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ENHANCEMENT OF NUTRIENT UPTAKE IN PEACH ROOTSTOCK WITH ARBUSCULAR MYCORRHIZAL FUNGI AND PLANT-GROWTH PROMOTING RHIZO-BACTERIA INOCULATION IN NURSERY

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Abstract

Arbuscular mycorrhizal fungi (AMF) and plant-growth promoting rhizobacteria (PGPRB) contribute to plant nutrient uptake by increasing the availability of nutrients and the root adsorbing surface. The aim of this study was to determine the effect of the inoculation of two AMF (*Glomus fasiculatum* and *Gigaspora* sp.) and three PGPR (*Azospirillum+ Frateuria aurentia + Bacillus megaterium*) on development and nutrient uptake of *Prunus persica* plantlets grown in different culture substrates [sand (S1), coarse peat (S2) and a mixture of sand: coarse peat: organic matter (1:1:1, v:v:v) (S3)]. Our results demonstrate a significant positive increasing on the levels of shoot tissue N content (*p=0.05*) in response to AMF and PGPRB inoculation. The AMF inoculated plantlets grown in sandy substrate presented an increase of 12.5% and 38% of their root tissues levels of K and Mg respectively when compared to the control plantlets. In addition, leaf tissue total N, P and Mg content of the AMF inoculated plantlets were significantly higher than the non inoculated one in all substrates. Root tissue P and Ca levels increased significantly with PGPRB inoculation in S1 and S2 substrates. The Mg migration through plantlets tissues was performed by PGPRB inoculation in S1 and S2 simultaneously. In conclusion, our results confirm the suitability of the application of AMF and PGPRB to improve growth and nutrition of *Prunus persica* plantlets during the nursery phase. The absence of references about this microbial interaction in ‘Garnem’ allows us to propose for the first time this method for the improvement of sustainability of *Prunus* cropping systems.

Keywords*: AMF, PGPRB, mineral nutrition, Garnem*

Introduction

Peach *(Prunus persica* (L.) Batsch), an economically important plant in the semi arid area, is the third cultivated temperate fruit tree species in the world (Mamouni, 2006). The productivity and the sensorial and nutritional quality of peaches are determined through interactions between different factors such as rootstock and cultivar interactions, training system, etc. (Gullo et *al*., 2014). However, many biotic and abiotic stress factors affect plant growth and fruit quality (Pérez-Jiménez et *al*., 2014) which allowed interest to rootstocks selection. In fact, the hybrids of almond x peach are largely used as rootstocks for peach trees in the Mediterranean countries, because they are tolerant to lime induced chlorosis (Moreno et al., 1994). In Tunisia (Mediterranean country), peach rootstocks that are used because of their adaptation to climatic and soil conditions and for their survival after transplanting are: almond, and hybrids obtained from cross almond x peach. The cultivar ‘Garnem’ is one of the most used peach rootstock characterized with its vigor and iron chlorosis tolerance. However, Font i Forcada et al. (2012) demonstrated that ‘Garnem’ trees showed the highest tree mortality rate which may be essentially attributed to nutritional deficits.
In this research we are focusing on the enhancement of the mineral nutrition of ‘Garnem’ peach rootstocks with inoculation in nursery using Arbuscular Mycorrhizal Fungi (AMF) and

Plant Growth Promoting Rhizobacteria (PGPRB) on growth and mineral nutrition of young ‘Garnem ‘plantlets. Indeed, AMF and PGPRB contribute to plant nutrient uptake by increasing the availability of nutrients and the root adsorbing surface. In fact, these microbes can promote plant growth by regularing nutritional and hormonal balance, producing plant growth regulators, solubilizing nutrients and including resistance against plant pathogens (Nadeem et *al*., 2014). Inoculation of plant stock with selected bacterial or fungal strains has often demonstrated to improve its physiological quality and to ameliorate the survival and development of plants after planting (Rincón et *al*., 2006). AMF establish beneficial symbiosis with most plants and have gained a growing interest as agro-ecosystem service providers able to sustain productivity and quality (Pellegrino and Bedini, 2014) and the symbiotic association generated by fungi with plants roots (mycorrhizae) increases the root surface area, and therefore enables the plant to absorb water and nutrients more efficiently from large soil volume (Nadeem et al., 2014). PGPRB facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Ahemad and Kibert, 2014). Our aim was to determine the microbe inoculation efficacy on mineral uptake by plantlets under nursery conditions.

Materials and methods

Experiment was conducted in 2011 under nursery conditions in a commercial woody nursery in the North-Est of Tunisia (36°45’11’’N, 10°13’8’’E, 18 m altitude)

The experimental site is characterized with a semi-arid climate with a rainfall rate varying from 275 to 515 mm and mean air temperature varying from 6.8°C to 17.9°C.

An almond x peach hybrid [*Prunus amygdalus* Batsch x *Prunus persica* (L.) Batsch] cultivar ‘Garnem’ was considered in this study. All plantlets had been obtained from the *in vitro* culture. The experimental treatments consisted on commercial inoculums of two strains of AMF G*lomus fasiculatum*+ *Gigaspora* species (T1) and three strains of PGPRB *Azospirillum, Frateuria* and *Bacillus megaterium* (T2) or non inoculated=control (Tc).

Each treated young rootstock was transferred into 3 liter pots. Three different growing substrates were used (S1: sandy, S2: coarse peat and S3: (1:1:1) (v:v:v) (sand + peat + organic matter). The experiment was set as a completely randomized design with five replicates. All plantlets were placed under nursery conditions for four months and fertilized with a commercial controlled release fertilizer (Osmocote) (10 g/pot).

Four months after inoculation, whole plantlets were uprooted and cuted into roots, shoots and leaves for mineral analysis. At sampling, fine roots were rinsed, stained with Trypan bleu and observed using a microscope at 40x and 20x amplifications. 80 segments were considered for each treatment in each substrate. Three treatments [the control, non inoculated (Tc), AMF inoculation (T1) and PGPRB inoculation (T2)] were compared by the contrast method. The variation in nutrient content attributed to the effects of growing substrates and inoculums were assessed via the analysis of variance procedure using the SPSS program 19.0. When the *F* test was significant, means were separated by Duncan’s multiple range test $(P \leq 0.05)$.

Results and discussion

Based on statistical analysis of data (ANOVA) subjected to *F*-test, obtained results showed positive effects on nutrients uptake by plantlets in nursery conditions by AMF and PGPRB inoculations (table1).

Effect of the AMF inoculation

Shoots total nitrogen content was significantly improved with AMF inoculation and percentage increases were 1.39, 1.49 and 2.02% in S1, S2 and S3 respectively. In addition,

leaf total nitrogen content was significantly enhanced on inoculated plantlets growing in coarse peat and mixture substrates (3.20 and 3.33% respectively). These results confirms those enounced by Thomson et *al*. (1996) and Taylor and Lukey (2001) showing that arbuscular mycorrhizal fungi enhanced significantly the nitrogen concentration in tomatoes and strawberry tissues respectively. Moreover, mycorrhized plantlets growing in sandy substrate presented a significant increase of 12.5% and 38% of their root tissues levels of K and Mg respectively when compared with the non-inoculated plantlets. Results are inconsistent with those established in strawberry seedlings in which shoots K concentration was not affected by AMF inoculation (Taylor and Lukey, 2001). In fact, K^+ is equally important for maintaining the turgor pressure in plants under drought and salinity stresses (Heidari and Karami, 2014)

In all substrates, leaf P and Mg concentrations of the inoculated plantlets were significantly higher than concentration in leaves of the non inoculated planlets. This confirms findings of Asery et al. (2008) who attributed the highest levels of P to the enhancement of phosphatase activity, P mobilization and P absorption resulting to the atmospheric N_2 fixation increased by AMF inoculation.

Effects of the PGPRB inoculation

PGPRB inoculation enhanced significantly total nitrogen assimilation by plantlets in sand and coarse peat growing medium that may be due to the PGPRB abilities to fix atmospheric N_2 and to assist in resource acquisition (Ahemad and Kibret, 2014). In addition, nitrogen mobilization through plantlets tissues was enhanced by rhizospheric inoculation and rootstocks growing on substrate (S2) showed the greatest levels of total N in their leaf tissues. This result may be attributed to the inhibitory effects of the mineral nitrogen to the rhizobial inoculation, especially in the growing substrate with organic matter (S3) (Erman et *al*., 2011).

Root tissue P and Ca levels increased significantly with PGPRB inoculation in S1 and S2 because of rhizobacteria’s role in phostphate solubilizing. This is in line with researche conducted by Dey et *al*. (2004) who demonstrated that available phosphorus content in the soil, and the total phosphorus content in shoots and kernels of peanut increased significantly due to the PGPRB inoculation. The Mg migration through plantlets tissues was performed by PGPRB inoculation in S1 and S2 simultaneously.

Conclusion

Under nursery conditions, the efficiency of AMF and PGPRB inoculation on an hybrid almond x peach rootstock cv. ‘Garnem’ was investigated. It was found that AMF and PGPRB inoculation significantly increased nutrient content and mobilization of potted plantlets. However, the greatest effect was obtained in substrates with low minerals concentrations. Then, results suggest that rhizospheric microbes’s inoculation can significantly reduce the amount of chemical fertilizers if used as biofertilizers. However, in further research interest must be given to (i) the establishment of the potential competition between AMF, PGPRB and other rhizospheric strains (ii) the response of other rootstocks to AMF and PGPRB inoculation

Table 1: AMF and PGPRB effects on root, shoot and leaf nitrogen, phosphorus and potassium contents four months after inoculation in ‘Garnem’ plantlets

Substrate	Treatment	Total nitrogen (%)			Phosphorus (%)			Potassium (%)		
		Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf
Sand	Control	2.23±0042d	1.29±0.000b	3.27±0.029f	0.38±0.040a	0.096±0.019a	0.25±0.045a	0.64±0.035b	1.06±0.128b	3.63±0.208b
	AMF	1.90±0.066b	1.23±0.029a	3.13±0.042d	0.80±0.070cd	0.17±0.061a	0.23±0.149a	0.93±0.011e	1.36±0.208c	3.80±0.000bc
	PGPRB	2.37±0.042f	1.32±0.028b	3.043±0.043c	0.67±0.070bc	0.14±0.016a	0.23±0.018a	0.89±0.011d	0.86±0.005b	3.40±0.200ab
Peat	Control	1.76±0.035a	1.39±0.021c	2.83±0.028a	0.38±0.112a	0.14±0.038a	0.29±0.089a	0.72±0.020c	0.55±0.010a	3.80±0.346bc
	AMF	1.90±0.056b	1.49±0.029e	3.20±0.058e	0.93±0.058d	0.13±0.065a	0.54±0.41c	1.05±0.042f	1.51±0.180d	4.10±0.360c
	PGPRB	1.93±0.008b	2.02±0.035f	3.33±0.028g	0.72±0.100bc	0.11±0.043a	0.41±0.063b	1.04±0.035f	1.037±0.075c	3.53±0.115b
Mixture (1:1:1)	Control	2.39±0.064ef	1.40±0.024c	2.94±0.000b	0.61±0.020b	0.06±0.041a	0.23±0.029a	0.57±0.011a	0.90±0.077b	3.14±0.169a
	AMF	2.09±0.021c	1.41±0.021cd	3.48±0.029g	0.73±0.146bc	0.14±0.067a	0.39±0.079a	0.83±0.052d	0.83±0.070b	3.77±0.252bc
	PGPRB	2.28±0.035e	1.45±0.021de	3.03±0.058c	0.69±0.121bc	0.11±0.077a	0.46±0.011b	0.84±0.006d	1.47±0.202c	3.73±0.231bc

Means separation within columns was done by Duncan’s multiple range tests at ($p \leq 0.05$).

Table 2: AMF and PGPRB effects on root, shoot and leaf calcium and magnesium contents four months after inoculation in ‘Garnem’ plantlets

Substrate	Treatment	Calcium (%)			Magnesium (%)		
		Root	Shoot	Leaf	Roots	shoots	leaves
Sand	Control	0.093±0.011b	1.35±0.473fg	0.88±0.020a	0.042±0.001a	0.030±0.036ab	0.190±0.013a
	AMF	0.033±0.011a	1.26±0.298efg	1.85±0.405c	0.058±0.003b	0.046±0.003c	0.506±0.066c
	PGPRB	0.020±0.000a	0.64±0.025bc	1.147±0.142ab	0.055±0.003b	0.032±0.033ab	0.218±0.039a
Peat	Control	0.110±0.023b	0.23±0.036ab	1.23±0.145ab	0.056±0.009b	0.028±0.128a	0.229±0.046b
	AMF	0.020±0.000a	1.14±0.193def	1.39±0.0.363b	0.059±0.005b	0.043±0.09bc	0.498±0.040c
	PGPRB	0.020±0.000a	0.80±0.080cd	1.28±0.193	0.055±0.005b	0.032±0.043ab	0.215±0.020a
Mixture (1:1:1)	Control	0.160±0.020c	0.90±0.156cde	1.089±0.126ab	0.035±0.000a	0.035±0.005ab	0.381±0.060b
	AMF	0.090±0.011b	0.117±0.029a	1.253±0.300ab	0.039±0.010a	0.038±0.013ab	0.264±0.078a
	PGPRB	0.033±0.011a	1.65±0.388g	1.21±0.163ab	0.044±0.000a	0.043±0.005bc	0.651±0.075d

AMF: Arbuscular Mycorrhizal Fungi
PGPRB: plant Growth Promoting rhizobacteria

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