Advances in Environmental Biology, 7(9): 2166-2174, 2013
ISSN 1995-0756

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Mycorrhizal dependency in the olive tree (*Olea europaea*) across a xeric climatic gradient

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ABSTRACT

The vesicular-arbuscular mycorrhizas (AM) are very significant in the life of most plants including olive tree (*Olea europaea* L.), which is of great economic importance. However, due to its wide distribution, especially, in the Mediterranean, it is subject to various environmental stresses, particularly drought and aridity. This study aims to evaluate the percentage of root colonization of olive by arbuscular fungi along a climatic gradient (sub-humid, semi-arid and arid), and to estimate how this varies depending years (2010-2012) and seasons (fall, winter, spring and summer). Results showed that the roots of the olive tree are colonized by arbuscular endomycorrhizal fungi under the three types of climate, whatever the season or the study years. Mycorrhization parameters revealed a frequency of colonization (*F%*) of up to 83%, mycorrhizal intensity (M%) reaching 42% and a rate of arbusculars in the mycorrhizal parts of root fragments (*a%*) of 4%. Mycorrhizal colonization varies between bioclimatic zones. It is more frequent and intense in the sub-humid climate. Mycorrhiza also varies according to the season; it is more important in spring and slight in winter. This study allowed the characterization, for the first time, of the mycorrhizal status of olive in the northeastern Algeria, and estimate variations of that colonization depending on seasons and climate types.

Key words: Arbuscular mycorrhizae, fungal colonization, *Olea europaea*, aridity, Northest Algeria.

Introduction

In natural systems, a lack of nutrients often limits plant growth. Therefore plants supplement their nutrient requirements by mycorrhizae [30]. This symbiotic association, formed by a fungus and plant roots, affects 95% of the known plants [38]. Better fed, a mycorrhized plant grows more, fructifies abundantly and, above all, acquires improved resistance to environmental stresses such as drought [18], cold [46] and root pathogens [1]. These mycorrhizae are hence an integral part of the root system [19].

Among the types of mycorrhizae observed in nature, there is one that is found on the vast majority of cultivated plants, it is the arbuscular mycorrhizal (AM). These are symbiotic associations observed in 200 botanical families representing 1,000 genera and about 300,000 plant species [6] including angiosperms, gymnosperms, pteridophytes, and some bryophytes with a limited range of fungi belonging to a single order, that of Glomeromycota [37].

This means that in the plant world mycorrhizal symbiosis is the rule rather than the exception [14]. These associations are the most prevalent in the roots [9]. They are found in all climate zones [29], ecosystems [37], vegetation types [23] and growing conditions [14], regardless of soil types [47,21].

The cultivated olive (*Olea europaea* L.) is a tree that can live for thousands of years. It easily adapts to poor stony soils even in drylands. With roots that can dive deep, the olive find the needed elements for its growth [3]. Furthermore, the species occupies an important place in the global fruit tree cultivation, notably in the Mediterranean. Its cultivation in the world covers about 8.6 M ha and produces 17.3 M tons of olives [25].

The olive tree is known to be drought tolerant, but few studies have been conducted under this topic [20,4]. In Algeria, the olive crops cover from the east and west some 300,000 ha. This area occupies the entire area of the Tell up to 1000 m of elevation a.s.l. The olive cultivation is practiced under three types of climates, even in the Sahara Desert, especially with the encouragement of the Algerian government since

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the establishment of the famous National Agricultural Development Plan “PNDA” since 2000 [22].

This study aims to quantify the spatiotemporal variation of mycorrhizal colonization in the olive in many types of climates. In this study, we focused on the northeastern Algeria, in which no previous study has been carried out to examine the myccorhizal status on olive tree that it is within a framework of an escalating extensive agriculture programs in all Algeria.

Assuming that increasing the impact of environmental stresses, mainly drought, increases the myccorhizal status in the same plant species [18], we hypothesized that myccorhization characteristics and parameters would differ in their responses to climate patterns (e.g. climatic gradient varying from mesic/wet and cold climate into dry and hot climate) and temporal variables (e.g. monthly, seasonal, yearly … variations). We predicted that myccorhization parameters broadly decreases with drought decrease (with the improvement of climate conditions) as precipitation amount gets bigger and environmental stresses decrease.

Materials And Methods

Study area:

The current study focuses on the provinces of Souk Ahras and Tebessa located in eastern Algeria. In this area, three sites were chosen, firstly for their olive oil vocation, and secondly according to an aridity gradient ranging from mesomediterranean climate to desertic climate (UNESCO, 1963; Fig. 1). At each site an olive orchard of about five hectares was sampled.

- The first site "Machrouha", located in Souk Ahras (36°21'46.44"N, 7°49'33.26"E) is an orchard located in a forest scrub characterized by a mesomediterranean climate with long dry season, which is considered subhumid climate, whose dry period according to the diagram ombrothermic of Gaussen [5] extends over four months (June-October). The hottest month is August with an average temperature of 25.5 °C and the coldest month is January with an average temperature of 6.7 °C. Annual rainfall varies between 118.3 and 864.6 mm with an average of 552 ± 172.4 mm. Characteristic forest species such as *Quercus ilex* and *Cistus monspeliensis* dominated the vegetation of the region.

- The second site is Morsott (35°33'54.68"N, 7°55'8.49"E). It represents a steppe ecosystem characterized by a thermomediterranean climate with short dry season, which is the equivalent of semi-arid climate whose dry season lasts five months (May-October). The hottest month is July with an average temperature of 26.6 °C and the coldest month is January with an average temperature of 6.6 °C. Annual rainfall varies between 191.8 and 636.7 mm per year with an average of 366.6 ± 101.1 mm. The vegetation is typical of semi-aride steppe rangelands and is represented mainly by *Atriplex halimus* and *Stipa tenacissima*.

- The third sampled site is located in Ferkane (34°29'13.32"N, 7°28'6.91"E) at 182 km south of Tebessa. The site is characterized by a desert (hot arid) climate; with a dry period which extends over eleven months (February-December). The hottest month is July with an average temperature of 26.4 °C and the coldest month is January with an average temperature of 6.6 °C. Rainfalls are erratic and have large interannual fluctuations ranging between 43.2 and 408.5 mm per year with an average of 175.2 ± 83.7 mm. The vegetation is very sparse characterizing desert steppe. The olive orchard sampled in this area is mixed with a culture of date palm (*Phoenix dactylifera*).

Collection of roots:

As roots can be harvested at any time of the year to estimate the endomycorrhizal colonization [36], we conducted our sampling over the four seasons during three years 2010-2012 at sites previously described. In each orchard oliver, five olive subjects were randomly sampled. After removing the surface litter of the ground, we dug in three places around each tree according to a triangle whose angles are distant at least one meter in order to obtain a representative set of the entire root system. Then we performed root samples, taking care to select the finest roots. The three lots of roots of the same tree were pooled into a single sample.

Preparation of roots and highlighting the endomycorrhizal colonization:

In the laboratory the staining technique used is that recommended by Phillips and Hayman [31] and Vierheilig *et al*. [41]. It consists in (i) identifying small fine and unlignified roots, (ii) thorough cleaning to get rid of any particle of soil, (iii) cut them into pieces of about 1 cm long and (iv) put them in a first time in a water bath in a solution of 10% KOH for an hour of time to empty cells of their cytoplasmic contents in order to improve the observation of the symbiotic fungus. After that, root fragments were rinsed with water to remove all traces of KOH, then they were immersed successively in 10 vol H_2O_2 for 40 minutes and 2% HCl for 30 minutes to clarify the roots, then a new rinsing with water is performed. The thinned root fragments were stained for one hour of time using black chlorazol [9] heated to 90 °C in a water bath. Roots thus colored were stored in labeled pillboxes containing glycerol.

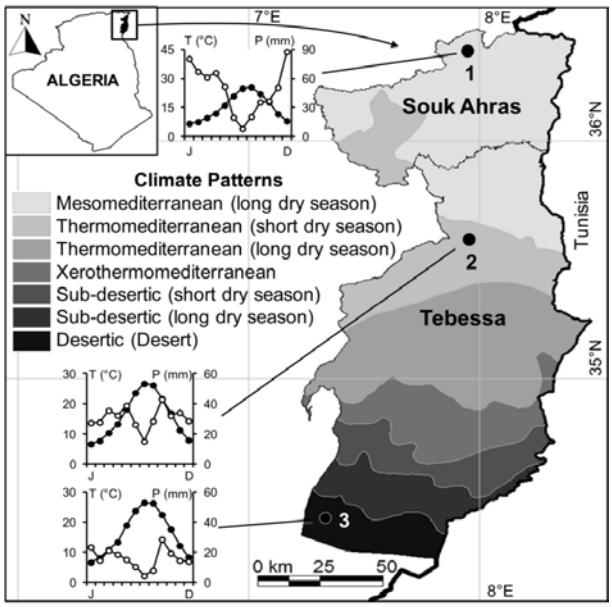

Fig. 1: Geographic location and climate patterns map of the study area including the climatic diagrams (solid circlesre present temperature and empty circle symbolize precipitations) of the sampled sites (1: Machrouha, 2: Morsott, 3: Ferkane) in eastern Algeria. [40].

Estimation of mycorrhization:

The estimate of percentage of root colonization was calculated using the method of Trouvelot *et al*. [39]. This method is rapid and reflects as much as possible the potential and the activity status of the symbiosis. It consists in putting five fragments of the colored roots between strips in glycerol and then observe them using a light microscope, the operation was repeated twice. The observed fragments were noted in a grid based on a scale class; this scale provides quick estimate of degrees of mycorrhizal colonization and richness of arbuscular colonization of each fragment. Using this method of colonization, five parameters were calculated as follows:

- Frequency of mycorrhizal colonization (*F%*): rate of the number of endomycorrhized root fragments, it reflects the importance of colonization of the root system.
- Intensity of mycorrhizal colonization in the root system (*M%*).
- Intensity of mycorrhizal colonization in the root fragments (*m%*).
- Abundance of arbuscular in the root system (*A%*).
- Abundance of arbuscular in mycorrhizal parts of root fragments (*a%*).

Calculation of those parameters was performed using the software program MYCOCALC available freely from http://www2.dijon.inra.fr/mychintec/

Analytical procedures:

Means and standard deviations of mycorrhization traits were given for each year, season and climate-type. Firstly, after assessing the normal distribution and homogeneity of data by the Shapiro-Wilk normality tests [34]; linear models (LMs) [12] were used to model the specific effects of each year, season and climate-type on mycorrhizal parameters. The LM summary was given of each mycorrhization traits including certain statistic outputs namely: Residual standard error (*RSE*), Multiple R-squared (MR^2), Adjusted R-squared (AR^2) and F-statistic (*F*) with p-value (*P*). In the second step, the said factors "year", "season" and "climate-type" were included in each model with their interactive effects after the application of contingency test that determine the dependency or the independency of the three factors. Then the assessed parameters were tested for significant differences using analyses of variance (ANOVAs) according to the said factors and their interactions [43]. All ANOVAs were performed by *type I* tests at alpha = 0.05.R-commander {Rcmdr} was used as a statistical package for computations.

Results:

Spatiotemporal variation of mycorrhization:

Olive roots collected at different sites during the four seasons and colored by black chlorazol, had a high colonization by AM fungi. Colonization was manifested by a large network of intra- and intercellular hyphae. The mycelium thickness varied between 10 to 20 μm. Many point entry of fungi in the root or *appressoria* were clearly visible. These structures are considered as the most crucial event in the development of endomycorrhizal colonization. In

addition, the endomycorrhizal colonization resulted in a high frequency of mycorrhization (*F%*) (Fig. 2A) and this whatever the sampled site. It was 58.34% in the semi-arid climate and about 67% in the sub-humid climate. The variation was also observed according to years (67.92% in 2010, 60.52% in 2011 and 65.87% in 2012), and also seasons (57.04% in winter and 72.50% in spring).

Regarding the mycorrhizal intensity, it was more or less low either in the root system (*M%*) or root fragments (*m%*). Depending on climate types, the intensity was low in semi-arid climate (*M%* = 2.65%, *m%* = 4.39%) but high in subhumid (*M%* = 7.94%, *m%* = 10.85%). The minimum values of mycorrhizal intensity were recorded in 2011 with *M%* = 3.35%, *m%* = 5.27% and it showed high values in 2012 with *M%* = 8.87%, and *m%* = 12.40%. According to the seasons, the most important values were estimated in spring *M%* = 10.16%, and *m%* = 13.34%. As for the lowest values, they coincide with winter *M%* = 2.77% and *m%* = 4.52% (Fig. 2B, 2C).

Arbuscular structures were also present in all sampled roots, especially in the site "Ferkane" characterized by hot arid climate, although their number was relatively slight. The abundance of arbuscular reflected very low levels, whether in the root system (*A%*) or mycorrhizal parts of root fragments (*a%*). The highest values were recorded in 2010 (*A%* = 0.56%, *a%* = 4.03%), in autumn (*A%* = 0.52%) and spring (*a%* = 3.17%) and in the arid bioclimatic (*A%* = 0.43%, *a%*= 3.59%). Moreover, the lowest values were reported in 2011 (*A%* = 0.07%, *a%* = 1.11%), during winter (*A%* = 0.02%, *a%* = 0.30%) in the semi-arid climate (*A%* = 0.06%, *a%* = 1.33%) (Fig. 2D, 2E).

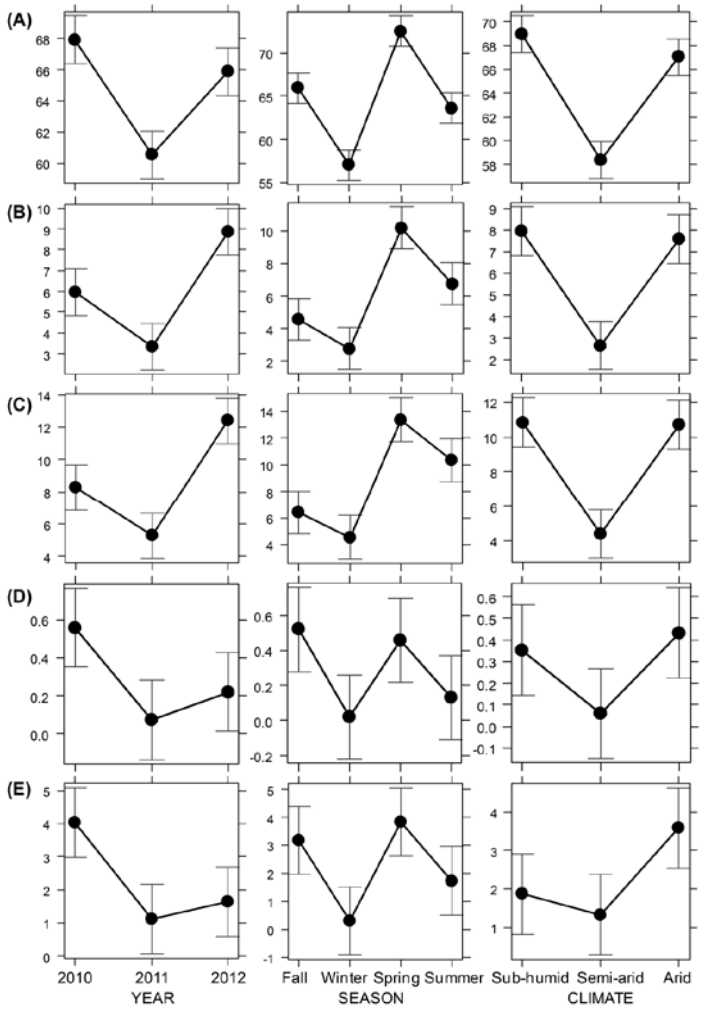

Fig. 2: Annual and seasonal variation and according to climate-type of mycorrhization parameters of the Olive tree roots. Graphs represent mean values of percentages with standard error bars. (A): Frequency of mycorrhizal colonization (*F%*), (B): Intensity of mycorrhizal colonization in the root system (*M%*), (C): Intensity of mycorrhizal colonization in root fragments (*m%*), (D): Arbuscular abundance in the root system (*A%*), (E): Abundance of arbuscular within mycorrhizal parts of root fragments (*a%*).

Factors affecting variation of mycorrhization parameters:

The linear models applied for all mycorrhization parameters shown a significant positive linkage with the arid climate, the year 2010 and the fall season, which all consist the model intercepts (Table 1).

The linear models revealed that the year 2011 was deemed negatively related to all mycorrhization parameters, whereas, during 2012 positive relationships were inferred for arbuscular abundances (A% and a%) and negative relationships for mycorrhization intensities (M% and m%). Frequency and intensity of mycorrhization were highly significantly associated with spring, while winter exhibited negative correlation with *F%* and arbuscular abundances. However summer was positively linked with arbuscular abundances and negatively with *M%*. In addition, all mycorhizal parameters were significantly negatively related with semi-arid climate (Table 1).

Table 1: Results from the linear models for the effects of individual year, season and climate type on the parameters of mycorrhization of olive tree in Eastern Algeria. Only significant terms are shown. (^{NS}: no significance, P >0.05; *: significant, P <0.05; **: highly significant, P <0.01; ***: very highly significant, P <0.001).

Variable	Estimate	*SE*	*t*-value	*P*	
F% (*RSE* = 6.03, MR^2 = 0.64, $F_{(7, 172)}$ = 43.6, *P*<0.001)					
Intercept	71.353	1.271	56.153	<0.001	***
2011	-7.399	1.100	-6.723	<0.001	***
2012	-2.046	1.100	-1.859	0.065	NS
Winter	-8.918	1.271	-7.019	<0.001	***
Spring	6.543	1.271	5.149	<0.001	***
Summer	-2.363	1.271	-1.859	0.065	NS
Sub-humid	1.931	1.100	1.755	0.081	NS
Semi-arid	-8.679	1.100	-7.887	<0.001	***
M% (*RSE* = 4.39, MR^2 = 0.50, $F_{(7, 172)}$ = 24.68, *P*<0.001)					
Intercept	5.970	0.926	6.448	<0.001	***
2011	-2.597	0.802	-3.239	0.001	**
2012	2.927	0.802	3.651	<0.001	***
Winter	-1.786	0.926	-1.929	0.055	NS
Spring	5.606	0.926	6.054	<0.001	***
Summer	2.176	0.926	2.350	0.020	*
Sub-humid	0.360	0.802	0.449	0.654	NS
Semi-arid	-4.933	0.802	-6.152	<0.001	***
m% (*RSE* = 5.53, MR^2 = 0.50, $F_{(7, 172)}$ = 24.68, *P*<0.001)					
Intercept	8.091	1.166	6.941	<0.001	***
2011	-3.008	1.010	-2.979	0.003	**
2012	4.121	1.010	4.082	<0.001	***
Winter	-1.878	1.166	-1.611	0.109	NS
Spring	6.937	1.166	5.951	<0.001	***
Summer	3.936	1.166	3.376	0.001	***
Sub-humid	0.137	1.010	0.136	0.892	NS
Semi-arid	-6.322	1.010	-6.262	<0.001	***
A% (*RSE* = 0.82, MR^2 = 0.15, $F_{(7, 172)}$ = 4.34, *P*<0.001)					
Intercept	0.945	0.172	5.503	<0.001	***
2011	-0.486	0.149	-3.268	0.001	**
2012	-0.339	0.149	-2.280	0.024	*
Winter	-0.499	0.172	-2.905	0.004	**
Spring	-0.060	0.172	-0.351	0.726	NS
Summer	-0.387	0.172	-2.253	0.026	*
Sub-humid	-0.080	0.149	-0.539	0.591	NS
Semi-arid	-0.374	0.149	-2.514	0.013	*
a% (*RSE* = 4.15, MR^2 = 0.21, $F_{(7, 172)}$ = 6.58, *P*<0.001)					
Intercept	6.277	0.876	7.168	<0.001	***
2011	-2.930	0.758	-3.863	<0.001	***
2012	-2.399	0.758	-3.163	0.002	**
Winter	-2.875	0.876	-3.283	0.001	**
Spring	0.660	0.876	0.754	0.452	NS
Summer	-1.447	0.876	-1.653	0.100	NS
Sub-humid	-1.720	0.758	-2.268	0.025	*
Semi-arid	-2.259	0.758	-2.979	0.003	**

The linear models presented above provided useful comparisons of the relationship between individual factors and mycorhizal traits, but multivariate models are needed to assess the combined impact of different factors taking into account their covariations. For the frequency of mycorrhizal colonization (*F%*), the ANOVA revealed that there is a very highly significant difference and this depending on climate type, year and season of the study (P <0.001). The interactions of factors, year*season, season*climate, or year*season*climate has highlighted that there is a highly significant difference (P = 0.002). For mycorrhizal intensity parameters, whether in the root system (*M%*) or root fragments (*m%*), the ANOVA revealed very highly significant effects (P <0.001), and this according year, season and climate, as well as the interactions of these three factors. In addition, arbuscular abundance in the root system (*A%*) varies statistically significantly depending on the climate (P = 0.021), and highly significantly between years (P = 0.002) and seasons (P = 0.004), and the interaction year*season (P = 0.003). As for the arbuscular abundance of mycorrhizal parts in root fragments (*a%*), ANOVA revealed highly significant differences for all sources of variation of the statistical model except with the interaction season*climate (Table 2).

Table 2: Modeling the effects of "year", "season" and "climate type" on the parameters of mycorrhization in Olive trees planted in Eastern Algeria using multi-way ANOVA tests.

		Multi-way ANOVA outputs of mycorrhizal colonization parameters									
		F%		*M%*		*m%*		*A%*		*a%*	
Sources	*Df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Year	2	32.2	<0.001	95.9	<0.001	94.1	<0.001	6.4	0.002	11.5	<0.001
Season	3	67.5	<0.001	95.3	<0.001	86.1	<0.001	4.6	0.004	8.8	<0.001
Climate type	2	70.5	<0.001	109.8	<0.001	100.0	<0.001	4.0	0.021	6.6	0.002
Year * Season	6	3.7	0.002	30.8	<0.001	29.9	<0.001	3.5	0.003	3.3	0.005
Year * Climate	4	1.9	0.111	32.0	<0.001	27.7	<0.001	2.4	0.057	6.2	<0.001
Season * Climate	6	3.8	0.002	16.9	<0.001	14.5	<0.001	0.9	0.506	1.8	0.107
Year*Season*Climate	12	2.8	0.002	11.4	<0.001	10.3	<0.001	1.2	0.266	2.8	0.002
Residuals	144	(SS=3912.7)		(SS=688.1)		(SS=1176.2)		(SS=84.4)		(SS=1833.6)	

Discussion and conclusion:

The endomycorrhizal colonization occurs in the three study sites located within three different bioclimatic zones and even as important in all study climates. This finding is consistent with that of Caravaca *et al*. [11] and Binet *et al*. [7], and shows the dependence of the olive vis-à-vis the AM fungi. Indeed, several authors have shown the importance and necessity of AM fungi for the development and survival of the olive tree, even under drought stress [32,28,24].

The endomycorrhizal colonization is higher in spring (period of vegetative developed par excellence) than in summer (period of fruit set and fruit growth) and autumn (growth period of fruit and branches) [27]. Indeed, the extension of the intense activity of photosynthesis during the growing season "spring" provides more carbon to the roots that allows greater development of AM [8]. By cons, in winter that represents the period of vegetative dormancy in which metabolic requirements are low, the days become shorter which could reduce photosynthesis and the rate of carbon transfer towards the roots. Consequently less carbon reaches for AM, thus colonization becomes less important during that season [42].

Our findings revealed that the endomycorrhizal colonization is more important in the sub-humid than in the semi-arid climate. Indeed, drought (or aridity in general) has a negative effect on mycorrhizal development, even if the drought does not completely prevent the growth of mycorrhizae. However, it causes an increase in level of root dormancy and decreases elongation rates of mother roots [16]. It is worth mentioning that the same plant develops more roots in a humid climate than in a dry climate [16,26]. The more developed roots will therefore be more likely to meet the AM fungal spores present in the soil, hence a large colonization [45].

However colonization is more important in arid climate that under semi-arid climate, which has resulted a higher values of frequency, intensity and number of arbuscules, this may be explained by the fact that endomycorrhizaes contribute to tolerate water stress by improving tissue hydration and physiology of plants [35,18,10,15]. Moreover, it should also be noted that the number of arbuscules present in the arid climate is sometimes more important than in sub-humid climate, this demonstrates the importance of endomycorrhizae role, especially arbuscules that represent sites of functional exchanges between the endomycorrhizal fungus and roots of the host plant [17].

Mycorrhizal parameters of the olive tree planted in arid areas are very important from a point of view endomycorrhizal frequency and intensity, and also number of arbuscules. These arbuscules are probably the result of one or more genera of fungal spores that have been developed in this harsh climate, which allows endomycorrhizas to perform a kind of adaptation under difficult conditions.

AM Colonization is less important for the year 2011 that is the year when there was more precipitation, as for the years 2010 and 2012, and this

can be explained by the fact that trees grow more endomycorrhizal colonization within adverse conditions to compensate the water deficit [13,2,35].

This study highlighted olive root colonization by arbuscular fungi in Northeast Algeria. The results demonstrate the dependence of the olive tree to arbuscular mycorrhizal fungi, in particular under arid conditions. Indeed, it is apparent that olive roots are all the time colonized by arbuscular fungi, and that under all climate types, but with higher colonization levels in sub-humid climate compared to semi-arid and arid climates; as well through all seasons where the colonization is higher in spring compared to other seasons. This study could be complemented by the isolation and the identification of fungi involved in the mycorrhization of olive in order to select those best adapted to climate conditions or environmental factors.

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