Effects of arbuscular mycorrhizal fungi on growth and physiology of date palm seedling under phosphorus deficit

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ABSTRACT

Arbuscular mycorrhizal fungi are endophytic fungi that enhance plant growth and biomass production in arid and sub arid areas where phosphorus is massively under insoluble forms in soil. Earlier works established that the enhanced biomass results from improved nutrient status. In particular, Arbuscular mycorrhizal fungi mediate phosphorous supply. The objective of this study is to evaluate the role of arbuscular mycorrhizal fungi (Glomus manihotis) in solubilizing rock phosphate enhancing thereby growth of date palm (*Phoenix dactylifera* L.) seedlings under phosphorus deficit. Young germinations of date palm were grown on inert substrate containing 5g of rock phosphate and inoculated or not with Glomus manihotis. Cultures were irrigated with Hoagland solution containing or not KH_2PO_4 as phosphorus source. After two months, growth, physiological and biochemical parameters were assessed in mycorrhizal (AM-plants) and non mycorrhizal plants (Non-AM). Obtained results showed that mycorrhizal colonization induces an increase in growth parameters (fresh weight and plant height) and biomass production (shoot and roots dry weights) regardless phosphorus treatment. In the presence of rock phosphate as the only source of phosphorus, mycorrhizal date palm seedlings showed increased acid phosphatase, activity as well as higher level of soluble sugar, higher relative water content and higher stomatal conductance compared to non-inoculated plants. However, the guaiacol peroxidase and polyphenoloxidase activities were highest in non-inoculated seedlings under phosphorus deficit.

Keywords: date palm seedlings, arbuscular mycorrhizal fungi, Phosphorus deficiency, rock phosphate solubilization.

1. INTRODUCTION

Plants of arid and semi-arid areas including date palm are often faced with the combined effect of several biotic and abiotic stresses. In addition to the lack of water, which is the main limiting factor of growth, soils are generally poor in essential nutrients such as phosphorus (Diem *et al.,* 1981; Mikola, 1987). Under such difficult environmental conditions, the quality of the root system and the efficiency of its association with the soil microorganisms may play an important role in plant development. In these extreme conditions, plants survival is likely due to the result of the symbiotic association between root and soil borne arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhizal symbiosis is known to benefit mineral nutrition and to provide improved water relations thereby enhancing host plant protection against the detrimental effects of environmental constraints such as salinity (Klironomos *et al.,* 2001; Giri *et al.,* 2003), water and nutrients deficiency

(Aqqua *et al.,* 2010; Faghire *et al.,* 2010 ; Baslam *et al.,* 2014) and resistance to pathogenic soil microorganisms (Garmendia *et al.,* 2004). The present work aims to evaluate the effect of arbuscular mycorrhizal fungi on growth and physiology of date palm seedlings under phosphorus deficit.

2. MATERIAL AND METHODS

Seeds of date Palm were disinfected with sodium hypochlorite (20%) for 20 minutes and germinated in sterile wet sand at 38°C in the dark. Two weeks later, germinating seeds were transplanted in pot containing 1kg of inert substrate containing or not 5 g of rock phosphate and inoculated or not with 5 g of inoculum of *Glomus manihotis* consisting of a soil mixture of spores and mycorrhizal roots fragments of barley (Meddich et al. 2000). Cultures were then irrigated with Hoagland solution containing or not KH_2PO_4 as phosphorus source. After four months date palm plantlets were harvested and growth parameters (shoot height and shoot fresh weight) as well as biomass production (shoot and roots dry weights) were measured. Plantlets water status was examined through the evaluation of the relative water content (RWC) and stomatal conductance. Measurements of the stomatal conductance were carried out during a sunny day on the leaf surface of a single leaf per plant using a diffusion porometer SC-1.

Biochemical analysis including enzymes essays were carried out on crude extract obtained from 0.1g of date palm leaves that were ground with 0.02g of polyvinylpolypirrolidine (PVPP) in a cold mortar and soaked with 2 ml of phosphate buffer 0.1 M (pH 7.0) containing 0.0168g $EDTANa_2$ and 0.1 g of polyvinylpolypirrolidine (PVPP) at 1%. The mixture was then centrifuged (15 000xg) for 15 min at 4 °C and the supernatant recovered was stored at 4 °C until analysis of the various enzymatic activities. Acid phosphatase activity was determined by measuring the amount of para-nitrophenol (pNP) released by using a UV-visible spectrophotometer at 405 nm (Araùjo *et al.,* 2008). Activity of guaiacol peroxidase (G-POX) was determined according to the method of Putter (1974) by following the increase of the absorbance caused by the appearance in the medium of oxidized guaiacol. Polyphenol oxidase as (PPO) activity was determined in a reaction mixture containing 200 µL of phosphate buffer (0.1 M), 500 µL of catechol (10 mM), 100 µL of enzyme extract and 250 mL of H_2O_2. After 3 min of incubation at ambient temperature, the absorbance was determined at 410 nm. Total soluble sugars were determined by the method of Yemm et Willis (1954). It is to take 50µL of plant extract, 450µL of phosphate buffer and 3 mL of anthrone reagent in clean glass tubes. The tubes are placed in a water bath at 100 ° C for 15 min. Reading the absorbance at 620 nm is performed. The values obtained are reported in the standard range.

Statistical analysis

The results were statistically analyzed using SPSS software. This analysis includes an ANOVA 2 followed by means comparison using LSD test at 5%.

RESULTS

3.1. Growth parameters

Plant growth parameters varied significantly depending on the phosphorus treatment and mycorrhizal inoculation (Table 1). Plan height and shoot and root fresh weights were greatly reduced in plantlets irrigated with nutrient solution without soluble phosphorus compared to those irrigated with complete nutrient solution regardless mycorrhizal inoculation. This reduction was more pronounced in non-inoculated than in mycorrhizal plants. In the absence of soluble P, plants cultivated on substrate added with rock phosphate showed higher plant growth parameters than those cultivated without any source of P. Moreover, in the presence of rock phosphate as the only source of P, mycorrhizal plants grow higher compared to the respective non-mycorrhizal plants and to the plants irrigated with nutrient solution without P. The lack of any source of P generates a significant reduction in biomass production in both mycorrhizal and non-mycorrhizal plants (Table 1). In mycorrhizal plants, the addition of rock phosphate allows a more relevant increase of root and shoots dry weights than in non-mycorrhizal plants. Non-inoculated plants and plants cultivated without any source of phosphorus produce less dry material (Table 1).

3.2. Physiological parameters

Values of relative water contents vary significantly regarding phosphorus treatments (Table 2). Water accumulated by the leaves of date palm seedlings remains increasingly important in the presence of phosphorus. Mycorrhizal plants showed the highest values of RWC regardless phosphorus status.

Stomatal conductance varies significantly with phosphorus treatment and mycorrhizal status (Table 2). Under phosphorus deficiency mycorrhizal plants recorded higher stomatal conductance value compared to non-inoculated plants (Table 2).

Activity of acid phosphatase was lower in plants treated with complete nutrient solution. The highest acid phosphatase activity was recorded in mycorrhizal plants with the rock phosphate (RP) as the only source of phosphorus. However, the guaiacol peroxidase and polyphenoloxidase activities were highest in non-inoculated seedlings regardless phosphorus treatments. The peroxidase and polyphenoloxidase activities were increased by P deficit regardless mycorrhizal status. Under phosphorus

deficit soluble sugar content was lower in leaves of non-inoculated plants than in mycorrhizal ones (Table 2).

DISCUSSION

This study investigated the influence of arbuscular mycorrhizae on the growth and physiology of date palm seedlings under phosphorus deficit. The results showed that plants growth and biomass production varied significantly with the applied phosphorus treatments. The highest values were recorded in AM-plant compared to NM-plant under all phosphorus treatments. The same results were observed by Bowen et Théodorou (1967) who showed an increase in dry material of *Pinus radiata* seedlings inoculated under phosphorus fertilization compared to non-inoculated seedlings. This increase was related to the role of arbuscular mycorrhizal fungi in enhancing uptake of $H_2PO_4^-$ (Gillespie and Pope, 1991).

On the other hand, values of the stomatal conductance vary differently according to mycorrhizal status and phosphorus treatments. Under phosphorus deficit stomatal conductance was highest in mycorrhizal seedlings. Similar results were reported by Augé et al (1986) showing low osmoticums in non-mycorrhizal plants under phosphorus deficiency. The enhanced stomatal conductance go along with increased levels of relative water content (Allen and Boosalis, 1983 ; Bildusas et al, 1986) as well as increased soluble sugar concentration (Suresh and Bagyaraj 1984).

Activity of acid phosphatase was lowest in plants treated with complete nutrient solution and highest in mycorrhizal plants with RP as the only source of P. This was probably due to the lack of soluble phosphorus in the soil and the availability of other forms of insoluble phosphorus (RP) which requires high activity of acid phosphatase for its solubilization (Mousain et Salsac 1986). The peroxidase and polyphenoloxidase activities were increased by P deficit regardless mycorrhizal status. Peroxidase and polyphenoloxidase activities were highest in non-inoculated seedlings. Similar result were reported by Avdiushko et al (1993) and Zheng et al (2005), suggesting the contribution of these enzymes in the catalysis of the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure.

CONCLUSION

Phosphorus deficiency is one of the major problems in palm grove ecosystems that affect plants growth and productivity. However, this investigation showed that arbuscular mycorrhizal colonization can improve date palm growth and biomass production under phosphorus deficit through, 1) enhancing plants water status, 2) increasing phosphorus availability by enhancing acid phosphatae activity that contributes to phosphorus solubility, and 3) the activation of enzymes catalyzing the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure.

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Tables

Table 1 : Effect of arbuscular mycorrhizal fungi on growth and biomass production in date palm seedlings under phosphorus deficit

P Treatment	Mycorrhizal status	% M	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Complete nutrient solution	NM	0	30,1bc	19,3b	15,5b	12,4ab	12,0bc
	AM	63.3b	35,8a	22,8a	17,7a	14,1a	14,5a
Nutrient solution without P + Rock P	NM	0	28,8bc	14,9cd	14,7bc	10,9cd	11cd
	AM	69a	31,9ab	15,6c	15,9b	11,8bc	12,4b
Nutrient solution without P	NM	0	26,0c	12,7d	12,7d	9,5d	9,6e
	AM	60c	28,4bc	13,3cd	13,7cd	10,1cd	10,1de

Table 2: Effect of arbuscular mycorrhizal fungi on physiology of date palm seedlings under phosphorus deficit

P treatments	Mycorrhizal status	Stomatal conductance (µmol/m2)	Relative water content (%)	Acid phosphatase activity (UE/ mg of protein)	Guaiacol Peroxidase activity (UE/ mg of protein)	Polyphenol activity (UE/ mg of protein)	Soluble sugar (µg/ mg of fresh material
Complete nutrient solution	NM	115,47e	64,44a	0,47e	77,63c	57,29f	62,30bc
	AM	161,27c	82,97ab	0,27d	36,81ab	37,72e	67,39ab
Nutrient solution without P + Rock P	NM	125,03de	55,62b	0,68c	102,93a	108,50b	58,62c
	AM	216,03b	60,98ab	2,79a	54,21bc	67,37d	71,15a
Nutrient solution without P	NM	136,00d	46,70b	0,41d	106,58a	168,90a	30,91e
	AM	233,67a	50,66b	1,85b	74,37ab	77,62c	45,22d