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Influence of culture substrates based on local materials on the growth and mycorrhizal status of cork oak (*Quercus suber* L.) grown in nurseries

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Key words: Cork oak, Ectomycorrhizae, Cork granule, WM containers, Mycorrhization.

Abstract

The cork oak forests of Northeastern Algeria are in a state of increased dieback; their natural regeneration is difficult following a series of constraints. It is therefore, necessary to intervene for their enhancement by reforestation with good quality cork oak seedlings. This work is an attempt to search for the proper substrate prepared from local materials for the cork oak seedlings production conforming to commonly accepted standards. In this context, four substrates were tested. They are made of materials such as cork granules, pine bark, apricot kernel and olive pomace, used as aerators mixed with forest humus for water retention. The experiment was carried out in WM containers with 32 replicates for each treatment subject to the forest nursery conditions. The influence of these substrates was evaluated on the one hand on the growth of 9-month old cork oak plants by measuring the following parameters: height of the stem (H), collar diameter (D), H/D ratio , aboveground and underground biomass, number of leaves, chlorophyll content, and on the other hand by the estimation of their mycorrhizal status. The obtained results make it possible to conclude that the plants grown in the substrates of cork granules and those of pine bark have the best performances, which are expressed by better growth in height(37.66 cm and 39.08 cm) respectively and collar diameter (3.5 mm and 3.8 mm), hence better H/D ratio(17.52; 16.57), as well as higher chlorophyll content (47.24; 47.39) spad respectively. The cork granules substrate also offers the best root biomass (13.85 g) and the highest mycorrhization rate (63.55%).

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Introduction

The current situation of the Algerian cork forests shows a state of alarming dieback due to the convergence of several factors (repeated fires, overexploitation of cork, diseases etc.). Mismanagement combined with ongoing climate change has profoundly affected the health of the cork forest, making it less resistant to fire and insect and pest attacks. To these problems, is added that of the conifer afforestation of the area of the cork oak following the reforestation programs with the pine of the years 1970 to 2000(Nasrallah and Kefifa, 2015).

This regressive evolution may jeopardize the sustainability of the cork heritage (Roula and Chouial, 2005) and cause detrimental effects on the country's economy following the reduction of national production of raw cork and the decline export of these processing products.

To remedy and valorize these declining ecosystems and in order to rehabilitate and restore them, Algeria launched a plantation program of 20,000 ha between the years 2003 - 2007 (D.G.F., 2003). However, according to Messaoudene (2011), the reforestation strategies adopted were marked by an important failure, particularly related to the seedlings quality, of which 75% had binding defects of root system.

Indeed, in addition to the genetic heritage, to the planting technique and the environmental characteristics of the planting site, the success of reforestation and the recovery of seedlings in open ground according to Lemaire (2003), depend mainly on their morpho-physiological quality and according to Blanc (1987), require the development of technically and economically efficient farming systems. Thus, the production of good quality plants requires, as suggested by Lamhamedi (2006a), an optimization of the different production phases from seed to planting. This will significantly reduce the costs of repeated replenishments. Many researchers have been interested in improving and modernizing cropping systems among other things, the design of a good breeding container (Wilson, 1977, Reidacker, 1978, Landis, 1990, 2010) and the production of culture substrates, by presenting physical and chemical properties that meet the requirements of the forest seedling and giving it the ability to withstand transplant stress (Miller and Jones, 1995, Lamhamedi *et al*., 2000, 2006a, and 2011; M'sadak *et al.,* 2012b, 2014).

In this context, several substrates have been used, in particular blonde peat associated with vermiculite, which is the most appreciated organic component, used as a reference substrate. However, this resource is not easily renewable and its selling price in non-producing countries continues to increase (Ammari *et al.,* 2003). For this purpose, special attention has been paid to research of peat substitutes and to the composting of forest biomass (pine bark, acacia and scrub branches) in nurseries (Ammari *et al.,* 2003).

In the extension, this present work is a contribution to the research of substitute products made with available local materials. It aims at the need to produce cork oak plants of morpho-physiological quality in accordance with standards and with good natural mycorrhizal potential.

To do this, the influence of four culture substrates on the growth and development of cork oak plants was studied in parallel with the determination of their natural ectomycorrhizal colonization rate and the morphotypic diversity of associated ectomycorrhizae. The materials used are cork granule (CG), pine bark (PB), apricot kernel (AK) and olive pomace associated with cork granule (OP + CG).

Materials and methods

Plant material

Cork oak acorns are taken from a single tree in the region of Jijel (northeastern Algeria). After a flotation sorting operation, the acorns judged to be healthy treated by the combination of two fungicides: a prior soaking in a solution of benomyl at 0.4 g/l, followed after a light wiping of adjusting with Tiramis to 2 g/kg acorns in order to inhibit the development of fungi including *Aspergillus glaucus* at low humidity, and at higher humidity that of *Ciboria batschiana*, responsible for black rot (Chouial, 2010). At the time of use, theacorns that were stored at +2°C are soaked in lukewarm water at 40°C to break dormancy and kill insect larvae if present and then disinfected with water oxygenated at 30 V for 15 minutes and rinsed thoroughly with sterile distilled water. They are then stratified in sterile sand until the appearance of the radicle to determine their germination capacity. The pre-germinated acorns are sown 1 to 2 cm deep at the rate of one acorn per container.

The container

The containers used are those in "WM" 400 cm³ofRiedacker (1978). Designed to impose a vertical root development and prevent the formation of buns. They are arranged in perforated plastic boxes, raised 80 cm from the ground to allow self-root pruning of the pivot (Benseghir, 2006). Watering is done manually according to the water needs and weeding is undertaken as needed.

The culture substrate and the conduct of the test

The culture substrate

The culture substrate is composed of:

(i) A retentive element consisting of a slightly acidic forest humus (FH) (pH = 6) originating from Brabtia's cork oak forest (Wilaya El Tarf, northeastern Algeria) and removed from the topsoil (10-20 cm) under cork oak.

(ii) An aerator element which by definition, should be chemically neutral, not involved in the mineral diet of the plant and having a certain stability and high permeability (Crozon and Neyroud, 1990). Four different aerators were used:

Incinerated cork granules (CG) : These materials from the cork industry of the city of Jijel, are used for various works notably chipboard. As stated by Chouial *et al.* (2004), their passage in the oven is considered to be a sterilization to eliminate phytotoxins and high levels of tannin. They are characterized by a high porosity (96%), a particle size of 4 to 12 mm, a retention capacity of 3% and an electrical conductivity of 0.07 ms / cm (Chouial *et al.,* 2001).

The pine bark (PB) composted : These are heterogeneous particles from the bark of maritime pine, diameter of 04 and 15 mm. This bark is characterized by a neutral pH, a very high organic matter rate (OM) (92.81%), a very low nitrogen content (0.35%) and a very high C/N ratio (132, 57). Composting has the effect of stabilizing and homogenizing the product (Blanc, 1987).

Composted olive pomace (OP) : These are residues from oil mills in the Guerbès region (Ben Azzouz, Wilayaof Skikda). They have been stored in clusters returned several times for 1 to 3 years to reduce the acids rate and toxic compounds that may exist (Chouial *et al.,* 2004). Olive pomaces are characterized by high nitrogen content (2.03) and a C/N ratio of 19.48. The diameter of their particles is 1 to 4 mm.

The apricot kernels (AK) crushed: From the agri-food industry, the apricot kernels are reduced to more or less coarse fragments of 1 to 10 mm. They are characterized by a very high rate of nitrogen (42.9).

Thus, 4 types of binary substrates were used. The proportions of the retainer and aerator inspired by Chouial *et al.* (2004) are Substrate 1: 50% FH + 50% CG, Substrate 2: 50% FH + 50% PB, Substrate 3: 50% FH + 50% AK and- Substrate 4: 40% FH + 20% OP + 40% CG. In the rest of the text, each substrate will be designated by the initials of the aerator used.

Experimental design and data collection

The culture substrates are distributed in WM containers of 400 cm³ and sown with an acorn pre-germinated aseptically per container with a repetition of 32 per treatment. The containers of each treatment are collected in a box and placed in an educational nursery at ambient conditions but sheltered from the rain.

After 9 months of breeding, the seedlings survival rate is previously calculated for each treatment. The growth of 5 randomly collected plants per treatment is evaluated on the basis of morphological and physiological criteria namely stem height (H), collar diameter (D), H/D ratio, fresh and dryaerialbiomasses (FAB, DAB) and belowground biomass (FBB), the number of leaves and the chlorophyll content.

Dry biomass is estimated only on the aerial part after drying in the oven at 63 °C for 72 hours as it is destructive on the roots.

The leaves chlorophyll content is measured using a SPAD 502 PLUS chlorophyll meter. For each substrate, the total nitrogen (N) content is evaluated using the Kjeldahl method, that of assimilable phosphorus (P_2O_5) by the molybdenum Blue method (EPA spectrophotometry 365.2+3, APHA 4500-P E and. DIN EN ISO 6878. D11), and the assimilable potassium content (K_2O) is evaluated by spectrophotometry (sodium tetraphenylborate method).

The rate of ectomycorrhization (M%) per plant is calculated on about 50 root fragments approximately 5 cm in length randomly collected from the entire root system and the number of ectomycorrhizal apexes is evaluated under Stereomicroscope on a Total of 500. Similar ectomycorrhizal apexes are grouped to determine the morphotypic diversity per substrate.

Statistical analysis

Data obtained for each parameter are interpreted by an analysis of the variance (ANOVA) using the software XLSTAT (2014). This analysis is supplemented by a comparison multiple test of means using the Newman and Keuls (NSK) test, for estimating the significance and homogeneity between the different treatments. The Pearson correlation coefficients between the different appraisal parameters are calculated to study the strength of the bond that can exist between these different variables, confirmed by a PCA (Principal Component Analysis).

Results

Chemical characteristics of substrates

The chemical analysis reveals that all the substrates used are characterized by aslightly acidic pH with the exception of the PB whose pH is neutral (Table1).

Total nitrogen is considered low at the OPCG substrate, very low for the other substrates, and greatly reduced at the CG substrate.

The contents of P_2O_5 are high, they are very high in K_2O for all the substrates. The OPCG substrate has a medium availability of mineral elements but the highest compared to the others. However, it has an excess of potassium.

Table1.pH and NPK content of the substrates.

Parameters substrates	pH	Total nitrogen (N) mg kg^{-1}	Available phosphorus (P_2O_5) mg kg^{-1}	Available potassium (K_2O) mg kg^{-1}
CG	6.20	36	198.49	768
PB	6.60	248	210.22	816
AK	6.15	242	146.05	758.4
OPCG	6.48	570	163.76	1108.8

CG: Cork Granule, PB: Pine Bark, AK: Apricot Kernel, OP: Olive pomace.

Development of seedlings of cork oak according to the culture substrates

Survival rate

After six months of breeding, pine bark (PB) and apricot kernels (AK) allow for the highest survival rates with values of 84.37% and 80.64% respectively followed by a mixture of granular cork and olive pomace (OPCG) with an acceptable rate of 68% survival and finally cork granules (CG) with 62.5%.

Plant growth

The data of Table 2 show the effect of the substrate on the growth parameters of cork oak (C.O) seedlings.

Height of the stem (H)

Overall, the average aerial development of C.O plants is high for all treatments 47.47 ± 12.57 cm. Statistical analysis with $F_{(3,16)} = 6.95$ and $P = 0.003$ reveals significant differences between treatments at the 5% threshold.

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The application of the Newman-Keuls test (SNK) makes it possible to discern 2 groups: the first one corresponds to the substrates AK and to the substrate OPCG with respectively average heights of 58.52 cm and 54.60 cm. This height is admitted as being a high growth.

The second group is represented by the substrates PB and CG with successively lower heights of 39.08 cm and 37.66 cm respectively. The best height (66.5 cm) is observed at the AK, while the lowest height of 25.6 cm is found at the level of CG (Table 2).

It is obvious that aerial over Head development is not limited to the stem elongation. We also observe in more than 50% of the plants lateral branches where the PB substrate is ranked first followed by AK and OPCG which are close. The CG, on the other hand, does not present any lateral branches.

Table 2. Effect of different substrates on the development of cork oak seedlings.

Culture substrate	Average height H (cm)	Average collar diameterD (mm)	Ratio H/D	Belowground fresh biomass FBB (g)	Aerial fresh Biomass FAB (g)	Aerial dry biomass DAB (g)	Number of leaves	Chlorophyll
CG	37.66 ± 8.97 b	3.5 ± 0.35 a	10.85 ± 2.92 a	13.85 ± 5.46 a	2.63 ± 0.62 b	1.47 ± 0.23 c	25.0 ± 6.59 b	47.24 ± 2.68 a
PB	39.08 ± 8.83 b	3.8 ± 0.75 a	10.74 ± 3.89 a	7.31 ± 1.84 b	5.04 ± 1.43 a	2.37 ± 0.51 b	39.4 ± 14.04 ab	47.39 ± 5.09 a
AK	58.52 ± 7.81 a	3.6 ± 0.54 a	16.57 ± 3.36 a	7.17 ± 1.22 b	6.46 ± 1.30 a	3.53 ± 0.58 a	60.2 ± 21.86 a	43.24 ± 2.87 a
OPCG	54.60 ± 10.30 a	3.2 ± 0.44 a	17.52 ± 4.68 a	12.39 ± 2.75a	4.63 ± 1.18 a	2.48 ± 0.60 b	49.4 ± 21.37 ab	36.47 ± 2.20 b
Mean ± SD	47.47 ± 12.57	3.52 ± 0.55	13.92 ± 4.73	10.18 ± 4.28	4.69 ± 1.77	2.46 ± 0.89	43.50 ±20.60	43.58 ± 5.51
Fisher's F	6.95	1.04	4.62	5.61	9.01	13.89	3.81	11,345
Significance (P)	0.003**	0.401^{NS}	0.016*	0.008**	0.001***	0.000***	0.031*	0.000***

For each column, the mean values followed by different letters indicate that the values are significantly different (P <0.05) according to the Newman and Keuls multiple comparison test. (* p ≤ 0.05, ** p ≤0.01, *** p ≤0.001), NS: not significant; SD: standard deviation; CG: Cork granule; PB: Pine bark; AK: Apricot kernel; OPCG: Olive pomace + Cork granule.

Collar diameter (D)

It is generally observed that the plants radial development in all the treatments is relatively small (3.52 ± 0.56 mm) (Table 2). However, the PB, AK and CG mixtures seem to favor better radial development although not significantly ($F_{(3.16)} = 1.04$, NS) with average diameters of 3.8, 3.6 and 3.5 mm respectively compared to OPCG which provides the smallest diameter of 3.2 mm. Regarding the individual diameters of treatments previously mentioned, values ranging from 3 mm to 4.5 mm are noted.

The H/Dratio

The values of the H/D ratio vary significantly between the different modalities($F_{(3,16)}$ = 4.62, P=0.016). Unlike to the Newman-Keuls test, Fisher test defines two homogeneous groups, namely group A consisting of OPCG and AK with respectively (17.52, 16.57) and group B containing CG and PB with respectively (10.85 and 10.74) (Table 2).

Fresh belowground biomass (FBB)

The root system is examined by the evaluation of its fresh material and also the description of its quality i.e. its architecture. Analysis of variance (ANOVA) reveals significant differences between the different treatments ($F_{(3, 16)}$ = 5.61, P = 0.008). The SNK test distinguishes three homogeneous groups; the first corresponds to the CG substrate with a very high biomass of 13.85 g followed by the second group, represented by the OPCG substrate with a fairly satisfactory biomass of 12.39 g. While the 3rd group, combines the PB and AK substrates with respective values 7.30 and 7.17 g which are significantly weak compared to the first two treatments. Individual measurements of root biomass fluctuate over awide range from the lowest value (4.44g) recorded in the PB to the remarkable biomass of 20.20 g recorded at the CG substrate (Table 2).It should be noted that the root systems that have the best biomass are characterized by dense hair with many branches of fine roots.

Table 3. Distribution of ectomycorrhizal morphotypes (ECM) detected according to culture substrates.

	Culture substrates			
	CG	PB	AK	OPCG
ECM morphotypes				
ECM1	+	+	+	+
ECM2	+	+	+	+
ECM3	+	-	+	-
ECM4	+	-	-	-
ECM5	+	-	-	+
ECM6	+	+	+	+
ECM7	+	+	-	+
ECM8	-	-	-	+
ECM9	-	-	+	-
ECM10	+	-	-	-
ECM11	+	-	+	-
ECM12	-	-	-	+
ECM13	+	-	-	+
ECM14	+	+	-	+

+ present, - absent.

Freshaerial biomass (FAB)

The results for fresh aerial biomass, whose average for all treatments being 4.69 ± 1.77, are consistent with height growth with some minor exceptions. Indeed, the substrate AK gives the largest biomass (6.46 g) as for the height and forms with PB and OPCG a homogeneous group. This group is significantly different ($F_{(3,16)}$ =9.01, P = 0.001) of CG, its mean fresh aerial biomass and height are the lowest (2.63 g, 37.66cm) respectively (Table 2).

Number of leaves per seedling

The number of leaves produced per seedling is variable and is on average 43.50 ± 20.60. ANOVA (Table 2) reveals significant differences ($F_{(3,16)}$ = 3.81, P=0.03) depending on the nature of the aerator. According to the Newman-Keuls test, the results are perfectly consistent with the results of aerial fresh biomass (AFB). In fact, the best yields are recorded in the seedlings obtained on the AK substrate with a high average number of 60.20 and an individual value of 88 leaves (the highest). OPCG and PB substrates have 49.40 and 39.40 respectively. Finally, the CG with 25 leaves on average and the lowest individual value of 17 leaves.

Table 4. Correlation matrix of parameters for the seedling quality assessment.

Variables	M (%)	Nbr (ECM) /seedling	H (cm)	D (mm)	H/D	FBB (g)	FAB (g)	N Nbr of l leaves/seedling	Chlorophyll
M (%)	1								
Nbr (ECM) /seedling	0,269	1							
H (cm)	-0,025	-0,376	1						
D (mm)	0,014	0,018	-0,295	1					
H/D	-0,030	-0,303	**0,903*****	**-0,658****	1				
FBB (g)	0,300	**0,765** ***	-0,136	0,092	-0,151	1			
FAB (g)	-0,030	**-0,539** *	**0,682****	0,128	**0,496***	-0,357	1		
Nbr of leaves/seedling	-0,079	-0,326	**0,612****	-0,117	**0,583****	-0,166	**0,750*****	1	
Chlorophyll	0,236	0,008	**-0,516***	0,401	**-0,595****	-0,222	-0,185	-0,390	1

The values in bold (r) are different from 0 to a level of significance alpha = 0.05; * p ≤0.05, ** p ≤0.01, *** p ≤0.001.

Chlorophyll content

For all treatments except OPCG, the leaves of cork oak seedling are well supplied with chlorophyll with an average of 43.58 ± 5.51 spadunits. ANOVA (Table 2) reveals that leaf chlorophyll contents vary significantly between culture substrates ($F_{(3.16)}$ = 11.35, P =0.000), The Newman-Keuls test classifies substrates in two homogeneous groups, group A containing the substrates PB, CG and AK with high and close contents of the order of (47.39, 47.24 and 43.24) spad respectively and the group B corresponding to the substrate OPCG with the lowest content of 36.47 spad. A maximum individual value (50.73) was recorded at the CG, and the lowest (34.5) at the OPCG.

Mycorrhization of cork oak seedlings according to culture substrates

Stereoscopic observation and mycorrhizal rate estimation show that regardless of the nature of culture substrate, all cork oak seedlings have a root system that is naturally colonized by ectomycorrhizal fungi (EMF). The average mycorrhizal intensity is (M %) = 52.85 ± 16.03. Statistically, the nature of the substrate appears to have no significant effect on the levels ECM colonization (M %) of the roots (F (3.16) = 2.26, ns). However, thanks to Fisher's test, three homogeneous groups emerge. Indeed, the M% of seedling is more important at the substrate level of the CG (M% = 63.55%) without, however, being significantly different from other PB (M% = 55.53), AK (M% = 52.45) and OPCG (M% = 39.86) substrates (Fig.1a). The highest mycorrhizal value (85.26%) is observed on a subject of the PB substrate, while the lowest (32.02%) is observed with OPCG.

Regarding the average number of ectomycorrhizal morphotype (Nbr ECM)/plant (Fig.1b), the ANOVA reveals that the differences between treatments arevery highly significant ($F_{(3.16)}$) =10.53, P=0.000***). In addition, the SNK test identified 4 substrates groups. The Nbr ECM / plant recorded at the level of the CG substrate was significantly higher (6.6) than the other substrates (4.6, 3.4 and 2.4) respectively OPCG, PB and AK.

These results are in accordance with those of the total number of morphotype / treatment, particularly for CG and OPCG. Indeed, during the examination of the root systems, 14 morphotypes could be detected, distributed differently on the four treatments. The CG substrate is marked by the greatest morphotypic diversity, represented by 11 morphotypes, 9 are associated with OPCG seedlings, whereas with AK and PB, this number is 6 and 5 morphotypes of ectomycorrhizae (ECM) only.

Distribution of ectomycorrhizalmorphotypes according to the culture substrates

The interpretation of the results shown in Table 3, allow to distinguish cosmopolitan morphotypes found in all substrates and whose presence does not characterize any of them, this is the case of ECM **1** (*Ceococcumgeophilum* Fr.) , ECM **2** (*Thelephora terrestris* like) and ECM **6**. There are also widespread species only on 3 or 2 substrates. Some morphotypes such as ECM **4** and **10**are exclusively present in CG, ECM **9** is AK-specific and ECM **8** and ECM **12** are present in OPCG.

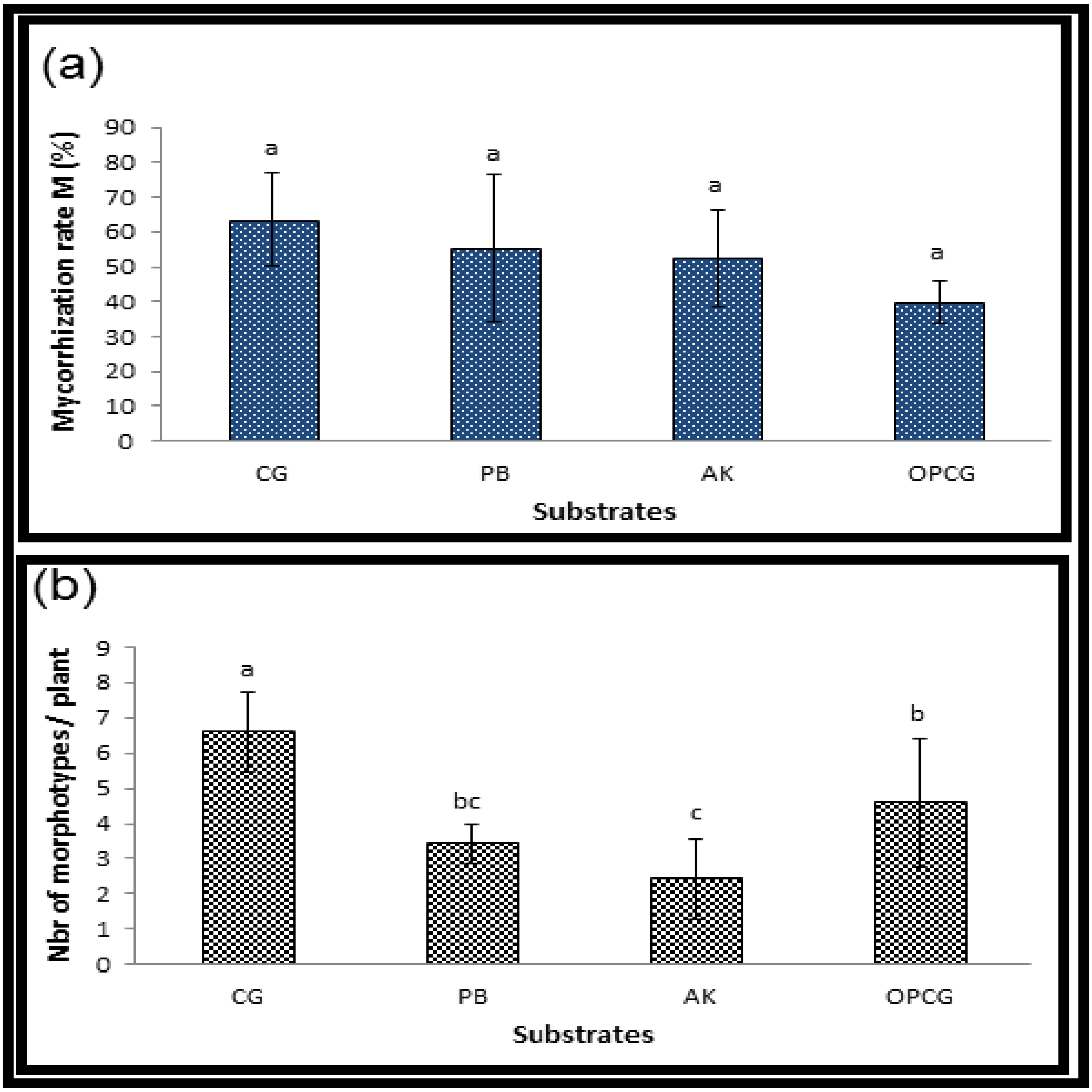

Fig. 1. Effects of substrates on: (a) Mycorrhization rate M (%) and (b) Average number of morphotypes / cork oak plant. Bars represent mean values ± standard deviation. The different letters indicate significant differences (P <0.05) according to the Newman and Keuls multiple comparison test.

Correlation between seedling appreciation parameters

As shown in Table 4, Pearson's correlation coefficient, calculated between different seedling quality assessment parameters, shows a strong statistically significant positive correlation between ECM morphotype number/plant and FBB ($r = 0.765$, $p < 0.0001$). The plant height (H) is moderately correlated with the number of leaves / plant ($r = 0.612$, $p = 0.004$). Whereas, Nbr ECM / plant and FAB are inversely correlated ($r = - 0.539$, $p = 0.014$), the same goes for H and chlorophyll ($r = - 0.516$, $p = 0.020$) (Table 4). It is moreover observed that the Pearson coefficient does not show any correlation between the M% and the growth parameters (Table4, Fig.2).

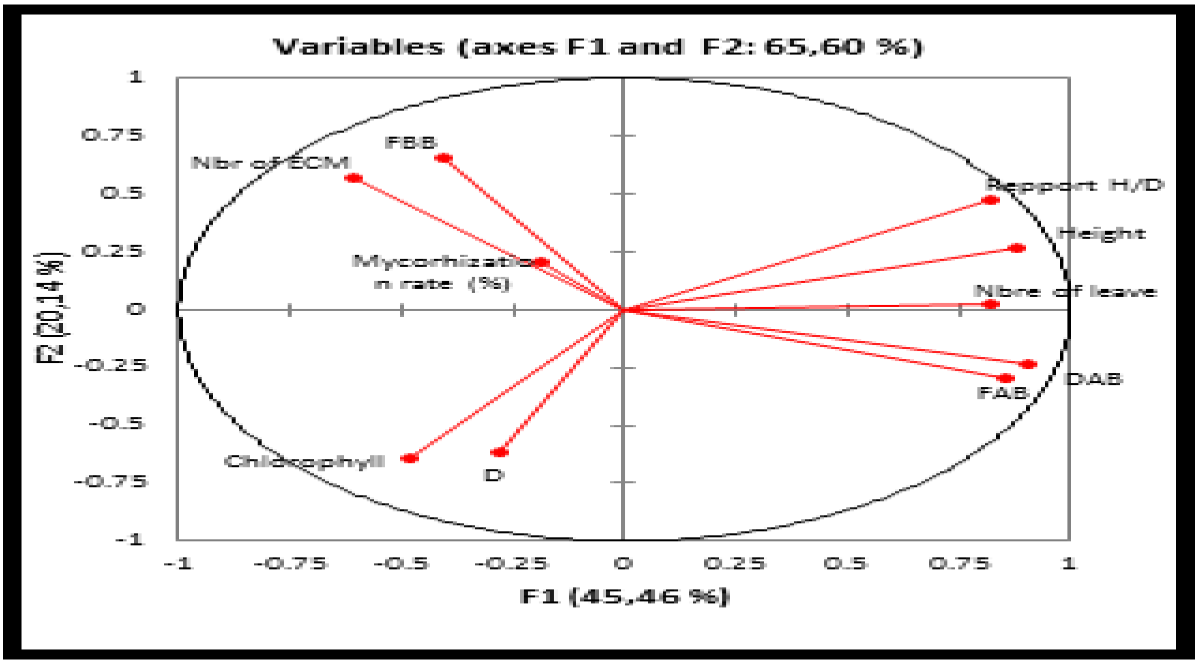

Fig.2. Principal component analysis (PCA) of assessment parameters.

Discussion

Forest humus under cork oak is favorable to the cultivation of the seedlings Kahia *et al.* (2004). However, it has an average performance in terms of total porosity and aeration rate. This problem can be corrected by adding aerator elements such as CG, PB, AK and OPCG in the case of this study.

Except the PB substrate characterized by the highest pH 6.6 due to the probable presence of pine bark, the other culture substrates have optimal pH values. De Rijck (1997) in Robles (1999) considers that the effect of pH is a determining factor for the availability of nutrients in the soil. Indeed, a pH above 6.5 negatively affects the availability of mineral elements in the rhizosphere, photosynthesis, growth of roots and aerial parts of forest seedlings (Lamhamedi *et al.*, 2006 a, Ammari *et al.*, 2007).

Development of the cork oak seedlings depending on the culture substrates

Important mortality was noted during summer season. Indeed, the seedlings grown on PB and AK were the mostheat-resistant with a high survival rate (84.37%, 80.64%) respectively, while the CG had the lowest rate (62.5%). According to Kahia *et al.* (2004), the survival rate must exceed the 80% threshold for it to be economically profitable.

Seedling growth

In general, the height of C. O seedlings is quite high. It is significantly the largest for AK and OPCG substrates. This explains the positive effect played by these materials marked by a high rate of nitrogen, thus favoring the growth of seedlings in height (Chouial *et al*., 2004).

These results are in agreement with those of Chouial (2010) who found that the growth of C.O is improved by anintake of olive pomace. However, the high growth in height of C.O plants could also be explained by the use of WM 400 cm^3 of small section causing a sowing density effect. Indeed, the smaller the container, the higher the density of the roots, the greater the growth of the seedlings. Conversely, the increase in the container section, which determines the number of plants per m^2, has an influence on contrary on the height of the seedlings (Benseghir *et al*., 2006).

If we refer to the dimensional standards of forest seedlings established in 1990 by the Directorate of Rural Space and Forest (DERF) and which impose a plant height less than double thecontainer height, the majority of seedlings will be marked by a shoot-root imbalance resulting in excessive aerial development which does not meet dimensional standards. On the other hand, Balleux (2009) limits this standard to a maximum height less than four times that of the container for hardwoods. According to Boufares (2012), more stems are short, less seedlings expenditure of energy for supplying the nutrients of the underground part towards the apical part. Residual energy can be used for other purposes.
A more recent standard indicates that the height of a C.O plant of one year maximum, packed in 400 cm^3 container, can be up to 60 cm (DRAAF Regional Directorate for Food, Agriculture and Forestry, 2014). While, the number of lateral stems per plant allows having an idea about the growth quality of the plant. The stem abundance enables many seedlings to supply water and minerals (Chaumeton *et al.,* 2006). It is estimated that the PB followed by AK and OPCG best meet these criteria.

It should be noted that the seedlings had a remarkable elongation and this at the expense of the diameter. The standard specifies a minimum collar diameter of 5 mm (DRAAF, 2014).

Furthermore, if we refer to Jaenicke (2006), we deduce that the best H/D ratios, which are obtained with the substrates PB and CG (10.74 and 10.85), since the growth in height is more weak and the diameter is stronger. However, these H/D ratios remain high, since according to the same author, a quotient greater than 6 is undesirable. A small quotient indicates a robust seedling with higher survival chance.

As regards fresh root biomass, the statistical analysis reveals in particular the positive effect of cork granules and, to a lesser degree, the mixture of cork granules and olive pomace on the development of the root system. The cork granule common to both substrates seems to optimize root growth because of its good porosity due to the obvious correlation between its proportion and the production of root biomass. This result corroborates those of Chouial *et al.* (2004) and Chouial (2010) confirming the close relation between the total porosity and theroot system quality. OPCG enhances height growth of C.O through the olive pomace it contains. It also improve root growth due to its granulated cork content.

The production of leaves has been considerable, mainly for the AK substrate. It is accepted that the leaves have an important role in gas exchange and production of organic matter through photosynthesis (Boufares, 2012). Estimating leaves number is a good indicator of the plant's assimilative capacity and biomass production (Fischesser *et al.,* 1996).

Finally, the chlorophyll content is an indicator of good health of plants. According to Blackmer *et al.* (1994) and Scharf (2001), there is a strong correlation between SPAD measurements and leaf nitrogen content, since according to Paiement (2011), nitrogen plays an important role in the photosynthesis cycleand promotes the development of chlorophyll. Thus by measuring the latter, we measure the plants photosynthetic potential and indirectly the amount of nitrogen in the plant. The classical found values are between 0 and 50 SPAD units (Benmati, 2014). Tahri *et al.* (1997) in Benmati (2014) state that water stress causes a decrease in total chlorophyll pigment levels (chlorophylls a and b). This leads us to believe that plants deficient in chlorophylls such as those of OPCG suffered from water stress probably due to a lack of water retention.

Mycorrhizal status of cork oak plants according to culture substrates

The average rate of mycorrhization M% for all treatments is acceptable (52.85%). This shows that the forest soil used (non-sterilized) mixed with the different aerators would contain ectomycorrhizal fungi (EMF) strains of sufficient infectivity.

In general, quite important differences are recorded in the mycorrhization of seedlings from different substrates and compounds of the same natural inoculum. CG treatment seems to favor the best mycorrhization, the highest morphotype number / seedling and the greatest morphotypic diversity of ectomycorrhizae.

This is probably the result of the symbiotic interaction with a wide range of ectomycorrhizal fungi. Although OPCG is well classified in terms of morphotypic diversity and ECM number / plant, it nevertheless records the lowest M%. The PB and AK, meanwhile, occupy the last positions concerning morphotypic diversity and ECM number / plant and an intermediate position for M% with quasi-equivalent values.

Trying to explain this variability between the different treatments, it is imperative tolook for a correlation between the chemical properties of the substrate, especially its NPK content and the mycorrhizal state of the seedlings.

Mycorrhization of seedlings and mineral nutrition

According to the literature, mycorrhization is a function of several factors. The concentration of substrates in mineral elements may be a limiting factor. In general, the high phosphorus (P)concentrations of the substrates seem to have a repressing effect, and negatively affect the colonization of the plants even if it is estimated that the latter was average for the CG substrate. Amijee *et al.* (1989) confirm that at a P concentration of 50 mg / kg, mycorrhizal colonization is maximal, whereas it is reduced by doubling this concentration. It is possible that the very low nitrogen (N) content that distinguishes the CG substrate has reduced the inhibitory effect of its high P concentration and is therefore responsible for the better colonization and greater diversity of EMF associated to C.O.EMF's in the case of CG are probably required to provide the plants with nitrogen, the levels of which are considerably higher in other substrates.

Boukcim and Mousain (2001) claim that the level of mineral or organic fertility of the culture substrate has a major effect in the establishment of seedling mycorrhization. According to many authors Conjeaud (1996), Grant *et al.* (2005), Breuillin *et al.* (2010), Cripps and Grimme (2011), Lonergan and Cripps (2013), Waligora (2016),this effect generally results in a decrease of the rootcolonization degree (M%) by the ectomycorrhizal fungus under the inhibitory effect in particular of P and / or N in sufficient or high amountin the soil.

Distribution of ECM saccording to culture substrates

The detected ectomycorrhizae are distributed differently in the four treatments. This reflects a behavioral heterogeneity of the fungal strains towards the substrate especially their fertility levels. This is very well illustrated by fungi such as *Thelephora terrestris*, which according to Le Tacon *et al.* (1997) has a remarkable ability to recolonize disinfected soils and to maintain a high level of fertility. This fungus plays a vital role in nurseries around the world. Tomás (1999) associates the abundant presence of *Cenococcum geophilum* Fr. (CEM 1 in our study) with the dieback of trees. Peter *et al.* (2016) and Jany (2002) confirm the beneficial role of *Cenococcum*on its host during severe droughtperiods. Moreover, according to Machado and Santos (2002), the great diversity of mycorrhizal species is an indicator of tree health. On the other hand, the lack of correlation between M% and the growth stimulation, in particular the height and the aboveground biomass of the seedlings, is consistent with that of Pierart (2012).

As for the strong link between the number of morphotypes / seedling and the fresh biomass of its roots, it has been supported in particular by Cárdenas (2010), which suggests that the diversity of natural mycorrhization offers to plants a greater capacity to exploit the N and other resources, improving their plant productivity.

Mycorrhizal status and root growth

CG seedlings and to a lesser extent OPCG have the best root biomasses. These results can be attributed to several reasons and lead to think of several hypotheses:

It is probably, as mentioned above, the effect of the physical properties (porosity of the cork granule) or chemical (mineral fertility level) of the substrate on the root growth: because, with respect to this last point, mineral element deficiency conditions, especially in phosphorus and nitrogen, induce plants to favor root development to the detriment of the aerial parts (Améziane *et al.*, 1995, Poorter *et al.*, 1995) thus increasing the possibilities of mycorrhizal colonization to improve the acquisition of mineral elements. This may be true for N-deficient CG seedlings with the best M% and important root biomass gains. But, does not apply to the OPCG which is more filled with mineral elements and whose seedlings are less mycorrhizal. Cárdenas (2010) believes that in the presence of resources that are difficult to access, plants develop more their fine roots as a physiological response to the lack of resources.

There is a relationship between root development, availability of mineral elements and natural mycorrhization. This relationship is concretized by the production of strigolactones, multifunctional plant hormones produced mainly in the roots and playing a role in the establishment of mycorrhizal relationships (Saint Germain *et al.,* 2016, Saint-Germain, 2014). Strigolactone exudation is negatively regulated by the presence of high phosphorus concentration (Yoneyama *et al.*, 2007a, 2012; Umehara *et al.*, 2010). Both P and N deficiency favours the strigolactones production (Yoneyama *et al.*, 2007a). Thus, the plant not only promotes the establishment of mycorrhizal interaction but it also promotes the lateral roots and absorbent hairs development while inhibiting the branching of aerial parts (Balzergue, 2012).

Mycorrhizal status of plants and growth in height

Except other substrates, CG seedlings do not show stem branching and produce a reduced number of leaves. However, the control of plant branching is provided by strigolactones, which act as an inhibitory signal by migrating into the stem towards the axillary buds thus preventing them from growing (Saint Germain *et al.*, 2016; Saint-Germain, 2014). This branching inhibition of CG seedlings would be due to a high concentration of strigolactones in favor of good root development and good colonization in order to better explore the environment. Despite the low M% at the OPCG, and to a lesser extent the AK, the results for the aboveground biomass produced show that it is important.

It is possible, as suggested by Cárdenas (2010), that the seedling have rather invested in height growth and green leaves needed to increase photosynthesis levels. Cárdenas (2010) indicates that this strategy is favorable for mycorrhizae, because the increase in photosynthesis implies an increase in the production of carbon products essential for the associated ECM fungi survival (Finlay, 2005).

Conclusion

The results of this preliminary work demonstrate globally that the development of cork oak seedlings has been significantly affected by the type of substrate. Thus, among the aerators tested, cork granules, just like the pine bark, can be used in the preparation of culture substrates and allow optimal growth of the cork oak. The CG comprising cork shows the best performance with the highest rate of natural mycorrhization M%, the greatest morphotypic diversity and the fresh root biomass probably due to its ability to improve certain physicochemical properties, including aeration and density of the substrate. However, by a simple comparison, it appears that the performances of the PB were very close to those of the CG (the collar diameter, the rate of the chlorophyll and the quotient of robustness H/D).In terms of the biomass aboveground production, the performances of the two substrates CG and PB are appreciably lower than the other substrates but this, not being a bad sign, is on the contrary in favor of a good balanceshoot-root and a good quotient of robustness. Moreover, despite the signs of maximum aerial biomass production, the seedlings of AK and OPCG remain marked by an unsustainable growth (a quotient of robustness too high which generates a fragile plant at the time of transplantation). Future work could be envisaged in order to continue the research with the aim of a more in-depth physicochemical characterization of the growth substrates (cation exchange capacity, C/N ratio, total porosity...), both in the initial state and at the end of the growth period to evaluate the evolution of their physicochemical properties.

Important information that can be drawn from the overall results of the mycorrhizal part, is that the mycorrhizal status of the seedlings is affected by the nature of the substrates and that the natural mycorrhization seems to be inversely correlated with the availability of mineral elements in the substrate.

However, we cannot conclude on this point, since the association establishment and the ECMs distribution are controlled not only by the chemical properties of the substrates, but also by genetic factors specific to each fungal specie, its requirement and its tolerance to excesses or deficiencies of mineral elements.

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