Journal of Materials and Environmental Science
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2021, Volume 12, Issue 9, Page 1165-1175

http://www.jmaterenvironsci.com

Morphological Responses of *Quercus Suber* L. and *Q. Coccifera* L. Seedlings to Mycorrhization with Desert Truffle *Terfezia boudieri* Chatin

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Received 09 July 2021,
Revised 08 Sept 2021,
Accepted 09 Sept 2021

Keywords
- ✓ *Morphology*
- ✓ Cork,
- ✓ Inoculation,
- ✓ Growth,
- ✓ Truffle.

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Abstract

The present study investigates the effect of mycorrhizal edible desert truffle *Terfezia boudieri* Chatin on the growth of forestry cork *Quercus. suber* and *Q. coccifera* seedlings grown in pots in greenhouse. For both species, mycorrhizae improved significantly stem length, leaf number, leaf dry weight and moisture content of the associate seedlings compared to controls ones. Inoculation with *T. boudieri* changed root characters; mainly it increases length size classes 4 and 5 (0-10mm). Survey of mycorrhizae characteristic proved that mycorrhizae length was approximately double in *Q. coccifera* than *Q. suber* ones. Mycorrhizae width was equal in two plant species. Mycorrhization of both *Quercus* species with *T. boudieri* inoculums enhanced morphological plant status. Results highlighted the efficacy of using this inoculum to promote plants behavior and their tolerance to climatic conditions by enhancing the growth of plants in particular roots properties beside protecting and growing edible truffle species.

1. Introduction

Desert truffles, which are ascomycete fungi, form hypogeous ascomata. These edible fungi are associated with numerous plants species especially with Cistacea shrubs [1]. Among desert truffle, *Terfezia boudieri* Chatin is commonly known and consume in arid and semi-arid of Mediterranean regions. *T. boudieri* establish endomycorrhiza or ectendomycorrhiza. Mycorrhiza type depends on associate plant species, soil type and the culture conditions [2]. The percentage of fine roots colonized by the fungal species is used as the main criteria for quality evaluation of mycorrhized plants.

In Mediterranean forest grows several plant species from these *Quercus*. sp. belonging to Fagaceae family. The ecological importance of cork appears in soil conservation, fight against desertification, the recharge of water reserves and the control of runoff [3, 4].

Deforestation actually is the major environmental problem added to climatic changes and anthropogenic proceedings. Thus *Quercus* sp. are threatened by disappearance like all forestry species besides problems related to cork: pathogens attacks [4], the reduced natural regeneration and the high rate of seedling mortality after transplanting [5]. In Tunisia, cork oak forests cover an area of 70 000 ha, ie 42.6% of the country's hardwood forests [6].

Mycorrhizal fungi associate with approximately 10% of the world's flora, mainly with Fagaceae species (oak, chestnut, beech…), [7] and [8] indicated that these fungi could connect with oaks, pines and cedars in the mountains of Algeria. In Tunisia, symbiotic and parasitic association between several desert truffles and other fungi species and *Q. coccifera* and *Q. suber* was confirmed [9] but not with *Terfezia* species. It is well known the mycorrhizal part in plant growth and nutrition [10,11,12].

Actually, new approach based on production of mycorrhizal seedlings in Mediterranean semi-arid areas occurs to increase plant installation, survive and growth, rehabilitation process in addition to truffle cultivation aims [13,14]. Mycorrhizal potency of two desert truffles species *Terfezia leptoderma* Tulasne and *Tirmania pinoyi* Maire Malençon on the growth of two forest seedlings species *Quercus ilex* L. and *Pinus halepensis* M was studied [15]. However, no studies are available on the mycorrhizal association between *T. boudieri* and *Quercus coccifera* and *T. boudieri* / *Q. suber*. In this study we focused on the determination of mycorrhization effect on *Q. coccifera* and *Q. suber* growth mainly on plant elongation, leaves aspect and number and across evaluation of roots difference through inoculation treatment.

2. Methodology

2.1 Fungal Material

Terfezia boudieri ascomata were collected in February 2019 from Ben Guardane region (33_170N, 10_460E; southeast Tunisia). Ascomata were dried during 2 months and stored at 20°C.

2.2 Plant Material

Quercus suber acorns were collected in November 2019 from Ain Drahem region (36°46'7.76"N; 8°47'47.76"E, 266m Northwestern Tunisia). *Q. coccifera* ones were collected from Beja region in November 2019 (39__062N, 10_494, 322m).

2.3 Substrate

For the realization of mycorrhizal syntheses, we used soil from Ain Drahem zone in North-West of Tunisia. This soil was sterilized before mycorrhizal essays.

2.4 Inoculation and plant cultivation

Inoculation was performed according to the method of [13] using sterile solution of *Terfezia boudieri* ascospores. Ten pots were prepared similarly for each plant species (Plastic pots (1-L volume, 7.5 cm base and 12 cm top diameters) and treatment. Three acorns for each plant species were sown in every pot. Treatments were based on mycorrhization with *T. boudieri* inoculums for mycorrhizal seedlings (M) and without inoculums add for non-mycorrhizal ones (NM). Mycorrhization was conducted according to [16]. Plants were grown in a greenhouse. Irrigation was applied twice a week with tap water during the experimental period.

2.5 Plant growth measuring

2.5.1 Determination of growth characteristics

Plant height and leaf number were measured after 115 days from sowing date for five inoculated and five control seedlings. Ten days interval measurements were then made until the end of the experiment (180 days).

2.5.2 Leaves characterization

Seedlings used in leaves characterization were aged 6 months. For the two *Quercus* species, fresh weight of 15 leaves randomly collected from M and NM seedlings was determined. Mean leaf area was measured using MESURIM software for 15 scanned leaves (Canon solution; Canoscan 4400F). Leaves were next dried at 65°C for 48h. Dry weights of the various samples were defined and moisture content was then calculated.

2.5.3 Root characterization

After six months from sowing date, total root systems of four plants in every treatment and species were carefully isolated, washed and scanned using scanner (Scan Snap SV 600 FUJITSU). Photographs were analyzed by WR-RIPL 2.0 software. Collected data by this software were used to calculate means of total roots length. Sizes classes [17] were fixed on class 1 with length roots between 23 and 31mm; class 2 (from 16-23mm); class 3 (10-16mm) and classes 4 and 5 (0-10mm).

2.6. Mycorrhiza length and width

From each plant species, 10 mycorrhizal seedlings were harvested at the end of the experiment. Sampled roots were washed free of soil and each plant root system was observed in distilled water under stereoscopic loupe (Leica, M205C), 20 mycorrhizas were randomly chosen and their length and width were measured using the loupe automatic software. Results are mean of 20 mycorrhizas.

2.7 Statistical analysis

The variance of multiple parameters (plant length, leave number and area, leaves dry weight and moisture content, length root classes, mycorrhiza length and width) was analyzed with the generalized linear model (GLM) using the SAS statistical software (version 9.0). Multiple comparison of means was performed using the Newman–Keuls method with a threshold p value of 0.05.

3. Results and Discussion

3.1 Effect of mycorrhiza on Q. suber and Q. coccifera growth, leaves and roots characteristics

3.1.1 Plant length

Mycorrhizal seedlings of two *Quercus* studied species have the higher stem length compared to NM ones (Fig. 1a and b). This distinction was continuous in all measured dates. Stem length for M and NM seedlings was more important in *Q. suber* species than *Q. coccifera*. Statistical analysis showed a high significance in the variation of stem length according species and treatment ($p<0.0001$).

3.1.2 Leaves number and area

Leaves number was higher for *Q. suber* M plants than NM in all measure dates (Fig. 2a). Correspondingly, M plants of *Q. coccifera* have a higher number of leaves than NM ones (Fig. 2b). Difference between two *Quercus* species was recorded in M and NM seedlings leaf number; identical to stem length, *Q. suber* has numerous leaves number than *Q. coccifera*. ANOVA's data showed high signification of leaves number variation ($p<0.0001$)

Results demonstrated the main effect of mycorrhization on leaf area characteristics for both species (fig. 3). This statement was confirmed by statistical analysis ($p<0.001$). Mycorrhization enlarge leaf area about twice times for the two studied species. Leaf's area was superior in *Q. coccifera* than *Q. suber*. Data affirm that mycorrhization by *T. boudieri* increases leaves number and area of *Q. suber* and *Q. coccifera* associated plants. Stem length, leaves number and area were enhanced with mycorrhization as previously described [1]. These authors showed that mycorrhizal *Helianthemum sessiliflorum* seedlings were characterized by a higher growth and numerous leaves than controls. While, leaves of inoculated plants of *H. salicifolium* and *H. ledifolium* by *T. boudieri* were longer, bigger and more numerous than non-inoculated ones [18].

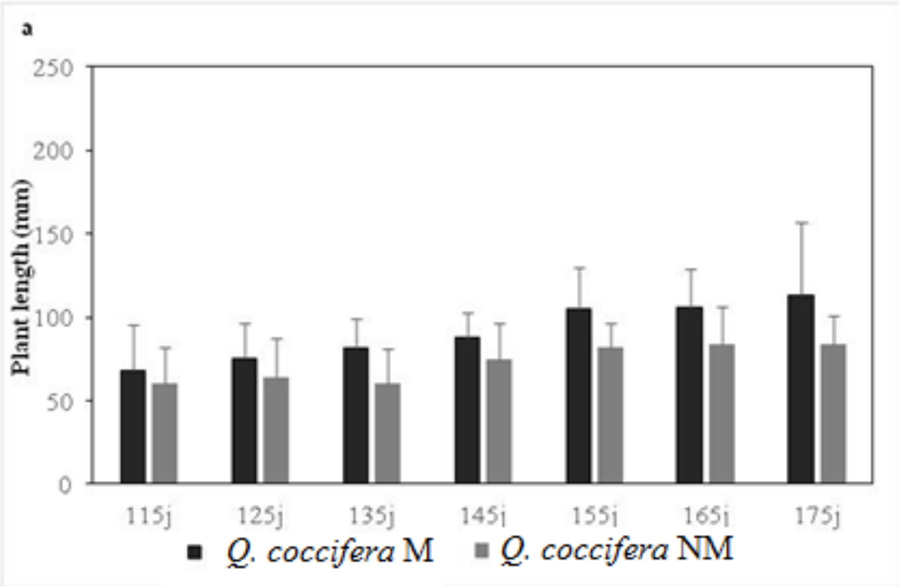

Figure 1a. Variation of plant length according time of mycorrhizal (M) and control (NM) seedlings of *Quercus coccifera*. Errors bars represented the SD of replicates

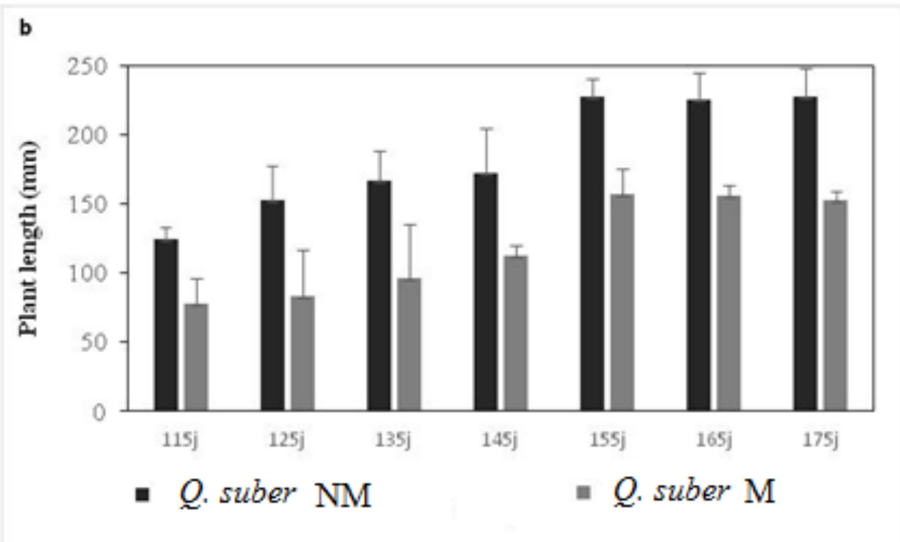

Figure 1b. Variation of plant length according time of mycorrhizal (M) and control (NM) seedlings of *Q. suber*. Errors bars represented the SD of replicates

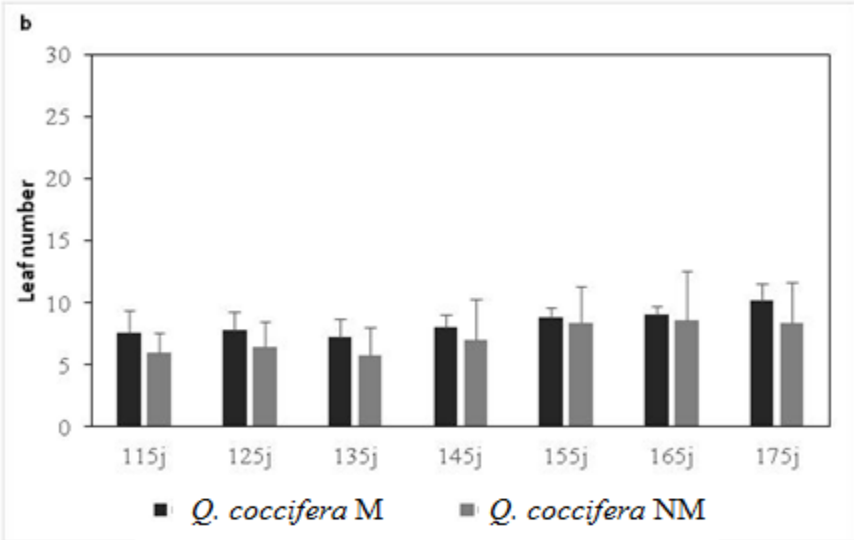

Figure 2a. Variation of leaves number according time of mycorrhizal (M) and control (NM) seedlings of *Q. coccifera*. Errors bars represented the SD of replicates

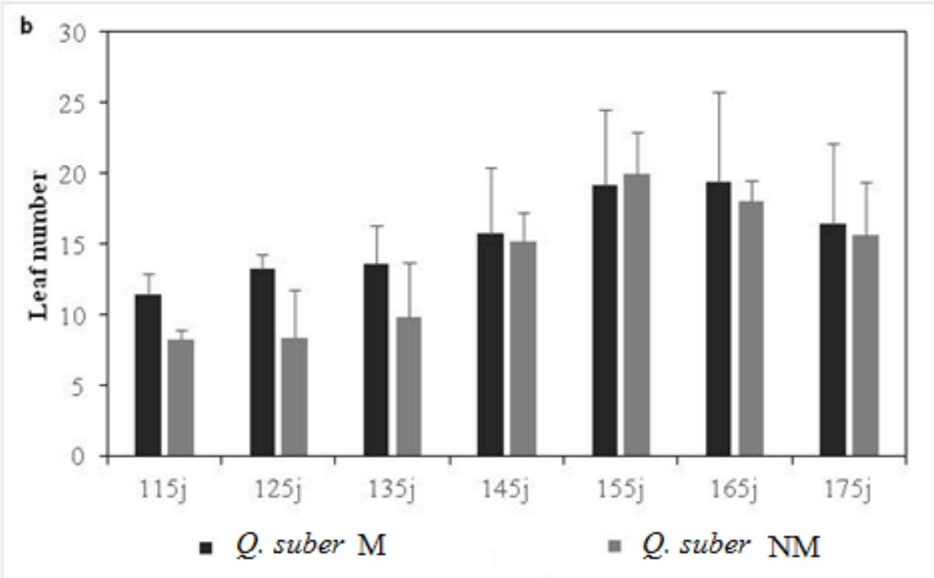

Figure 2b. Variation of leaves number according time of mycorrhizal (M) and control (NM) seedlings of *Quercus suber* (b). Errors bars represented the SD of replicates

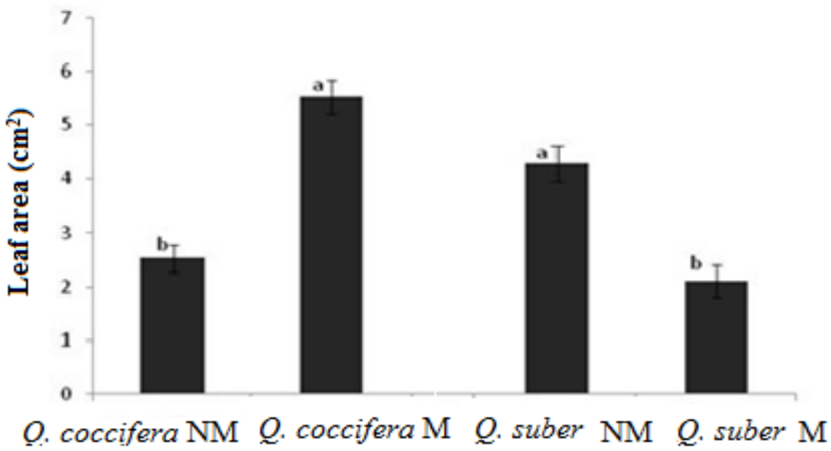

Figure 3. Variation of leaf area in mycorrhizal (M) and control (NM) seedlings of *Q. suber* and *Q. coccifera*. Errors bars represented the SD of replicates. Different uppercase letters above values, SNK groupements.

3.1.3 Leaf dry weight

Mean of 15 leaves dry weight results (Fig.4a) proved that M seedlings of both *Quercus* species have the highest values than NM ones. *Q. coccifera* has the largely mean leaf dry weight compared to *Q. suber* specie. Leaves moisture content data (Fig.4b) corroborate previous conclusion about the beneficial effect of mycorrhization for studied plant. Mean difference analysis for leaves moisture content between species and treatment is significant (p<0.001) however dry weight mean variation according species and treatment was less significant (p<0.01).

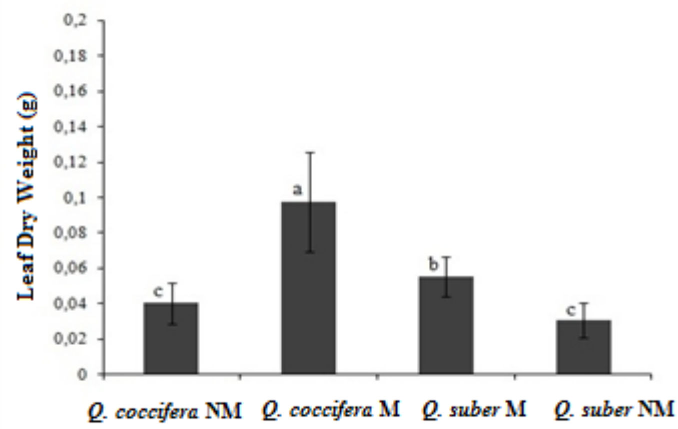

Figure 4a. Variations of leaf dry weight for mycorrhizal (M) and control (NM) seedlings of *Q. suber* and *Q. coccifera*. Errors bars represented the SD of replicates. Different uppercase letters above values, SNK groupements.

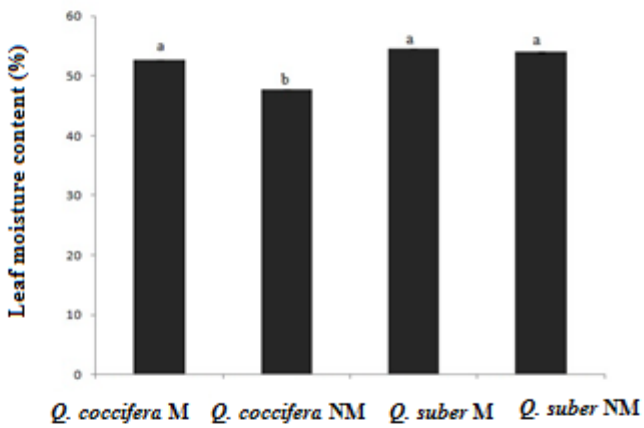

Figure 4b. Variation of moisture leaves content for mycorrhizal (M) and control (NM) seedlings of *Q. suber* and *Q. coccifera*. Errors bars represented the SD of replicates. Different uppercase letters above values, SNK groupements.

3.1.4 Roots characteristics

Roots class sizes variation (Fig. 5), showed a significant effect according treatment (p< 0.05). For both *Quercus* species, roots class 4 and 5 (0-10mm) increase with mycorrhization. Knowing that mycorrhizae structures are represented in this class we can deduce that increase was due to mycorrhiza establishment. Roots class 3 (10-16mm) and 1 (23 - 31mm) were highest in M seedlings of *Q. coccifera* dissimilarly to *Q. suber* ones. Difference for root class 2 (from 16-23mm) was not significant between species and treatment. This class is not affected by mycorrhization. Globally ANOVA's test proved non signification of variation of class size parameter on species and treatment but correlation results state that mycorrhization modify principally root class 4 and 5 (0-10mm) and fewer class 2 (R^2=1 for both classes). This modification depends also on plant species.

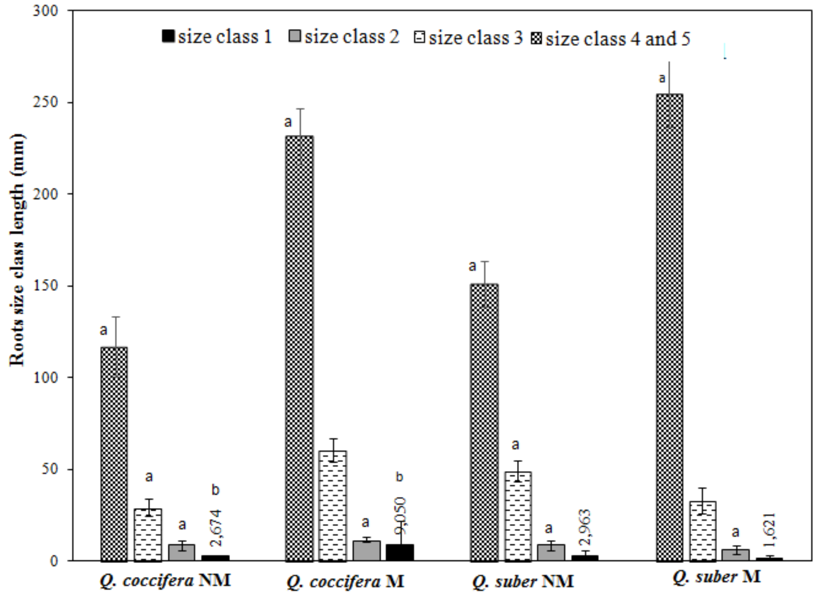

Figure 5. Length of different root classes for mycorrhizal (M) and control (NM) seedlings of *Quercus coccifera* and *Q. suber*. Errors bars represented the SD of replicates. Different uppercase letters above values, SNK groupements.

It is important to notify that destructive practice to detach roots could also modify some records and probably statistic conclusions.

Root length is considered as an important parameter to evaluate root functions and plant vigor [19]. This study has demonstrated that root classes 2 and 3-4 were more represented and greatest in M seedlings of *Quercus suber* and *Q. coccifera* species compared to NM ones. Our results corroborate those of [18] and [20], declaring that roots of *Helianthemum* species were more developed in inoculated plants by *T. boudieri* than non-inoculated ones. It is known that mycorrhization essays roots induction depends on substrate, hormone content (auxin) and container plant size[21]. According to [22] and [23], thin roots ameliorate the surface area and the exchange between plant and soil for water and nutrient. Fine roots (diameter ≤ 2 mm) are responsible of water and nutrients uptake, and participate in the nutrient cycling and energy flow of terrestrial ecosystems[24]. Related observation was announced by [25] whom exhibited that mycorrhizal plants of pepper (*Capsicum annuum* L.) inoculated with the arbuscular mycorrhizal fungi *Glomus intraradices* maintained greater root and biomass at many tested salinity levels compared to control plants. These confirmations valorize our results about *T. boudieri* part in root formation and therefore water and nutrient uptake in mycorrhizal seedlings. Roots increase owing to mycorrhization ameliorates water and nutrient uptake and consequently the growth and vigor of *Q. suber* and *Q. coccifera* seedling.

It's commonly known that elevation of plant biomass and total root length enhance leaf area [26,27,28]. Correspondingly, it was shown that the survival of *Q. suber* seedlings after 10 weeks of drought in semi-controlled conditions was assigned to "osmotic adjustment through soluble sugars and proteins accumulations in leaves and to the increase in carbon discrimination which enhances the water use[29]. These beneficial effects on plant growth has been also demonstrated in mycorrhizal association holm oak / *Terfezia leptoderma* and holm oak / *Tirmania pinoyi* [15] and in association holm oak / European truffles (*Tuber melanosporum, Tuber aestivum* and *Tuber magnatum*) [30]. Similar positive effect of further desert truffle species on the growth of their associated plants has been also demonstrated [13,31,32]. Growth of host plants upgraded thankful to their symbiotic partner. Not only in favorable conditions but mycorrhizal associations promise also the development and survive of their host plants in harsh climates [20].

3.2 Mycorrhiza between Quercus sp./ T. boudieri size

Results about variation of mycorrhiza length and width (Fig. 6) attest that this last parameter depends on species. Stereoscopic observation and counting of mycorrhiza width and length of 20 mycorrhizae characteristic showed that mycorrhizae length was approximately double in *Q. coccifera* than *Q. suber* ones. Data variation of mycorrhiza length was significant (p=0.006). Mycorrhizae width value was not far from in two plant species. Variation of this last parameter according species was not significant. According to [33] for *T. aestivum* species, diameters and length of mycorrhiza at 12 months age strawberry seedling was different from those at 6 months, while only diameter parameter differs over time for *T. melanosporum* fungus. Possibly, *T. boudieri* has a lower affinity for *Q. suber* than *Q.coccifera*. However, further mycorrhization trials of hyphal and ultrastuctural root cells in different growth conditions should be carried out to explain this difference. Mycorrhization with ascocospores and hypha in truffle fructification needs further monitoring to respond to [34] hypothesis about truffles cultivation interest as “new exotic crops”.

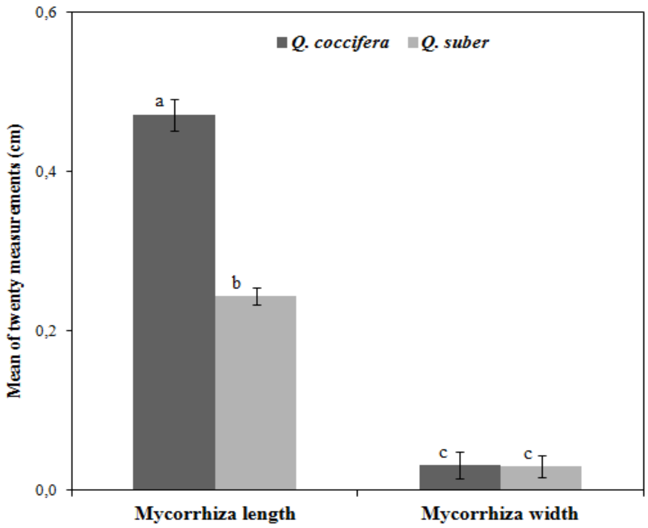

Figure 6. Mycorrhiza width and length on mycorrhizal (M) seedlings of *Q. coccifera* and *Q. suber*. Errors bars represented the SD of replicates. Different uppercase letters above values, SNK groupements

Conclusion

The cultivation of well-mycorrhized plants, followed by their planting into appropriate sites, is the crucial method in recent mycorrhization cultivation. This ecological approach combined with our finding recommends the use of *T. boudieri* inoculums to improve *Q. suber* and *Q. coccifera* morphological performance. Transplantation and utilization of mycorrhizal cork in reforestation could enhance plant behavior and re-equilibrate forest area by increasing plants carbon dioxide sequestration and reducing the effects of climatic change. However, in this equilibrium part of mycorrhizal plant species needs more investigation to explain exactly their contribution. In addition many harsh forest areas suffer of diminution of financial source. Such process could help economical attributes for local habitants in these lands by growing truffle mushrooms.

Acknowledgement

Authors acknowledge the laboratory support of Ms F. Ayari of National Researches Institute of Rural engineering, Water ad Forests (INRGREF).

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

References

[1] A., Slama, M. Gorai, Z. Fortas, A. Boudabous, and M. Neffati, "Growth, root colonization and nutrient status of *Helianthemum sessiliflorum* Desf. inoculated with a desert truffle *Terfezia boudieri* Chatin," *Saudi Journal of Biological Science,* 19 (2012) 25–29.

[2] A. Morte, M. Zamora, A. Gutiérrez, and M. Honrubia, "Desert Truffle Cultivation in Semiarid Mediterranean Areas," Chapter 15,. 221-233, in: C. Azcón-Aguilar et al. (eds.) "Mycorrhizas Functional Processes and Ecological Impact," Springer-Verlag Berlin Heidelberg (2009).

[3] R. Nsibi, N. Souayah, M.L. Khouja, A. Khaldi, A. Skhiri, and S.Bouzid, "Caractérisation de l'état de sénescence de la subéraie Tunisienne," *Integrated Protection in oak forests,* 28 (2005) 14-2.

[4] A.C.L. Da Costa, D. Galbraith, S. Almeida, B.T.T. Portela, M.D. Costa, J.A.S. Junior, A.P. Braga, P.H.L. Gonçalves, A.A.R. Oliveira, R. Fisher, O.L. Phillips, D.L..B Metcalfe, P Levy, and P. Meir, "Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest," *New phytologist,* 187 (2010) 579-591, https://doi: 10.1111/j.1469-8137.2010.03309.x

[5] M.N. Bugalho, M.C. Caldeira, J.S. Pereira, J. Aronson, and J.G. Pausas, "Mediterranean cork oak savannas require human use to sustain biodiversity and ecosystem services," *Frontiers in Ecology and the Environment*, (2011) 9 (5): 278-286, https://doi:10.1890/100084

[6] H. Khiari, "Conséquences des régimes contrastés d'eau et d'azote sur la croissance et la photosynthèse des plants de chêne liège (*Quercus suber* L.)," Dissertation, University of Tunis El Manar (2010).

[7] F. T.Reis, C. Valdiviesso, R. M. Varela Tavares, P. Baptista, and T.Lino-Neto, "Ectomycorrhizal fungal diversity and community structure associated with cork oak in different landscapes," *Mycorrhiza,* 28 (2018) 357–368.

[8] A.D. Chatin, "La truffe". Ed. J.B. Baillière (1892), Paris 370p.

[9] G. Malençon, "Contribution à l'étude des champignons de la Kroumirie," *Bulletin de la Société Botanique de France,* 99 (1952) 33-52.

[10] D. Southworth, E.M. Carrington, J.L. Frank, P. Gould, C.A. Harrington, and W.D. Devine, "Mycorrhizas on nursery and field seedlings of *Quercus garryana*," *Mycorrhiza,* 19 (2009)149–158.

[11] A. Fini, P. Frangi, G. Amoroso, R. Piatti, M. Faoro, C.Bellasio, and F.Ferrini, " Effect of controlled inoculation with specific mycorrhizal fungi from the urban environment on growth and physiology of containerized shade tree species growing under different water regimes," *Mycorrhiza,* 21 (2011) 703–719.

[12] T. Turgeman, J. Ben Asher, N. Roth-Bejerano, V. Kagan-Zur, Y. Kapulnik, and Y. Sitrit, "Mycorrhizal association between the desert truffle *Terfezia boudieri* and *Helianthemum sessiliflorum* alters plant physiology and fitness to arid conditions," *Mycorrhiza,* 21 (2011) 623-630.

[13] Z. Fortas, and G. Chevalier, "Effet des conditions de culture sur la mycorhization de l'*Helianthemum guttatum* par trois espèces de terfez des genres *Terfezia et Tirmania d'Algérie* ", *Canadian Journal of Botany,* 70 (1992) 2453-2460, https://doi.org/10.1139/b92-303

[14] A. Morte, M. Honrubia, and A. Gutierrez, "Biotechnology and cultivation of desert truffles," 467–483, in: Varma A, (ed.) "Mycorrhiza: state of the art genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics". Springer- Verlag, Berlin, Heidelberg (2008).

[15] S. Dib, and Z. Fortas, "Inoculation with desert truffles increases growth of the forest seedlings *Quercus ilex* L. and *Pinus halepensis* M. " *Asian Journal of Microbiology and Biotechnology and Environnemental Science,* 21 (2019) 907-914.

[16] G. Chevalier, and J. Grente, "Application pratique de la symbiose ectomycorhizienne : production à grande échelle de plants mycorhizés par la truffe, " *Mushroom Science* 10 (2) (1979), 483-505.

[17] http://www.nzsoils.org.nz/Topic-Describing_Soils/Size_and_Abundance

[18] M.S. Awameh, "The response of *Helianthemum salicifolium* and *H. ledifolium* to infection by the desert truffle *Terfezia boudieri*," *Mushroom Science*, 11 (1981) 843-853.

[19] T. Gaiser, U. Perkons, P.M. Küpper, T. Kautz, D. Uteau-Puschmann, F. Ewert, A. Enders, and G. Krauss, "Modeling biopore effects on root growth and biomass production on soils with pronounced subsoil clay accumulation". *Ecological Modelling,* 256 (2013) 6-15.

[20] A. Morte, C. Lovisolo, and A. Schubert, "Effect of drought stress on growth and water relations of the mycorrhizal association *Helianthemum almeriense-Terfezia claveryi,*" *Mycorrhiza,* 10 (2000) 115–119.

[21] R. Santelices, and G. Palfner, "Controlled Rhizogenesis and Mycorrhization of Hazelnut (*Corylus avellana* L.) Cuttings with Black Truffle (*Tuber melanosporum* Vitt.)," *Chilean Journal of Agrigultural Research,* 70 (2010) 204-212.

[22] Y. Waisel, and A. Eishel, "Functional diversity of various constituents of a single root system," 157-174, in Y. Waisel, A. Eshel, and U. Kafkafi, eds. "Plant roots: The hidden half," New York (2002).

[23] A. Estrada-Luna, F. J. Davies, and J. Egilla, "Mycorrhizal fungi enhancement of growth and gas exchange of micropropagated guava plantlets (*Psidium guajava* L.) during ex vitro acclimatization and plant establishment," *Mycorrhiza,* 10 (2000) 1–8.

[24] W. Liu, "Correlation between specific fine root length and mycorrhizal colonization of maize in different soil types," *Frontiers of Agriculture in China,* 3 (2009) 13-15.

[25] J. Beltrano, M. Ruscitti, M. C. Arango, and M. Ronco, "Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in

pepper grown under different salinity and P levels," Journal Soil Science and Plant Nutrition, 13(2013) 123-141.

[26] H.R. McCarthy, R. Oren, A.C. Finzi, D.S. Ellsworth, H-S. Kim, K. Johnsen, and B. Millar, "Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO2," *Global Change Biology,* 13 (2007) 2479–2497

[27] S.G. Pritchard, A.E. Strand, M.L. McCormack, M.A. Davis, A.C. Finzi, "Fine root dynamics in a loblolly pine forest are influenced by free-air-CO2-enrichment: a six-year-minirhizotron study," *Global Change Biology,* 14 (2008) 588–602.

[28] D.S. Ellsworth, R. Thomas, K.Y. Crous, S. Palmroth, E. Ward, C. Maier, E. Delucia, and R. Oren, "Elevated CO2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke Face". *Global Change Biology* 18 (2012) 223–242.

[29] H. Daoudi, A. Derridj, L. Hannachi, and J. P. Mevy, "Growth, ectomycorrhization and biochemical parameters of *Quercus suber* L. seedlings under drought conditions," *African Journal of Biotechnology* 15 (2016) 2082-2090.

[30] G. Chevalier, H. Dessolas, and J-CL Pargney, "Nouveau manuel de trufficulture". Réédition du manuel du Docteur Pradel (1914). Ed. Dessolas, Paris (France), (2008) 308p.

[31] A. Navarro-Ródenas, M. Pérez-Gilabert, P. Torrente, and A. Morte, "The role of phosphorus in the ectendomycorrhiza continuum of desert truffle mycorrhizal plants," *Mycorrhiza,* 22 (2012) 565-75.

[32] F. El-Houaria Zitouni-Haouar, Z. Fortas, and G. Chevalier, "Morphological characterization of mycorrhizae formed between three *Terfezia* species (desert truffles) and several Cistaceae and Aleppo pine," *Mycorrhiza,* 24 (2014): 397-403.

[33] F. Ori, M. Leonardi, A. Faccio, et *al.* "Synthesis and ultrastructural observation of arbutoid mycorrhizae of black truffles (*Tuber melanosporum* and *T. aestivum*)," *Mycorrhiza,* 30 (2020) 715 –723.

[34] A. Morte, V. Kagan-Zur, A.Navarro-Ródenas, and Y. Sitrit, "Cultivation of Desert Truffles—A Crop Suitable for Arid and Semi-Arid Zones,". *Agronomy*, 11 (2021) 1462.

(2021) ; http://www.jmaterenvironsci.com