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Original article

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.

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ABSTRACT

The carob tree (*Ceratonia siliqua* L.) is an important component in semi-arid Mediterranean ecosystems, particularly in Morocco where it plays a considerable socio-economic role. This species is widely used in the reforestation programmes and in the rehabilitation of degraded soils serving both environmental and socio-economic objectives. In spite of these assets, this species is suffering the particular climatic conditions, rare and irregular rains, long hot and dry summers, generally, leading to desertification processes. To withstand these contrasting conditions, selected arbuscular mycorrhizal fungi (AMF) were tested for their contribution to the growth, nutrient uptake and photosynthesis improvement of the carob tree *C. siliqua* under nursery conditions.

The objective of this study was, to evaluate the effects of some arbuscular mycorrhizal fungi complexes isolated in different Mediterranean ecosystems compared to single-species isolates selected using morphological tools on the growth, mineral nutrition, and chlorophyll content of *C. siliqua* seedlings.

The results indicate that all the used AMF *inocula* stimulated significantly the height of *C. siliqua* seedlings after eight months under nursery conditions. An increase in plant height between 33% and 70% compared to a control without inoculation was recorded. Similarly, the aerial dry weight recorded an increase of 62% to 124% comparing inoculated and non-inoculated seedlings. The root dry weight has shown an increase rate of 24% to 86% compared to the control. The analysis of mineral contents in plant tissues, showed a highly significant increase in P. N. K. Ca and Mg levels of the aerial parts compared to the control. A significant increase in chlorophyll contents was noticed when inoculated seedlings were compared to non-inoculated ones. This study had confirmed the importance of AMF improving the growth of *C. siliqua* seedlings; the AMF complexes remain to have the important growth and mineral nutrition responses. However some single- species have shown similar magnitude to the complexes for all analysed parameters. A large biofertilizer potential of the single-species isolates in the inoculation of *C. siliqua* is demonstrated for the first time.

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1. Introduction

The Mediterranean ecosystems are considered among the most original of the planet and the richest in biodiversity thank to the diverse biogeographical specificities (climates, soils, landscapes) in this region (Blondel and Aronson, 2015). Currently, the environmental conditions in Mediterranean areas are becoming severe and are affecting vegetation and soil quality (structure, micro and macro-element contents, etc.) (Mace and Masundire, 2005; Cuttelod et al., 2008). Thus this area is considered to be one of the most vulnerable regions (Zaimeche and Sutton, 1997). Plants are facing several difficult situations such as stress and overexploitation of natural resources, leading to delicate and impoverished soils. The development and production of the most tree plants in these arid and semi-arid zones are limited, leading to their degradation (Zaimeche and Sutton, 1997; Duponnois et al., 2013). In Morocco, the carob tree is an important thermophilic species (Rankou et al., 2017). The carob tree is present in the country in natural stands or in artificial plantations up to 1150 m (Emberger and Maire, 1945; Batlle and Tous, 1997; Baumel, 2018). This species has the capacity to develop in such a contrasting environment, which supposes its high degree of adaptability (Ozturk et al., 2010). It contributes to the preservation of the landscape and plays considerable socio-economic roles. The concern in this plant has increased incredibly in these regions due to the development of the food industry and the growing demand for carob products (Gubbuk et al., 2010). All the components of the tree (foliage, flowers, Pods, wood, bark, and roots) are beneficial, and have been evaluated. *C. siliqua* pods, pulp, seeds and gum are widely traded and used in the food industry (Biner et al., 2007; Dakia et al., 2007; Papaefstathiou et al., 2018). Despite these assets, this species is subject to aggressions due to biotic and abiotic constraints, such as rare and irregular rainfall, long hot and dry summers. These conditions have led to intense destruction of forests and deterioration of ecosystems and have often limited the performance of reforestation tasks (Rankou et al, 2017). This degradation is accompanied by an increase in the impact of erosion on the surface stratum of soil, leading to a decrease in the soil physicochemical, and biological fertility (Requena et al., 2001). Nowadays, maintaining the quality of soil is a key issue to optimize the stability and the productivity of natural ecosystems (Buscot, 2005). Soil microorganisms are responsible for essential ecosystem functions such as biogeochemical nutrient cycling, maintenance of plant health and soil quality (Barea et al., 2005; Richardson et al., 2009; El Kinany et al., 2018). Initiatives have been undertaken to domesticate this forest species in nurseries and plantations, in reforestation programs serving both environmental and socio-economic objectives (Ouahmane et al., 2012; Manaut et al., 2015). *C. siliqua* is used to develop marginal lands (Batlle and Tous, 1997; Janick and Paull, 2008). Among the successful revegetation strategies in these ecosystems, those based on the use of mycorrhizal symbiosis are widely adopted (Azcón-Aguilar et al., 2003; Duponnois et al., 2007). Two strategies are generally proposed depending on the level of environmental degradation (Duponnois et al., 2013), the management of mycorrhizal soil infectivity through the establishment of indigenous and mycotrophic plant species (Azcón-Aguilar et al., 2003; Ouahmane et al., 2006) and/or the prior inoculation of plants *via* selected high-performance fungal strains (Caravaca et al., 2003; Manaut et al., 2015). The carob tree is indeed highly dependent for its growth on the efficiency of interactions with soil mycorrhizal fungi (Ouahmane et al., 2012; Jadrane et al., 2021). Arbuscular mycorrhizal fungi (AMF) improve the efficiency of nutrient uptake by plants in low fertility soils by increasing the area of uptake and mobilization of nutrient sources (Smith and Read, 2008). They help plants to tolerate biotic (Pozo et al., 2010) and abiotic stress (Wu et al., 2013; Jadrane et al., 2021). They influence the structure and activity of microbial communities in the mycorrhizosphere (Marschner and Timonen, 2005; Dabire et al., 2007) and also promote coexistence between plant species (Hart et al., 2003). In a nutrient-poor environment, AMF contribute not only to plant establishment but also to soil improvement and protection (Caravaca et al., 2003). In Mediterranean ecosystems, many researchers have recognized that the mycorrhizal symbiosis is a key factor in the facilitation of plants establishment on degraded soils by the use of native AMF in controlled mycorhization practices (Ouahmane et al. 2007). In addition, the use of native species and drought-tolerant plants has been reported in many studies to accelerate succession and restore functional plant cover (Caravaca et al., 2003; Alguacil et al., 2005).

The objective of this study was to evaluate and compare the effects of Arbuscular mycorrhizal fungi complexes and single-species isolates from different Mediterranean ecosystems on the growth, on the mineral nutrition and on the photosynthetic activity of *C. siliqua* under nursery conditions. The ultimate goal was to use these fungal isolates as biofertilizers in reforestation programs based on the production of *C. siliqua* seedlings inoculated with efficient and competitive arbuscular mycorrhizal fungi in controlled mycorrhization manipulations.

2. Materials and methods

2.1. Study sites

This study was conducted in five areas located in the regions of Marrakesh -Safi and Zagora in Morocco. The climate is arid to semi-arid. Samples were conducted under different climates and soil conditions. The following representative plants were sampled, *Argania spinosa, C. siliqua, Phoenix dactylifera and Retama monosparma).* Some extreme stressed sites were sampled like salty soil and abundant mine site. (Fig. 1) (Table 1).

2.2. Soil samples

The soil samples used in this study were obtained from the above-mentioned sites. At each site, approximately 10 kg of soil close to the root system was collected from 10 randomly selected trees. The samples were taken at a depth of 10 to 30 cm and homogenized to obtain a representative sample of the entire site.

2.3. Preparation and multiplication of the mycorrhizal inoculum

The Corn (*Zea mays*) was used in trap culture for the production of endomycorrhizal inoculum for mycorrhizal complexes. For this, the corn seeds were disinfected in a 30% (v/v) hydrogen peroxide solution for 30 min and rinsed several times with sterile distilled water and then germinated in plastic pots containing soil from the various stations. The complexes were classified as A1 (rhizosphere soil of the Argan tree), A4 (Abandoned mine), A7 (Rhizosphere soil of the Carob tree), A9 (Salty soil) and A10 (Rhizosphere soil of the date palm tree) (Table 1).

2.4. Spore extraction and preservation

Spore suspensions from (*Zea mays*) culture were prepared using two techniques. One was based on the wet sieving and decantation of the soil samples (Gerdemann and Nicolson, 1963). The second one consists in concentrating the spores by centrifugation on a sucrose solution (Brundrett et al., 1996). Recovered spores were stored in polyvinyl-lacto-glycerin (PVLG) medium (Estaún et al., 1997).

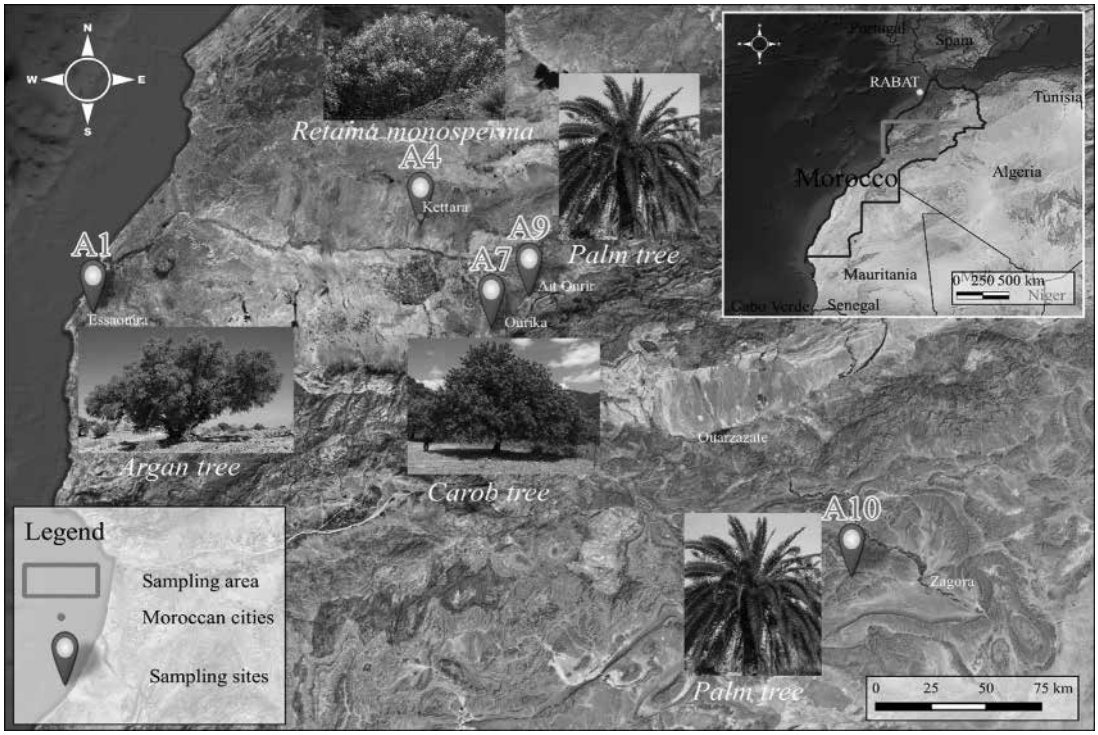

Fig. 1. Geographical position of the sampling sites.

Table 1
Geographical position, physicochemical parameters and fungal spore richness of the sampling sites. Mean values ± SE in the same column followed by the same lower case letters are not significantly different according to Tukey (HSD) test ($P < 0.05$).

Sites	Rhizosphere soil	Localization	pH	Conductivity ($ms.cm^{-1}$)	TOC (%)	N (%)	P(ppm)	Number of fungal spores/100 g of soil
Essaouira (A1)	**Argan tree**	31.49 ± 9.71	8.09^{b} ± 0.02	0.35^{d} ± 0.01	4.56^{a} ± 0.2	1.38^{a} ± 0.12	64.76^{a} ± 3.23	1240^{a} ± 62
Abandoned mine kettara (A4)	**Retama shrub**	31.87 ± 8.18	6.81^{d} ± 0.1	44.13^{b} ± 2.2	0.95^{c} ± 0.03	0.93^{b} ± 0.03	2.28^{e} ± 0.23	470^{c} ± 58
Ourika valley (A7)	**Carob tree**	31.43 ± 7.84	8.06^{b} ± 0.03	0.41^{d} ± 0.12	5.07^{a} ± 0.2	1.59^{a} ± 0.23	47.28^{b} ± 2.67	970^{b} ± 80
Salty site (A9)	**Date palm tree**	31,57 ± 7.67	7.05^{c} ± 0.05	118.03^{a} ± 1.97	3.67^{b} ± 0.43	0.46^{c} ± 0.14	12.24^{c} ± 1.11	425^{d} ± 70
Zagora (A10)	**Date palm tree**	30.38 ± 6.21	8.23^{a} ± 0.12	7.75^{c} ± 0.22	0.96^{c} ± 0.01	0.32^{d} ± 0.21	4.45^{d} ± 0.89	520^{c} ± 15

2.5. Single-species culture

The selection process of AMF, was based on the most abundant morphotypes among the spore community forming the complexes (A1, A4, A7, A9, A10). Indeed, the major morphotypes in the complexes might be the most effective fungal species colonizing and promoting the growth of the sampled host plant (Table1).The production of monospecific isolate from single spore suspension was applied using the technique of micropipette tips proposed by (Feldmann., 1998) and well detailed by (Tchabi et al., 2009). Monosporal culture process usually starts with carefully selected healthy and infectious spore originated from rhizosphere soils. The success varies greatly depending on the type of soil, the amount of organic matter, the environmental conditions and the season. Sandy soils with low organic matter content seem to give the best results. More consistent results were obtained by using spores from potted crops for obvious reasons because many spores are newly formed and therefore of closer age and morphological distinctions between species are easier to detect (minimizing contamination) (Tchabi et al., 2009). The single-species culture can then be summarized in the following six points. Isolation of AMF spores from trap cultures, e.g. by sucrose-gradient technique, collection of spores in a Petri dish. Set up of pipette tip microcosms to promote mycorrhizal formation due to the narrow space of leek roots and AMF spore. After 2 months the bottom of the pipette tip is cut, to enable the plant to expand when it is transferred to a bigger soil volume. The transfer of pipette tip into bigger soil volume (100 ml) to promote the multiplication of spores. Additionally, more leek plants are added to the soil (Maize, sorghum, wheat can be added as well). After another 3 months culture, the soil can be dried out and all pots can be screened if the mycorrhizal establishment was successful, regular success rate about 10–15 % was recorded. The soil of positive samples can be used as *inocula* to set up bigger pots for spore multiplication (1–2 L). Finally six refined monospecific isolates were produced (A1RF, A1RC, A4RF, A7RF, A9RF, A10RF).

2.6. Experimental protocol

In order to compare the effects of different *inocula*, termed as complexes A1, A4, A7, A9, A10 and fungal isolates A1RF, A1RC, A4RF, A7RF, A9RF, A10RF on *C. siliqua* plants in controlled mycorrhization experiment, fungal isolates were placed against the root system of the carob seedlings at planting time when the germinated seeds were 1 to 2 cm long. For this, 2 g of root fragments extracted from the three months Maize culture containing the multiplied fungus were used as inoculum. Sterilized *C. siliqua* rhizospheric soil was used as substratum in the experiment (Fig. 2).

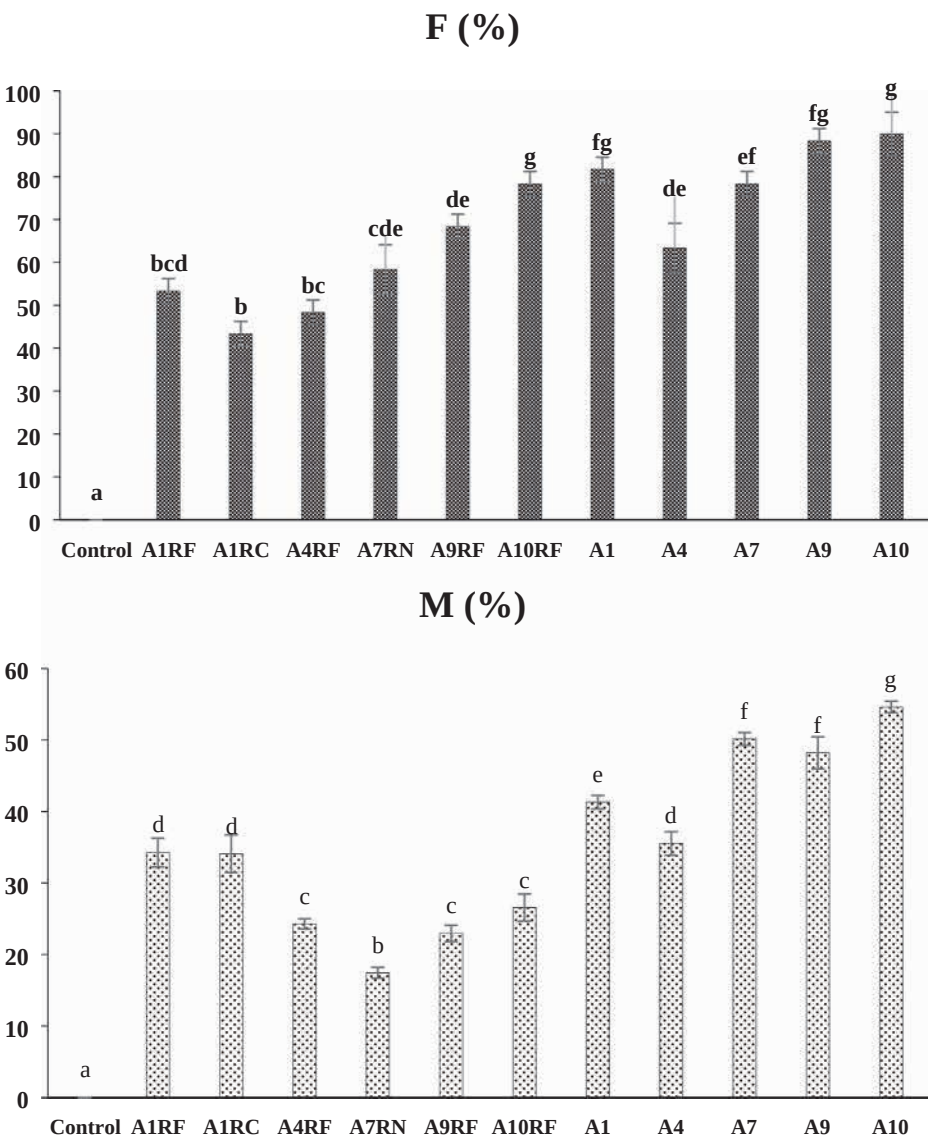

Fig. 2. Roots infection rate in *C. siliqua* seedlings after inoculation with different fungal complexes and isolates. Mycorrhizal frequency (F%) and colonization intensity (M%). *The values represented by the same letter are not significantly different according to Tukey's test at* $p \leq 0.05$.

Pots containing 2 kg of soil collected under Carob tree and sterilized (140 °C for 6 h) were used in the experiment. The mean soil characteristics were pH (8.06), clay% (18), silt% (40.9), sand% (41.1), C% (5.11), N% (1.07), and P mg.Kg-1 (18). The experiment was conducted under greenhouse conditions at the Cadi Ayyad University of Marrakesh. The average day/night temperature was 36/25 °C, the relative humidity (RH) was 55/86 % and the photoperiod was about 16 h light / 8 h dark. Seedlings were daily irrigated with tap water. Ten repetitions for each treatment were set. Five repetitions were used for the analysis of growth, and mineral nutrition parameters.

2.7. Parameters assessment

2.7.1. Determination of root colonization and plant biomass production

A fraction of the roots, from the lateral root system, was carefully washed, cleared with 10% of KOH at 90 °C for 30 min, then acidified with 1% HCl for 10 min and was stained with Trypan blue at 90 °C for 20 min (Phillips and Hayman, 1970). AMF infection frequency and intensity were evaluated in root fragments by the method of Trouvelot (Trouvelot, 1986). The response of plants to mycorrhizal inoculation was evaluated after 8 months of cultivation by determining the biometrical data (i.e., plant height, dry weight of shoots and roots, the root collar diameter)

2.7.2. Photosynthetic pigments

Photosynthetic pigments were extracted from leaf samples in 80% acetone as described by Arnon (Arnon, 1949). The extracted material was centrifuged at 10 000 × g for 10 min. The optical density of the supernatants was recorded at 480, 645 and 663 nm using a UV–vis spectrophotometer (UV-3100PC spectrophotometer) and a blank containing 80 % acetone was used as a control. Chlorophyll *a*, b and total chlorophyll contents were determined.

2.7.3. Nutrient analysis

To determine the mineral contents, oven-dried shoots and roots were powdered, and digested with 98% H_2SO_4 and 30% H_2O_2. The phosphorus (P) content was estimated after biomass calcination using the Olsen method (Olsen, and Dean, 1965) and the total

nitrogen was measured by the Kjeldahl method. The contents of Na^+, K^+ and Ca^{2+} in the seedlings were measured by flame photometry (JENWAY, PFP7) as described by Wolf (Wolf, 1982).

2.7.4. Statistical analyses

The data were processed with Principal Component Analysis (PCA) to separate the different treatments and determine the groups according to the measured parameters. The PCA was completed by a variance analysis (ANOVA) at the 5% threshold with the XL stat software, followed by the Tukey test for a comparison of the means which allows to classify the different inoculums and to know which ones are the most adapted to carob trees in terms of growth improvement.

3. Results

3.1. Mycorrhizal inoculants

From the trap culture five mycorrhizal complexes were obtained and numbered A1, A4, A7, A9 and A10 (Table 1). Generally these complexes were consisted of different genera in different rates, such as *Glomus* (82–86%), *Rhizophagus* (10–14%) *Claroideoglomus* (1–2%), *Sclerocystis* (0.5–2%), *Paraglomus* (0.5–1%), *Gigaspora* (0.5–1%), *Acaulospora* (0.5–1%).

From the monosporal culture, six monospecific isolates were produced (A1RF, A1RC, A4RF, A7RF, A9RF, A10RF) from respective complexes and were assimilated to (*Glomus sp*1, *Glomus sp*2, *Glomus sp*3, *Glomus sp*4, *Glomus sp*5, *Glomus sp*6).

3.2. Evaluation of arbuscular mycorrhizal fungi infection

Microscopic observation of the roots of inoculated seedlings has shown inequalities in the behaviour of these seedlings with respect to different isolates and fungal complexes, which confers that *C. siliqua* is very receptive to colonization (Fig. 3). The mycorrhizal frequency was higher than 43% for all the tested *inocula*. Mycorrhizal complexes were more infectious, the mycorrizal frequency was higher than 63% especially A10 and A9 showed a frequency exceeding 88%, the native carob complex A7 recorded a mycorrhizal frequency of 78%). The monospecific isolates tested were less infectious with the exception of the A10RF and A9RF isolates which have recorded a high frequency of 65% (Fig. 3). Similarly the mycorrhizal colonization intensity of seedling roots was higher for the different AMF inoculants, and the mycorrhizal complexes presented higher intensities than mono-specific isolates.

3.3. Effect of different fungal inocula on the growth of Ceratonia siliqua

After 8 months of cultivation, the results obtained are shown in (Fig. 2). Generally, inoculation with the different tested fungal *inocula* had a marked effect on plant growth, especially on height. The best stimulation was obtained when the seedlings were in association with the A9, A10 complexes. The height is larger than 34% compared to the control. For the fungal isolates A9RF and A10RF an important improvement in height was recorded (more than 24%). In addition the root collar diameter was higher in mycorrhizal seedlings than in the control test. The highest root collar diameters were observed in plants inoculated with the mycorrhizal complexes (A9, A10) (Fig. 4).

Overall, the measurement of fresh and dry shoot and root weights had shown a positive effect of the inoculation of the seedlings with the different isolates compared to the control. However, it was noted that the shoot and root dry weight was the best criterion for growth estimation. In this case, it was observed that the dry weight is increased for over 50% in mycorrhizal seedlings compared to the control. The best values were obtained using the complexes A9 and A10.

3.4. Effects of different isolates on the nutrient contents

The different *inocula* had significantly improved the nutritional pool of the carob tree in a wide range of ways (Fig. 5). Phosphate nutrition was significantly higher when plants were inoculated with the complexes especially with A9 and A10 and with the isolate A9RF compared to the control seedlings. For nitrogen nutrition, the inoculation of the plants significantly induced an increase in nitrogen content and the best values were recorded with the A9 complex and also with the A9RF isolate (p greater than 0.05) (Fig. 4). With respect to other nutrients, and more specifically potassium (K) and calcium (Ca) levels, the efficacy of the arbuscular mycorrhizal fungi was observed in plants inoculated with the different complexes and isolates. For magnesium (Mg) nutrition, a significant improvement was recorded compared to the control, especially with the mycorrhizal complexes (Fig. 5).

3.5. The effect of different isolates on chlorophyll contents

All the used mycorrhizal fungi *inocula* induced an increase in chlorophyll contents compared to the control. The best values were recorded in the inoculated plants with the complexes compared to the isolates. The complexes extracted from stressed environments (A4 abundant mine and A9 saline soil) had shown higher improvements of chlorophyll contents in plant tissues, compared to the control (Fig. 6).

4. Discussion

The arbuscular mycorrhizal fungi play an important role in the establishment and sustainability of plant cover. However, in degraded soils of Mediterranean ecosystems, the mycorrhizal infectious potential is very low. Hence the need to improve this potential in natural and artificial regeneration programmes in these arid and semi-arid ecosystems. Results obtained in the current study showed that the AMF colonization rates varie between different types of inoculations. Globally, this rate is improved in the case of inoculation with mycorrhizal complexes, particularly with AMF from stressed zones (A9 and A10) and in the case of simple inoculation by isolates, which allows a high colonization rate, especially with the isolates A9RF and A10R (Fig. 3).These results corroborate other studies carried out in the Mediterranean region on the carob tree (Ouahmane et al., 2012; Manaut et al., 2015; Jadrane et al. 2021) and also on other forest plants (Bouamri et al., 2006; Abbas et al., 2007) which have shown high mycorrhizal infection in these plants. On the basis of the previous results, it is noted that inoculum from stressed environments ((A10) or (A9)) have a high capacity to colonize roots of *C. siliqua* seedlings.

The results showed a positive effect of the different *inocula* on the growth parameters of *C.siliqua* , after eight months, (Fig. 4) which resulted in a twice as important increase in plant height compared to the control. Similar trend was recorded for the fresh and dry aerial and root biomass, which were significantly increased compared to the control. Consquently, the growth rate of the plants was significantly higher. In general, the highest values were recorded for complexes which stimulated strongly the plant growth compared to the other fungal isolates. Besides, inoculation with AMF isolates had an important effect compared to the control. Similar results were reported in other plants in arid and semi-arid regions (Abbas et al., 2007; Ouahmane et al., 2007).

After eight months of planting, P and N content of the leaves of the young carob seedlings was higher in the mycorrhized plants compared to the control (Fig. 5) (Manaut et al., 2015). Between

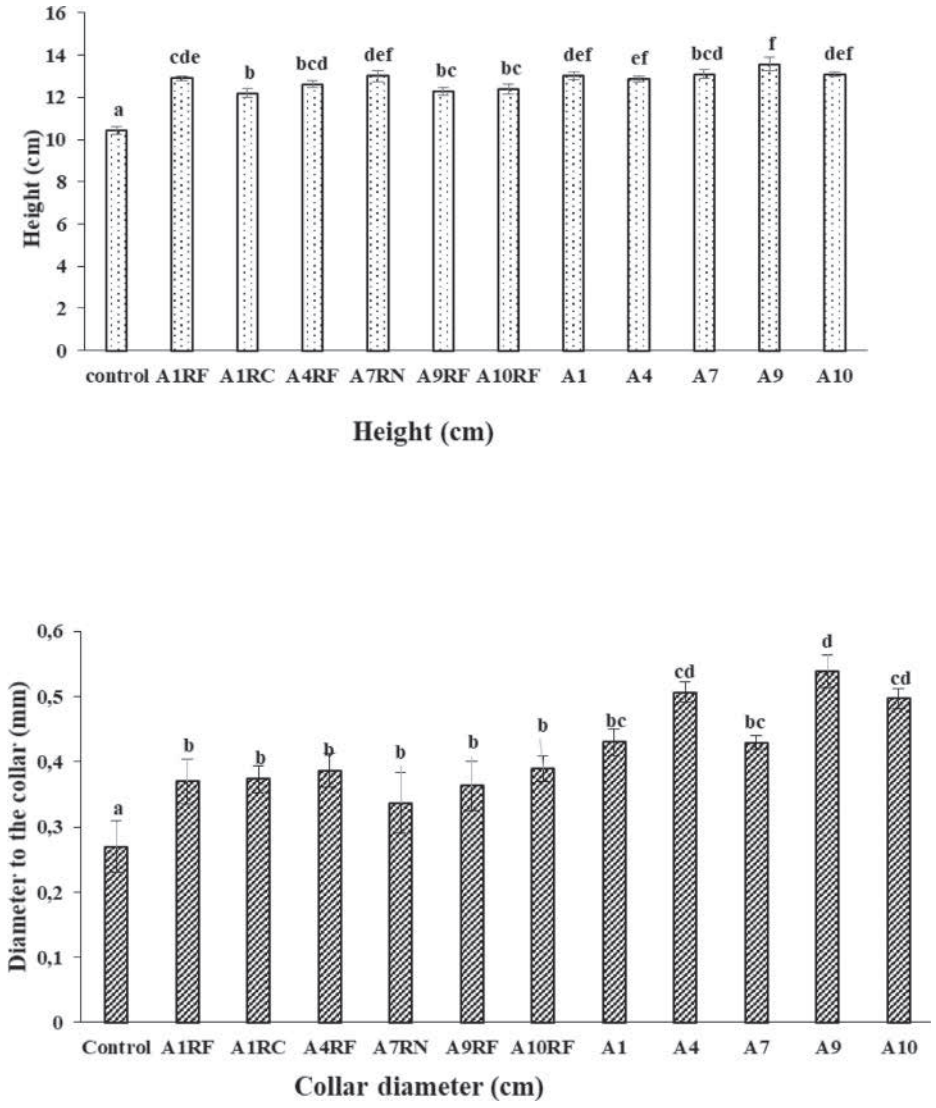

Fig. 3. Effects of different fungal inoculates on growth parameters of C. siliqua plants. The values represented by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

the different *inocula*, the highest values were obtained with comlpexes compared to isolates. The complexes A9 and A10 seemed to be the most efficient in this process by allowing a nitrogen increase twice as much as that encountered in the control plants (Nakmee et al., 2016). The autochtonus complexes from the stressed environments had a significant effect compared to the complex A7. The A9RF isolate showed a remarkable effect compared to the other isolates (Farzaneh et al., 2011; Richardson et al., 2009).

These results corroborate with other studies on carob (Ouahmane et al.,2012; Manaut et al., 2015; Jadrane et al. 2021) and other plants in the Mediterranean region (Ouahmane et al., 2007; Rooney et al., 2011; Abbaspour et al., 2012).

With respect to other nutrients; and more specifically the levels of Potassium (K) and calcium (Ca)) (Fig. 5), the efficiency of AMF was observed in the plants inoculated with the various isolates. Also, for both elements, the seedlings inoculated with the A9 and A7 comlexes remain important compared to those of the control (Abbaspour et al., 2012). For magnesium (Mg) content, an important improvement was recorded compared to control, especially for complexes (Wu and Xia, 2006). Therefore in carob seedlings, AMF had improved the absorption of mineral elements. This process is closely related to the origin of the fungal strains used.

Compared to the control without inoculation, the inoculated plants had shown a significant improvement of the chlorophyll *a*, b, and the total chlorophyll contents, especially for the complex A9 and for the isolate A9RF (Fig. 6). This increase is due to the absorption of mineral elements, mainly magnesium. Several authors reported that the increase in Mg content was necessary for the biosynthesis of chlorophyll (Chandrasekaran et al., 2019; Ratti et al., 2010). The results obtained were subjected to (PCA) analysis which allowed a distribution and grouping of different fungal inoculants with similar effects on the measured parameters (Fig.7). It can be seen that the complexes A9, A10, A4, A1 and A7 are more efficient compared to all the monospecific isolates and to the control, and their effects were tagged on the most analysed parameters. The PCA analysis has been completed by Anova analysis which allowed to classify these different *inocula* and to see the best performing ones by comparing the averages of the different parameters Table 2. It's obvious that complexes from stressed regions (A9, A10) performed better compared to the fungal complex A7 and also to the isolates ARF9 and ARF10, complexes from saline regions are very stimulating for *C. siliqua* seedlings (Table 2).

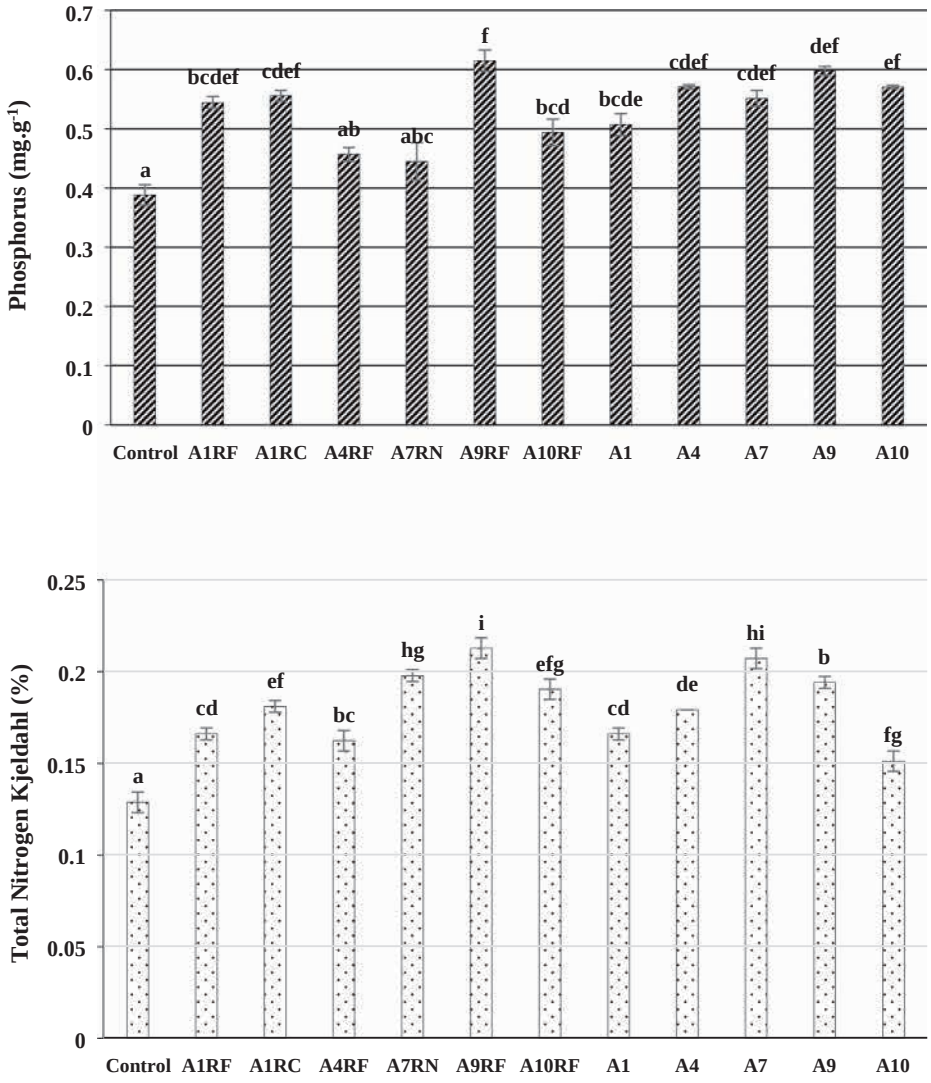

Fig. 4. The effect of different fungal complexes and single-species isolates on the nutrient contents. The values represented by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

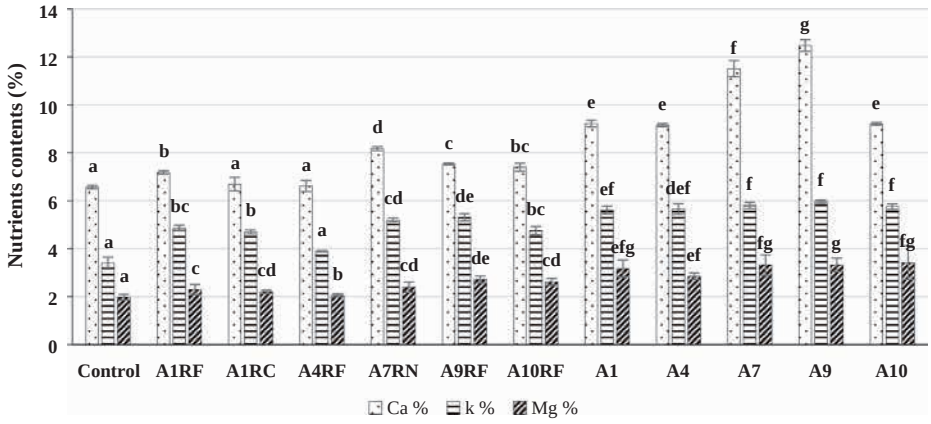

Fig. 4 (*continued*)

5. Conclusion

Ceratonia siliqua is a highly dependent plant species from mycorrhizal symbiosis. The use of AM fungal inoculant can be very effective improving the growth capacity of the carob seedlings. Mineral nutrient uptake and chlorophyll content, particularly with the complexes have shown a high potential. The current study has shown that it is therefore necessary to exploit more the mycor-

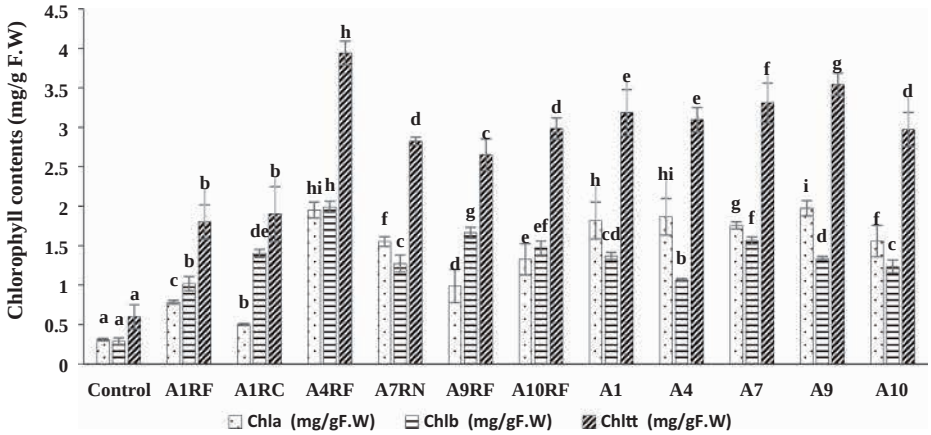

Fig. 5. Chlorophyll (a, b and total) contents of Carob seedlings inoculated or not with mycorrhizal complexes and monospecific isolates after eight months culture under greenhouse conditions. Graphs indexed by the same letter are not significantly different according to Tukey (HSD) test ($P < 0.05$).

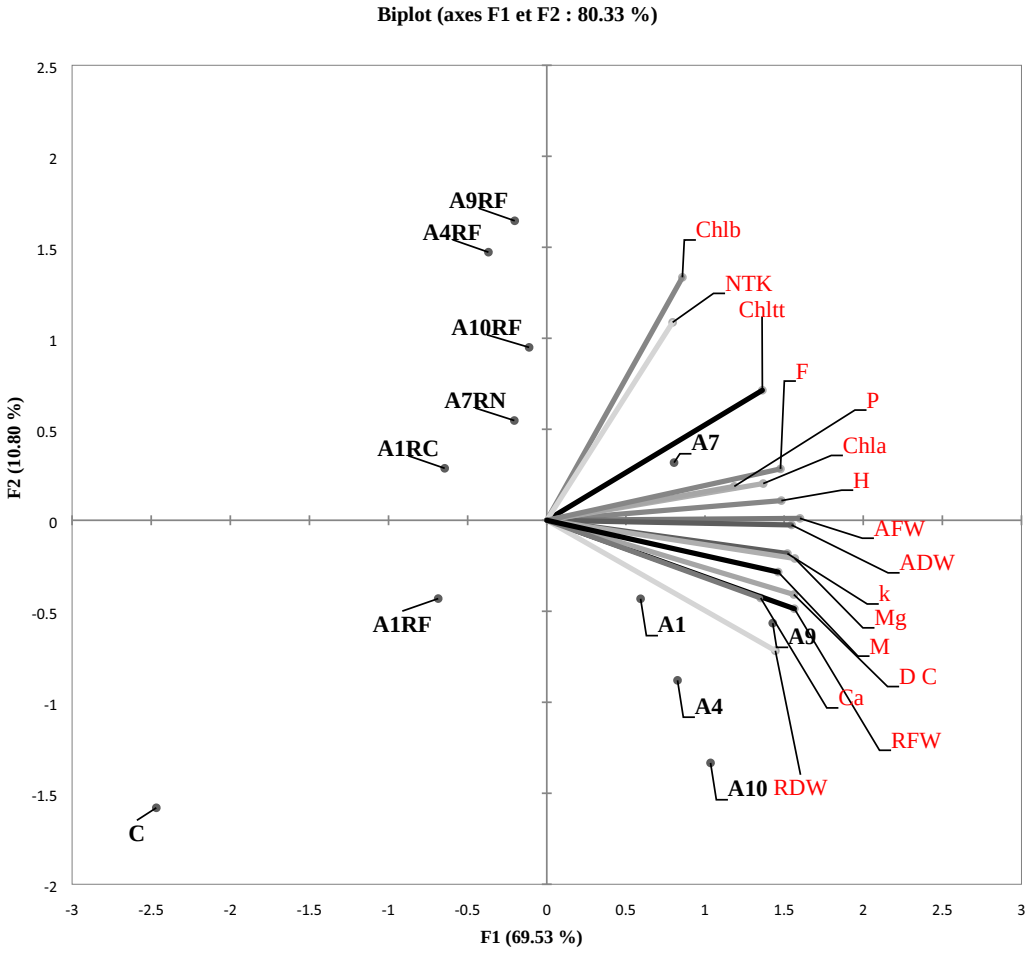

Fig. 6. Graphical representation of the principal component analysis (PCA) of the correlation between the effects of Arbuscular Mycorrhizal Fungi inoculates and the different growth and nutrition parameters of *C. siliqua* seedlings. A1, A4, A7, A9, A10: are AMF complexes; A1RF, A1RC, A4RF, A7RN, A9RF, A10RF: are monospecific isolates; control: (no-mycorrhizal plants).

rhizal fungi diversity in the Mediterranean area for forward use in domestication of *Ceratonia siliqua* and for revegetation of degraded ecosystems under harsh conditions using the carob tree seedlings inoculated with performing inoculants.

Authors' contributions

Ouahmane Lahcen conceived the idea and designed the study with substantial contributions. **Elmostapha Outamamat** PhD Stu-

Table 2
Statistical analysis of the effects of mycorrhizal inoculation of *C. siliqua* seedlings with various complexes and isolates on growth, mineral nutrition, chlorophyll contents and mycorrhizal traits after eight months culture using the ANOVA followed by the Tukey's test (HSD).

	H (cm)	RFW (g)	RDW (g)	DC (cm)	AFW (g)	ADW (g)	Chla (mg/g F.W)	Chlb (mg/ gF.W)	Chltt (mg/ gF.W)	NTK (%)	P(mg/g)	Ca %	k %	Mg %	F (%)	M (%)
A9	**13.56^{f}**	**1.44^{d}**	**0.52^{d}**	**0.54^{d}**	**3.02^{f}**	**1.25^{e}**	**1.94^{i}**	**1.37^{d}**	**3.56 ^{g}**	**0.19 ^{fg}**	**0.61^{ef}**	**12.31 ^{g}**	**6.01^{f}**	**3.10 ^{g}**	**81.66 ^{fg}**	**48.21^{f}**
A10	**13.1 ^{def}**	**1.47^{d}**	**0.53^{d}**	**0.49 ^{cd}**	**3.05^{f}**	**1.28^{e}**	**1.55^{f}**	**1.26^{c}**	**2.94^{d}**	**0.15^{b}**	**0.58^{def}**	**9.21^{e}**	**5.8^{f}**	**3.02 ^{fg}**	**88.3 ^{g}**	**54.61 ^{g}**
A7	**12.62^{bcd}**	**1.04^{bc}**	**0.47 ^{cd}**	**0.42^{bc}**	**2.72^{def}**	**1.12^{de}**	**1.75 ^{g}**	**1.55^{f}**	**3.34^{f}**	**0.2^{hi}**	**0.57^{cdef}**	**11.49^{f}**	**5.82^{f}**	**3.03 ^{fg}**	**71.66^{ef}**	**50.15^{f}**
A4	**13.48^{ef}**	**1.38^{d}**	**0.53^{d}**	**0.50 ^{cd}**	**2.94^{f}**	**1.17^{de}**	**1.88^{hi}**	**1.08^{b}**	**3.10^{e}**	**0.17^{de}**	**0.58^{cdef}**	**9.21^{e}**	**5.62^{def}**	**2.84^{ef}**	**63.3^{de}**	**35.55^{d}**
A1	**13.02^{def}**	**1.26 ^{cd}**	**0.47 ^{cd}**	**0.43^{bc}**	**2.73^{ef}**	**1.06 ^{cd}**	**1.83 ^{h}**	**1.36 ^{cd}**	**3.16^{e}**	**0.16 ^{cd}**	**0.52^{bcde}**	**9.22^{e}**	**5.66^{ef}**	**2.9^{ef}g**	**83.3 ^{fg}**	**41.33^{e}**
A10RF	**12.38^{bc}**	**0.94^{bc}**	**0.34^{ab}**	**0.39^{b}**	**2.64^{def}**	**0.94^{bc}**	**1.37^{e}**	**1.49^{ef}**	**2.95^{d}**	**0.19^{efg}**	**0.50^{bcd}**	**7.41^{bc}**	**4.79^{bc}**	**2.58 ^{cd}**	**88.33 ^{g}**	**26.56^{c}**
A9RF	**12.3^{bc}**	**0.83^{b}**	**0.27^{a}**	**0.36^{b}**	**2.39^{cde}**	**0.93^{bc}**	**0.97^{d}**	**1.65 ^{g}**	**2.67^{c}**	**0.21^{i}**	**0.63^{f}**	**7.58^{c}**	**5.32^{de}**	**2.69^{de}**	**63.33^{de}**	**22.95^{c}**
A7RN	**13.01^{def}**	**1.04^{bc}**	**0.39^{bc}**	**0.35^{b}**	**2.31^{bcd}**	**0.9^{bc}**	**1.52^{f}**	**1.26^{c}**	**2.82^{d}**	**0.19^{gh}**	**0.48^{abc}**	**8.21^{d}**	**5.18 ^{cd}**	**2.56 ^{cd}**	**58.33^{cde}**	**17.43^{b}**
A4RF	**12.62^{bcd}**	**0.77^{b}**	**0.37^{abc}**	**0.38^{b}**	**2.49^{de}**	**1.04 ^{cd}**	**1.91^{hi}**	**1.96 ^{h}**	**3.94 ^{h}**	**0.16^{bc}**	**0.45^{ab}**	**6.63^{a}**	**3.85^{a}**	**2.17^{b}**	**48.33^{bc}**	**24.28^{c}**
A1RC	**12.19^{b}**	**0.8^{b}**	**0.36^{abc}**	**0.37^{b}**	**2.06^{bc}**	**0.85^{b}**	**0.47^{b}**	**1.44^{de}**	**1.90^{b}**	**0.18^{ef}**	**0.57^{cdef}**	**6.71^{a}**	**4.65^{b}**	**2.54 ^{cd}**	**43.33^{b}**	**34.11^{d}**
A1RF	**12.9^{cde}**	**0.87^{b}**	**0.36^{abc}**	**0.37^{b}**	**1.90^{ab}**	**0.63^{a}**	**0.77^{c}**	**1.02^{b}**	**1.80^{b}**	**0.16 ^{cd}**	**0.55^{bcde}**	**7.18^{b}**	**4.87^{bc}**	**2.44^{c}**	**53.33^{bcd}**	**34.28^{d}**
Control	**10.41^{a}**	**0.42^{a}**	**0.26^{a}**	**0.27^{a}**	**1.54^{a}**	**0.58^{a}**	**0.32^{a}**	**0.28^{a}**	**0.57^{a}**	**0.12^{a}**	**0.39^{a}**	**6.60^{a}**	**3.42^{a}**	**1.95^{a}**	**0.0 ^{a}**	**0.00^{a}**

For each column, the values followed by the same letter are not significantly different ($p < 0.05$). A1, A4, A7, A9, A10: are AMF complexes and A1RF, A1RC, A4RF, A7RN, A9RF, A10RF are isolated strains; C: control (no-mycorrhizal plants).
H : Height (cm) ; RFW : Root fresh weight (g) ; RDW : Root dry weight (g) ; DC : Diameter to collar (cm) ; AFW : Aerial fresh weight (g) ; ADW : Aerial dry weight (g)

dent led the experiments and the writing of the manuscript. **Dounas Hanane** analysed the data, **Faissal Aziz, Adnane Barguaz, Robin Duponnois** contributed to the manuscript corrections. All authors gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abbas, Y., Ducousso, M., Abourouh, M., Azcón, R., Duponnois, R., 2006. Diversity of arbuscular mycorrhizal fungi in Tetraclinis articulata (Vahl) Masters woodlands in Morocco. Ann. For. Sci. 63, 285–291. https://doi.org/10.1051/forest.

Abbaspour, H., Saeidi-Sar, S., Afshari, H., Abdel-Wahhab, M.A., 2012. Tolerance of Mycorrhiza infected Pistachio (*Pistacia vera L.*) seedling to drought stress under glasshouse conditions. J. Plant Physiol. 169 (7), 704–709. https://doi.org/10.1016/j.jplph.2012.01.014.

Alguacil, M.M., Caravaca, F., Roldán, A., 2005. Changes in rhizosphere microbial activity mediated by native or allochthonous AM fungi in the reafforestation of a Mediterranean degraded environment. Biol. Fertil. Soils 41 (1), 59–68. https://doi.org/10.1007/s00374-004-0788-5.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase inbeta vulgaris. Plant Physiol. 24, 1–15. https://doi.org/10.1111/j.1399-3054.1988.tb09206.x.

Azcón-Aguilar, C., Palenzuela, J., Roldán, A., Bautista, S., Vallejo, R., Barea, J.M., 2003. Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. Appl. Soil Ecol. 22 (1), 29–37. https://doi.org/10.1016/S0929-1393(02)00107-5.

Barea, J.M., Pozo, M.J., Azcón, R., Azcón-Aguilar, C., 2005. Microbial co-operation in the rhizosphere. J. Exp. Bot. 56, 1761–1778. https://doi.org/10.1093/jxb/eri197.

Baumel, A., Mirleau, P., Viruel, J., Bou Dagher Kharrat, M., La Malfa, S., Ouahmane, L., Diadema, K., Moakhar, M., Sanguin, H., Médail, F., 2018. Assessment of plant species diversity associated with the carob tree (Ceratonia siliqua, Fabaceae) at the Mediterranean scale. Plant Ecology and Evolution 151 (2), 185–193.

Batlle, I. and, Tous, J., 1997. Carob tree Ceratonia siliqua L. -Promoting the conservation and use of underutilized and neglected crops. 17 (Vol. 17). Bioversity International.

Biner, B., Gubbuk, H., Karhan, M., Aksu, M., Pekmezci, M., 2007. Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua L.*) in Turkey. Food Chem. 100 (4), 1453–1455. https://doi.org/10.1016/j.foodchem.2005.11.037.

Blondel, J., Aronson, J., Bodiou, J.-Y., Boeuf, G., 2010. The Meditrerranean Region – Biological diversity in space and time. , pp. 27–28.

Bouamri, R., Dalpé, Y., Serrhini, M.N., Bennani, A., 2006. Arbuscular mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera L.* in Morocco. African J. Biotechnol. 5, 510–516. https://doi.org/10.5897/AJB05.412.

Brundrett, M., Bougher, N., Dell, B., Grove, T., Malajczuk, N., 1996. Working with mycorrhizas in forestry and agriculture (ACIAR Monograph 32). Aust. Cent. Int. Agric. Res, Canberra, Aust.

Buscot1, F., 2005. What are soils ? In: Buscot, F., Varma, S. (Eds.), Microorganisms in Soils: Roles in Genesis and Functions, 682. Springer-Verlag, Heidelbert, Germany, pp. 3–17.

Caravaca, F., Barea, J.M., Palenzuela, J., Figueroa, D., Alguacil, M.M., Roldán, A., 2003. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. Appl. Soil Ecol. 22 (2), 103–111. https://doi.org/10.1016/S0929-1393(02)00136-1.

Chandrasekaran, M., Chanratana, M., Kim, K., Seshadri, S., Sa, T., 2019. Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress–a meta-analysis. Front. Plant Sci. 10, 1–10. https://doi.org/10.3389/fpls.2019.00457.

Cuttelod, A., García, N., Abdul Malak, D., Temple, H., Katariya, V., 2008. The Mediterranean: a biodiversity hotspot under threat. 2008 Rev. IUCN Red List Threat, Species.

Dabire, A.P., Hien, V., Kisa, M., Bilgo, A., Sangare, K.S., Plenchette, C., Galiana, A., Prin, Y., Duponnois, R., 2007. Responses of soil microbial catabolic diversity to arbuscular mycorrhizal inoculation and soil disinfection. Mycorrhiza 17 (6), 537–545. https://doi.org/10.1007/s00572-007-0126-5.

Dakia, P.A., Wathelet, B., Paquot, M., 2007. Isolation and chemical evaluation of carob (*Ceratonia siliqua L.*) seed germ. Food Chem. 102 (4), 1368–1374. https://doi.org/10.1016/j.foodchem.2006.05.059.

Duponnois, R., Plenchette, C., Prin, Y., Ducousso, M., Kisa, M., Bâ, A.M., Galiana, A., 2007. Use of mycorrhizal inoculation to improve reafforestation process with Australian Acacia in Sahelian ecozones. Ecol. Eng. 29 (1), 105–112. https://doi.org/10.1016/j.ecoleng.2006.09.008.

Duponnois, R., Ramanankierana, H., Hafidi, M., Baohanta, R., Baudoin, É., Thioulouse, J., Sanguin, H., Bâ, A., Galiana, A., Bally, R., Lebrun, M., Prin, Y., 2013. 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. Comptes Rendus - Biol. 336, 265–272. https://doi.org/10.1016/j.crvi.2013.04.015.

El Kinany, S., Achbani, E., Faggroud, M., Ouahmane, L., El Hilali, R., Haggoud, A., Bouamri, R., 2019. Effect of organic fertilizer and commercial arbuscular mycorrhizal fungi on the growth of micropropagated date palm cv. Feggouss. Journal of the Saudi Society of Agricultural Sciences. 18 (4), 411–417. https://doi.org/10.1016/j.jssas.2018.01.004.

Emberger L. et Maire R., n.d. Catalogue des plantes du Maroc (Spermaphytes et Pteridophytes). Impr. Minerva.

Estaún, V., Savé, R., Biel, C., 1997. AM inoculation as a biological tool to improve plant revegetation of a disturbed soil with Rosmarinus officinalis under semi-arid conditions. Appl. Soil Ecol. 6 (3), 223–229. https://doi.org/10.1016/S0929-1393(97)00014-0.

Farzaneh, M., Vierheilig, H., Lössl, A., Kaul, H.P., 2011. Arbuscular mycorrhiza enhances nutrient uptake in chickpea. Plant, Soil Environ. 57 (No. 10), 465–470.

Feldmann, F., 1998. The strain-inherent variability of arbuscular mycorrhizal effectiveness: ii. Effectiveness of single spores. Symbiosis 25, 131–143.

Gerdemann, J.W., Nicolson, T.H., 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46 (2), 235–244. https://doi.org/10.1016/S0007-1536(63)80079-0.

Gubbuk, H., Kafkas, E., Guven, D., Gunes, E., 2010. Physical and phytochemical profile of wild and domesticated carob (*Ceratonia siliqua L.)* genotypes. Spanish. J. Agric. Res. 8 (4), 1129. https://doi.org/10.5424/sjar/2010084-1209.

Hart, M.M., Reader, R.J., Klironomos, J.N., 2003. Plant coexistence mediated by arbuscular mycorrhizal fungi. Trends Ecol. Evol. 18 (8), 418–423. https://doi.org/10.1016/S0169-5347(03)00127-7.

Jadrane, I., Al feddy, M.N., Dounas, H., Kouisni, L., Aziz, F., Ouahmane, L., 2021. Inoculation with selected indigenous mycorrhizal complex improves *Ceratonia siliqua*'s growth and response to drought stress. Saudi J. Biol. Sci. 28 (1), 825–832. https://doi.org/10.1016/j.sjbs.2020.11.018.

Janick, J., Paull, R.E., 2008. The Encyclopedia of Fruit and Nuts. CABI Publ.

Mace, G., Masundire, H., Baillie, J., 2005. Biodiversity. In: Hassan, R., Scholes, R., Ash, N. (Eds.), Ecosystems and human well-being: Current state and trends. Findings of the Condition and Trends Working Group.

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. Ecol. Eng. 79, 113–119. https://doi.org/10.1016/j.ecoleng.2015.03.007.

Marschner, P., Timonen, S., 2005. Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. Appl. Soil Ecol. 28 (1), 23–36. https://doi.org/10.1016/j.apsoil.2004.06.007.

Nakmee, P.S., Techapinyawat, S., Ngamprasit, S., 2016. Comparative potentials of native arbuscular mycorrhizal fungi to improve nutrient uptake and biomass of *Sorghum bicolor Linn*. Agric. Nat. Resour. 50 (3), 173–178. https://doi.org/10.1016/j.anres.2016.06.004.

Olsen, S.R., Dean, L.A., 1965. Phosphorus. Chemical and microbiological properties.". methods soil. Anal Part 2, 1035–1048.

Ouahmane, L., Hafidi, M., Plenchette, C., Kisa, M., Boumezzough, A., Thioulouse, J., Duponnois, R., 2006. Lavandula species as accompanying plants in Cupressus replanting strategies: Effect on plant growth, mycorrhizal soil infectivity and soil microbial catabolic diversity. Appl. Soil Ecol. 34 (2-3), 190–199. https://doi.org/10.1016/j.apsoil.2006.02.002.

Ouahmane, L., Hafidi, M., Thioulouse, J., Ducousso, M., Kisa, M., Prin, Y., Galiana, A., Boumezzough, A., Duponnois, R., 2007. Improvement of *Cupressus atlantica* Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. J. Appl. Microbiol. 103 (3), 683–690. https://doi.org/10.1111/jam.2007.103.issue-310.1111/j.1365-2672.2007.03296.x.

Ouahmane, L., Ndoye, I., Morino, A., Ferradous, A., 2012. Inoculation of *Ceratonia siliqua L.* with native arbuscular mycorrhizal fungi mixture improves seedling establishment under greenhouse conditions. African J. Biotechnol. 11, 16422–16426. https://doi.org/10.5897/AJB12.1163.

Ozturk, M., Dogan, Y., Sakcali, M.S., Doulis, A., Karam, F., 2010. Ecophysiological responses of some maquis (*Ceratonia siliqua L., Olea oleaster* Hoffm. & Link, *Pistacia lentiscus* and *Quercus coccifera L.*) plant species to drought in the east Mediterranean ecosystem. J. Environ. Biol. 31, 233–245.

Papaefstathiou, E., Agapiou, A., Giannopoulos, S., Kokkinofta, R., 2018. Nutritional characterization fPOPUof carobs and traditional carob products. Food Sci. Nutr. 6, 2151–2161. https://doi.org/10.1002/fsn3.776.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55 (1). https://doi.org/10.1016/S0007-1536(70)80110-3.

Pozo, M.J., Jung, S.C., López-ráez, J.A., 2010. Impact of Arbuscular Mycorrhizal Symbiosis on Plant Response to Biotic Stress : The Role of Plant Defence Mechanisms 193–207. https://doi.org/10.1007/978-90-481-9489-6

Rankou, H., M'Sou, S., Chadburn, H., Rivers, M., Ouahammou, A., Martin, G., 2017. Ceratonia siliqua. The IUCN Red List of Threatened species. https://doi.org . https://dx.doi.org/10.2305/IUCN. UK.2017-3.RLTS.T202951A112823254.en.

Ratti, N., Verma, H.N., Gautam, S.P., 2010. Effect of Glomus species on physiology and biochemistry of Catharanthus roseus. Indian J. Microbiol. 50 (3), 355–360. https://doi.org/10.1007/s12088-010-0012-2.

Requena, N., Perez-Solis, E., Azcón-Aguilar, C., Jeffries, P., Barea, J.-M., 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microbiol. 67 (2), 495–498. https://doi.org/10.1128/AEM.67.2.495-498.2001.

Richardson, A.E., Barea, J.-M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321 (1-2), 305–339. https://doi.org/10.1007/s11104-009-9895-2.

Rooney, D.C., Prosser, J.I., Bending, G.D., Baggs, E.M., Killham, K., Hodge, A., 2011. Effect of arbuscular mycorrhizal colonisation on the growth and phosphorus nutrition of *Populus euramericana* c.v. Ghoy. Biomass and Bioenergy 35 (11), 4605–4612. https://doi.org/10.1016/j.biombioe.2011.08.015.

Smith, S.E., David, R., 2008. Mycorrhizal Symbiosis. Third Edition 137 (3), 204. https://doi.org/10.1097/00010694-198403000-00011.

Tchabi, A., Burger, S., Coyne, D., Hountondji, F., Lawouin, L., Wiemken, A., Oehl, F., 2009. Promiscuous arbuscular mycorrhizal symbiosis of yam (*Dioscorea spp.*), a key staple crop in West Africa. Mycorrhiza 19 (6), 375–392. https://doi.org/10.1007/s00572-009-0241-6.

Trouvelot, A., 1986. Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une significantion fonctionnelle. Mycorrhizae Physiol. Genet, 217–221.

Wolf, B., 1982. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. Commun. Soil Sci. Plant Anal. 13 (12), 1035–1059. https://doi.org/10.1080/00103628209367332.

Wu, Q.-S., Xia, R.-X., 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J. Plant Physiol. 163 (4), 417–425. https://doi.org/10.1016/j.jplph.2005.04.024.

Wu, Q.S., Srivastava, A.K., Zou, Y.N., 2013. AMF-induced tolerance to drought stress in citrus: A review. Sci. Hortic. (Amsterdam) 164, 77–87. https://doi.org/10.1016/j.scienta.2013.09.010.

Zaimeche, S.E., Sutton, K., 1997. The future of the environment in Algeria, Morocco and Tunisia. J. North African Stud. 2 (1), 40–57. https://doi.org/10.1080/13629389708718287.

Further Reading

Subramanian, K.S., Santhanakrishnan, P., Balasubramanian, P., 2006. Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Scientia Horticulturae 107 (3), 245–253. https://doi.org/10.1016/j.scienta.2005.07.006.