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Research Article

Mycorrhization and root excision effects on morphological and biomass production of carob (*Ceratonia siliqua* L.)

Faten KANFOUD[1,2], Awatef SLAMA[1*], Issam TOUHAMI[1], Abdelaziz AYARI[1], Ines HAJLAOUI[1], Mohamed L. KHOUJA[1], Mohamed T. ELAIEB[1]

[1]*University of Carthage, The National Research Institute of Rural Engineering, Water and Forestry, INRGREF, Laboratory of Management and Valorization of Forest Resources, BP 10 Ariana 2080, Tunisia; fatenkanfoud85@gmail.com; slamaawatef@yahoo.fr (*corresponding author); issam_touhami@yahoo.fr; abdelazizayari@yahoo.fr; ines.hajlaoui@gmail.com; khoujalarbi@yahoo.fr; ayeb2022@yahoo.fr*

[2]*University of Tunis EL Manar, Faculty of Sciences of Tunis, Departement of Biology, Tunis 2092, Tunisia*

Abstract

Ceratonia siliqua L. plant (carob tree) is a Mediterranean species. In Tunisia, the natural distribution of this species is situated in humid zones in the north to the driest ones in the south of the country. *Ceratonia siliqua* regeneration depends on seeds quality and performances as well as on the practical vegetative propagation tools. Nowadays, carob tree cultivation is limited by the seedlings quality behind the juvenile stage caused by the deterioration of plant root system. This study investigates the effect of lone and mutual root excision technique and mycorrhization on carob seedlings growth performance. Two carob seeds origins were germinated then excised and/or mycorrhized using a sterile substate. For control seedlings, experiment was made without excision nor mycorrhization application. Mycorrhizae applied with excision ameliorated significantly plant length, leaf number, dry and fresh weight of external plant part and roots, collar root diameter and roots branching numbers. Survey of mycorrhizae effects proved also amelioration on carob growth and biomass production but indiscernibly than use of mycorrhization and excision techniques together. Excision alone does not take a part on seedling increasing performance. Mycorrhization of carob species applied with excision increased morphological plant status. Results highlighted the efficiency to use this process to promote plants behaviour by enhancing plant roots and growth in forest areas and agricultural lands.

Keywords: carob tree; inoculation; *Rhizophagus intraradices*; root excision

Introduction

Carob tree (*Ceratonia siliqua* L.), is a plant species belonging to Fabaceae family, endemic of the Mediterranean regions (Gharnit and Ennabili, 2015). This species grows naturally in several bioclimatic zones with a large spectre adaptation to soil nature. Carob distribution extends gradually with farming essays

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(Haddarah *et al.*, 2013; Boubkar *et al.*, 2021). This tree species is recently introduced and cultivated in Australia, Africa, and central America (Palaiogianni *et al.*, 2022).

In Tunisia this species has a great ecological and socio-economic interest (Rejeb, 1992; Dallali *et al.*, 2018) and is exploited for its various products (lumber, leaves, fruits, edible pods and seeds). Currently, carob bean gum and locust bean flour are becoming increasingly used in food, pharmaceutical and dietetic industries (Makris and Kefalas, 2004; Aissa *et al.*, 2021).

The carob tree is a dioic legume plant with some hermaphroditic species (Battle, 1997; Di Guardo *et al.*, 2019). The vegetative propagation of carob is naturally restricted due to its low adventitious rooting potential and that's why carob propagation is generally coupled to seeds uses. Carob trees have an extensive root system with a deep taproot and lateral roots. These plant species are resilient, drought and salt-tolerant, with a low orchard management requirement (Boutasknit *et al.*, 2021). Carob trees fight soil erosion and desertification processes therefore this plant species is commonly used as windbreaks around orchards.

Effect of symbiosis on the development of *Ceratonia siliqua* L. was confirmed previously (Cruz *et al.*, 2004; Aysan and Deemir, 2009). Several studies showed the positive effect of mycorrhizal symbiosis on the growth and production of plants in poor soils (Haro *et al.*, 2017; Dalli *et al.*, 2020). Arbuscular mycorrhizal fungi are symbiotic microorganisms associated with the majority of plant species in agro-ecosystems process (Ryan and Graham, 2018) to improve nutrition and growth of associated plants (Manga *et al.*, 2017; Outamamat *et al.*, 2021). Mycorrhizal potency of several arbuscular mycorrhizal fungi species on the growth of plant species was studied (Kchikich *et al.*, 2021, Liu *et al.*, 2021). However, no studies are available on neither root excision nor mycorrhization effects on carob morphological response. In this study we focused on the lone and mutual effects of root excision and mycorrhization on *Ceratonia siliqua* growth mainly on plant length, leaves number, biomass production, roots elongation and root branching number.

Materials and Methods

Plant materials

Carob seeds (Figure 1), used in this study, were collected in August 2019 from two Tunisian localities (Figure 2). First location was Zaghouan (localized in the North of Tunisia /Sub-humid) where seeds were provided by the General Direction of Forests (DGF). The second location was Sayada (Monastir /Semi-arid). Seeds were assembled from local orchards.

Figure 1. Carob seeds collected from two Tunisian provenances (Zaghouan and Mounastir)

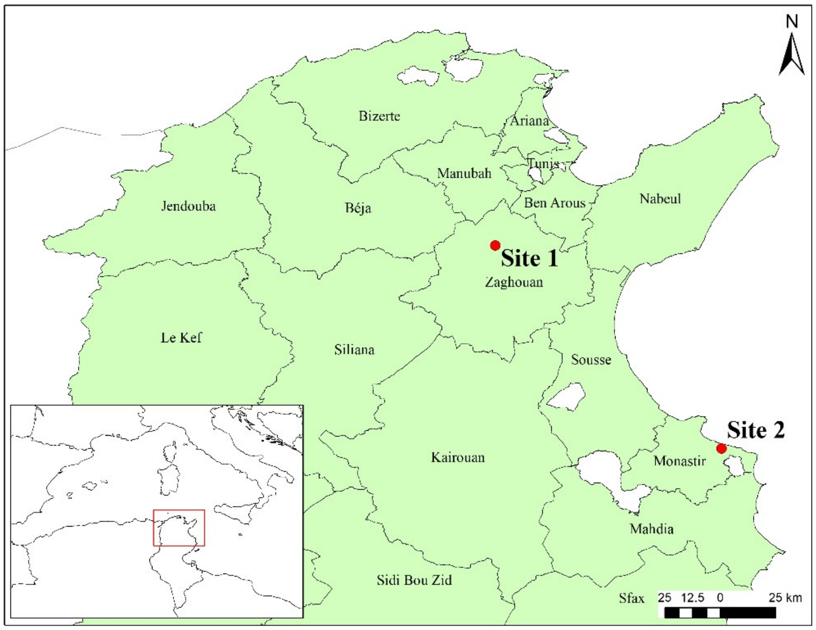


Figure 2. Location of Carob seeds sampling (Zaghouan and Sayada provenances)

Seeds treatment

For each carob origin site, 150 seeds were soaked in tap water for 24 hours without any pre-treatment, then placed in Petri dishes filled with sterile peat for germination essay. Petri dishes were incubated in obscurity at 25 °C in an incubator (Blender).

Roots excision

After one week, root excision was applied when the radical makes 10 mm of length. 50% of germinated seeds (75 seeds) were excised while others (75 seeds) were not and considered as control.

Plant mycorrhization

Mycorrhization was made using the commercial *Rhizophagus intraradices* (syn*: Glomus intraradices*) fungus species spores.

50% of excised and control seeds (37 seeds in each treatment) were mycorrhized with *Rhizophagus intraradices* species. Mycorrhization was made by the addition of 5 g of fungus spores below seven seeds lay in every pot filled with sterile substrate (five pots for each treatment). Substratum was composed of a mixture of sand and peat (2-1). For control seedlings, substrate were free from fungus spores. Thus, four groups of treatment were obtained for two carob seeds origins: control (not excised and not mycorrhizal seeds), excision (Ex-NM), mycorrhization (NEx-M) and mutual excision and mycorrhization treatments (Ex-M). Plastic pots (1l/volume) were used. Seedlings were maintained in greenhouse at ambient humidity and a growing temperature ranging from 25 °C to 30 °C. A manual irrigation with top water was applied twice a week.

Growth parameters measurements

Plant length, root collar diameter and leaves number of five carob seedlings were measured using a ruler every 15 days during 3 months, then a final measure at 9 months seedling age was recorded. This delay was chosen to evaluate excision and mycorrhization effects on carob juvenile stage of seedlings (after 9 months). In the same dates root collar diameter was calculated using a vernier caliper.

Fresh and dry weight of external plant part and roots

Three plants in each treatment and seed site were collected; root system was washed carefully with tap water. External plant part and roots were separated then weighted for counting fresh weights. Dry weights of same parts were determined after drying at 60 °C for 48 hours.

Total root system branching

All roots part of the three sampled seedlings used previously was washed with tap water. Total root system branching was determined by numbering of all root branches besides the principal root length was measured with ruler.

Statistical analysis

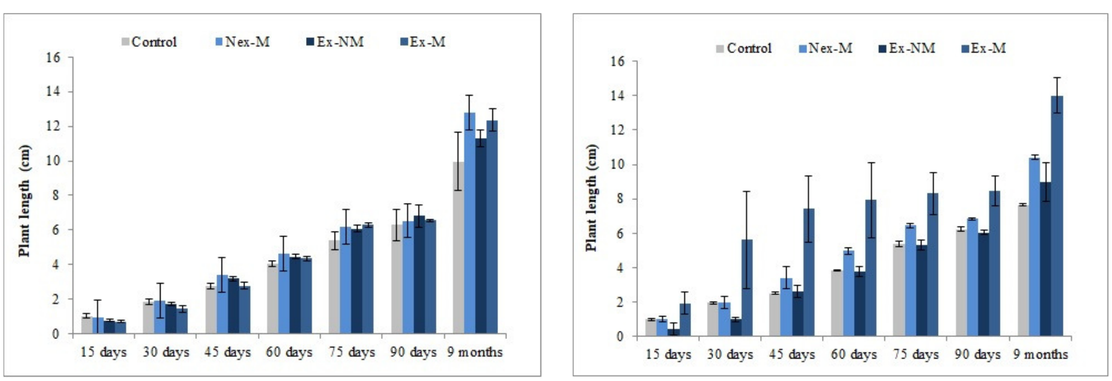
The variance of multiple parameters (plant length, leaf plant number, root length, root collar diameter, root branching number, fresh and dry external plant part and roots weights) was analysed with the generalized linear model (GLM) using the SAS statistical software (version 9.0). Multiple comparisons of means were performed using the SNK test with a threshold p value of 0.05.

Results

Effect of excision and mycorrhization on plant growth

Plant length

Results of the effect of roots excision and mycorrhization on the plant length of carob seedlings originated from Zaghouan (Figure 3A) and Sayada (Figure 3B) showed that variation of carob length increases with measurement dates for control in all tested treatments. A difference in carob origin sites according to treatment effect was registered. Mycorrhization was the significant treatment which affects carob seedlings length originated from Zaghouan. However, mutual effect of excision and mycorrhization provided longer seedlings than excision and mycorrhization applied separately. Length seedlings increased approximately 1/3 times between treated and control ones from the period from 3 to 9 months. Statistical analysis proved that the mean difference of seedlings length variation according to time were significant ($p<0.05$) and high significant according to treatment ($p=0.001$). No significance for seeds site was registered. For both carob origins the effect of mycorrhization and root excision applied together were discarded from uncombined treatment and from controls.



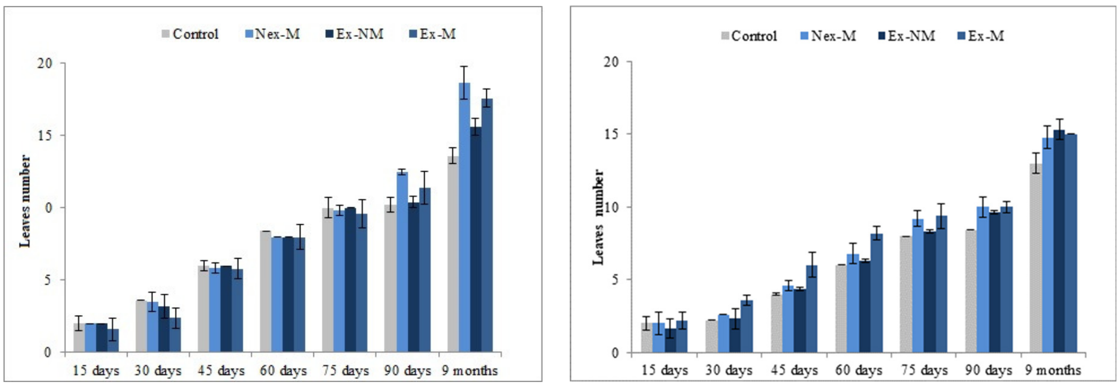
(A) (B)

Figure 3. Plant length of mycorrhized (Nex-M), excited (Ex-NM), excited and mycorrhized (Ex-M) and control seedlings of carob originated from Zaghouan (A) and Sayada (B) sites

Errors bars represented the SD of five replicates

<u>Leaves number</u>

Leaves number of treated and none treated carob seedlings from Zaghouan (Figure 4A) and Sayada sites (Figure 4B) increased with time. From early stage (15 days), effect of mycorrhization and excision combined on carob leaf number was higher than other treatments and control seedlings. After this date the remarkable effect was registered for the uniquely application of mycorrhization and for the combination of mycorrhization and excision. Statistical analysis showed that leaf number was not a discriminative factor between tested sites and treatments used. In fact, in experimental numbering, a leaf fall was noticed in several measurement dates. This loss of leaves could justify the statistical result found.



(A) (B)

Figure 4. Leaves number of mycorrhized (Nex-M), excited (Ex-NM), excited and mycorrhized (Ex-M) and control seedlings of carob originated from Zaghouan (A) and Sayada (B) sites

Errors bars represented the SD of five replicates

<u>Roots length</u>

Data of root length of control and treated carob seedlings (Figure 5) distinguished diversity through site origin. Zaghouan provenance seems more corresponding with root elongation variation compared with Sayada provenance. In both provenance combination of mycorrhization and excision permitted the best root system growth of carob seedlings followed by the separate application of mycorrhization and then excision. ANOVA' test proved that variation of root length was high significant according to time (p=0.02) and treatment (p=0.0002) and significant according to seeds origins (p<0.05). Among the used treatments, SNK test showed that Ex-M has the major treatment effect followed by NEx-M. Application of only root excision not influenced root length. Data with this treatment were not different from none treated carob seedlings (control) ones.

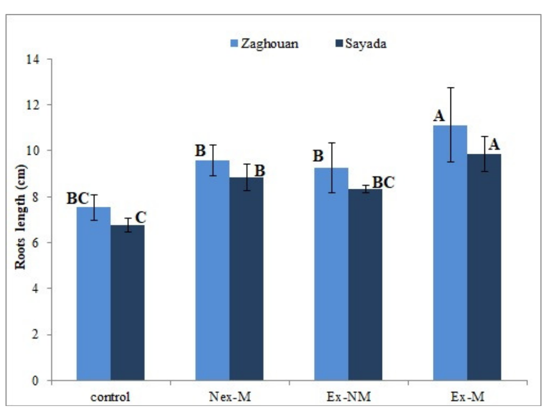


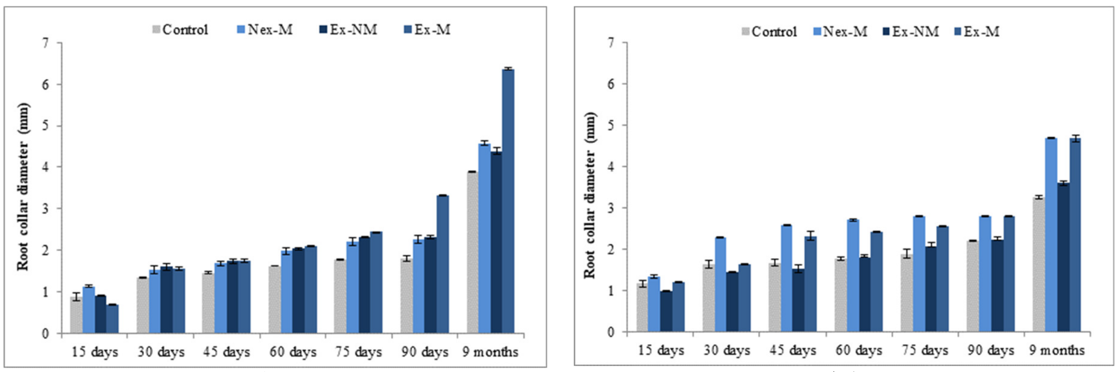
Figure 5. Roots length of mycorrhized (Nex-M), excited (Ex-NM), excited and mycorrhized (Ex-M) and control seedlings of carob originated from Zaghouan and Sayada sites

Errors bars represented the SD of triplicates. Different uppercase letters above values, SNK groupements.

Root collar diameter

The effect of root excision and mycorrhization of carob plants on their collar roots diameter (Figures 6A and 6B) showed that in all measure dates, control plants have the lowest diameter than treated ones. Application of mycorrhization and excision separately and mutually, ameliorated significantly collar roots diameter growth of carob seedlings. This amelioration was maintained with plants grow old.

After nine months of growing, control carob seedlings showed a collar root diameter of 3.89±0.021 mm and 3.26±0.04 mm respectively for Zaghouan and Sayada sites. This parameter increased respectively to 4.582±0.052 mm/4.693 ±0.012 mm with mycorrhization, 4.384±0.083 mm /3.611 ±0.025 mm with excision and to 6.37 ±0.027 mm /4.678 ±0.079 mm with combination of excision and mycorrhization respectively. Statistical analysis showed that variation of collar diameter was significant according to time and treatment ($p< 0.05$) and not significant according to seeds origins. Carob species don't depend on origin sites and responds similarly for both tested ones.



(A) (B)

Figure 6. Roots collar diameter (mm) of mycorrhized (Nex-M), excited (Ex-NM), excited and mycorrhized (Ex-M) and control seedlings of carob originated from Zaghouan (A) and Sayada (B) sites
Errors bars represented the SD of triplicates

Root branching number

The variation in the number of root branching with carob origin and applied treatments (Figure 7) demonstrated that the principal treatment that affects root branching number was the application of mycorrhization and excision mutually. Statistical analysis showed a high significance in the variation of root branching number according to time and treatment ($p<0.0001$) and not significance according to seeds sites.

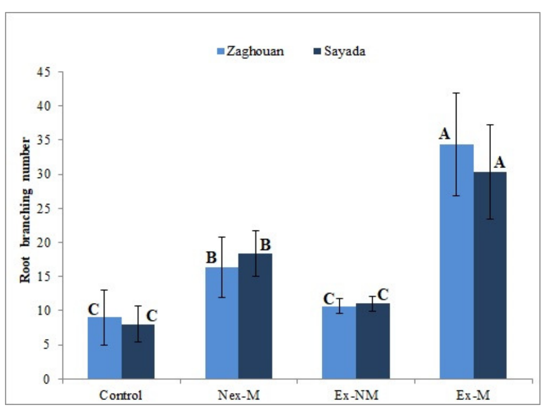
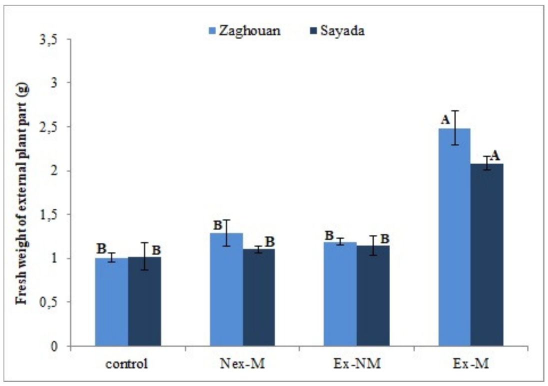


Figure 7. Branching number of mycorrhized (Nex-M), excited (Ex-NM), excited and mycorrhized (Ex-M) and control seedlings of carob originated from Zaghouan and Sayada sites
Errors bars represented the SD of triplicates. Different uppercase letters above values, SNK groupements.

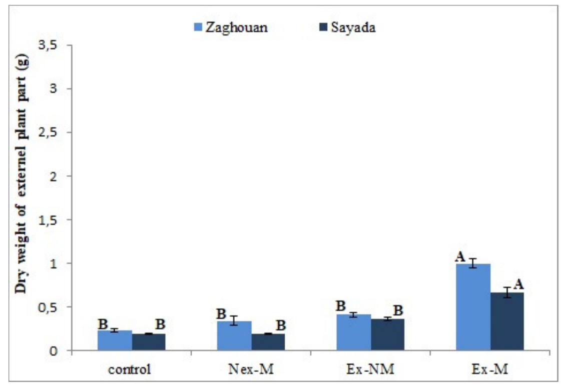
Plant weight

<u>External parts weight</u>

Fresh weight of carob external parts in control seedlings were approximately analogous for both tested seeds origin (1.008±0.05 g and 1.02±0.15g for Zagouan and Sayada sites respectively). These values increased weakly with the use of mycorrhization and excision separately but highly with the application of mutual treatments. Data of fresh and dry weights of external plant parts (Figures 8A and 8B) proved the advantageous effect of used treatments especially when proceeding mycorrhization and excision in the same time. Variation of dry weight of carob external parts was high significant according to time, treatment and seeds sites ($p<0.0001$) however variation of fresh weight depends only on treatment used and according to times ($p<0.0001$).



(A)

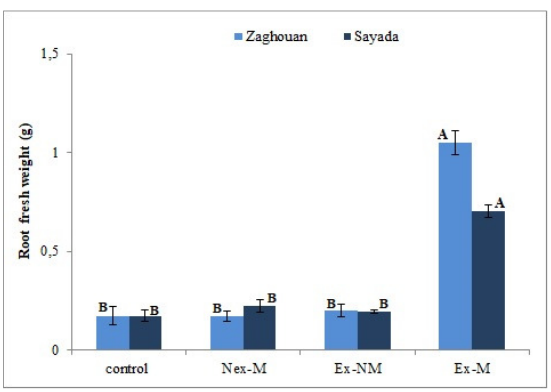


(B)

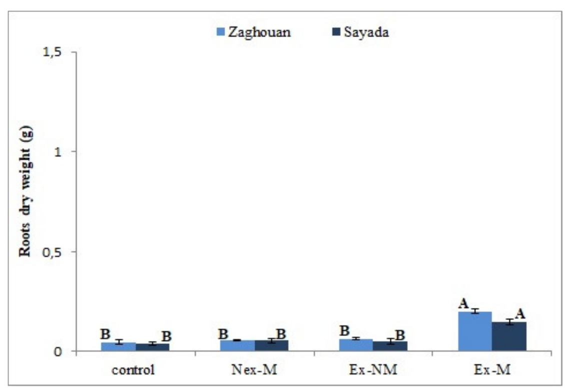
Figure 8. Fresh (A) and dry (B) weights of external part of mycorrhized (Nex-M), excited (Ex-NM), excited and mycorrhized (Ex-M) and control seedlings of carob originated from Zaghouan and Sayada sites
Errors bars represented the SD of triplicates. Different uppercase letters above values, SNK groupements

<u>Roots weight</u>

Carob seedlings originated from Zaghouan and Sayada were affected by the used treatments. Control seedlings showed the lowest fresh and dry weights among others (Figures 9A and 9B).



(A)



(B)

Figure 9. Roots fresh (A) and dry (B) weights of mycorrhized (Nex-M), excited (Ex-NM), excited and mycorrhized (Ex-M) and control seedlings of carob originated from Zaghouan and Sayada sites
Errors bars represented the SD of triplicates. Different uppercase letters above values, SNK groupements

Heavier plants were those obtained with mycorrhization and excision at the same time. Statistical analysis showed that variation of roots dry weight of carob was high significant according to time, treatment and seeds sites ($p<0.0001$). Also, variation of fresh roots weight was highly significant according to time and treatment ($p<0.0001$). Seeds origin is not a significant factor for the variation of root fresh weight ($p>0.01$).

Discussion

During the entire follow-up period of experiment, the root excision and mycorrhization of carob seedlings influences plants growth. Results showed that mycorrhizal and excised seedlings have the greatest collar root diameter than control ones and then those treated separately with mycorrhization or excision. Similar results are found by Haro *et al*. (2020) by proving an increase of 170.51% on plant length of inoculated plants compared to non-inoculated ones.

Leguminous plants are characterized by a high mycorrhizal dependence allowing a better soil use and absorption of water and nutrients (Kasaka *et al*., 2021). This enrichment on plant nutrition supports plant growth and biomass production. Indeed, the number of leaves, roots and plant length of carob seedlings were more distant with mycorrhization application than control and other treatments. In a Moroccan degraded site, uses of a complex native AM community (naturally associated with carob trees), improved carob trees growth and nutritional status and induced a positive soil microbial environment for nutrient cycling and resistance to environmental stress (Manaut *et al*., 2015). This positive effect observed during a mycorrhizal association on the biomass production of the aerial and the root parts, was earlier confirmed by Trouvelot *et al*. (1982) on wheat. These results are in agreement with Diop *et al*. (2013) ones who showed that the biomass production of vine was enhanced by mycorrhizal inoculation. Our results were also in accord with Zouari *et al*. (2020) confirmations about rooting and growth of carob plants improvement by establishing a successful artificial symbiosis with *P. tinctorius* fungus species.

Root length is considered as an important parameter to evaluate root functions and plant vigor (Nia *et al*., 2021; Slama *et al*., 2021). Excision of roots of young cork-oak plants give significantly better results than plants treated with physical treatment. Compensatory growth is the primary adaptive response to root restriction using regulation and increase of growth plasticity and nutrient and water uptake by plant (Yan *et al*., 2011). Maize stem and root growth was improved after root restriction (Zhang and Ning, 2022)

As we know, mutual effect of mycorrhization and root excision on plants isn't studied until now but, a positive effect of arbuscular mycorrhization on walnut micro propagated seedlings was demonstrated (Morier, 2021). It seems that physical stress could enhance plant capacity to establish connection and symbiosis with mycorrhizal fungi when excised or grafted

Excised roots in cork oak species developed lengthy and most branched roots (El Boukhari *et al*., 2013).

Root excision alone applied here did not have a great effect on root and plant differentiation from control but mutual application of root excision with mycorrhization promotes carob seedlings growth performance. The association with fungus partner in roots part accelerated plant root elongation and growth and support root branching. This effect of mycorrhizal fungi was earlier demonstrated by Slama *et al*. (2021, 2012) and Nouaim and Chaussod (1994) who showed that the root system becomes more efficient after treatments by mycorrhization. Commonly, these treatments amplify the surface percentage of soil use by fungal hyphae expanded area. Indeed, Riedacker (1979) confirmed that excision of the roots allows the regeneration of 2 to 8 of new roots. In our study, the average number of branches will ensure a better occupation of soil as well as a better supply of water and minerals. The branching of the underground part of carob tree is one of the characteristics linked to the species itself (Riedacker, 1979; Clarke and Mosse, 1981; Battle, 1997). Related observation was announced by Beltrano *et al*. (2013) whom exhibited that mycorrhizal plants of pepper (*Capsicum annuum* L.) inoculated with the arbuscular mycorrhizal fungi *Rhizophagus intraradices* maintained greater root and biomass at many tested salinity levels compared to control plants. These confirmations could valorise our results about *Rhizophagus* sp. 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 carob seedling. The same type of effects observed for the various types of treatments applied on the growth parameters (length, root collar diameter and number of leaves) on rhizogenesis, affects therefore fresh and dry plant biomass. After 9 months, plant behaviour and growth confirm the effectiveness of these two techniques uses to promote carob performance.

The possible genetic variability between seeds attributed to geographic regions (Nia *et al.*, 2021) do not generates difference on carob response against mycorrhization and root excision.

Conclusions

Plants mycorrhization is highly used in replanting programs. This study highlighted mycorrhization coupled to root excision effect on carob morphological responses and improve carob species growing. For this, it's recommended for agronomists, forest managers and nurserymen to simultaneously use of these two techniques in planting practice. Since, root excision effect on plant growth requires further experimentation to explain physical and enzymatic variations. Discernment of plant response system on carob rooting could resolve carob species installation and roots problems.

Authors' Contributions

Data analysis; Investigation; Manuscript redaction and corrections (AS and FK); Methodology; Project administration (MLK, AA and IH); Resources; Supervision; Validation (IT and MTE). All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aissa A, Chakroun I, Rejeb R, Ayed M (2021). Effect of partial dietary substitution of Carob (*Ceratonia siliqua* L.) to barley grains on diet digestibility in growing rabbits. Journal of New Sciences Agriculture and Biotechnology 79(1):4580-4585.

Aysan E, Demir S (2009). Using arbuscular mycorrhizal fungi *Rhizobium leguminosarum* and *Biovar phaseoli* against *Sclerotinia sclerotiorum* (Lib.) de Bary in the common bean (*Phaseolus vulgaris* L.). Plant Pathology Journal 8:74-78.

Battle I (1997). Current situation and possibilities of development of the carob tree (*Ceratonia siliqua* L) in the Mediterranean region. Rome, Italy.

Beltrano J, Ruscitti M, Arango MC, Ronco M (2013). 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 13:123-141. *http://dx.doi.org/10.4067/S0718-95162013005000012*

Boubkar F, Rachid F, Fatima A, Oudou IA, Saadia T, Said W (2021). Bioclimatic impact on the carob seeds morphological diversity in Morocco. Bioscience Biotechnology Research Asia 18(1). *http://dx.doi.org/10.13005/bbra/2908*

Boutasknit A, Baslam M, Ait-El-Mokhtar M, Anli M, Ben-Laouane R, Ait-Rahou Y, Meddich A (2021). Assemblage of indigenous arbuscular mycorrhizal fungi and green waste compost enhance drought stress tolerance in carob (*Ceratonia siliqua* L.) trees. Scientific Reports 11(1):1-23. *https://doi.org/10.1038/s41598-021-02018-3*

Clarke C, Mosse B (1981). Plant-growth responses to vesicular-arbuscular mycorrhiza. 12. Field inoculation responses of barley at 2 soil-p levels. New Phytologist 87(4):695-703. *https://doi.org/10.1111/j.1469-8137.1981.tb01704.x*

Cruz C, Green JJ, Watson CA, Wilson F, Martins-Loucào AA (2004). Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity. Mycorrhiza 14:177-184. *https://doi.org/10.1007/s00572-003-0254-5*.

Dallali S, Aloui F, Selmi H, Sebei H (2018). Comparison of the chemical composition and the antioxidant activity of the leaves of Carob tree (*Ceratonia siliqua* L.) collected in three sites of Djebel Zaghouan (Tunisia). Journal of New Sciences Agriculture and Biotechnology CIRS (21):3429-3438.

Dalli Y, Yahia N, Bekki A (2020). Diversity of arbuscular mycorrhizal fungi associated with carob trees (*Ceratonia Siliqua* L.) in Western Algeria. Plant Cell Biotechnology and Molecular Biology 180-193.

Di Guardo M, Scollo F, Ninot A, Rovira M, Hermoso JF, Distefano G, La Malfa S, Batlle I (2019). Genetic structure analysis and selection of a core collection for carob tree germplasm conservation and management. Tree Genetics & Genomes 15:41. *https://doi.org/10.1007/s11295-019-1345-6*

Diop I, Kane A, Krasova-Wade T, Sanon KB, Houngnandan P, Neyra M, Noba K (2013). Impacts of pedoclimatic conditions and cropping system on the response of cowpea (*Vigna unguiculata* L. Walp.) to inoculation with endomycorhizal *Rhizophagus irregularis*. Journal of Applied Biosciences 69:5465-5474. *https://dx.doi.org/10.4314/jab.v69i0.95072*

El Boukhari E, Gmira N, Brhadda N (2013). Effet des traitements physiques sur la croissance et le développement des semis de glands de chêne liège (*Quercus suber* L.) en pépinière forestière au Maroc. Geo-Eco-Trop 37(2):177-190.

Gharnit N, Ennabili A (2015). Categories of carob tree (*Ceratonia siliqua* L.) from Morocco. International Journal of Fruit Science 16(3):259- 274. *https://doi.org/10.1080/15538362.2015.1102674*

Haddarah A, Ismail A, Bassal A, Hamieh T, Ioannou I, Ghoul M (2013). Morphological and chemical variability of Lebanese carob varieties. European Scientific Journal 9(18):353-369. *https://doi.org/10.19044/esj.2013.v9n18p%p*

Haro H, Sanon KB, Le Roux C, Duponnois R, Traoré AS (2017). Improvement of cowpea productivity by rhizobial and mycorrhizal inoculation in Burkina Faso. Symbiosis 1-14. *https://doi.org/10.1007/s13199- 017-0478-3*

Haro H, Semde K, Bahadio K, Sanon KB (2020). Effect of mycorrhizal inoculation with arbuscular mycorrhizal fungi strains on *Mucuna pruriens* (L.) DC growth under controlled condition. International Journal of Biological and Chemical Sciences 14(3):1065-1073.

Kasaka D, Onautshu O, Muliwambene K, Lebisabo B, Baert G, Swennen R, Haesaert G, Dhed'a D (2021). Importance and diversity of Mycorrhizae under plantain cultivation in the slash-and-burn and non-burn cropping systems in the forest region of Kisangani, Tshopo Province, Congo. African Journal of Agricultural Research 17(6):809-821. *https://doi.org/10.5897/AJAR2020.15411*.

Kchikich A, Mrid RB, Kabach I, Nhiri M, Omari RE (2021). Arbuscular mycorrhizal fungi enhance sorghum plant growth under nitrogen deficient conditions through activation of nitrogen and carbon metabolism enzymes. International Journal of Agriculture and Biology 26:201-208. *https://doi.org/10.17957/IJAB/15.1825*

Liu RC, Xiao ZY, Hashem A, Abd Allah EF, Xu YJ, Wu QS (2021). Unraveling the interaction between arbuscular mycorrhizal fungi and Camellia plants. Horticulturae 7(9):322. *https://doi.org/10.3390/horticulturae7090322*.

Makris DP, Kefalas P (2004). Carob pods (*Ceratonia siliqua* L.) as a source of polyphenolic antioxidants. Food Technology and Biotechnology 42(2):105-108.

Manaut N, Sanguin H, Ouahmane L, Bressan M, Thioulouse J, Baudoin E, Galiana A, Hafidi M, Prin Y, Duponnois R (2015). Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. Ecological Engineering 79:113-119. *https://doi.org/10.1016/j.ecoleng.2015.03.007*

Manga A, Ndiaye F, Diop TA (2017). Le champignon arbusculaire *Glomus aggregatum* améliore la nutrition minérale d'Acacia seyal soumis au stress salin progressif. International Journal of Biological and Chemical Science 11(5):2352-2365. *https://doi.org/10.4314/ijbcs.v11i5.32*

Mortier E (2021). Impact de la fertilisation phosphatée et de la mycorhization arbusculaire sur le développement de porte-greffes de noyers fruitiers micropropagés (Impact of phosphate fertilization and arbuscular mycorrhization on the development of micropropagated walnut tree rootstocks). PhD Thesis. Franche-Comté Univ, Bourgogne.

Nia S, Addi M, Abid M, Belkoura I (2021). Effect of pre-sowing treatments and basal media on in vitro carob (*Ceratonia siliqua* L.) seed germination. Journal of Biotech Research 12:74-82.

Nouaim R, Chaussod R (1994). Mycorrizal dependency of micropropagate d'Argan tree (*Argania spinosa*). Growth and biomass production. Agroforestry Systems 27:53-65. *https://doi.org/10.1007/BF00704834*

Outamamat E, Dounas H, Aziz F, Barguaz A, Duponnois R, Ouahmane L (2021). The first use of morphologically isolated arbuscular mycorrhizal fungi single-species from Moroccan ecosystems to improve growth, nutrients uptake and photosynthesis in *Ceratonia siliqua* seedlings under nursery conditions. Saudi Journal of Biological Sciences. *https://doi.org/10.1016/j.sjbs.2021.11.049*.

Palaiogianni A, Stylianou M, Sarris D, Agapiou A (2022). Carob-agro-industrial waste and potential uses in the circular economy. In: Mediterranean Fruits Bio-wastes: Chemistry, Functionality and Technological Applications. Cham: Springer International Publishing, pp 765-797.

Rejeb MN (1992). Etude des mécanismes de résistances à la sécheresse du caroubier (*Ceratonia siliqua* L.) en Tunisie (Study of drought resistance mechanisms of the carob tree (*Ceratonia siliqua* L.) in Tunisia). PhD Thesis. Faculty of Sciences, Manar Univ, Tunis.

Riedacker A (1979). Etude de la déviation des racines horizontales ou obliques issues de bouture de peuplier qui rencontrent un obstacle: application pour la conception de conteneurs (Study of the deviation of horizontal or oblique roots from poplar cuttings which encounter an obstacle: application for the design of containers). Annales Sciences Forestières 351:18.

Ryan MH, Graham JH (2018). Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. New Phytologist 220:1092-1107. *https://doi.org/10.1111/nph.15308*

Slama A, Fkiri S, Fortas Z, Nasr Z, Khaldi A (2021). Morphological responses of *Quercus suber* L. and *Q. coccifera* L. seedlings to mycorrhization with desert truffle *Terfezia boudieri* Chatin. Journal of Material Environmental Science 12(9):1165-1175.

Slama A, Gorai M, Fortas Z, Boudabous A, Neffati M (2012). Growth, root colonization and nutrient status of *Helianthemum sessiliflorum* Desf. inoculated with a desert truffle *Terfezia boudieri* Chatin. Saudi Journal of Biological Science 19:25-29. *https://doi.org/ 10.1016/j.sjbs.2011.04.003*

Trouvelot A, Gianinazzi-Pearson V, Gianinazzi S (1982). Les endomycorhizes en agriculture; recherché sur le blé (Endomycorrhizae in agriculture: researched on wheat). In INRA (Eds). Les mycorhizes. France pp 251-255.

Yan H, Li K, Ding H, Liao C, Li X, Yuan L, Li C (2011). Root morphological and proteomic responses to growth restriction in maize plants supplied with sufficient N. Journal of Plant Physiology 168(10):1067-1075. *https://doi.org/10.1016/j.jplph.2010.12.018*

Zhang Y, Ning P (2022). Differential root response of maize inbred seedlings to root growth restriction and phosphorus availability. Biologia 1-9. *https://doi.org/10.1007/s11756-022-01174-9*

Zouari N, El Mtili N (2020). Effects of ectomycorrhizal fungal inoculation on growth and rooting of carob tree (*Ceratonia siliqua* L.). South African Journal of Botany 135:181-187. *https://doi.org/10.1016/j.sajb.2020.08.025*