

Effect of Arbuscular mycorrhizal fungi on growth of semi-woody olive cuttings of the variety "Sigoise" in Algeria

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Abstract

In Algeria, the olive is produced mainly in nursery by grafting oleaster, wild species, it contracted symbioses with certain soil fungi and form arbuscular mycorrhizae, these are little studied in Algeria. Two complex CMS and CMB of arbuscular mycorrhizal fungi and two species belonging to the genus *Glomus* (*Glomus* sp.1 and *Glomus* sp.2) isolated from the rhizosphere of two olive orchards of western Algeria were inoculated to herbaceous cuttings of variety "Sigoise" olive and raised under controlled conditions in a greenhouse for 3 months. The results show that inoculation of plants by the mycorrhizal complex significantly improves the growth of plants of this variety of olive hence the interest to consider its use in the nursery.

Key words: arbuscular mycorrhizal fungi; herbaceous cuttings; olive; Algeria.

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Introduction

Algeria has many varieties of native and introduced olive trees of which 11 are currently the reputation of the Algerian olive (Chaouia et al. 2003; Douzane et al. 2010) which is concentrated in the North, especially in the up Tell to 1000 m altitude and unevenly distributed in the central region (54%), East (29%) and West (17%) (Yakoub-Bougdal et al. 2007). South of the country *Olea europaea* subsp. *Laperrinei* Batt. & Trab. , Protected endemic species is found in the wild up to 2700m altitude in the mountains of the Hoggar and Tassili Ajers (Baali-Cherif and Besnard 2005; Baali-Cherif et al. 2008; Anthelme et al . 2008; Sahli 2009). The varieties "Chemlal" Kabylia variety and " Sigoise "in the Northwest of the country (Mendil and Sebri 2006) are the dominant varietal profiles, while other varieties are the rest of the olive varietal heritage Algeria (Sahli 2009). But in recent years, the cultivation of these varieties has declined considerably due to many demographic factors, especially technical and phytosanitary increased their susceptibility to fungal diseases such as verticillium wilt (Bellahcene et al. 2000; 2005a and 2005b),

leaf spot disease (Guechi and Girre 1994). Many studies have been done to identify and characterize the genetic varietal olive to improve and develop any resistant variety to both the diversity of agro-ecological zones of production and diseases abiotic and biotic factors that threaten the Algerian orchard (Mendil and Sabri 2006; Meddah-Hamza et al. 2010). Thus, to improve the situation of the olive whose consequences are felt not only in economic but also social level, Algeria in 2007 launched an extensive program of planting 15 million trees on both olive varieties "Chemlal" and "Sigoise" in 15 wilayas particularly in steppe and Saharan areas (in the Wilayat of Naama (374 ha), Ghardaia (1124 ha) and Tindouf) (Mendil 2009). Now, Algeria is produced olive nursery mainly by grafting onto oleaster (wild) and this mode of propagation is not very practical for obtaining intensive olive plantations (Abdul Hussain and Abdul Hussain 2004), to remedy this, some nurseries use micropropagation which provides very fast plants with a lower cost of production (Benderradji et al. 2007; Yakoub-Bougdal et al. 2007).

Otherwise, the olive tree is mycorrhizotrophe that form arbuscular mycorrhizae (AM) (Roldan-Fajardo and Barea 1986) and application of mycorrhization may enhance the production of olive in nursery.

Many studies have shown that controlled mycorrhization of semi woody cuttings of olive by AMF (arbuscular mycorrhizal fungi) allows better growth of this ligneous, promotes its adaptation to local soil conditions and acclimation (Rai 2001; Ganz et al. 2002; Porras Soriano et al. 2002; Estaun et al. 2003 ; Porras Soriano et al. 2010) and also its resistance to abiotic stresses (drought and salinity) and biotic attacks by pathogens and pests (verticillium, peacock spot (cycloconium), ringworm of the olive tree) after their planting (Caravaca et al. 2003; Castillo et al. 2006; Porras-Soriano et al. 2006 and 2009; Kapulnik et al. 2010). olive semi woody microcuttings of many varieties from greenhouse nebulization were mycorrhized with different *Glomus* species: "Cornicabra" variety with *Gl. intraradices*, *Gl. mosseae* and *Gl. claroidium* (Porras Soriano et al. 2002; Porras Piedra et al. 2005), and those varieties "Arbequina, Leccino and Picual" with *Gl. intraradices*, *Gl. mosseae* and *Gl. viscosum* (Calvente et al. 2004, Castillo et al. 2006), and vitro-plants of varieties "Agladau, Tench and Laragne" with *Glomus mosseae* (Binet et al. 2007).

In Algeria, arbuscular mycorrhizal fungi are little studied. Meddad-Hamza et al. (2010) have shown the effect of two native species of *Glomus* (*Gl. intraradices*, *Gl. mosseae*) isolated from the olive rhizosphere varieties "Blanquette and Rougette" of north eastern Algeria (Wilaya of Skikda) on growth and resistance to water stress during transplantation vitro-plants of the variety "Agladau" olive.

No study has been done on the characterization of local indigenous strains and their ability to improve production in the nursery softwood cuttings of the main olive varieties from Algeria where the objective of our research.

The purpose of this study is to test the controlled mycorrhization of softwood cuttings of the variety "Sigoise" olive (*Olea europea* L.) by CMA isolated from the rhizosphere of two groves in western Algeria and demonstrate their effectiveness on the growth and development of olive cuttings.

Materials and methods

Fungal material

Two complex mycorrhizal (CMS and CMB), and two species of *Glomus*, *Gl. sp1* and *Gl.sp2* (denoted (I1) and (I2), respectively) isolated by the method of wet sieving (Gerdemann and Nicholson 1963) from the rhizosphere soil of cultivated olive tree in two orchards of which one is located in Sig (CMS and I1) northwest Algeria (35 ° 31'44 .2 "N 0 ° 11'29" W) and the other at Bechar (CMB and I2) southwest (31 ° 37 '00 "N 2 ° 13 '00" W). CMS is composed by a diversity of 4 species of *Glomus sp. And Acaulospora sp.*, where CMB is constitute of 6 morphotypes of *Glomus* comprises *Gl. Macrocarpum* and *Gl. multicaulis* and other species of *Acaulospora sp.* and one *Scutellospora sp.*

AMF were multiplied by trap culture on leek (*Allium porrum* L.) in the greenhouse disinfected soil from the two olive orchards for four months.

Plant material

Semi-woody cuttings of the variety "Sigoise" rooted olive, aged of two months produced and acclimatized in a greenhouse at the experimental farm nebulization El Matmer (Relizane, North West Algeria) in a greenhouse were inoculated with AM fungi or not and grown in pots 400cm³.

Inoculation of semi-wood cuttings of olive

The semi-hardwood cuttings of olive were transferred into pots 12 cm high and 10 cm diameter polyethylene brown filled with 400 cm³ at mid-height of sterile substrate consisting of a mixture of sand and peat (V / V) previously disinfected (autoclaving at 120 ° C for 1h).

The inoculation of plants of "Sigoise" olive variety of 2 months old is by setting near each root system about 50 g of fungal inoculum consisted of a mixture of excised roots of leek, previously infected by 80% AMF (CMB, CMS, I1, I2) and a mixture of substrate from rhizosphere culture leek potted and containing spores and mycelia. After inoculation, the rest of the cultivation substrate disinfected is added until the pots are filled. We inoculated 50 cuttings of each series.

The plants were grown in the greenhouse without air conditioning (under normal daylight) and were watered regularly with 100ml of distilled water for three months.

Study of mycorrhizal associations

Associations were studied on plants aged three months. The observations were carried out using light microscopy on roots prepared according to the method of Phillips and Hayman (1970) and stained with Trypan blue.

Estimation of colonization AM

The rate of colonization AM was estimated using the method described by Trouvelot et al. (1986). The method is to select at random from each plant mycorrhiza 50 root fragments of about 1cm after Trypan blue staining. The fragments were mounted on a glass slide in a drop of lactoglycérol, with 10 fragments per slide and observed under light microscope. This method calculates five parameters of colonization:

F%: frequency of mycorrhizal colonization (% of mycorrhized root fragments), it reflects the importance of the colonization of the root system.

M%: Intensity of colonization of the root cortex (cortex colonized estimated proportion from the entire root system and expressed in %).

m%: Intensity of colonization developed in the mycorrhized root system (proportion of colonized cortex mycorrhiza in the root system expressed in%).

A%: Amount of arbuscular colonization brought the whole root system (proportion of the root system containing arbuscules, expressed in%).

a%: Content arbuscular colonization of mycorrhiza in the root system (proportion colonized containing arbuscules,%).

The calculation of these parameters is carried out using the calculation formulas developed by Trouvelot et al. (1986).

The effectiveness of the inoculum or efficiency was expressed as the percentage gain inoculated plant biomass (fresh and dry weight of the aerial part of plants) compared to non-inoculated control and other parameters of plant growth (height, number of stems and leaves root length)

For the measurement of dry weight, plants were dried in an oven at 105 ° C for 24 hours (Oihabi and Meddich 1996).

Mycorrhizal dependency index (MDI) of plants is determined from the average biomass of mycorrhizal plants and aerial control plants following formula Plenchette et al. (1983):

$$\text{MDI} = 100 \times (\text{dry mass of mycorrhizal plants} - \text{dry mass of control plants}) / (\text{dry mass of mycorrhizal plants})$$

The results were subjected to analysis of variance (ANOVA) using the Statistica software and mean comparisons using the software STATISTICA (6.0), the differences between treatments were confirmed by the Newman-Keuls test at 5% ($p < 0.05$) (Guissou 2009).

Results

Estimating the parameters of endomycorrhizal infection olive plants

The rate of mycorrhization (F) varies from 30 to 58% respectively with inocula CMS and CMB is very low (<10%) with I1 and I2 (Fig. 1). As the intensity of colonization of the root cortex (M), they oscillate from 11.14% to 13.04% with CMS and CMB are almost zero with I1 and I2 (0.74% to 1%). The rate of the root system of arbuscular part mycorrhiza (a%) are 81.7% to 59.13% with CMS and CMB and almost zero with the other two inocula (I1 and I2). These results highlight the potential of mycorrhizal colonization of AM fungi inocula contained in the CMB and CMS.

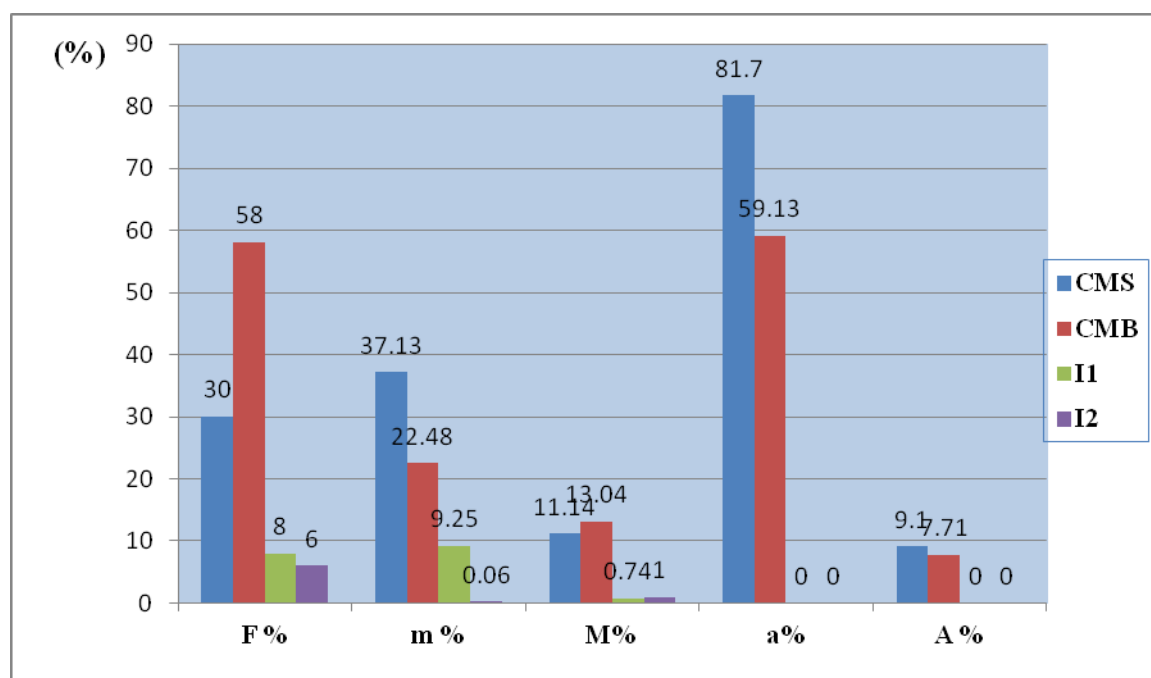


Fig. 1: Parameter Estimation of mycorrhizal infection olive plants. F: Frequency of mycorrhizal. M and m: mycorrhization intensity relative and absolute respectively, compared to cortex colonized the entire root system; arbuscular content of the root system (A) and Part mycorrhiza (a).

Growth of olive plants

The inoculation of olive plants by arbuscular mycorrhizal fungi isolated from soil improved plant growth. After three months of growth, CMB particularly complex and CMS as I1 and I2 species tested were effective in improving growth (Fig. 2). Indeed, statistical analysis of all measured parameters (root length, number of leaves and stems, fresh weight and dry weight of the aerial part) revealed significant differences between inoculated and control plants (Fig. 3 to 8). Gain fresh biomass of aerial parts of inoculated plants ranged from 11 to 79%.

These results show that the complex mycorrhizal inoculated CMS and CMB are more efficient than species I1 and I2 in improving the growth of plants. Their positive impact on growth would be due to the diversity of arbuscular mycorrhizal fungi in these inocula.

Estimation of mycorrhizal dependency olive plants

Inoculated plants respond differently to mycorrhization by fungal inoculum. Mycorrhizal dependency index (MDI) is high (72.9%) with CMB inoculum which shows the effectiveness of the inoculums; it is average with I2 and CMS (20% and 34.18% respectively) and low with I1 (10%) (Fig. 9).

Morphology arbuscular endomycorrhizal

Exploring the roots of olive plants inoculated showed a characteristic colonization of arbuscular mycorrhizae (AM). This colonization is manifested by a typical AM mycelium and numerous vesicles and arbuscules.

Vesicles with regular shapes, ovoid or elongated (Fig. 10a and b). Intercellular mycelium, fine and coarse longitudinally traverses the root cortex and enters the cells, either in the form of arbuscules, this is the type "Arum" (Fig. 10 c), or wraps inside cells (Fig. 10d) The appressoria were also observed in the root cortex. All these structures confirm the intense colonization of olive plants inoculated by the AM.

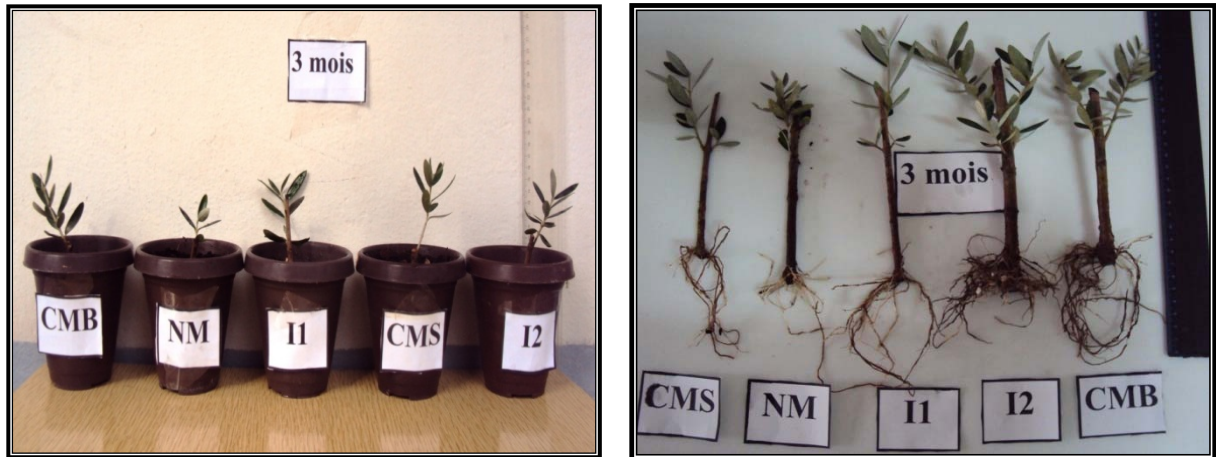


Fig. 2: Effect of mycorrhizal inoculation on the growth of olive plants. a: height of the aerial part of plants B: morphology of plants, NM: non-mycorrhizal plant.

Discussion

Plants of the variety "Sigoise" olive form endomycorrhizal type "Arum" regardless of the inoculum. These results confirm those of many authors who showed that AM of *Oleaceae* form type "Arum" (Smith and Smith 1997; Wubet et al. 2003; Azcon-Aguillar et al. 2003; Ahulu et al. 2005; Dickson et al. 2007). According to Smith and Smith (1997) and Yamato (2004), this type of mycorrhizae is controlled by the host plant.

Mycorrhizal dependency is more important in olive plants inoculated CMB and CMS than those inoculated with *Gl.* sp.1 and sp.2 (I1 and I2). It seems to be determined by the fungal inoculum used. These results confirm those of Meddad-Hamza et al. (2010) who reported that the olive tree shows greater dependence vis-à-vis *Gl. mossae* (73.9%) than *Glomus intraradices* (67.3).

The inoculation of semi-hardwood cuttings of the variety "Sigoise" olive complex by mycorrhizal CMB and CMS significantly improved height growth and biomass of olive plants. Both inocula CMS and CMB in particular have proved most effective in improving growth.

These results confirm those of Calvente et al. (2004), Porrás Piedra et al. (2005) and de Rougemont (2008) have shown the benefits of mycorrhization on semi woody cuttings of varieties "Cornicabra, Arbequina, Leccino and Picual" olive trees inoculated with four species *Glomus* (*Gl. mossae*, *Gl. intraradices*, *Gl. claroideum* and *Gl. viscosum*),

CMB inoculum seems to have a greater mycorrhizal effect on "Sigoise" olive variety, this is due to the diversity of mycorrhizal fungi arbuscular contained in the inoculum. This preference between mycorrhizal inoculants and olive has been demonstrated by Caravaca et al. (2003) and Porras-Soriano et al. (2009) who compared the efficiency of the same two species of *Glomus* (*G. intraradices* and *G. mosseae*) on the variety "Cornicabra" olives. The superiority of the effect of *Glomus mosseae* on olive varieties was also reported by Binet et al. (2007) and Porras-Soriano et al. (2009). Calavente (2004) also described the superiority of *G. intraradices* on varieties 'Arbequina' and 'Leccino' olive trees.

Mycorrhizal plants of the variety "Sigoise" increased branching root systems that have a root hair especially important with CMS and CMB. These results are consistent with those described for some mycorrhizal fruit species such as *Olea europea* L. (Citernesi et al. 1998; Dag et al. 2009; Meddad-Hamza et al. 2010), *Prunus cerasifera* L. (Berta et al. 1995), *Vitis vinifera* L. (*Vitaceae* family) (Schellenbaum et al. 1991), date palm (Dreyer et al. 2009) and *Fragaria ananassa* Duch. (*Rosaceae*) (Norman et al. 1996). According to some authors, this stimulation of root growth (change in color (into yellow) and root morphology, increased their number and their ramifications, and total absence of hairs roots) improves the absorption of water and mineral nutrition (Kaldorf and Ludwig -Müller 2000; Fidelibus et al. 2000; Fester et al. 2002; Derkowska et al. 2008).

Smith and Read (1997) suggest that mycorrhizal symbiosis can improve the quality of the root system by increasing the survival of plants transplanted to the field. This suggestion is confirmed by Guissou (2009) for fruit trees according to which, mycorrhization not improve stress tolerance of these but stimulates their growth and mineral nutrition.

Conclusion

This work has shown the existence of a complex isolated indigenous mycorrhizal rhizosphere variety "sigoise" olive with a power mycorrhizal significant. The inoculation of olive plants with a complex of native mycorrhizal gives better results compared to inoculation with a single species. The evaluation of the effectiveness of AM fungi showed that they significantly improve growth.

The choice of native fungi as inoculum seems to be the best, because they are better adapted to local environmental conditions (arid and semi-arid), which reduces the problems associated with the introduction of exogenous species in equilibrate habitats. It would be interesting to inoculate plants from semi-hardwood cuttings of the variety of olive sigoise combining these complexes mycorrhizal inoculum selected and evaluate their effectiveness in improving growth.

To conclude, the environmental policy of Algeria is in favor of sustainable development, operating symbiotic systems in general and mycorrhizal system in particular, is a promising alternative for improvement of production while maintaining environment.

The inoculation of herbaceous cuttings of olive by AMF isolates (isolate I1 or I2 previously selected and propagated in culture leek) or complex mycorrhizal indigenous soil orchards Sig (CMS and CMB) showed favorable effects on the growth of plants that form mycorrhizae natural analogues AM.

We concluded that inoculation of olive plants with a mixture of native species gives better results compared to inoculation with a single species.

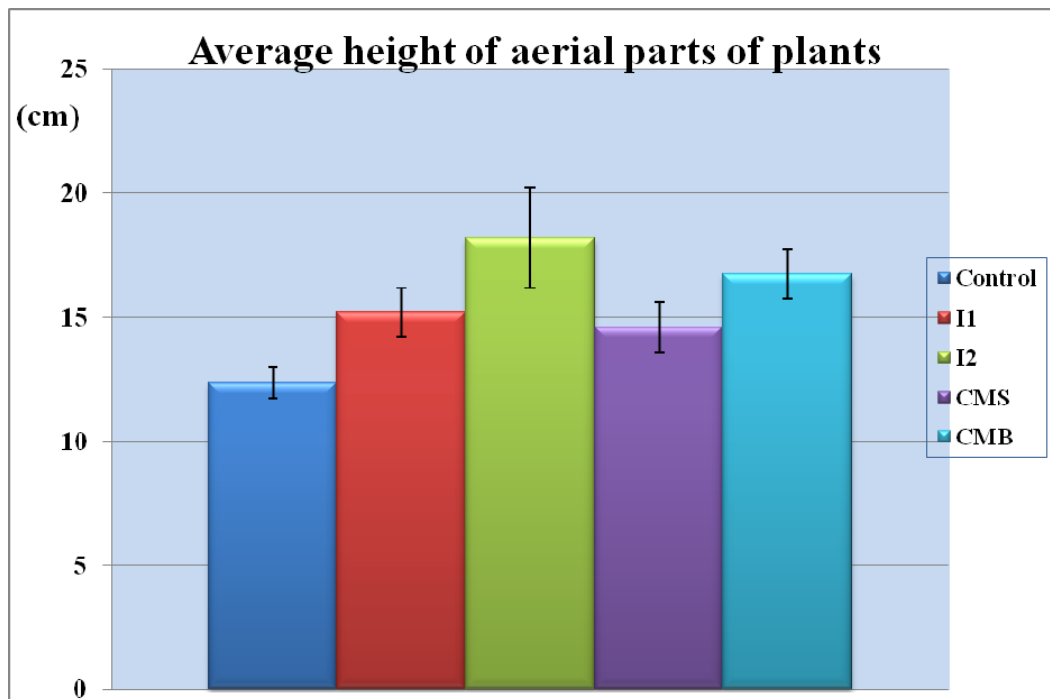


Fig. 3: Estimate the height of mycorrhizal olive cuttings. (I1: isolate 1, I2: isolate 2; CMS complex mycorrhizal Sig; CMB: complex mycorrhizal Bechar).

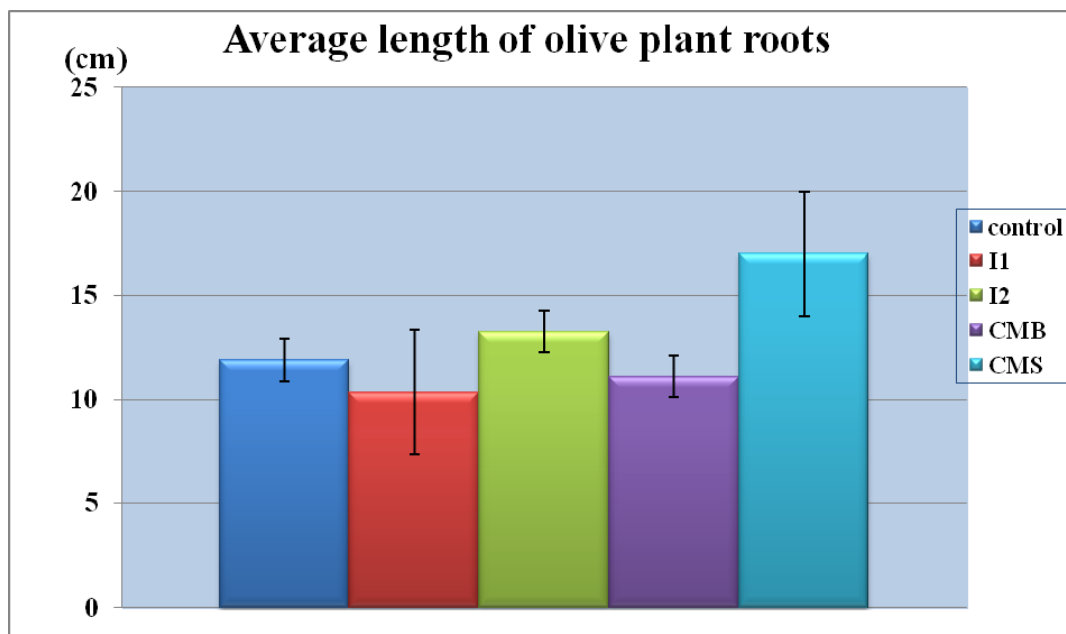


Fig. 4: Estimate length of the roots of mycorrhizal olive cuttings.

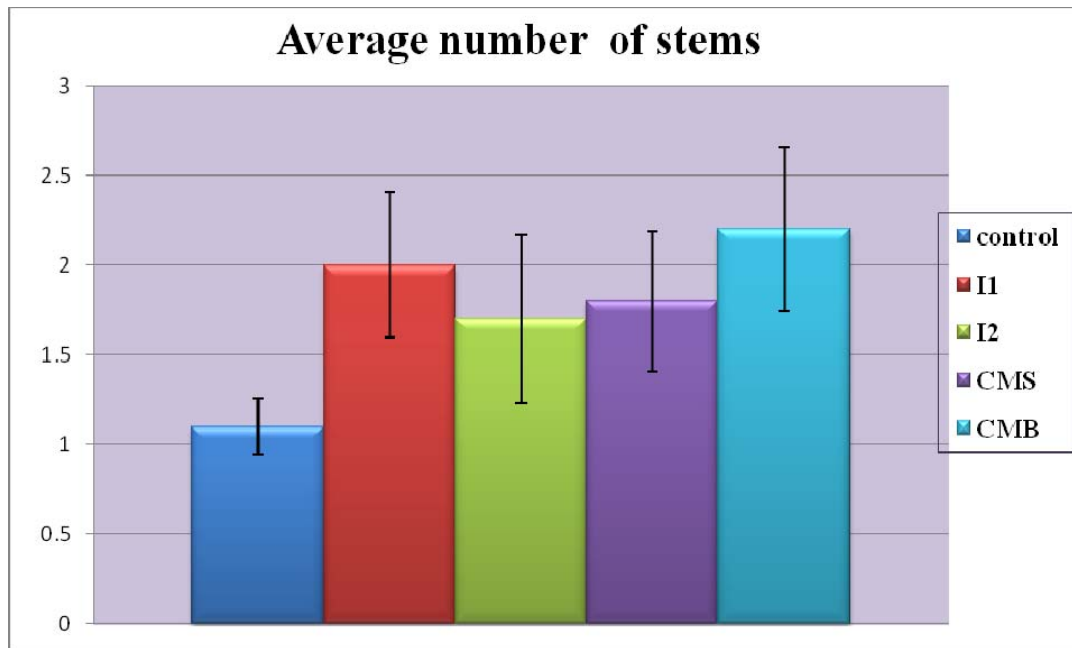


Fig. 5 : Estimate of the number of stems cuttings olive mycorrhizal.

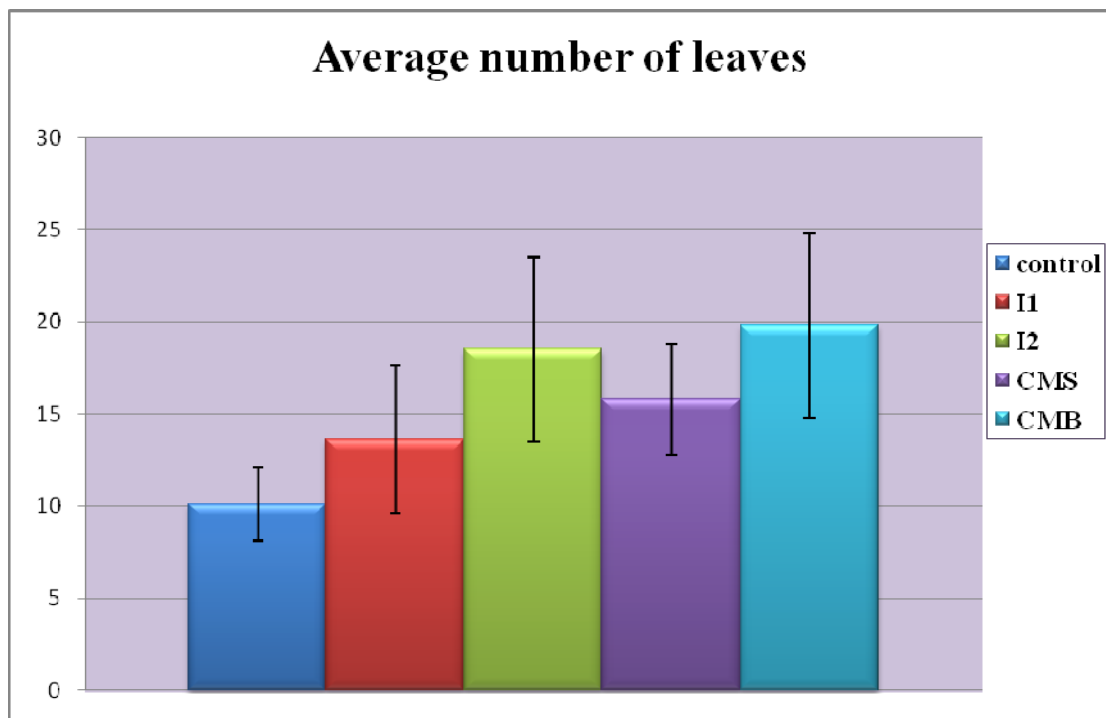


Fig. 6 : estimate number of leaves of mycorrhized olive cutting.

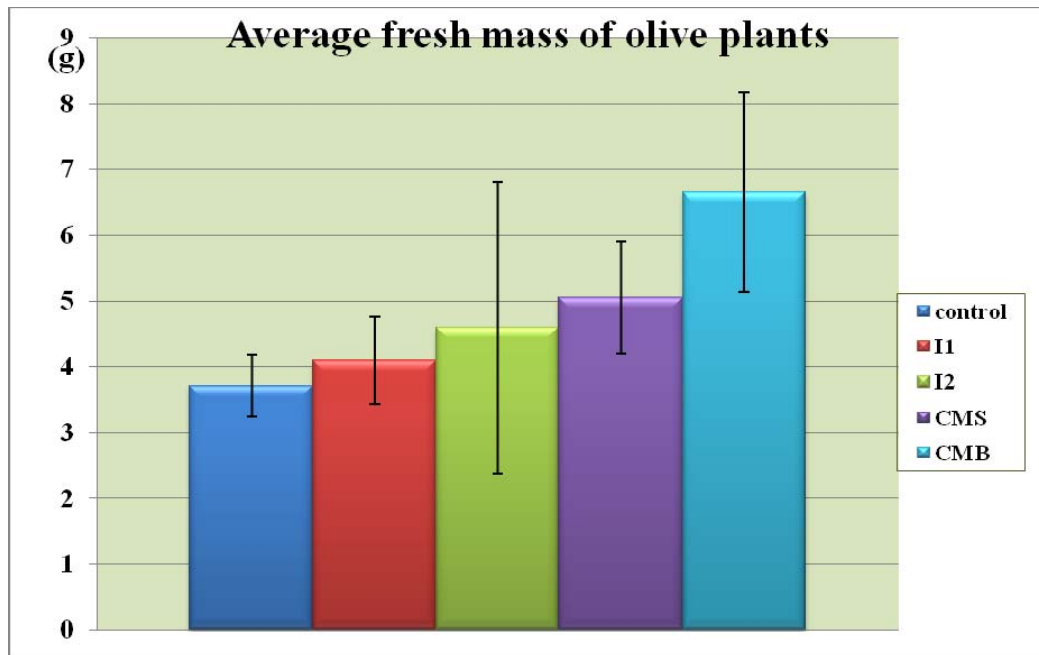


Fig. 7 : estimate fresh weight of aerial parts of olive cuttings.

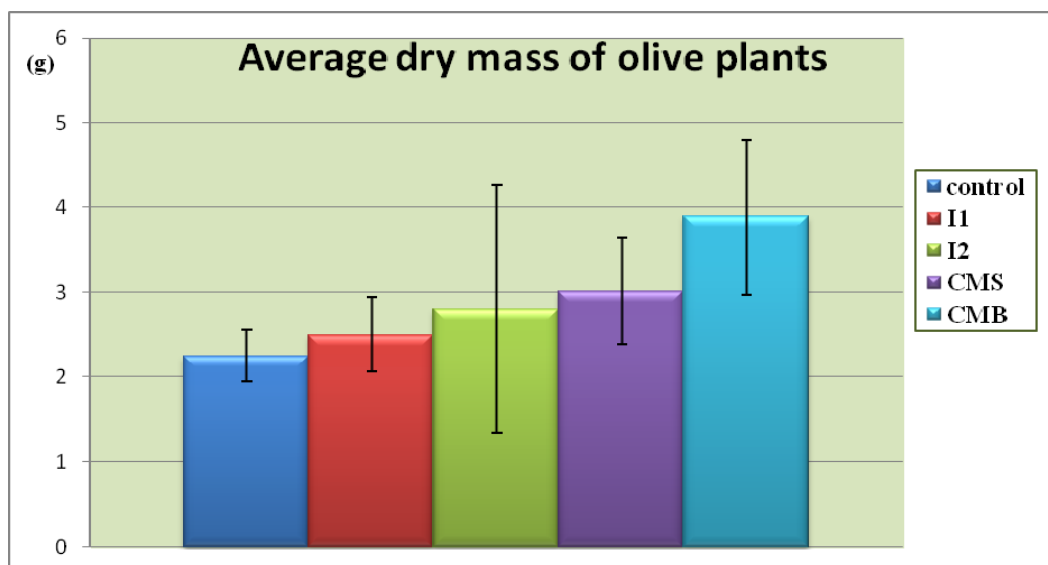


Fig. 8 : Estimate of dry weight of aerial parts of olive cuttings mycorrhizal.

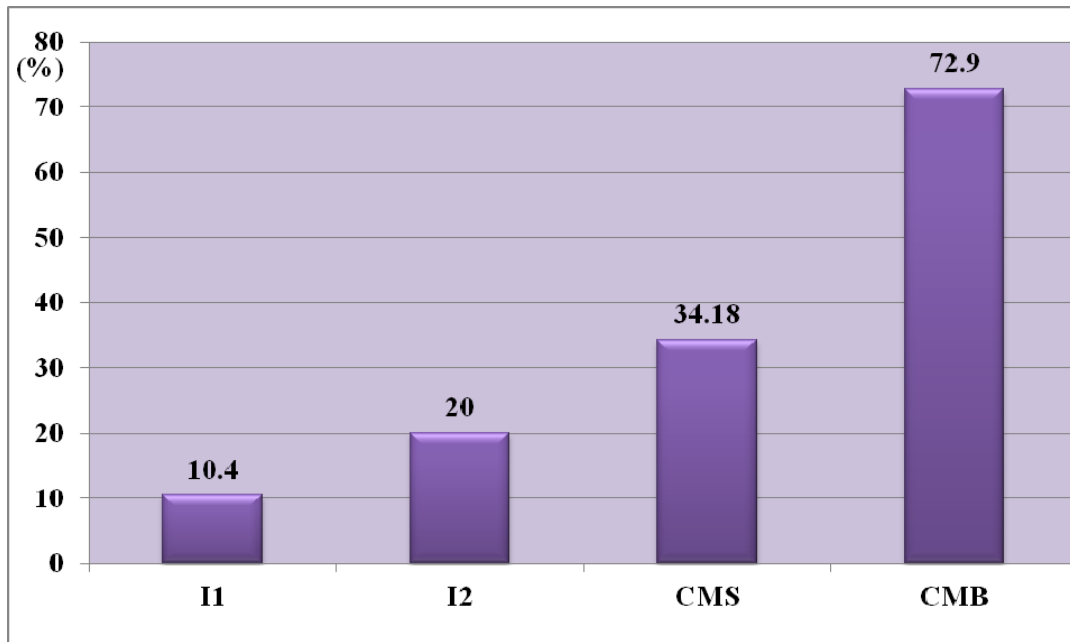


Fig. 9: Degree of mycorrhizal dependency olive plants in the four sets of inoculation.

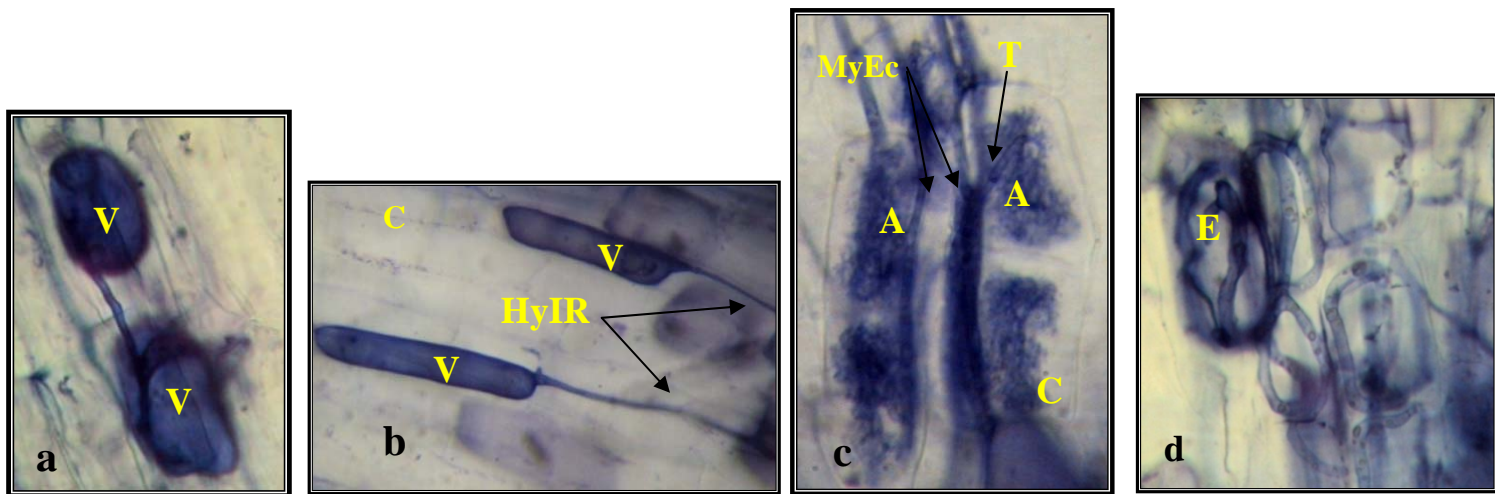


Fig. 10: Appearance of intra-root structures formed in the association AM/ wood cuttings of Sigoise olive variety. G × 400.

a and b: mycorrhiza showing ovoid or elongated vesicles (V), C: cortex, HyIR: intra-radical hyphae. c: mycorrhiza showing arbuscules. A: arbuscules; T: trunk; MyEc: extracellular mycelium; d: mycorrhizae showing intracellular coils (E).

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