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Rock phosphate and arbuscular mycorrhiza effects on growth and mineral nutrition of *Acacia gummifera* Wild.

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ABSTRACT: The influence of arbuscular mycorrhizal fungi (AMF) and rock phosphate (RP) was studied on *Acacia gummifera*, an endemic and Moroccan spontaneous species that is experiencing a regression. They are also a source of firewood, charcoal and precious air fodder in the dry season. The response of *Acacia gummifera* to a mixture of two species of arbuscular mycorrhizal fungi (*Glomus intrardices* and *Glomus mossae*) and two levels of rock phosphate (9 and 37 % of P equivalent of 0,25 and 0,5g P/kg of soil) was evaluated under greenhouse conditions.
The arbuscular mycorrhizal root colonization varied from 10 % to 25 % when rock phosphate applications increased. Acacia seedlings grew poorly without arbuscular mycorrhizal fungi and without rock phosphate applications. However, AMF plants with RP applications achieved better results in terms of P and N concentration in shoot and root. In contrast, there were not additive effects of inoculation and phosphate application on total biomass. However, inoculation of acacias took up more P and N at 0.25 g P kg-1 of soil and above. These results suggest that AMF are able to absorb P from soil and rock phosphate for a better mineral nutrition of *Acacia gummifera*.

KEYWORDS: Rock phosphate, arbuscular mycorrhizal fungi and *Acacia gummifera.*

1 INTRODUCTION

Acacia is an economically and ecologically important species. They are indeed anti-erosion agents because they help to fix the soils with their dense and deep root system. Moreover, by their symbiotic associations, they promote the improvement of these soils in nitrogen [1]. In traditional medicine, acacias are widely used as antitussive and antirheumatic or against eye diseases, diarrhea, jaundice, lung diseases [2]. Furthermore, *Acacia gummifera* is the only endemic and spontaneous Moroccan species, which is experiencing a regression [3]. In the past, A.gummifera occupied all the plain Haouz, Rehamna, Tadla, Chaouia until the plain Doukkala. Gradually following the extension and intensification of crops and human activities, this species is currently only seen in the peripheral regions of Haouz to the Tadla hinge to the east and in the area of argan [3]. Today it is found among the genetic resources that are threatened with extinction in the long term [4].

West African soils are structurally poor in phosphorus [5]. This low availability of phosphorus limits considerably the plant growth and its productivity in many ecosystems. Poor availability of P is due to the presence of Ca in neutral to alkaline pH and Fe and Al in acidic soil, which leads to the fixation of P and thus unavailable to the plant [6]. Arbuscular mycorrhizal fungi (AM) are known to play an important role in phosphorous (P) supply to plants in a sustainable manner in P deficient soils. It is well known that natural mycorrhizal associations are more common in soils poor in minerals. Their role in the mineral nutrition of the host plant is essential especially when the elements involved are not very mobile in the soil such as phosphorus [7]. AM-

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fungi increase mineralization of organic phosphate and increase phosphate availability. They may also solubilize some unavailable form of mineral phosphate like natural phosphate to available form increasing its availability in favour of plants. However, when phosphorus increases in the soil and becomes directly available to the plant, the intensity of root colonization by AM is reduced [8].

The aim of this study is to investigate the simultaneous effects of phosphorus fertilization and arbuscular mycorrhizal fungi on growth and nutrition of P and N *Acacia gummifera* seedlings

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The seeds of *Acacia gummifera*, from the commune of Mechrâa ben Abbou in the region of Settat-Marrakech, were provided by the regional station of forest seed of Marrakech. Seeds are dried and scarified through treatment with concentrated sulfuric acid for one hour, followed by several washes with sterile distilled water. Pre-germinated seeds were cultured in sterile peat. Watering was done daily during two months for multiplication of plants.

2.2 AMF INOCULUM

A mixture *Glomus intrardices* and *Glomus mossae* was used in our experiment. The both species mycorrhizal inocula were prepared through the trap culture using maize (Zea mays L) as host plant The uninoculated plants were supplied with filtered washing to provide the associated microorganisms other than mycorrhizal propagules. The control plants were kept without AMF for preserving the naturally-occurring microbial association and used for control treatments. The trap culture was conducted in the greenhouse of the Moulay Ismail University located, under natural daylight of 14 hours, a daily average temperature of 26-22°C and relative humidity of 60-70%.

After 12 weeks of culture, the potential of inoculants produced was measured by evaluation of spore density and root colonization based on Gerdemann and Nicolson [9].

2.3 FERTILIZATION WITH ROCK PHOSPHATE AND INOCULATION OF ACACIAS

Acacia plants were transplanted to pots containing 1 Kg of sterile soil, previously autoclaved at to remove the native microflora. It was the composition: 18% coarse silt, 28% coarse sand and 12%, fine sand, 2,95‰ N, 0,017% P, 0,003% K, 0,027% Ca, 0,00052% Na, pH (H20) 7,86. Rock phosphate is provided by the OCP Khouribga, which has the composition in the following table was added to two different concentrations (0.25 and 0,5 g P.kg-1 of soil).

Table 1. Composition of the rock phosphate

	O2	F	Na	Mg	Al	P	S	Sn	Ca
%	56	2,42	1,81	1,94	2,03	9,73	0,77	0,12	16,35

In mycorrhizal treatments, 2 g of AM inoculants (mixture of colonized root and sand containing spore and mycelium) were added to pots at the time of transplanting. In non mycrrhizal treatments, the inoculants was a mixture of non-colonized root and sand. Pots were irrigated every two days and maintained for three months in greenhouse conditions

2.4 MYCORRHIZAL COLONIZATION

Root samples were prepared for analysis by boiling in 10% KOH and staining with trypan blue according to technique of Phillips & Hayman [10]. Root fragments were mounted on glass slides and examined under a light microscope. Photographs of mycorrhizal structures were taken by a digital camera. Mycorhizal colonization was estimated according to the method described by Trouvelot et al [11].

2.5 EVALUATION OF BIOMASS

After three months of growth, the shoots and roots of acacia plants were collected, washed in distilled water, and allowed to dry in forced air circulation oven at 60°C, in order to obtain shoots dry matter (SDM) and roots dry matter (RDM)

2.6 DETERMINATION OF NITROGEN AND P CONCENTRATION

Dried shoot and root samples were ground to pass a 0.5-mm screen, mixed thoroughly. The ground material was mixed thoroughly, and samples of 1.0 g were ashed for five hours at 550°C in a muffle furnace, and then the ash was dissolved in 2N HCl for determination of the concentration of P. Phosphorus was determined according to the yellow phosphorus vanado-molybdate complex method by using spectrophotometer [12]. Nitrogen concentration in shoots was determined by using the micro-Kjeldhl method. Mineral contents were calculated by multiplying of mineral concentration by corresponding dry weight of shoots.

2.7 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design consisted of completely randomized blocks with 4 replicate. Data were statistically analyzed using analysis of variance procedures, and differences among treatments were compared using probabilities of significance and least significant difference (LSD) values ($P<0.05$).

3 RESULTS AND DISCUSSION

3.1 PERCENTAGE OF INFECTION

All inoculated and randomly sampled plants revealed the presence of at least one apex mycorrhizal. No mycorrhiza contaminant was observed on the roots of control plants (Figure 1).

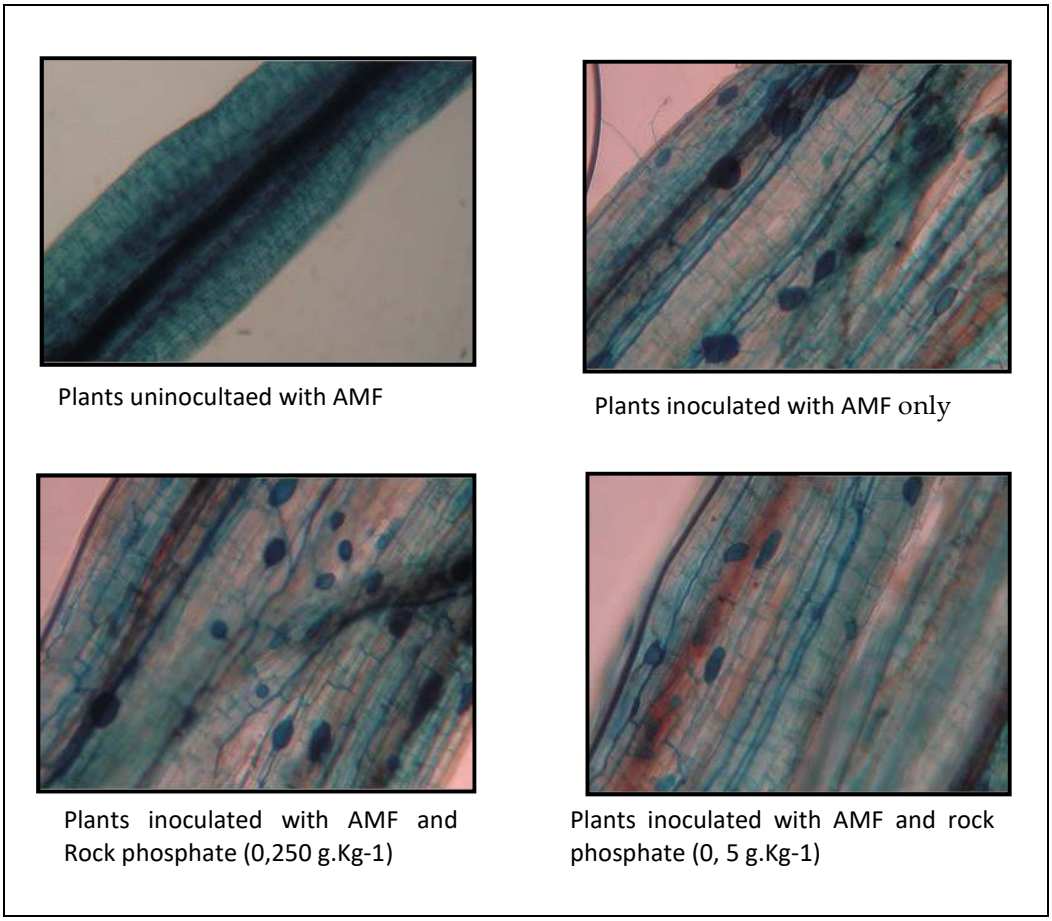

Fig. 1. Microscopic observation magnification (250 ×) of the mycorrhizal colonization of Acacia gummifera

Counts of mycorrhizal apex showed a significant improvement in the degree of mycorrhizal roots consecutively to a moderate intake of phosphorus (0.25 g P. kg-1 of soil) relative to unfertilized plants P (Figure 2).

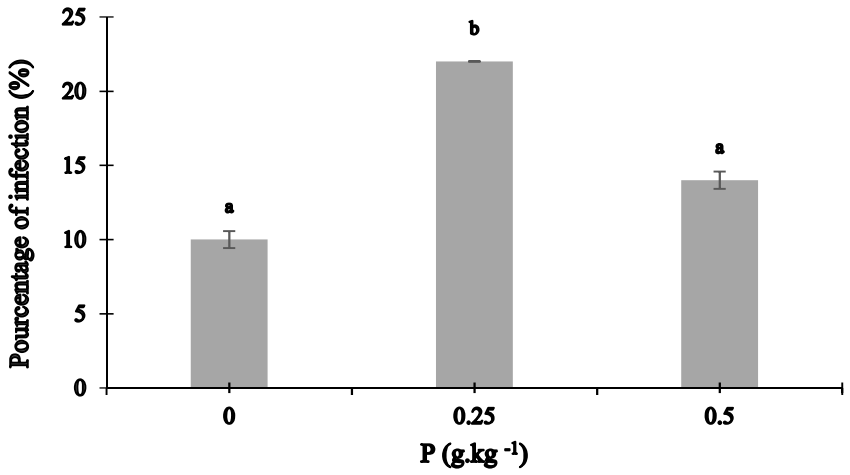

Fig. 2. Effect of P fertilization on the degree of mycorrhizal roots of Acacia gummifera

In semi-arid conditions, it is well known that AM fungal inoculum potential is very low and it was already shown that AM inoculation of the plant is very effective in establishing plants on disturbed soils [13, 14]. However A high P concentration (0.5 g P. kg-1 of soil) did not affect significantly the percentage of mycorrhizal apex compared to the unsupplemented medium P.

3.2 BIOMASS OF THE SHOOT AND ROOT OF ACACIA GUMMIFERA

The AM have different strategies to colonize the roots that help in reducing the need for fertilizer plants [15] and improve both the increasing biomass of roots and aerial parts of the plants [16, 17]. This is consistent with our results that indicate that the biomass of mycorrhizal plants is significantly higher than those non mycorrhizal. Furthermore, total biomass of a non-inoculated acacia increase with the dose of PN compared to inoculated acacias (Table 2).

Table 2. Effect of phosphate fertilization on dry matter production of shoot and root of Acacia gummifera

	Shoot dry weight ($g.plant^{-1}$)		Root dry weight ($g.plant^{-1}$)	
P (g. kg-1 of soil)	**Inoculated**	**Non inoculated**	**Inoculated**	**Non inoculated**
0	**2,30±0,57$^{(a)}$**	**2,50±0,5$^{(b)}$**	**2,00±0,1$^{(a)}$**	**1,93±0,11$^{(b)}$**
0,25	**5±0,28$^{(a)}$**	**4,66±0,57$^{(b)}$**	**3,33±0,57$^{(a)}$**	**2,16±0,28$^{(b)}$**
0,5	**5,30±0,57$^{(b)}$**	**5,80±0,25$^{(a)}$**	**3,50±0,57$^{(b)}$**	**3,56±0,4$^{(a)}$**

Biomasses followed by the same letter in the same row or column (letters in brackets) are not significantly different (Duncan's test, $p<0.05$).

This suggests that the mycorrhizal compensates the growth deficit induced by the absence of phosphorus. Our results are also consistent with those of Ekblad et al [18], which showed that a low intake of P stimulate the production of biomass plants of Pinus sylvestris inoculated without changing the biomass of the aerial parts to the roots. The lack of effect of P fertilization on the biomass of plants inoculated was also observed by other authors. [19].

3.3 CONTENT OF NITROGEN AND TOTAL PHOSPHORUS IN ACACIA GUMMIFERA

The percentage of mycorrhizal infection is not affected by the higher doses of P. Although RP and AM inoculation produced a higher growth and nutrient uptake that the non AM inoculation [20]. As expected the nitrogen fertilization was the same for all plants. Inoculation of Acacia by endomychorize arbuscular improves significantly ($p < 0.05$) total nitrogen contents in their roots in the presence of a phosphorus external intake (table 3).

From the high dose of phosphorus (0, 25 g P.kg-1 of soil), there was a significant increase ($p < 0.05$) in total nitrogen contents in the shoot and root of plants following inoculation. The effect of nitrogen nutrition on mycorrhization of plants inoculated, in the presence or absence of external P was demonstrated in maritime pine but the direction (positive or negative) and the intensity of this effect will vary depending on the fungal species inoculated and the contribution or not of P [21].

Furthermore, glomus promotes accumulation of nitrogen in plant roots when P fertilization is high (Table 3). This accumulation of nitrogen in the root can result from translocation to either the underground parts or an absence of translocation to the above ground parts or a remobilization of N from the aerial parts to the roots [22, 21].

Table 3. Effect of phosphate fertilizer on the nitrogen in A. gummifera

	Nitrogen in the shoot (%)		Nitrogen in the root (%)	
P (g. kg-1 of soil)	**Inoculated**	**Non inoculated**	**Inoculated**	**Non inoculated**
0	**1,22±0,1$^{(a)}$**	**1,1±0,1$^{(a)}$**	**1,12± 0,03$^{(a)}$**	**1,3±0,03$^{(a)}$**
0,25	**1,68±0,01$^{(b)}$**	**1,12±0,04$^{(a)}$**	**1,57±0,02$^{(b)}$**	**1,37±0,03$^{(a)}$**
0,5	**1,72±0,02 $^{(b)}$**	**1,56±0,05$^{(a)}$**	**2,02±0,06$^{(c)}$**	**1,44±0,04$^{(a)}$**

The nitrogen percent in shoot and root followed by the same letter in the same row or column (letters in brackets) are not significantly different (Duncan's test, p=0,05).

On the other hand, the concentration of P in shoots and roots of the mycorrhizal and the non mycorrhizal plants increased significantly with the addition of phosphate to the soil (table 4).

Table 4. Effect of phosphate fertilizer on the total phosphorus content in A. gummifera

	Phosphorus in the shoot (%)		Phosphorus in the root (%)	
P (g. kg-1 of soil)	**Inoculated**	**Non inoculated**	**Inoculated**	**Non inoculated**
0	**0,3±0,03**	**0,25±0,025**	**0,35±0,11**	**0,2±0,03**
0,25	**0,57±0,11**	**0,35±0,06**	**0,57±0,1**	**0,36±0,06**
0,5	**0,4±0,05**	**0,37±0,14**	**1,06±0,028**	**0,61±0,03**

However, the mycorrhizal plants had higher contents of P in both roots and shoots than those in the non mycorrhizal plants. These results are in agreement with those found by Abdel Fattah et al, 2014 and Zhang et al, 2015 [23, 24]. AM fungi associated with the addition of the rock phosphate give a significant increase in the concentration of phosphorus compared to the control but the application of high concentration of P, reduced the mycorrhizal growth. This result support the previous finding which indicated that are in accordance with [25, 23], which show that highest phosphorus uptake occurs in mycorrhizal plants with added low P than non–mycorrhizal as well as plants without added P. Mycorrhizal inoculation consistently accumulated more quantities of phosphorus in their root than shoot.

4 CONCLUSION

This study showed that AM inoculation associated with the rock phosphate influence significantly on the uptake of growth and nutrients by A. gummifera. Indeed, the experiment showed a significant improvement in growth of the mycorrhizal plants. The variation of the concentration of P indicated that there is no change of the biomass of the mycorrhizal plants acacias. However, mycorrhizal acacias absorb more effectively the phosphorus and nitrogen as non-mycorrhizal, so it is possible to speculate that the use of AM on the presence of the RP could improve the feeding value and production of *Acacia gummifera*

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REFERENCES

[1] K. Fikri Benbrahim , H. Berrada , N. El Ghachtouli, M. Ismaili. Les acacias: des plantes fixatrices d'azote prometteuses pour le développement durable des zones arides et semi-arides. I.J.I.A.S, vol 8 pp 46-58. 2014

[2] M. Hmaouchi. Les plantes médicinales et aromatiques marocaines. Fedala, 390p. 1999.

[3] A. Benabid. Flore et écosystèmes du Maroc, Evaluation et préservation de la biodiversité. Ibis Press, Paris 2000.

[4] A. Harfouche, A. Nedjahi, M. Ellatifi, H. Daly-Hassen. Les ressources génétiques forestières nord-africaines et leur conservation. Revue forestière française, vol 57 no 1, pp 15-32. 2005.

[5] T. Guissoua, A. Moustapha, B.S. Guinkob, R. Duponnois, C. Plenchette. Influence des phosphates naturels et des mycorhizes à vésicules et à arbuscules sur la croissance et la nutrition minérale de Zizyphus mauritiana Lam. dans un sol à pH alcalin. J. Annales des sciences forestières, vol 55, no 8, pp 925-931. 1998.

[6] C.P. Vance, C. Uhde-Stone, D.L. Allan. Phosphorus Acquisition and Use: Critical Adaptations by Plants for Securing a Nonrenewable Resource. J. New. Phytologist, vol 157, pp 423-447. 2003.

[7] P. Doubkováa, J.Sudaa, R Sudováa. The symbiosis with arbuscular mycorrhizal fungi contributes to plant tolerance to serpentine edaphic stress. J. Soil Biol Biochem , vol 44, pp 56-64. 2012.

[8] S. Dickson, S.E. Smith, F.A. Smith. Characterization of two arbuscular mycorrhizal fungi in symbiosis with Allium porum : colonization, plant growth and phosphate uptake. J. New Phytologist, vol 144, pp 163-172. 1999.

[9] J.W. Gerdemann, H.S. Nicolson. Spore of mycorrhizal endogen species extracted from soil by wet sieving and decanting. Transactions of the British mycological society, vol 46, pp 235-244. 1963.

[10] J.M. Phillips, D.S. Hayman. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. J.Trans. Br. Mycol. Soc. Vol 55, pp 159–161. 1970.

[11] A. Trouvelot, J. Kough, V. Gianinazzi-Pearson. Evaluation of VA infection levels in root systems. Research for estimation methods having a functional significance. In: V. Gianinazzi-Pearson and S. Gianinazzi (eds.) Physiological and Genetical Aspects of Mycorrhizae. INRA Press, Paris, France. pp 217–221. 1986.

[12] E.J.M. Temminghoff, V.J.C. Houba. Plant analysis procedures seconde edition. Klower academic publishers. ISBN 1-4020-2976-4. 2004.

[13] V. Estaun, R.. Save, C. Biel. AM inoculation as a biological tool to improve plant re-vegetation of a disturbed soil with Rosmarinus officinalisunder semi-arid conditions. J. Appl. Soil Ecol. Vol 6, pp 223–229. 1997.

[14] R. Duponnois, A. Colombet, V. Hien, J. Thioulouse. The mycorrhizal fungus Glomus intraradice and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of Acacia holosericea. J. Soil Biol Biochem , vol 37, pp 1460-1468. 2005.

[15] D.M. Weller. Biological control of soil pathogens in the terminal plant rhizosphere with bacteria. J. Phytopathoogy, vol 26, pp 379-407. 1988.

[16] B. Schippers. Exploitation of microbial Mechanisms to Promote plant health and plant growth. J. Phytoparasitica, vol 21 pp 275-279. 1993.

[17] B. Benmasaoud, L. Nassiri, J. Ibijbijen. Effects of Rhizobia and Mycorrhizae Inoculations on the Growth and Nodulation of Chamaecytisus proliferus. Int.J. A.S.N.R, vol 2 no 2, pp 28-35. 2015.

[18] A. Ekblad, H. Wallander, R. Carlsson, K. Huss-Danell. Fungal biomass in roots and mycelium extramatrical in relation to macronutrients and plant biomass of ectomycorrhizal Pinus sylvestris and Alnus incana. J. New Phytol, vol 131, pp 443-451. 1995.

[19] W. Khabou, B. Hajji, M. Zouari, H. Rigane. Arbuscular mycorrhizal fungi improve growth and mineral uptake of olive tree under gypsum substrate. J. Biol. Eng, vol 73, pp 290-296. 2014.

[20] M.A. Satter, M.M. Hanafi, M.M. Mahmud, H. Azizah.. Influence of arbuscular mycorrhizaandphosphaterockonuptake of major nutrients by Acacia mangium seedlings on degraded soil. J. Biol Fertil Soils, vol 42, 345-349. 2006.

[21] C. Conjeaud, P. Scheromm, D. Mousain. Effects of phosphorus and ectomycorrhiza maritime pine seedlings were (Pinus pinaster). J. New Phytol, vol 133 pp 345 -351. 1996.

[22] R. Améziane, M.A. Limami, G. Noctor, J.F. Morot Gaudry. Effect of nitrate concentration During growth is carbon partitioning and sink strength in chicory. J. Exp. Bot, vol 46 (Suppl.), pp 1423 -1428. 1995.

[23] G.M. Abdel-fattah, A.A. Asrar, S.M. Al-Amri and E.M. Abdel-salam. Influence of arbuscular mycorrhiza and phosphorus fertilization on the gas exchange, growth and phosphatase activity of soybean (Glycine max L.) plants. Photosynthetica. vol 52, no 4, pp 581-588. 2014.

[24] S. Zhang, L. Ma.F. Wang, , X. Zhang, Z. Li, S. Li, X. Jiang. Can arbuscular mycorrhiza and fertiliser management reduce phosphorus runoff from paddy fiels ? J. Env. Sci, vol 33, pp 211-218. 2015.

[25] A. Tanwar, A. Aggarwal, N. Kadian, A. Gupta. Arbuscular mycorrhizal inoculation and super phosphate application influence plant growth and yield of Capsicum annuum. J. of Soil Science and Plant Nutrition, vol 13 no. 1, pp 55-66. 2013.