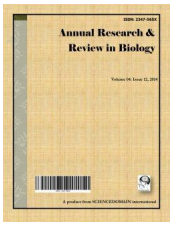

Annual Research & Review in Biology

13(5): 1-11, 2017; Article no.ARRB.33873
ISSN: 2347-565X, NLM ID: 101632869

Effect of Endomycorrhizal Inoculation on the Young Cork Oak Plants (*Quercus suber)* Growth

Otman Hamidi[1], Zouheir Talbi[1], Mohamed Chliyeh[1], Amina Ouazzani Touhami[1], Karima Selmaoui[1], Rachid Benkirane[1] and Allal Douira[1*]

[1]Laboratory of Botany, Biotechnology and Plant Protection, Faculty of Sciences, University Ibn Tofaïl, B.P. 133, Kénitra, Morocco.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/33873
Editor(s):
(1) Prof. George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Arun Karnwal, Lovely Professional University, India.
(2) Sanjay Mishra, IFTM University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/19617

Original Research Article

Received 1st May 2017
Accepted 4th June 2017
Published 19th June 2017

ABSTRACT

Inoculation with endomycorrhizae had a significant effect on the cork oak (*Quercus suber*) seedlings growth. The average length (55.37 cm) and weight (12.6 g) of the aerial part, the length (41 cm) and weight (26.41 g) of the root system, the rod diameter (0.56 cm) and the leaves number (120) of the plants inoculated with mycorrhizae AM were higher than those of control, respectively, 41.75 cm; 10.1 g; 28.5 cm; 16.75 g; 0.53 cm; 112.75. Frequency (90%) and intensity (71%) of mycorrhization were higher at the root levels of plants inoculated with endomycorrhizae than those of control plants that did not show any mycorrhization.

However, the study of morphological spores criteria of AM fungi isolated from the rhizosphere of plants inoculated with endomycorrhizae made it possible to identify 18 different species (9 species belong to *Glomus* genus, 4 belong to *Acaulospora* genus, 2 belong to *Scutellospora* genus and one to *Gigaspora* genus, *Diversispora* genus and *Redeckera* genus).

Keywords: Quercus suber; plants; inoculation; endomycorrhizae; growth; mycorrhization parameters.

**Corresponding author: E-mail: douiraallal@hotmail.com;*

1. INTORDUCTION

Mycorrhizal associations play a key role in the functioning and stability of terrestrial ecosystems that are heavily involved in the mechanisms governing the spatiotemporal evolution of ecosystems [1]. Some plant species have the property of contracting symbiotic relationships with the two types of fungal symbiosis; Mycorrhizal arbuscular fungi (endomycorrhizae) and ectomycorrhizae [2,3,4]. These include the *Acacia* genus from Australia, *Casuarina, Eucalyptus,* and *Quercus* [5,6,7,8], *Salicaceae* [9,10], *Populus* [11,12].

In the establishment of the mycorrhizal procession, the arbuscular mycorrhizal fungi settle early and are followed by the ectomycorrhizal symbionts [13]. Ducousso et al. [14] showed that the double inoculation of *Acacia holosericea* reduced by 39% the rate of ectomycorhization by *Pisolithus* sp. in the presence of *Glomus mosseae* (endomycorrhizae arbuscular). On the contrary, Chen et al. [6] observed an increase in the rate of ectomycorhization at *Eucalyptus globulus* and *E. urophylla* inoculated with ectomycorhizal fungi (*Laccaria* genus) in the presence of arbuscular mycorrhizal fungi (*Glomus invermaium, Acaulospora laevis* or *Scutellospora calospora)*. On the other hand, Founoune et al. [7] showed that the dual inoculation improved the development of *Acacia holosericea*, compared to the growth of the plants inoculated with one or more of the symbiotes.

Similarly, other studies have shown that specific ectomycorrhizal fungi are able to improve the juvenile growth of certain forest species and mitigate the effects of the transplant crisis [15-20]. In addition, the cork oak (*Quercus suber*) is a mycotropic tree, frequently associated with endomycorrhizae and/or more rarely with ectoendomycorrhizae. The arbuscular mycorrhizae are dominant in young seedlings while Ectomycorrhizal are mainly detected in adult trees [21,22].

In this context, the objective of this work was to study the effect of endomycorrhizae on the mycorrhizal parameters and growth of cork oak seedlings *(Quercus suber).*

2. MATERIALS AND METHODS

2.1 Plant Material

The study was carried out on six-month-old cork oak seedlings, which were brought from a nursery located in the region of Kenitra in the north-west of Morocco.

2.2 Production and Multiplication of the Endomycorrhizal Inoculum

An endomycorrhizal inoculum was collected from the rhizosphere of carob tree (soil and roots) from different Moroccan regions (Taroudant, Khénifra, Afourar, Nador and Ksiba). This inoculum, made up by 30 endomycorrhizal species [23,24], was multiplied on two mycotrophic species: maize and sorghum. The seeds of these two species were disinfected with sodium hypochlorite (5%) for two minutes, then rinsed with tap water and sown in pots containing mycorrhizal soil and carob tree root fragments. These pots were placed under greenhouse and watered regularly with distilled water. After three months of cultivation, mycorrhizal roots of maize and sorghum were used to inoculate cork oak plants.

2.3 Enodmycorrhizal Inoculation

Inoculation consists of cork oak transplanted seedlings in pots filled with the disinfected Mamora's sand forest containing a layer of mycorrhizal roots. The control plants are transplanted into pots containing only the soil of the disinfected Mamora forest. All plants are watered with distilled water to facilitate the installation of mycorrhizae.

2.4 Experimental Apparatus

The experiment was carried out between January and June 2015. The experimental design was designed in random blocks with one plant per pot and eight replicates for each treatment. The pots were placed in a greenhouse whose temperature varies between 18 and 25 C°.

Lot 1: Control plants (T).

Lot 2: Plants inoculated with AM fungi (Myc).

2.4.1 Evaluation of cork oak plants agronomical parameters

After 5 months of cultivation, the pots were brought back from the greenhouse and the cork oak plants were cut at the collar. The growth parameters evaluated were: the number of leaves, number of branches, length and fresh weight of the aerial part, diameter of the main

stem, fresh weight and length of the root part and the percentage of leaves that show symptoms of chlorosis or necrosis, calculated according to the formula:

$$\%F_{sym} = \frac{N_{F_{sym}}}{N_{TF}} \times 100$$

%Fsym: Percentage of leaves with symptoms.
$N_{F_{sym}}$: Number of leaves showing symptoms.
N_{TF} : Total number of leaves.

2.4.2 Evaluation of mycorrhization parameters

2.4.2.1 Mycorrhizal roots

After five months of inoculation, the colonization of the cork oak plants roots by AM fungi was carried out using the root staining technique of [25]. The roots were recuperated from the substrate and washed with water. The finest roots were cut into pieces of 1 cm length, soaked in a solution containing 10% KOH and a few drops of hydrogen peroxide (H_2O_2), and placed in an oven at 90 C° for 45 min. These fragments were then rinsed with distilled water and heated to 90 C° for 15 min in the Cresyl blue.

Thirty randomly selected fragments were used for microscopic observation and for the calculation of mycorrhizal parameters, namely mycorrhizal frequency (MF%), mycorrhizal intensity (MI%), arbuscular and vesiclar contents, according to index Mycorhizal of Trouvelot et al. [26].

The mycorrhizal frequency (M.F. %) reflects the importance of the colonization of the root system and was calculated using the following formula:

M.F. % = 100 × (N - n0) / N

N: Number of observed fragments, n0: Number of non-mycorrhizal fragments.

The mycorrhizal intensity (M.I.%) (Cortex colonized estimated proportion from the entire root system and expressed in %) was determined as follows:

M.I. % = (95 n5 + 70 n4 + 30 n3 + 5 n2 + n1) / N

The numbers n5, n4, n3, n2, and n1 denote the number of recorded fragments 5, 4, 3, 2 and 1 estimating the proportion of root colonized by mycorrhizae according to the scale.

2.4.2.2 Spore's extraction

Spores are extracted according to the wet sieving method described by Gerdemann and Nicolson [27]. Indeed, in a 1 L beaker, 100 g of each composite sample of the soil is immersed with 0.5 L of running water and stirred for 1 minute with a spatula. After 10 to 30 seconds of decanting, the supernatant is passed through two superposed sieves with decreasing meshes (315 and 50 µm). This operation is repeated twice. The contents retained by the sieves are distributed in tubes and centrifuged for 5 min at 2000 towers/min. The supernatant is discarded and a viscosity gradient is created by adding 20 ml of a 40% sucrose solution to each centrifuge tube [28]. The mixture was rapidly stirred and the tube returned to the centrifuge for 1 min at 3000 turns/min.

Unlike the first centrifuging operation, the supernatant is poured onto the 50 µm mesh sieve. The substrate obtained is rinsed with distilled water to remove the sucrose and then disinfected with an antibiotic solution of Streptomycin at 10 mg / L. The spores were then recovered in an Erlenmeyer flask with a little distilled water. The AM fungi are then identified based on their morphological characters.

2.5 Statistical Analysis

The statistical treatment of the results obtained involved the analysis of the variance with a single classification criterion (ANOVA 1) at the 5% threshold using the STATISTICA software.

3. RESULTS

3.1 Evaluation of Cork Oak Plants Agronomical Parameters

The lengths and average fresh weights of aerial and root parts of the cork oak plants, after 5 months of inoculation, vary according to the type of treatment (Fig. 1). Plants inoculated with mycorrhizae showed the longest aerial part (55.37 cm) compared to controls (41.75 cm).The aerial part weight was higher in plants inoculated with AM fungi (12.6 g) than in control plants (10.1 g). The same result was recorded for the average root length and average fresh weight in the plants inoculated with endomycorrhizae (41 cm and 26.41 g, respectively) compared to controls (28.5 cm and 16.75 g).

The effect of AM (Myc) fungi on the development of the aerial and root parts of the cork oak plants after 5 months of inoculation compared to the control is shown in Fig. 2.

The effect of cork oak plants mycorrhization on the average values of the branches number, the diameter of the stems, the number and percentage of leaves presenting symptoms are given in Table 1.

The plants inoculated with endomycorrhizae have the average numbers of the highest branches (11.25), stem diameter (0.56 cm) and number of leaves (120) with respect to those recorded in the control (9.87 respectively; 0.53 cm and 112.75). The percentage of leaves with symptoms was higher in control plants (12.12%) than in plants inoculated with endomycorrhiza (8. 37%).

3.2 Evaluation of Mycorrhization Parameters

3.2.1 Roots mycorhization

The microscopic observation of the root fragments after 5 months of inoculation (Fig. 3) revealed the presence of different mycorrhizal structures, namely: arbuscules (A, E), vesicles (D), Hyphae (B) and endophytes (C).

The frequency and intensity of the cork oak roots mycorrhization (Fig. 4) were elevated in roots inoculated with mycorrhiza (90%, 71%, respectively). Whereas the roots of the control plants were not mycorrhizal. On the other hand, the contents of arbuscules and vesicles in the inoculated roots were respectively in the order of (60%; 25%) compared to the roots of the control plants which had neither arbuscules nor vesicles (Fig. 4).

Moreover, the number of spores (114 spores / 100 g of soil) was high in roots inoculated with mycorrhizae while their density in the control plants rhizosphere was nulle.

3.2.2 B-extraction of spores

The study of the morphological criteria of the AM fungi spores isolated from the inoculated plants rhizosphere, allowed identifying 18 different species. Nine species belong to the *Glomus* genus, four *Acaulospora*, both to *Scutellospora* and one to *Gigaspora*, *Diversispora* and *Redeckera*. *Glomus etunicatum* was the most abundant species, with a frequency of occurrence which reached 20% (Fig. 5).

4. DISCUSSION

In this work, the beneficial effect of endomycorrhizal fungi on the growth of the cork oak plants has been proved. This effect was mainly due to an increase in biomass, axial, and root growth. This is consistent with the work done on tomato plants [29,30], the carob tree [31] and *Eucalyptus* [32] which also demonstrated that plant inoculation with AM fungi stimulated the weight and length of the aerial and root parts of these plants. Indeed, the development of the root system is due to the formation of a greater number of rootlets, confirming that the AM fungi increase the rooting zone [33].

The exploration of the soil volume by extrametrical mycelium and its ability to mobilize nutrients from primary minerals promote phosphate plant nutrition [34,35,36]. This improvement in plant mineral nutrition also concerns other macro elements and trace elements [37,38]. On the other hand, mycorrhizal associations play a significant role in decomposition and mineralization of organic matter from land-based nutrients and mobilize the benefit on the host plant [39] and an improving water nutrition of the host plants in exchange of photosynthates [39,40].

In addition, our results showed that inoculation with AM fungi reduced the percentage of leaves showing symptoms relative to control, which would be partly responsible for growth stimulation in these plants. Many results attribute to mycorrhizal symbiosis a bioprotective effect via a pathogen reduction effect of certain plant-agents [41,42,43].

The seedlings roots of *Quercus suber* showed the presence of different structures of arbuscular mycorrhizae (arbuscules, vesicles, hyphae), with a high frequency and intensity of mycorrhization, while the control plants roots were not mycorrhizae. Ian et al. [44], working on the seedlings roots of *Quercus rubra*, noted the abundance of hyphae and vesicles, and the absence of arbuscules. The same result was recorded on *Q. imbricaria* [45].

However, other authors have reported the presence of arbuscules on *Quercus rubra* and *Q. falcata* [46], on *Q. rubra* and *Q. palustris* [47]. Moreover, other studies on *Eucalyptus* revealed the presence of different structures of arbuscular mycorrhiza [32,48,49,50,51]. On the other hand, microscopic observation revealed the

presence of 18 species, whose *Glomus etunicatum* is the most dominant. This is consistent with the results of El Asri et al. [23], who noted the dominance of *Glomus etunicatum* in the carob tree's rhizosphere in five regions of Morocco. The same result was recorded at the maize rhizosphere [31]. Chliyeh et al. [30] also noted the dominance of *Glomus etunicatum* at the root zone of the olive tree. This specie also dominates in the rhizosphere of the plant species in the Atlantic forest of Brazil [52].

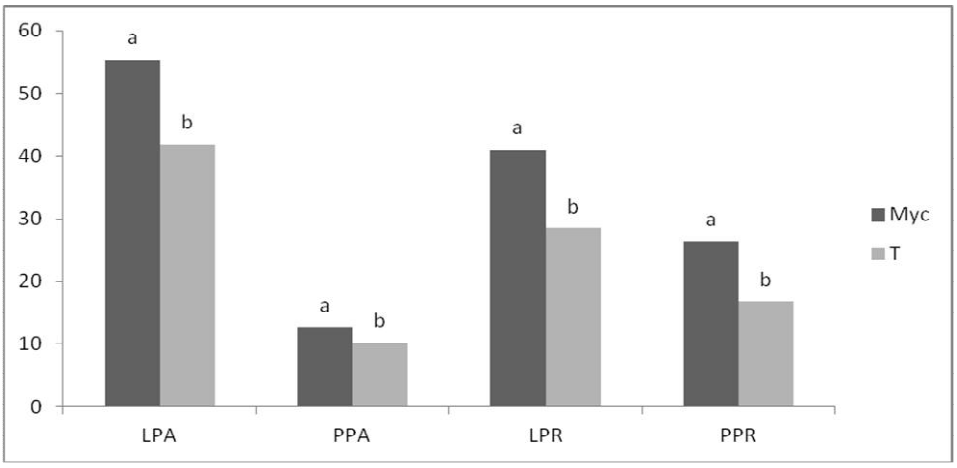

Fig. 1. Effect of AM (Myc) fungi on average length (cm) (LPA, LPR) and weight (g) (PPA, PPR) of the aerial part and root of the cork oak plants after 5 months of Inoculation compared to control (T)

Two affected results of the same letter of the same parameter do not differ significantly with the threshold from 5%, according to ANOVA test

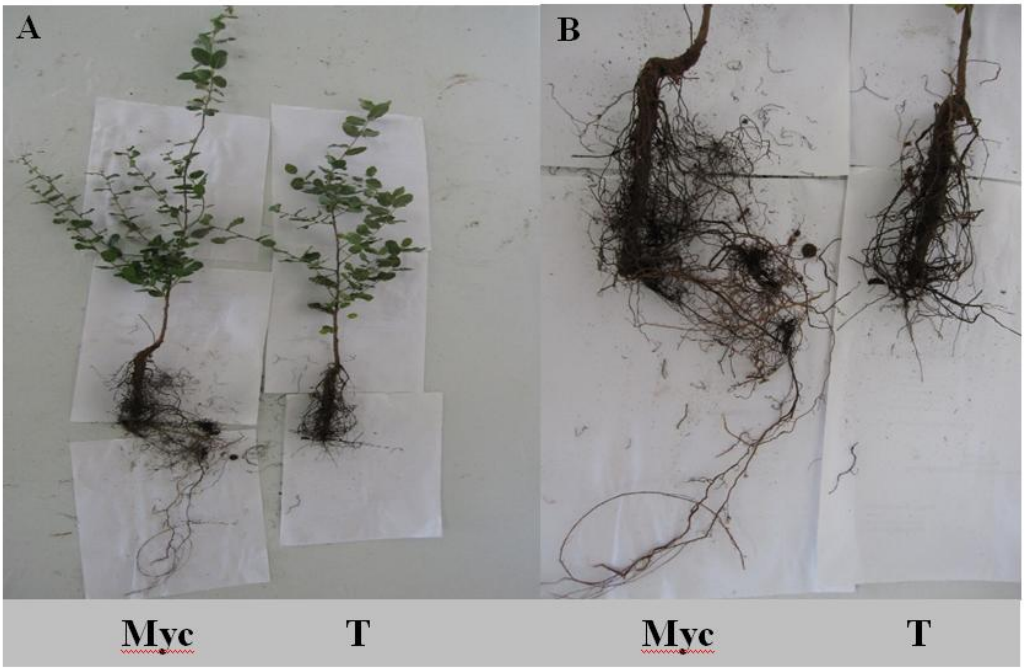

Fig. 2. Effect of AM (Myc) fungi on the development of aerial (A) and root (B) parts of cork oak plants after 5 months of inoculation compared to control plant (T)

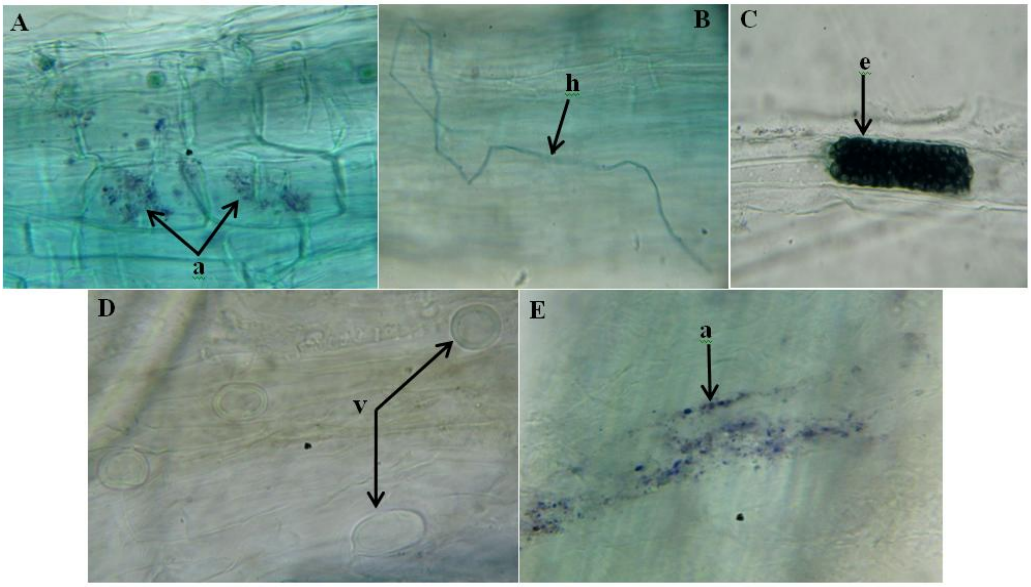

Fig. 3. Different structures of arbuscular mycorrhizae at the roots of *Quercus suber* plants inoculated with mycorrhizae. A, E: Arbuscule (a, e); B: hyphae (h); C: Sclerotia of endophyte (e); D: vesicle (v); (G. × 400)

Table 1. Effect of mycorhization on average values of the branches number, the stems diameter, the number of leaves and the percentage of leaves presenting symptoms, after 5 months of culture

	Number of branches	Stem diameter (cm)	Number of leaves	Percentage of leaves showing symptoms (%)
Myc	11.25[a]	0.56[a]	120.37[a]	8.37[b]
Control	9.87[b]	0.53[b]	112.75[b]	12.12[a]

Myc. Mycorrhizal plants

Two affected results with the same letter in the same column do not differ significantly with the threshold from 5%, according to ANOVA test

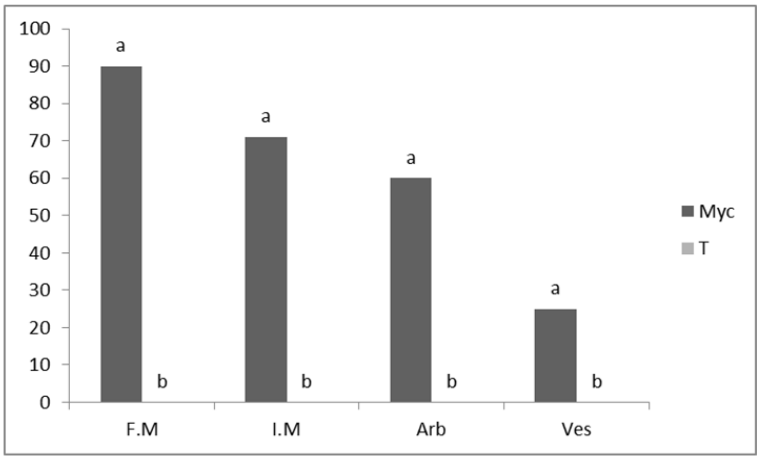

Fig. 4. Mycorrhizal frequency and intensity, the arbuscular and vesicular contents, of the *Quercus suber* roots after 5 months of culture. M.F: Mycorrhizal frequency; M.I.: Mycorrhizal intensity; Arb: arbuscule; Ves: vesicle; C: control; Myc: arbuscular mycorrhiza

For a given parameter, two affected results of the same letter do not differ significantly with the threshold from 5%, according to ANOVA test

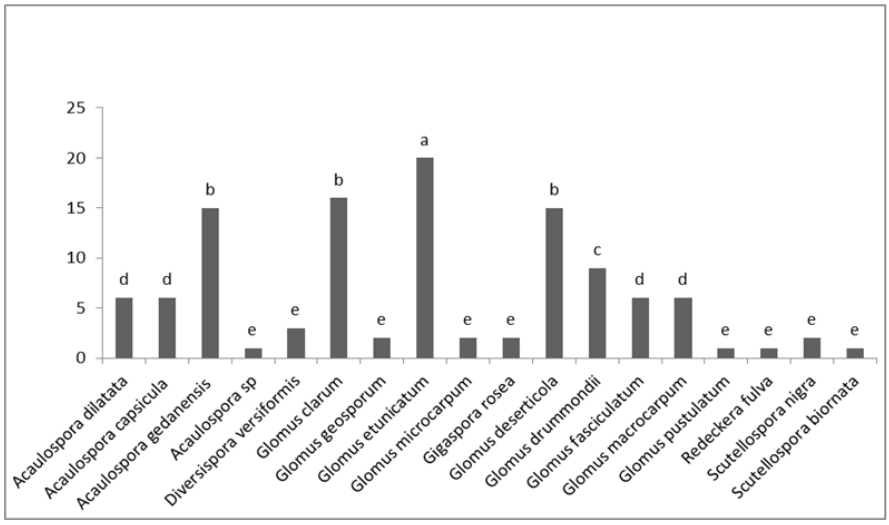

Fig. 5. Occurrence frequency of endomycorrhizal species isolated from the rhizosphere of *Quercus suber* plants inoculated with AM fungi

For a given parameter, two affected results of the same letter do not differ significantly with the threshold from 5%, according to ANOVA test

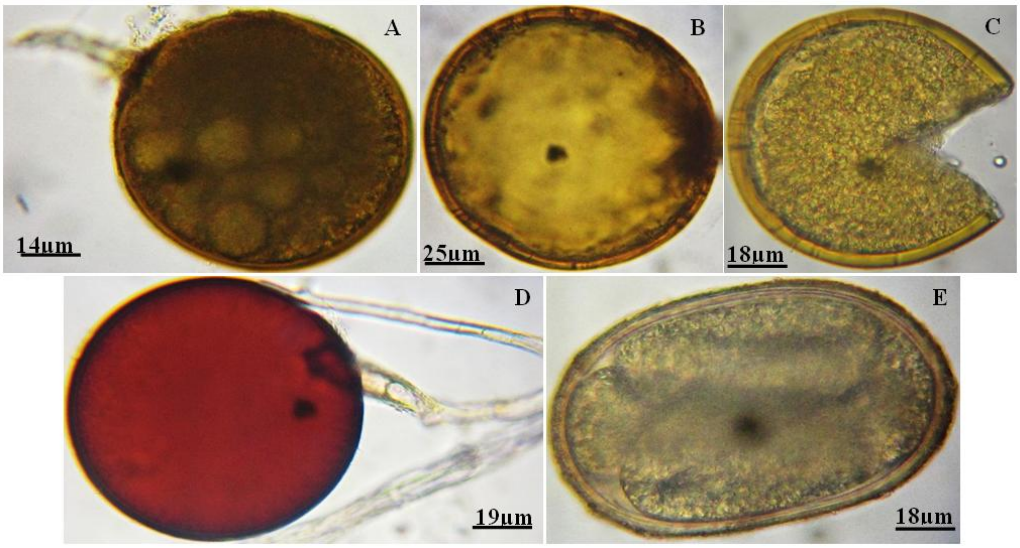

Fig. 6. Some species of endomycorrhizal fungi isolated from the rhizosphere of *Quercus suber* plants after 5 months of cultivation. (A) *Glomus clarum*, (B) *Acaulospora gedanensis*, (C) *Glomus macrocarpum*, (D) *Glomus deserticola*, (E) *Redeckera fulva*

Among the vascular plant species that simultaneously or successively harbor endomycorrhizae and ectomycorrhizae, *Eucalyptus* [53,32], *Populus* [11,12] and *Quercus* [46]. This double colonization increases the ecological amplitude of the plant host by improving its mineral supply: Endomycorrhizal and ectomycorrhizal symbionts facilitate respectively phosphate and nitrogen nutritions [54,55]. Indeed, although *Quercus* and *Eucalyptus* are typically ectomycorrhizal, some authors [56,57] reported that endomycorrhizal fungi are predominantly present in the root system of young plants and when these plants develop, endomycorrhizae are reinforced by ectomycorrhizae.

5. CONCLUSION

The obtained results show the potential effect of inoculation with mycorrhizae on the growth and development of cork oak plants. Thus, the use of a functional inoculums based on AM fungi as a biotechnological technique allows better exploitation of nutrients from culture substrates in order to obtain a better improvement in the growth of horticultural crops.

This inoculum can also be used in reforestation, restoration of degraded ecosystems and enhancement of cork oak plants strength in nurseries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Duponnois R, Ramanankierana H, Hafidi M, Baohanta R, Baudoin E, Thioulouse J, Sanguin H, Ba B, Galiana A, Bally R, Lebrun M, Prin Y. Des ressources végétales endémiques pour optimiser durablement les opérations de réhabilitation du couvert forestier en milieu méditerranéen et tropical: Exemple des plantes facilitatrices vectrices de propagation des champignons mycorhiziens. C. R. Biologies. 2013; 336: 265–272.
2. Lodge DJ. Negative associations among VA-mycorrhizal fungi and some ectomycorrhizal fungi inhabiting the same root system. Oikos. 1990;57:347-356.
3. Lopez Aguillon R, Garbaye J. Some aspects of a double symbiosis with ectomycorrhizal and VAM fungi. Agricul. Ecosyst. 1990;29:263-266.
4. Van der Heidjen L. Mycorrhizal symbioses of Salix repens. Ph.D. Thesis, Wageningen. 2000;289.
5. Brundrett M, Bougher N, Dell B, Malajczuk N. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Canberra, Australia. Australian Center for International Agricultural Research. 1996; 374.
6. Chen TL, Brundrett MC, Dell B. Effects of ectomycorrhizas and vesicular arbuscular mycorrhizas, alone or in competition, on root colonisation and growth of *Eucalyptus globulus* and *E. urophylla*. New Phytologist. 2000;146:545-556.
7. Founoune H, Duponnois R, Bâ AM, El Bouami F. Influence of the dual arbuscular endomycorrhizal/ ectomycorrhizal symbiosis on the growth of *Acacia holosericea* (A. Cunn. ex G. Don) in glasshouse conditions. Annals of Forest Sciences. 2001;59:93-98.
8. Ramanankierana N, Ducousso M, Rakotoarimanga N, Prin Y, Thioulouse J, Randrianjohany E, Ramaroson L, Kisa M, Galiana A, Duponnois R. Arbuscular mycorrhizas and ectomycorrhizas of *Uapaca bojeri* L. (*Euphorbiaceae*): sporophore diversity, patterns of root colonization, and effects on seedling growth and soil microbial catabolic diversity. Mycorrhiza. 2007;17:195-208.
9. Dominik T. Synopsis of a new classification of the ectotrophic mycorrhizae established on morphological and anatomical characteristics. Mycopathologia. 1956;11 (4):359-367.
10. Malajczuk N. Presence of vesicular arbuscular mycorrhizes in *Eucalyptus* spp. and *Acacia* sp. and their absence in *Barkia* sp. After inoculation with *Glomus fasciculatus*. New Phytol. 1981;87:567-572.
11. Gardes M, Bialet E, Binet E, Brousseau C, Carré F, Charcosset JY, Fradet N, Griffith P, Gryta H, Laquerbe M, Martinez C, Millot S. Les symbiotes mycorhiziens du peuplier noir (*Populus nigra* L.): La spécificité des assemblages fongiques en milieu riverain. Les Actes du BRG. 2003;4:453-466.
12. Talbi Z, Chliyeh M, Selmaoui k, Ouazzani Touhami A, Benkirane R, Douira A. Mycorrhizal status of *Populus alba* and accompanying species of Riparian Forest in the reserve of Sidi Boughaba (Northwest of Morocco). International Journal of Plant, Animal and Environmental Sciences. 2014; 4:126-133.
13. Selosse MA, Richard F, He X, Simard SW. Mycorrhizal networks: Des liaisons dangereuses? Trends in Ecology & Evolution. 2006;21:621-628.
14. Ducousso M, Colonna JP, Badji S, Thoen D. Influence de l'azote et du phosphore sur l'établissement de la symbiose quadripartite: *Acacia holosericea* / *Bradyrhizobium* sp. / *Glomus mosseae*/ *Pisolithus* sp. in: Physiologie des Arbres et Arbustes en zones arides et semi-arides. Groupes d'Etude de l'Arbre. Paris, France. 1991;1-14.

15. Castellano MA, Molina R. Mycorrhizae. In: The biological component: Nursery pest and mycorrhizae manual, Landis TD (ed). Agric. Handbook 674. USDA Forest Service, Washington, DC. 1989;5:101-167.
16. Kropp BR, Langlois CG. Ectomycorrhizae in reforestation. Canadian Journal of Forest Research. 1990;20:438-451.
17. Marx DH. The practical significance of ectomycorrhizae in forest establishment. Ecophysiology of Ectomycorrhizae of Forest Trees, Marcus Wallenberg Foundation Symposia Proceedings. 1991; 7:54-90.
18. Garbaye J, Churin JL. Growth stimulation of young oak plantations inoculated with the ectomycorrhizal fungus *Paxillus involutus* with special reference to summer drought. Forest Ecology and Management. 1997;98:221-22.
19. Duponnois R, Founoune H, Masse D, Pontanier R. Inoculation of *Acacia holosericea* with ectomycorrhizal fungi in a semi-arid site in Senegal: Growth response and influences on the mycorrhizal soil infectivity after 2 years plantation. Forest Ecology and Management. 2005;207:351-362.
20. Duponnois R, Plenchette C, Prin Y, Ducousso M, Kisa M, Bâ AM, Galiana A. Use of mycorrhizal inoculation to improve reforestation process with Australian *Acacia* in Sahelian ecozones. Ecological engineering. 2007;29:105-112.
21. Dos Santos VL, Muchoveg RM, Uchoveg RM, Borges AC, Neves JCL, Kasuya MCM. Vesicular-arbuscular/ectomycorrhiza succession in seedlings of *Eucalyptus* spp. Brazilian Journal of Microbiology. 2001;32: 81-86.
22. Egerton-Warburton L, Allen MF. Endo- and ectomycorrhizas in *Quercus agrifolia* Nee. (*Fagaceae*): Patterns of root colonization and effects on seedling growth. Mycorrhiza. 2001;11:283-290.
23. El Asri A, Talbi Z, Ait Aguil F, Chliyeh M, Sghir F, Touati J, Ouazzani Touhami A, Benkirane R, Douira A. Arbuscular mycorrhizal fungi associated with rhizosphere of Carob tree (*Ceratonia siliqua* L.) in Morocco. Int. J. Pure App. Biosci. 2014;2(3):286-297.
24. Talbi Z, EL Asri A, Touati J, Chliyeh M, AIT Aguil F, Selmaoui K, Sghir F, Ouazzani Touhami A, Benkirane R, Douira A. Morphological characterization and diversity of endomycorrhizae in the rhizosphere of carob tree (*Ceratonia siliqua*) in Morocco. Biolife. 2015;3(1):196-211.
25. Philips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 1970;55 (1):158-161.
26. Trouvelot A, Kough JL, Gianinazzi-Pearson V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. Physiological and Genetical Aspects of Mycorrhizae (Gianinazzi-Pearson V & Gianinazzi S, eds), INRA Press, Paris. 1986;217–221.
27. Gerdemann JW, Nicolson TH. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 1963;46(2):235–244.
28. Walker C, Mize CW. Population of endogonaceous fungi at two locations in central Iowa. Can. J. Bot. 1982;60(12): 2518-2529.
29. Mwangi MW, Monda EO, Okoth SA, Jefwa JM. Effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on growth in tomato (L*ycopersicum esculentum* mill) seedlings, napier *(Pennisetum purpureum* l) and tea (C*amellia sinensis* l) cuttings. Tropical and Subtropical Agroecosystems. 2011;11:423–429.
30. Chliyeh M, Ouazzani Chahdi A, Selmaoui K, Ouazzani Touhami A, Filali-Maltouf A, El Modafar C, Moukhli A, Oukabli A, Benkirane R et Douira A. Effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi against Verticillium wilt of tomato. International Journal of Recent Scientific Research. 2014;5(2):449-459.
31. Talbi Z, Chliyeh M, Mouria B, El Asri A, Ait Aguil F, Ouazzani Touhami A, Benkirane R, Douira A. Effect of double inoculation with endomycorrhizae and *Trichoderma harzianum* on the growth of carob plants. IJAPBC. 2016;5(1):44-58.
32. Nounsi A, Outcoumit A, Talbi Z, Touati J, Ait Aguil F, El Asri A, Ouazzani Touhami A, Benkirane R, Douira A. Effect of endomycorrhizal inoculation on growth of *Eucalyptus* plants. Biolife. 2015;3(3):583-594.
33. Boureima S, Diouf M, Diop TA, Diatta M, Leye EM, Ndiaye F et Seck D. Effects of

arbuscular mycorrhizal inoculation on the growth and the development of sesame (*Sesamum indicum* L.). African Journal of Agricultural Research. 2008;3(3):234-238.

34. Manjunath A, Hue NV, Habte M. Response of *Leuceana leucocephala* to vesicular-arbuscular mycorrhizal colonization and rock phosphate fertilization in an Oxisol. Plant & Soil. 1989;114:127-133.
35. Landeweert R, Hoffland E, Finlay RD, Kuyper TW, Van Breemen N. Linking plant to rocks: Ectomycorrhizal fungi mobilize nutrients from minerals. Trends in Ecology & Evolution. 2001;16:248-255.
36. Strullu DG. Les mycorhizes des arbres et des plantes cultivées. Lavoisier Paris. 3eedit. 1991;249.
37. Bâ AM, Sanon BK, Duponnois R, Dexheimer J. Growth responses of *Afzelia africana* Sm. seedlings to ectomycorrhizal inoculation in nutrient-deficient soil. Mycorrhiza. 1999;9:91-95.
38. He X, Nara K. Element biofortification: Can mycorrhizas potentially offer a more effective and sustainable pathway to curb effective and sustainable pathway to curb human malnutrition? Trends in Plant Science. 2007;12:331-333.
39. Lambers H, Raven JA, Shaver GR, Smith SE. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology & Evolution. 2008;23:95-103.
40. Joner EJ, Leyval C. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environmental Science & Technology. 2003;37:2371-2375.
41. Duponnois R, Cadet P. Interactions of *Meloidogyne javanica* and *Glomus* sp. On growth and N_2 fixation of *Acacia seyal*. Afro Asian Journal of Namatology. 1994;4:228-233.
42. St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA. Inhibition of *Fusarium oxysporum* f. sp. dianthi in the non-VAM species *Dianthus caryophyllus* by co culture with *Tagetes patula* companion plants colonized by *Glomus intraradices*. Canadian Journal of Botany. 1997;75:998-1005.
43. Pozo MJ, Azcón-Aguilar C, Dumas-Gaudot E, Barea JM. b-1,3-Glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica*: Time course analysis and possible involvement in bioprotection. Plant Sci. 1999;141:149–157.
44. Ian AD, Roger TK, Aaron CF. Vesicular–arbuscular mycorrhizal infection of *Quercus rubra* seedlings. New Phytologist. 2001;151:257–264.
45. Rothwell FM, Hacskaylo E, Fisher D. Ecto- and endomycorrhizal fungus associations with *Quercus imbricaria* L. Plant and Soil. 1983;71:309–312.
46. Grand LF. A beaded endotrophic mycorrhiza of northern and southern red oak. Mycologia. 1969;61:408–409.
47. Watson GW, Von der Heide-Spavka KG, Howe VK. Ecological significance of endo-/ ectomycorrhiza in the oak sub-genus *Erythrobalanus*. Arboriculture Journal. 1990;14:107–116.
48. Chen YL, Liu S, Dell B. Mycorrhizal status of *eucalyptus* plantations in South China and implications for management. Mycorrhiza. 2007;17:527–535.
49. Bellei MM, Garbaye J, Gil M. Mycorrhizal succession in young *Eucalyptus viminalis* plantations in Santa Catarina (southern Brazil). For. Ecol. Manage. 1992;54:205–13.
50. Oliveira VL, Schmidt VDB, Bellei MM. Patterns of arbuscular and ectomycorrhizal colonization of *Eucalyptus dunnii* in southern Brazil. Ann. For. Sci. 1997;54: 473–481.
51. Campos DTS, Da Silva MCS, Da Luz JMR, E Maria RJT, Kasuya CM. Colonização Micorrízica Em Plantios De Eucalipto. Revista Árvore, Viçosa-MG. 2011;35(5): 965-974.
52. Souchie EL, Saggin-Júnior OJ, Silva EMR, Campello EFC, Azcón R, Barea JM. Communities of P-solubilizing bacteria, fungi and arbuscular mycorrhizal fungi in grass pasture and secondary forest offungi and arbuscular mycorrhizal fungi in grass pasture and secondary forest of Paraty, RJ – Brazil. Anais da Academia Brasileira de Ciências. 2006;78(1):183-193.
53. Lapeyrie FF, Chilvers GA. An endo-mycorrhiza-ectomycorrhiza succession associated with enhanced growth of *Eucalyptus dumosa* seedlings planted in a calcareous soil. New Phytologist. 1985; 100:93-104.
54. Govindarajulu M, Pfeffer, PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar Hill Y. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature. 2005;435:819-823.

55. Subramanian KS, Charest C. Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought stressed and wellwatered conditions. Mycorrhiza. 1999;9:69-75.
56. Adouane M, Beddiara A. La gestion des subéraies et la qualité du liège. Deuxièmes Rencontres Méditerranéennes Chercheurs-Gestionnaires-Industriels Jijel. 2011;17-19.
57. Boudarga K, Dexheimer J. Sur la mycorhization contrôlée de semis d' *Eucalyptus camaldulensis* Dehnardt par *Gigaspora margarita* Becker & Hal. Annales Des Sciences Forestières. 1989; 4 Competing interests 6(2):131-139.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19617