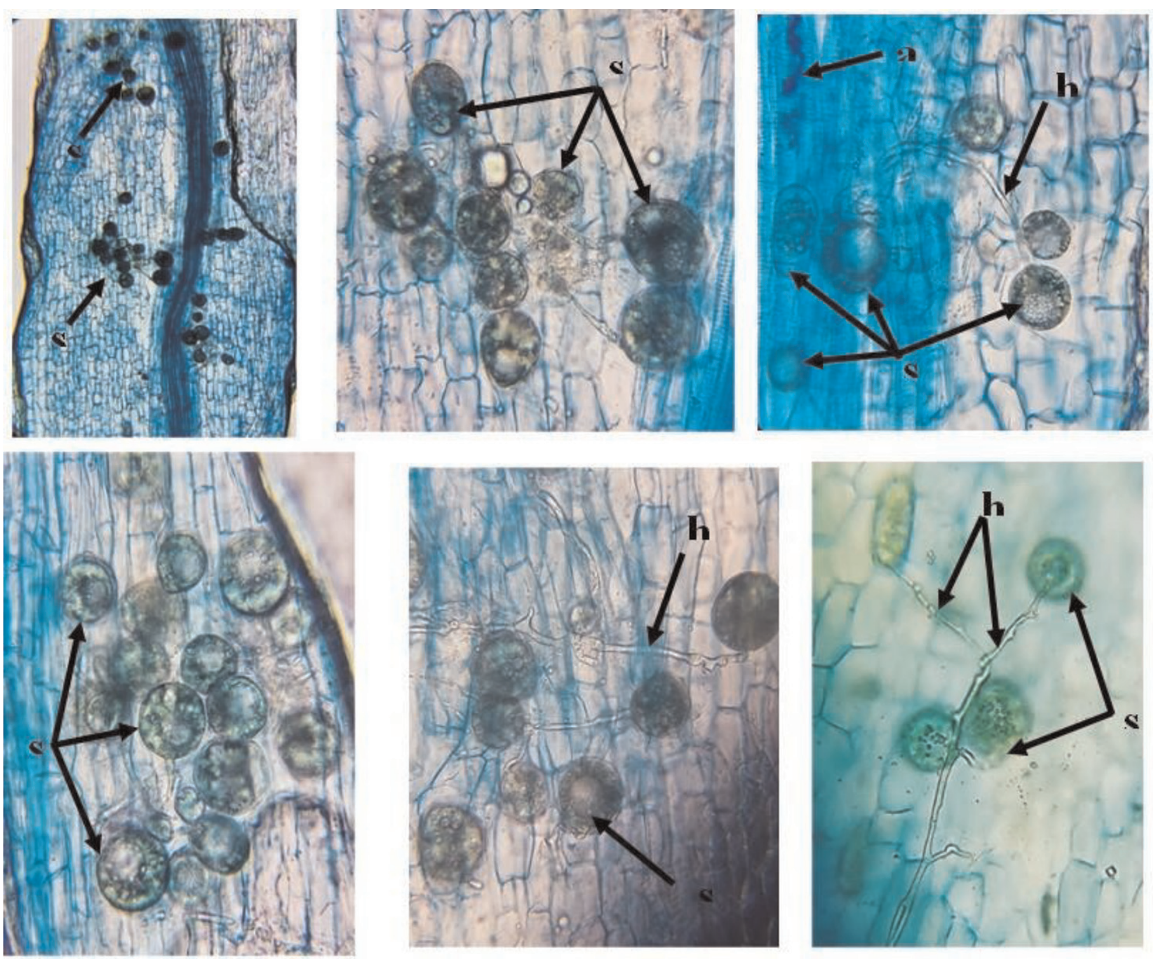

Figure 7.
Different structures of endomycorrhizal fungi colonizing the roots of Saffron plants 4 months after corms inoculation: Arbuscules (a), Spores (s) and intra-radicular hyphae (h) (G × 400).

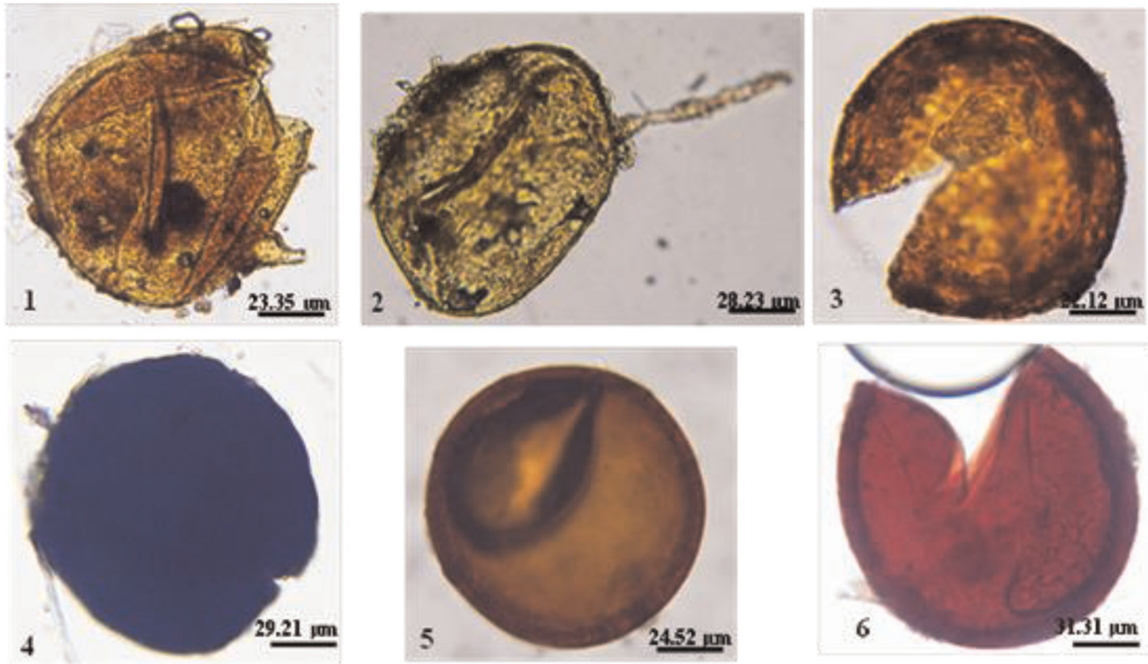

Figure 8.
Quelques morphotypes de spores rencontrés au niveau de la rhizosphère des plants du safran provenant des cormes traitées avec un inoculum endomycorhiziens (S4), après quatre mois de culture. 1: Rhizophagus intraradices*, 2:* Glomus lamellosum, 3: Funneliformis geosporum, 4: Densicitata nigra, 5: Glomus microcarpum:, 6: Glomus deserticola.

4.3 Root colonization with AMF after corms inoculation

The positive influence of AMF on plant is otherwise viewed in roots, at this level, mycorrhized plants are shown if they have been able to establish a strong mutualistic relationship with composite AMF inoculum. Four months after inoculation in potted culture, the roots of the plants from the inoculated corms are richly mycorrhized and showed numerous arbuscules and vesicles with extended hyphal colonizing groots. Arbuscules and spores were the most dominant structures (**Figures** 7 and **8**).

5. Conclusion

The growing interest in knowing and safeguarding the biological diversity associated with certain arid environments may contribute to improving ecologically the yields of agricultural crops. In the case of saffron, the rhizospheric soil harbored a diversified AMF community and its application as composite inoculum in substrate culture pertains a potential performance on growth and plant succession. As a perspective, future studies should be focused to know the influence of AMF on the growth of the saffron plant in open field conditions.

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