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RESEARCH ARTICLE

ROLE OF PHOSPHATASE OF ECTOMYCORRHIZAL FUNGI IN GROWTH AND PHOSPHORUS ENRICHMENT OF PINUSHALEPENSIS MILL

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ABSTRACT

The mycorrhization of *Pinushalepensis* had been investigating dusing different autochthonous ectomycorrhizal (ECM) fungi isolated from soils collected at the Haouz region. The obtained results showed that *Hebelomamesophaeum*, *Suillusbellini*, *Suilluscollinitus*, the isolate 3530 of *Suillusmediterraneensis* and *Tricholomaterreum*has led to growth improvements depending on the fungal speciesand the nature of phosphate (P) source. The culture of Aleppo pine seedlings in the presence of organic (phytate) or mineral (KH_2PO_4) P source is reflected by a very significant increase of acid phosphatase activity in cytoplasmic and membrane-bound fractions. This phosphatase induction was found to be more important in the presence of organic phosphate. In the presence of phytate, a positive correlation was observed between high levels of phosphatase activity and the improvement of growth and P nutrition of mycorrhized Aleppo pine seedlings.

INTRODUCTION

Aleppo pine (*Pinushalepensis* Mill.) constitute a very important reforestation essence in many mediterranean countries with more than 65.000 ha in our country (Belghazi *et al,* 2000). Because, of the soil and severe climatic constraints the reforestation success were very limited. Mycorrhization process of plants in controlled conditionsimproved the quality seedling (Boukcim *et al,* 2002). It is well known that this symbiotic assotiation improve the hydro-mineral nutrition of the host plants. The large volumes of soil explored by fungal hyphae increase the absorption of water and nutrients. This contribution is a crucial importance in limiting conditions (Baxter and Dighton, 2005; Turjaman *et al,* 2006; Garcia *et al,* 2014). Many authors' reported that tree species such as pines are highly dependent on their fungal symbionts (Ouahmane *et al,* 2009; Brundrett, 2002). ECM fungi provides mineral nutrients to their hosts, mainly N and P, which otherwise would not be available (Smith and Read, 2008). In addition, ectomycorrhizae have been shown to enhance resistance of hosts plants to biotic and abiotic stress such as drought (Kipfer *et al,* 2012; Worchel *et al,* 2013), degraded soil (Rincón *et al,* 2007) and burnt soil (Sousa *et al*,2011), high salt concentrations (Langenfeld-Heyser *et al*,2007; Luo *et al,* 2011), or metal contamination (Kzrnaric *et al,* 2009).The P nutrition is a limiting factor for plants growth in wide regions of the globe(Persson *et al*,2000; Hrynkiewicz *et al*,2009; Nygren and Rosling, 2009).Most of phosphorus in soils is present in unavailable forms to plants as insoluble mineral and/or organic. The ECM symbiosis are often reported to play a key role in improving P nutrition of the host plant (Wallander, 2000a, 2000b; Tatry *et al,* 2009; Jouranda *et al,* 2014).Through this symbiosis, hosts plants develop numerous adaptation strategies (morphological, physiological or biochemical) which enhance the acquisition of inorganic P and/or improve the effectiveness of its internal use (Nehls *et al,* 2001; Rosling and Rosenstock, 2008). Indeed, Mycorrhizal fungi are known to enhance nutrient uptake, particularly of P. either (i)by increasing the absorbing surface and also by explorating larger soil volumes (ii)the small hyphal diameter leading to an increased P-absorbing surface area and, compared to non-mycorrhizal roots, higher P influx rates per surface unit, (iii) the production of organic acids and phosphatases, which catalyze the release of P from organic complexes, and (iv) the

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formation of polyphosphates by mycorrhizal fungi and hence low internal P concentrations (Marschner and Dell, 1994).

In poor P soils, hydrolysis of organic phosphorus is attributed to the excretion of fungal phosphatases that increase the amount of orthophosphate available in the soil solution and expand the range of degradable phosphorylated substrates (Mousain *et al*, 1997; Alvarez *et al*, 2005). Hydrolytic enzymes of ECM fungi play a paramount role in the mutualistic relationship between the fungus and their hosts in mobilizing nutrients from organic sources (Talbot *et al*, 2008; Pritsch and Garbaye, 2011). The synthesis of these enzymes and their regulation appear critical for access to the organic P and improving P nutrition of ectomycorrhized plants. Despite the importance of this phenomenon, few studies have attempted to elucidate the mechanisms regulating the activity of these enzymes as well as the influence of the soil environment conditions. According to Read and Perez-Moreno (2003), ECM fungi have the potential to be directly involved in attack both on structural polymers, which may render nutrients inaccessible, and in mobilization of N and P from the organic polymers in which they are sequestered. Furthermore, the production of phosphatases has been often reported as variable depending on the ECM species (Antibus *et al*, 1997; Eaton and Ayres, 2002). The selection of efficient strains so a particular importance for the production of quality plants can adapt to severe conditions of the arid zones. The present work fits into this context and aims to study the physiological (growth and mineral nutrition) and biochemical (acid phosphatase activity) behavior in mycorrhized or non-mycorrhized Aleppo pine using five autochthonous ECM fungi depending on the nature of Psource, organic (phytate) or inorganic (KH_2PO_4).

MATERIALS AND METHODS

Plant and fungal material

The Aleppo pine seedlings grown from seeds were kindly provided to us by the Regional Forest Research Centre of Marrakech.

Five autochthonous ECM fungi: *Suilluscollinitus*(Fr.) O. Kuntze, *Suillusbellini* (Inz.) watl, *Hebelomamesophaeum*(Pers.) Quélet, *Tricholomaterreum*(Sch.: Fr.) Kummer and the isolate 3530 of *Suillusmediterraneensis*(Jaquet. and Blum) Redeuilh collected from the Haouz forests of Marrakech province (Toufliht, Amezmiz and Tizrag) (Oihabi, 1998) constitute the fungal inoculum used in this work.

Conduct of cultures

Mycorrhization with five ECM fungi was carried out according to the technique of solid inoculum (Marx and Bryan, 1975). The mycorrhizal inoculation was realized just after germination of Aleppo pine seeds by mixing the inoculum with a proportion of 10% culture substrate. Cultivation of plants was conducted on a single type of artificial substrate (peat) disinfected for 4 hours at 150°C. Fifty repetitions have been prepared for each symbiotic species. Culture plants was conducted in a growth chamber at temperature 22/18°C and photoperiod 16/8 hours (day/night) and under a ceiling light with a light intensity of 240 µmol.m^{-2}.s^{-1}. The irrigation of plants was effected by tap water (pH 7), three times per week. The periodic control of mycorrhized root systems development, allows following of growth and mycorrhizal colonization. Seven months after the inoculation, all plants proved mycorrhizal colonization. The mycorrhizal intensity was determined according to the technique described by Nezzar-Hocine (1998). The root systems of plants were washed and examined under the microscope, and then were cut into segments of 2cm in length. The counting was conducted on segments randomly chosen to achieve 100 root tips per plant. The mycorrhizal intensity was expressed as a percentage of mycorrhized short roots contribution to the total number of short roots. After seven months of culture, mycorrhized and non-mycorrhized (control) aleppo pine seedlings by the five symbiotic fungi were transplanted in jars of 500 ml containing 400 ml of vermiculite saturated with the mineral components of the nutrient solution of melinNorkrans (Norkrans, 1949) in the presence of organic P(phytate [100mg/l]) or mineral (KH_2PO_4 [500mg/l]). The substrate thus prepared is autoclaved for 20 min at 120 °C. In each container, three plants are transplanted and six replicates were performed for each treatment and symbiotic species. The seedlings are then incubated in the same conditions of temperature and photoperiod described above. The watering of plants was repeated three times per week with 20 ml of mineral solution prepared of melinNorkrans containing organic or inorganic P. Physiological and biochemical parameters were determined in mycorrhizal and non mycorrhizal plants according a kinetics0, 15, 30 and 45 days of treatment.

Measured parameters

Estimating Growth

The growth of harvested seedlings is assessed by measuring the rate of dry matter (DM) of both aerial and root parts after drying for 48h at 80°C.

Minerals contents

Dried samples of the aerial parts of Aleppo pine seedlings of various treatments have been mineralized according to the method described by Harvey and Fox (1973). The determination of the phosphorus is performed by colorimetrically using the AFNOR T90-23 technique. The sodium, calcium and potassium contents were determined using flame emission spectrophotometry (Rodier, 1984). For the determination of magnesium content anatomic absorption spectrophotometry analysis was performed (Perkin-Elmer, 1966). The total amount of nitrogen (N) is measured using the Kjeldahl method (Rodier, 1984).

Phosphatase activity determination

Extraction of phosphatases was performed on the roots of mycorrhizal and non mycorrhizal plants at15th, 30th and 45th day of treatment. The extraction and fractionation procedures were based on the method of Straker and Mitchell (1986), as modified by McElhinney and Mitchell (1993). The samples of 200 mg of each species are ground in mortar in 1.5 ml ice-cold distilled water. The homogenate was then centrifuged twice at 12000g for 15 min at 5°C. The supernatant were bulked to constitute the cytoplasmic fraction. The residue was re-suspended in 1.5 ml ice-cold 0.2% Triton X-100 solution for 2 hours. The homogenate is centrifuged at 12000g for 15 min at 5°C. The supernatant collected constitutes the membrane-bound fraction.

Determination of acid phosphatase activity was carried out using p-nitrophenolP (pNPP) as a substrate (20 mg of pNPP were solubilized in 15 ml of distilled water). A mixture of 0.1 ml enzyme extract, 0.9 ml 0.1 M of sodium acetate buffer (pH 4.6) and 1 ml 3.6 mM-pNPP as the substrate was incubated for 30 min at 25 °C and the reaction was stopped by adding 5 ml 0.1 M NaOH (Antibus *et al,* 1992). A control without the enzyme (0.1 ml distilled water) was always included to measure non-enzymatic hydrolysis of the substrate. Absorbance at 410 nm was measured and converted to units of µmol p-nitophenol (PNP) released. The results of the specific activities are expressed as a percentage change compared to the control.

Protein determinations

Total protein of each root fraction was assayed by the method of Bradford (1976) using bovine serum albumin as the standard.

Statistical analysis

Statistical analyses were carried out using the STAT-ITCF program (Anonymous, 1988), and means were separated according to the Newman-Keuls test ($P \leq 0.05$).

RESULTS

The mycorrhizal colonization of Aleppo pine by different root symbionts of the Haouz region is expressed by the mycorrhizal intensity. The evaluation of mycorrhizal intensity in seedlings of Aleppo pine shows a difference in the ability of colonization of different symbiotic species used. The most important colonization capacities were observed in the presence of *Hebelomamesophaeum*, the isolate 3530 of *Suillus mediterraneensis* and *Suilluscollinitus* species, 74%, 73% and 70% respectively (Table 1). *Suillusbellini* presented the lowest mycorrhizal capacity (40%). The *Tricholomaterreum* species presented the intermediate performance of approximately 60%. The mycorrhizalintensity of Aleppo pine seedlings after 45 days of culture in the presence of both forms of Phave remained virtually unchanged. The type of organic P (phytate) or mineral (KH_2PO_4) used in the culture medium seems to have no effect on the mycorrhizal intensity after 45 days of treatment. The mycorrhization by all fungal species tested resulted in significant improvements of the production of shoot and root biomass for both organic and inorganic P sources (Fig.1). The improvements for biomass production vary in the case of mineral P nutrition between 100 and 180% for aerial parts and 120-230% in the roots (Fig. 1 A and C). It is important to note that these improvements rates have virtually equivalent to those observed at time T0 corresponding to the time of the contribution of the mineral P. These improvements in biomass production would consequently be due solely to the mycorhization and non to the contribution of inorganic P. The *Suillusbellini* species is characterized by the lowest rate of improvement unlike *Hebelomamesophaeum*, *Suilluscollinitus* and the isolate 3530 of *Suillusmediterraneensis* species that leads to the highest rate of improvement. The application of organic P source resulted in improvement rate of shoot and root biomass production increasing as a function of the treatment time and the used symbiotic species (Fig. 1 B and D). These increases reached after 45 days more than 260% for dry shoot matter with *Hebelomamesophaeum*, Suilluscollinitus, Tricholo materreum and the isolate 3530 of *Suillusmediterraneensis* species and more than 350% for dry root matter with *Hebelomamesophaeum*, *Suilluscollinitus* and the isolate 3530 of *Suillusmediterraneensis*.

The analysis of the results of various mineral elements contents (K^+, Na^+, Ca^{2+} and Mg^{2+}) in the aerial parts of mycorrhized and non-mycorrhized plants, after 45 days of culture in the presence of organic (phytate) or inorganic (KH_2PO_4) P shows that mycorrhization enhances the absorption of these minerals in a more or less important according to the symbiotic species and the used P form (Table 2). In the presence of inorganic P, most symbiotic species have caused no improvement in the absorption of the different elements examined. Only the *Hebelomamesophaeum* and *Suilluscollinitus* species seem to promote significantly the absorption and accumulation of these elements. In the presence of phytate, all symbiotic species induced highly significant improvements in mineral nutrition in mycorrhized plants, when we noticed a marked decrease in the ionic status in control plants (non-mycorrhized) compared to control plants grown in the presence of KH_2PO_4.

We also note that both *Hebelomamesophaeum* and *Suilluscollinitus* species proved to be the most effective inducing higher levels of minerals in mycorrhized plants. Concerning the two elements nitrogen and phosphorus known to be the most limiting growth and biomass production, the improvements registered in the case of phytate must come through an induction of synthesis and secretion of enzymes capable of mineralizing this organic form of P. Knowledge of the kinetics of absorption and accumulation of P and nitrogen would inquire on performance and speed of adaptation to different symbiotic species.

Table 1 Mycorrhization intensity of Aleppo pine seedlings, determine dat 0th (T0) and 45th (T45) day after treatment in the presence of organic (phytate) or inorganic (KH_2PO_4) phosphate.

	Treatments	T0	T45
Inorganic phosphate (KH_2PO_4)	*Sc*	69,50 ± 6,36	70,50 ± 2,12
	Sb	40,00 ± 4,24	31,00 ± 5,66
	Sm3530	73,00 ± 4,24	71,50 ± 4,95
	Hm	74,00 ± 2,83	73,00 ± 5,66
	Tt	59,50 ± 4,95	51,50 ± 3,54
Organic phosphate (phytate)	*Sc*	69,50 ± 6,36	74,50 ± 4,95
	Sb	40,00 ± 4,24	46,50 ± 3,54
	Sm3530	73,00 ± 4,24	79,00 ± 1,41
	Hm	74,00 ± 2,83	78,50 ± 4,95
	Tt	59,50 ± 4,95	64,50 ± 3,54

Sb : *Suilluscollinitus*(Fr.) O. Kuntze
Sc : *Suillusbellini* (Inz.) watl
Sm3530 : *Suillusmediterraneensis*(Jaquet. & Blum) Redeuilh isolat 3530
Hm : *Hebelomamesophaeum*(Pers.) Quélet
Tt : *Tricholomaterreum*(Sch. : Fr.) Kummer

Table 2 Some mineral Contents (mg/g DM) of the mycorrhized and non-mycorrhized Aleppo pine seedlings shoots, after 45 days supply of organic (phytate) or inorganic (KH2PO4) phosphate.

	Treatments	Na^+	K^+	Ca^{2+}	Mg^{2+}
Inorganic phosphate (KH_2PO_4)	*Control*	1,78 b	6,97 b	1,21 a	3,58 b
	Sc	2,23 a	7,88 a	1,34 a	3,83 a
	Sb	1,78 b	7,23 ab	1,21 a	3,66 b
	Sm3530	2,00 ab	7,42 ab	1,30 a	3,82 a
	Hm	2,18 a	7,75 a	1,34 a	3,86 a
	Tt	1,89 ab	7,49 ab	1,25 a	3,75 a
Organic phosphate (phytate)	*Control*	0,96 b	5,75 b	1,07 b	2,90 b
	Sc	2,24 a	7,68 a	1,30 a	3,83 a
	Sb	1,84 a	7,23 a	1,16 ab	3,64 a
	Sm3530	2,01 a	7,42 a	1,25 ab	3,79 a
	Hm	2,23 a	7,55 a	1,30 a	3,83 a
	Tt	1,84 a	7,36 a	1,16 ab	3,73 a

The results followed by different letters for each phosphate treatment differ significantly at 5%.

3530 of *suillusmediterraneensis*s owed significantly improved nitrogen nutrition.

The results of the evolution of phosphorus levels as a function of time and the nature of Psource show higher rates of phosphorus in mycorrhized plants compared to control plants (Table 4).

In the case of the mineral P nutrition, different fungal species have resulted in practically equivalent improvements in absorption and accumulation of P in Aleppo pine plants. In contrast, the use of phytate causes a significant decrease in the levels of phosphorus in the control plants. After 45 days of culture, the phosphorus content in these plants are reduced by 20% compared to initial contents. Mycorrhized plants were able to maintain their phosphorus levels at

Table 3 Total nitrogen Contents (mg/g DM) in mycorrhized and non-mycorrhized Aleppo pine seedlings shoots, determinedat 0th, 15th, 30th and 45th day after supply of organic (phytate) or inorganic (KH_2PO_4) phosphate.

NTK	Inorganic phosphate				Organic phosphate			
Treatments	T0	T15	T30	T45	T0	T15	T30	T45
Control	3,16 bc	3,09 b	3,21 bc	3,22 d	3,16 bc	3,14 b	3,09 d	3,05 d
Sc	3,46 a	3,28 ab	3,44 a	3,51 b	3,46 a	3,36 a	3,42 b	3,46 ab
Sb	3,14 bc	3,12 b	3,11 c	3,19 d	3,14 bc	3,16 b	3,21 cd	3,23 cd
Sm3530	3,26 b	3,21 ab	3,29 b	3,39 c	3,26 b	3,30 ab	3,33 bc	3,37 bc
Hm	3,50 a	3,35 a	3,50 a	3,67 a	3,50 a	3,42 a	3,58 a	3,60 a
Tt	3,21 b	3,16 b	3,23 bc	3,29 cd	3,21 b	3,19 b	3,23 cd	3,29 bc

The results followed by different letters differ significantly at 5%.

Table 4 Phosphorus contents (mg/g DM) in mycorrhized and non-mycorrhized Aleppo pine seedlings shoots, determinedat 0th, 15th, 30th and 45th day after supply of organic (phytate) or inorganic (KH_2PO_4) phosphate.

P	Inorganic phosphate				Organic phosphate			
Treatments	T0	T15	T30	T45	T0	T15	T30	T45
Control	0,33 d	0,30 d	0,35 c	0,39 b	0,33 d	0,31 d	0,31 c	0,26 d
Sc	0,38 a	0,36 a	0,41 a	0,42 a	0,38 a	0,36 ab	0,37 ab	0,39 ab
Sb	0,35 c	0,31 c	0,35 c	0,38 b	0,35 c	0,35 bc	0,35 b	0,36 c
Sm3530	0,37 bc	0,36 ab	0,39 b	0,40 ab	0,37 bc	0,34 c	0,35 b	0,35 c
Hm	0,38 ab	0,36 a	0,39 b	0,41 a	0,38 ab	0,37 a	0,38 a	0,39 a
Tt	0,36 c	0,36 ab	0,38 b	0,40 b	0,36 c	0,35 bc	0,35 b	0,37 bc

The results followed by different letters differ significantly at 5%.

Total nitrogen contents determined in the aerial parts of plants indicate that ectomycorrhizas improve nitrogen nutrition in the presence of two P forms (Table 3). This nutrition is influenced most at least importantly according to symbiotic species. Monitoring levels of total nitrogen for 45 days of treatment in the presence of both forms of P shows a significant improvement in mycorrhizal plants by contribution to the control plants.

In mycorrhized plants, it seems that the uptake and accumulation of nitrogen in the aerial parts of Aleppo pine was independent of the nature of the P source. Conversely in control plants, in the presence of phytate, the kinetics of uptake and accumulation of nitrogen decreased. At the beginning of treatment, only *Hebelomamesophaeum* and *Suilluscollinitus* have significantly improved the levels of total nitrogen.

In the presence of phytate and after 45 days of treatment all symbiotic species have significantly improved nitrogen nutrition with the exception of the *Suillusbellini* species. oppositly, in the presence of KH_2PO_4, only the symbiotic species *Hebelomamesophaeum, Suilluscollinitus* and the isolate

comparable valuesas those registered in the case of inorganic P source. Indeed, comparison of the phosphorus content of mycorrhized plants can be observed practically no significant differences depending on the nature of the used P.

The acid phosphatase activity was measured in the presence of two types of substrates (KH_2PO_4 and phytates) in different cell fractions (cytoplasmic and membrane-bound). The evolution of this activity as a function of time wasalmost identical for the different fractions but differ depending on the type of substrate (Fig. 2). The results show that the inorganic P induces virtually no stimulation of this activity. Mycorrhized plants have higher activities compared to non-mycorrhized plants;differences exceeding 70% were registered. The mycorrhization by *Hebelomamesophaeum*, *Suilluscollinitus* and the isolate 3530 of *Suillusmediterraneensis* has led to the most important phosphatase activities. In contrast to the inorganic P, the phytate induced significant stimulations of phosphatase activities resulting in continued increases as a function of time. These improvements of over 200% indicate a net stimulation. The ECM fungi *Hebelomamesophaeum*, *Suilluscollinitus* and the isolate 3530 of *Suillusmediterraneensis* have demonstrated the best performing.

DISCUSSION

The mycorrhization of Aleppo pine seedlings by autochthonous fungi of the Haouz region has led to an improvement in their growth for both Psources. The improvement of plant growth by ECM symbiosis as indicated by significant increases in shoot and root biomass has been widely reviewed (Marschner, 2012; Jouranda *et al*, 2014).Stimulation of dry matter production was much greater in the presence of organic P, the same finding was reported by several authors (Lunt and Hedger, 2003; Baxter and Dighton, 2005). No additional improvement in biomass production has been registered after application of KH_2PO_4. This result would probably be the result of favorable and non-limiting culture conditions before treatment with P sources.

Ekblad *et al* (1995) reported that mycorrhized *Pinussylvestris* seedlings by the ECM fungus *Paxillusinvolutus*, leads to 7 times greater improvements in growth in cases where the availability of P is limiting for growth. The best productions of aerial dry matter are observed with *Hebelomamesophaeum*, *Suilluscollinitus*, the isolate Sm3530 of *Suillusmediterraneensis* and *Tricholomaterreum* species. The ECM fungi *Hebeloma Mesophaeum*, *Suilluscollinitus* and the isolate Sm3530 of *Suillusmediterraneensis* have also promoted root dry biomass production. These differences in the induction of biomass as a function of the mycorrhizal species can be explained by the high carbon costs of mycorrhization establishment and development (Lindahl *et al*, 2002). Finlay and Söderström (1992) estimated that around 20% of the assimilated carbon from the plant is translocated to the fungal symbiont in ECM associations Bücking and Heyser (2001) added that during the ECM symbiosis, a bidirectional transfer of carbohydrates and P occurs across the same interface

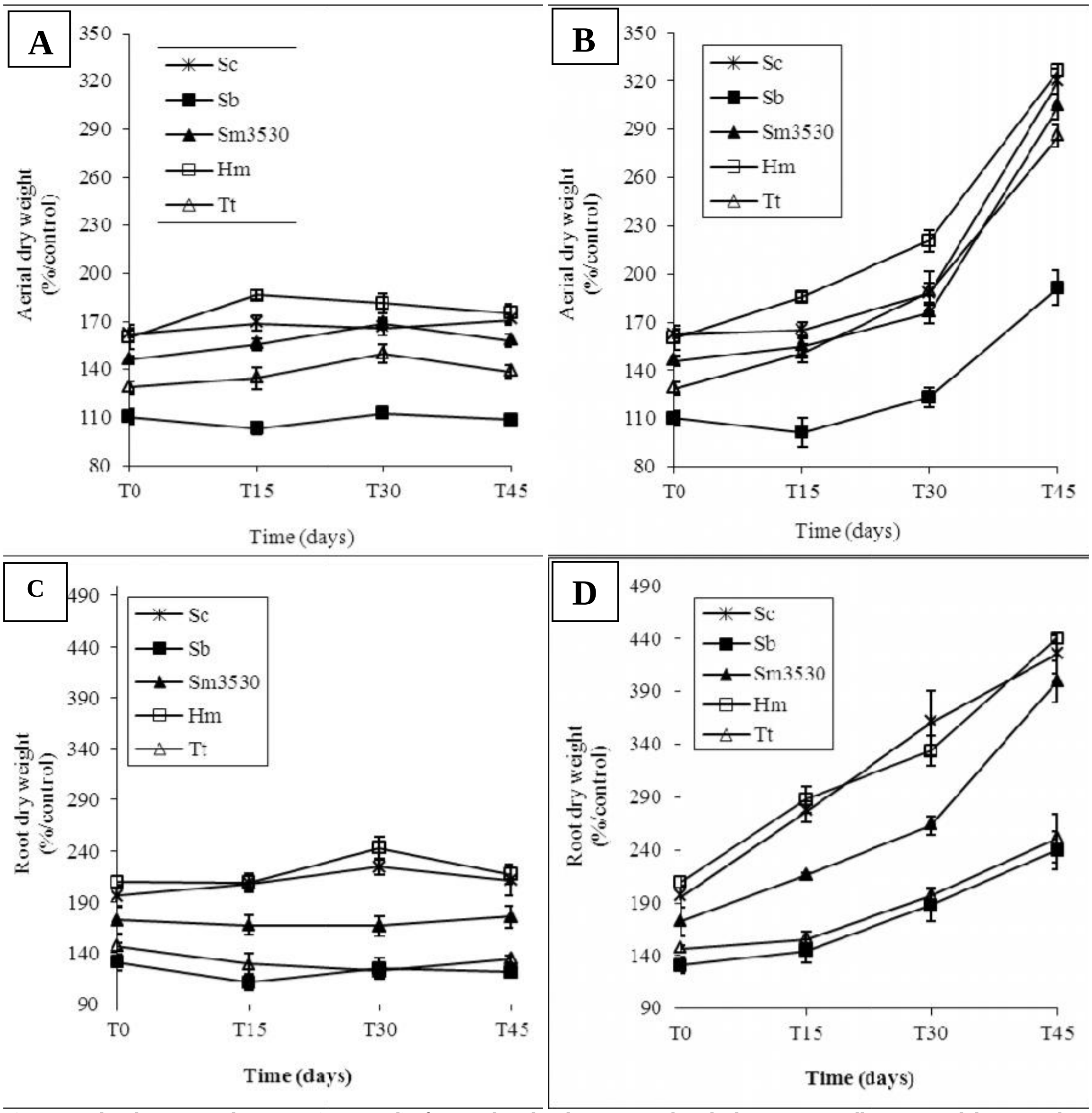

Fig. 1 Aerial and root parts dry matter (%/control) of mycorrhized and non-mycorrhized Aleppo pine seedlings, treated during 45 days with different P sources.

The aerial part **A:** in the presence of KH_2PO_4 or **B:** in the presence of phytate and the root part **C:** in the presence of KH_2PO_4 or **D:** in the presence of phytate.

The values represent the mean of 6 individual measures.

structure. In addition, the relatively short duration of treatment with P sources (45 days) would probably explain the absence of effect on the mycorrhizal intensity. Nevertheless, several studies reported a decrease in the degree of root colonization by ECM fungus to high levels of fertility particularly in nitrogen and phosphorus (Conjeaud, 1996; BoukcimandMousain, 2001; Ingleby *et al,* 2001; Brearley *et al,* 2007).

Generally, the mycorrhization of Aleppo pine induced a significant improvement of mineral nutrition including nitrogen, magnesium, potassium, sodium and calcium. Several studies have demonstrated the crucial role of ECM fungi in improving the acquisition of nutrients from the host plant (Baxter and Dighton, 2005; Turjaman *et al,* 2006). Thus, the significant increase in the assimilation of nitrogen was reported for conifer mycorrhizal seedlings (Finlay *et al,* 1988), As well as for sodium (Bücking and Heyser, 2000b), calcium (Blum *et al,* 2002) and potassium (Bücking and Heyser, 2000b). According to Garcia *et al* (2014) the potassium nutrition of mycorrhizal pine plants was significantly improved under potassium-limiting conditions.

Our results demonstrate an influence of the P form on the mineral nutrition of mycorrhizedand non-mycorrhized plants. In the presence of phytate, the mycorrhization has improved nutrition in nitrogen, magnesium, potassium, sodium and calcium. By contrast, in the presence of KH_2PO_4, calcium nutrition registers no difference between mycorrhized and non-mycorrhized plants. However, in some cases a dilution effect would explain the lower levels in mycorrhized plants. Despite higher absorbed amounts of a growth-limiting nutrient, its concentration on a fresh or dry weight basis may not change appreciably because the resulting stimulation in growth causes dilution (Oihabi, 1991; Syvertsen et Graham, 1999; Blum *et al,* 2002). This dilution effect reduces the value of the mineral analysis of leaf tissue as an indicator of nutrient status.

Mycorrhization induces a significant improvement in the P nutrition. This improvement depends very significantly on the symbiotic species and the used P form. Non-mycorrhized plants have proved unable to absorb organic P which had for consequences a reduction in their growth (Haran *et al,*2000; Baxter and Dighton, 2005).

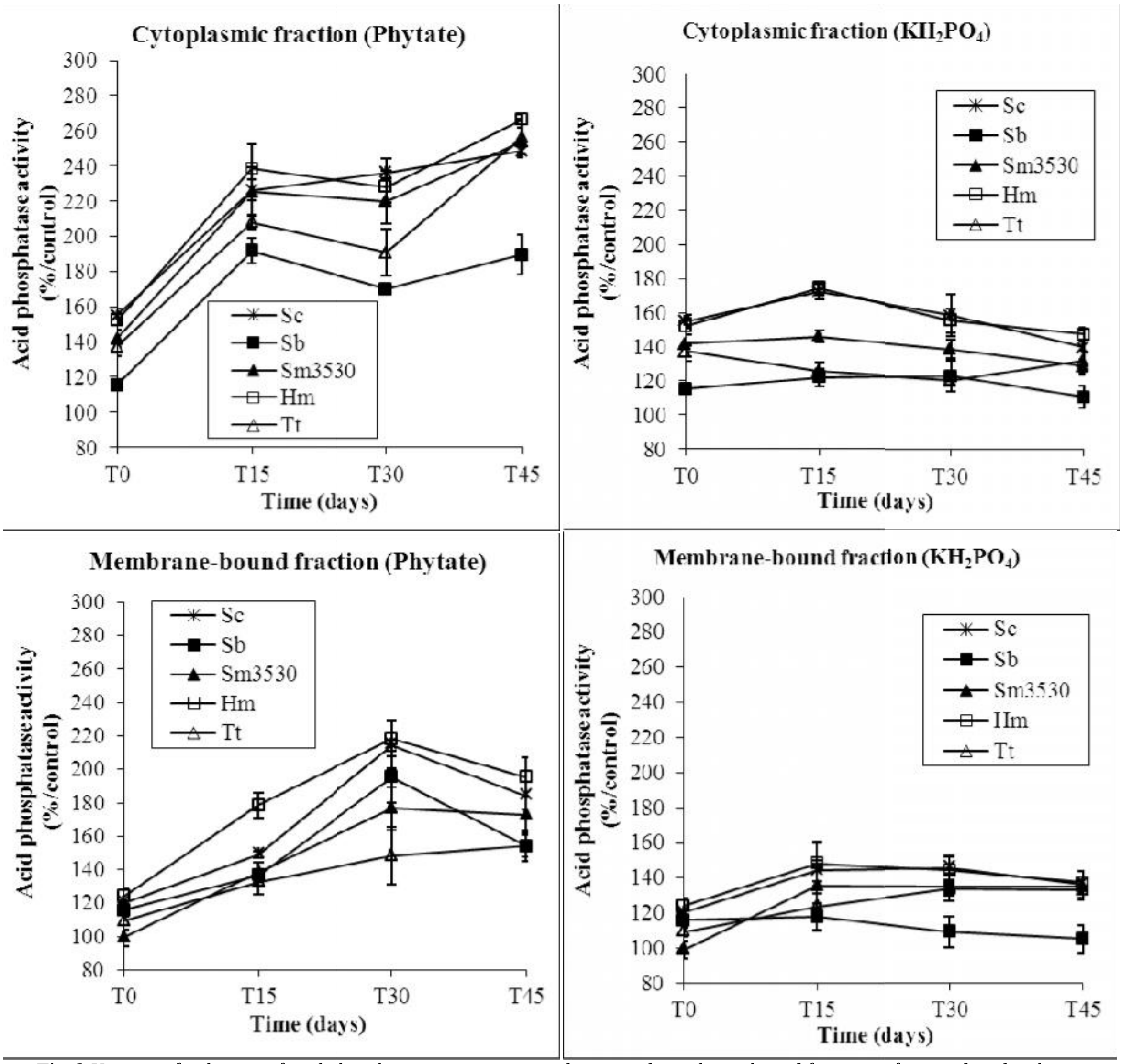

Fig. 2 Kinetics of induction of acid phosphatase activity in cytoplasmic and membrane-bound fractions of mycorrhized and non-mycorrhized Aleppo pine seedlings in the presence of phytate or KH2PO4 as the phosphate source.

The values represent the mean of 6 individual measures

This reduction of growth affects more the aerial parts; it has been concluded that root growth, and the resulting extension of the absorbing surface, occurs at the expense of further shoot growth and is an adaptive mechanism of plants subjected to P limitation (Bücking and Heyser, 2001).Different ECM species tested showed abilities to use organic P and improve P nutrition of host plants. This ability well known for mycorrhizal fungi (Lunt and Hedger, 2003; Read et Perez-Moreno 2003; Baxter and Dighton, 2005; Courty *et al,* 2005; Lambers *et al,* 2009) Allows to improve the plants growth in environments with low availability of inorganic nutrients (Lindahl *et al,* 2002; Alguacil, 2003).

The mechanisms of acquisition and transfer of organic P of the mycorrhizal fungus to the host plant pass through plant-fungi interactions. It has been suggested that P transfer resulting from mineralization of organic forms to the host plant is regulated by the intracellular P concentration in the hyphae of the Hartig net (Bücking and Heyser, 2000a). Cairney and Smith (1992) explain that the P demand of the host plant regulates P uptake by a mycorrhizal fungus. Regulation by the host would ensure that the mycorrhizal root absorbs P with the greatest efficiency when the plant is under P limitation (Bücking and Heyser, 2001; Casarin *et al,* 2004). Plants grown under P limiting conditions showed several physiological changes that reflect the metabolic systems activity, induced by P starvation, suspected of playing a crucial role in maintaining the supply of plant nutrients (Duff *et al,* 1994). Previous works suggest that P starvation increases the synthesis of a P-carrier complex that is postulated to be involved in the P-uptake process (Furihata *et al,* 1992; Shimogawara and Usada, 1995). In addition to increasing their capacity for P uptake, roots may alter chemical equilibria in the rhizosphere and access P-containing pools not readily available to the plant (Cumming, 1996).

Mycorrhization of Aleppo pine seedlings is also accompanied by improvements in acid phosphatase activity. This activity depends on the ECM species involved (Eaton and Ayres, 2002; Alvarez *et al,* 2003; Baxter and Dighton, 2005) and the availability of P (Pasqualini *et al,* 1992; Cumming, 1993). The culture of plants in the presence of organic P (phytate) is reflected by a very significant increase of acid phosphatase activity in cytoplasmic and membrane-bound fractions. This demonstrates that plants respond to phosphorus limitation by increased exploitation of phosphorus-containing organic sources by ectomycorrhiza (Bernard *et al,* 2002; Hagerberg *et al,* 2003). This adaptation to phosphorus limiting conditions passes through a stimulation of phosphatase activities (Alvarez *et al,* 2006; Courty *et al,* 2006; Hrynkiewicz *et al,* 2009). In the presence of KH_2PO_4, phosphatases activities of mycorrhizal plants remained comparable to those of control plants. This suggests that at sufficient and supraoptimal P supply, a mycorrhizal infection has no positive effect on P absorption (Amijee *et al,* 1993). The high concentrations of orthophosphate repress the phosphatases synthesis by mycorrhizal fungi (Pérez-de-Mora *et al,* 2013). Kimmo *et al* (1994) also demonstrated a negative correlation between acid phosphatase activity and P concentration in Scots pine. The primordial advantage of mycorrhizal fungi is their secretion of phosphatase enzymes to mineralize the phosphorus-containing organic sources (Talbot *et al,* 2008; Pritsch and Garbaye, 2011). Similarly, Tibbett and Sanders (2002) reported the ability of *Hebelomasyrjense* to improve P nutrition of the plant through access to limited P organic resources. The performance of ECM species is closely related to the level of production and activity of these enzymes (Sinsabaugh and Moorhead, 1994). These activities vary depending on the ECM species involved. *Hebelomamesophaeum*, *Suilluscollinitus*, the isolate 3530 of *Suillusmediterraneensis* and *Tricholomaterreum* species were more effective in inducing acid phosphatase activities. In addition, our results show a positive correlation between high levels of phosphatase activity produced by mycorrhized plants and the improvements of growth and Pnutrition. These results confirm those of many authors reported in other models (Cumming, 1993; Mousain *et al,*1997; Lunt and Hedger, 2003).

CONCLUSION

Among the five studied autochthonous ECM species, four were particularly effective in improving the growth and P nutrition of Aleppo pine plants. Indeed, *Hebelomamesophaeum*, *Suilluscollinitus*, the isolate 3530 of *Suillusmediterraneensis* and *Tricholomaterreum* species led to the highest rates of mycorrhizal colonization, biomass production, improvement of the ionic status and acid phosphatase activities. In the presence of phytate, a positive correlation was observed between the high level of phosphatase activity produced by ectomycorrhized plants and phosphorus uptake. These results are of great interest, especially as these mycorrhizal fungi are not only a stimulatory effect of growth and mineral nutrition, but can also improve water status and also play roles as biological control. These studies must be deepened to provide additional elements regarding the understanding of induction and regulation mechanisms of phosphatase activity with a view to exploit the biological performance of these mycorrhizal fungi.

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