



Original Research Article

Beneficial effect of rhizobacteria inoculation on nutrition and mycorrhization of peanut grown in Morocco

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ABSTRACT

Keywords

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The aim of this study is to assess the effects of some Plant Growth Promoting Rhizobacteria (PGPR) strains on arbuscular mycorrhization and growth of KT22's peanut variety grown in Northwest of Morocco. The germinated seeds were inoculated with 5 bacterial strains (PP22, GP70, GR1, PR29 and GR70). Peanut plants were then grown in plastic pots in 2 unsterilized soils collected from subsistence farmers' fields of Laaouamra and Moulay Bouselham. Plant harvesting was made after 8 weeks of growth under chamber conditions. Results showed the positive and negative effects of these rhizobacteria on growth, mycorrhization and nodulation of peanut. Likewise, the origin of soils influenced the response of inoculated plants to bacterial strains. Highest stimulation was obtained with PP22 and GP70 that increased significantly the mycorrhizal colonization, nodulation, mineral nutrition (N, P and K) and leaf area in both soils. Also, PR29 improved leaf area, shoot length and mineral nutrition. On the other hand, GR70 reduced mycorrhizal infection and plant growth, especially on Moulay Bouselham's soil. This study reveals that GP70 and PP22 could be identified as Mycorrhiza Helper Bacteria (MHB). They can enhance growth, yield and nutrient uptake in combination with native rhizobia and arbuscular mycorrhizal fungi (AMF).

Introduction

The associated microflora with plant roots plays an essential role in nutrient cycling and ecosystem function. This is particularly the case of nitrogen (N₂) fixing microorganisms. They are generally categorized as symbiotic N₂-fixing bacteria including members of the rhizobiaceae family which forms symbiosis with leguminous plants (Ahemad and Khan, 2012) and non-symbiotic N₂-fixing forms such as

cyanobacteria, *Azospirillum*, *Azotobacter* and *Azocarus* (Bhattacharyya and Jha, 2012). These bacteria convert atmospheric nitrogen into available forms by biological N₂ fixation. This is also the case of AMF, which constitute critical microorganisms of the soil. They establish a symbiosis with 92% of plant families (Wang and Qui, 2006). Besides, they levy through specific transporters (Harrison 2005) phosphorus and

trace elements usually present in limited quantities and low mobility in soil. However, the long evolution of plants, in relationship with these plant-beneficial microorganisms, is probably not done independently of some bacteria which are particularly abundant in the rhizosphere. In fact, several rhizobacteria play a major role in enhancing the growth and health of plants. The beneficial effects of rhizobacteria have been attributed towards their ability to promote plant growth by either facilitating resource acquisition (nitrogen, phosphorus and other essential minerals) or modulating plant hormone levels. Because of their phytobeneficial activities, these bacteria are called PGPR. In addition, several researchers have noted that the establishment and functioning of mycorrhizal symbioses can be positively influenced by some bacterial strains named Mycorrhiza Helper Bacteria (MHB) (Frey-Klett et al., 2007). Consequently, the use of PGPR in interaction with indigenous rhizospheric microorganisms (rhizobia and AMF) seems to be a real agro-ecological alternative to chemical fertilization. Hence, the present study was carried out to evaluate the efficiency of some PGPR on mycorrhization, nodulation, mineral nutrition and plant biomass of KT22's peanut variety cultivated in Northwest of Morocco.

Materials and Methods

Plant material and soils used

Sampling of soils, cultivated previously by peanut, is performed in the first 20 centimeters deep on both sites of Laaouamra (clay 10.10%, silt 6.11%, sand 80.81%, pH (H₂O) 6.1, total organic matter 1.1%, P₂O₅ 97.47 ppm, total nitrogen 50 ppm) and Moulay Bouselham (clay 5.03%, silt 8%, sand 85.43%, pH (H₂O) 6.5, total organic matter 0.71%, P₂O₅ 62.91 ppm, total

nitrogen 35 ppm). The mixture of these samples, for each site, gave rise to the composite sample. The soil was air dried, sieved on 2 mm mesh sieves and placed in favorable conditions throughout the duration of the study. The plant material used in this study is KT22's variety of peanut (*Arachis hypogaea* L.). This peanut variety is a legume that belongs to the botanical group of "Virginia".

Inoculation of seedlings with PGPR

The bacterial strains used as inocula are isolated from the rhizosphere of 3 varieties of rice (Puntal, Elio, and Guadiamar). There are 3 *Pseudomonas* (PP22, GP70 and GR1) and 2 *Aeromonas* (PR29 and GR70) (Aarab., 2015a, 2015b). These bacterial strains are selected for their ability to solubilize the tricalcium phosphate and to secrete indole acetic acid (IAA). The peanut's seeds were surface sterilized by agitation in 0.5% sodium hypochlorite for 5 min, followed by 6 washings with sterile water. Seeds were then germinated in 1% agar water (w/v) plates for 72 h at 28°C. After germination, seedlings were planted in both soils of Laaouamra and Moulay bouselham. In fact, 2 groups of plastic pots (18 cm diameter, 20 cm height) were filled with 3 Kg of non sterilized soil. One germinated seed was sown in each pot, of both soils, and inoculated directly with 1.5 ml of bacterial culture (10⁸ cfu ml⁻¹) grown in TSB. All pots were maintained at 28 ± 2 °C with 16 hours photoperiod and a light intensity of 400 µE m⁻² s⁻¹. Four replications were maintained for each treatment.

Mycorrhizal parameters and plant growth

Plants were harvested 8 weeks after sowing in the growth chamber under controlled conditions. The leaf area was calculated by using the equation described by Ahmed and

Morsy (1999): Leaf area (cm^2) = 0.70 (length x width) – 1.06. Then, plants were uprooted carefully from the soil and washed with water. Also, shoot weight was measured after oven drying at 62°C for 72 hours. A part of the root of each plant was collected, cleared and stained as described by Phillips and Haymann (1970) and finally mounted on slides. Quantification of arbuscular mycorrhizal infection and colonization was performed using the notation scale described by Trouvelot et al. (1986). Parameters of mycorrhization were calculated with MYCOCALC software, available at:

<http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html>.

Plant mineral analysis (N,P,K)

Shoot samples were oven-dried at 68 °C for 48 hours, ground, and passed through a 1-mm sieve. Then, the Kjeldahl method was used to determine total nitrogen (N) after wet digestion with concentrated sulfuric acid. Also, Phosphorus (P) and potassium (K) were performed using the method « ICP: inductively coupled plasma spectrophotometer » at the National Center of Scientific and Technical Research (CNRST) in Rabat, Morocco.

Statistical analysis

Statistical analyses of the experimental data were performed using ANOVA test, p-value ≤ 0.05 was considered statistically significant. Data analysis was performed on mycorrhizal infection, vegetative growth and mineral nutrition.

Results and Discussion

Evaluation of plant mycorrhization

On the soil of Moulay Bouselham, inoculation of plants with GP70, GR1 and

PP22 improved the mycorrhizal frequency in root (F%) compared to control (Fig. 1). Among these 3 bacterial strains, GP70 and PP22 increased significantly arbuscular abundance in the mycorrhizal root cortex (A% and a%) (Fig. 1,2); while inoculation with GR1 promoted only the intensity of root cortex colonization (M% and m%). However, GR70 bacterial strain has a negative impact on mycorrhizal infection. For the soil of Laouamra, GP70 stimulated all mycorrhizal parameters of peanut. Besides, the PP22 favored only the abundance of arbuscules in the root cortex.

Inoculation effect on plant development

Inoculation of plants with GP70, PP22 and PR29 increased significantly peanut's shoot height on the soil of Moulay Bouselham. Similarly, these rhizobacteria improved also peanut leaf area on both soils (Table 1). Furthermore, we obtained a significant increase in nodulation and shoot weight on both soils after inoculation with PP22. Also, GP70 had the same effect but only on the soil of Laouamra. In contrast, GR70 bacterial strain reduced shoot weight on the soil of Moulay Bouselham.

Nutrient content of plants (N, P and K)

Peanut inoculation with 3 bacteria, GP70, PP22 and PR29 in both soils enhance plants content in potassium and phosphorus (Table 2). Moreover, this increase is accompanied by a significant improvement in plant nitrogen content. On the other side, GR70 has a negative effect on nutrient proportions of the plants cultivated on soil of Moulay Bouselham.

In this study, our interest was to assess peanut growth, KT22 variety, under the effect of 5 rhizobacteria estimated as PGPR, in interaction with indigenous nodulating

rhizobia and AMF. The results show that bacterial inoculation has a positive effect on nutrient uptake and yield of peanut. On the other hand, soils origin influences magnitude and inoculum impact degree on plant growth and symbiosis establishment between peanut and micro-organisms. The low levels of available soil phosphorus and the high peanut's rate mycorrhization reflect the richness of both soils of Moulay Bouselham and Laouamra in AMF propagules. Thus, it was shown that soils which contain low proportions of assimilable phosphorus, promote the growth and development of mycorrhizal spores (Katsunori et al., 2008).

Furthermore, the 3 *Pseudomonas* strains PP22, GP70 and GR1 had the highest capacity to enhance plant's mycorrhizal infection depending of crop soil. These bacteria might be favorable to mycorrhizal infection by producing organic acids and some growth factors such as IAA. Several studies have reported the ability of pseudomonads to act as mycorrhiza helper bacteria (MHB) (Frey-Klett et al., 2007; Naziret al., 2010). It has been previously demonstrated that some pseudomonads promote the saprophytic growth and root colonization by AMF (Pivato et al., 2009). Indeed, PP22 and GP70 can positively influence the efficiency of mycorrhization by increasing arbuscular exchange area. Likewise, phosphate solubilizing in the rhizosphere is one of the most important actions of these 2 bacterial strains (Aarab et al., 2015b). Thus, they are estimated as phosphate solubilizing bacteria (PSB). The release of phosphate from insoluble compounds in rhizosphere involves specific enzymatic processes. The phosphate-solubilization capacity by these PSB is widely associated to production of low molecular weight organic acids (Qureshi et al., 2012). The released acid chelate the

cations (Al, Fe, Ca) bound to the insoluble forms of inorganic phosphate and convert them into soluble forms with the consequent decrease in the pH of the medium (Stevenson 2005). Similarly, the fungal hyphae are capable of releasing the insoluble phosphate by secreting extracellular enzymes (phosphatase, phytase) (Gobat et al., 2003). Consequently, AMF improve the absorption of phosphorus and other nutrients by plants increasing the contact surface and the explored soil volume (He & Nara, 2007). Experiments have shown that the improvement in the absorption of phosphate is one of the mechanisms by which the AMF may improve the productivity of plant; they contribute to 90% in phosphate uptake by plants (Van der Heijden, 2006). In addition, the 2 *Pseudomonas* bacteria (PP22 and GP70) and indigenous AMF make phosphate available for symbiotic fixation of atmospheric nitrogen by rhizobia. Thus, a significant increase of nodulation and nitrogen content of plants was obtained. Also, the improvement in nodules number after inoculation by PP22 and GP70 can be attributed to a distinct change in the architecture of root system. Indeed, Stimulation of root hairs elongation, by secreting IAA, can provide more active sites and access to nodulation and mycorrhization by native micro-organisms. The ability of PGPR to affect root architecture is mainly related to their influence in the hormonal balance of plants, especially in the relationship between auxins and cytokinins (Vacheron et al., 2013). These 2 hormones stimulate the elongation of root hairs (Contesto et al., 2008; Galland et al., 2012) and increase number and size of secondary roots (Chamamet al., 2013). Fluorescent pseudomonads have been reported to promote nodulation in chickpea and thereby enhance biological nitrogen fixation (Parmar and Dadarwal, 2000). As suggested by Contesto (2008), the inhibitory effect of

peanut's nodulation by GR70 and PR29 could be explained by the competition with rhizobia for food and other nutrients. These bacteria may also inhibit nodule formation and growth in a given symbiotic relationship, depending upon the nature and concentration of secondary metabolites

released in rhizosphere (Contesto et al., 2008).It has been also demonstrated that inoculation of peanut by GR70 strain has favored the proliferation of nematodes in the root system of peanut's plants (Bouhraoua et al., 2015).

Table.1 Effect of PGPR inoculations on nutrient uptake and growth of peanut grown on both soils of Moulay Bouselham (M.B) and Laaoumra (Lmra)

Parameters	Soils	Control	GP70	GT1	PR29	GR70	PP22
Shoot height (cm)	M.B	38.50 cd	40.13 bc	39.63 bc	44.75 ab	33.63 d	49.38 a
	Lmra	37.38 b	44.38 a	37.88 b	39.25 ab	40.88 ab	42.50 ab
Leaf area (cm ²)	M.B	2.27 cd	3.92 b	2.75 cd	3.16 bc	2.01 d	6.73 a
	Lmra	2.49 b	3.71 a	2.16 b	3.44 a	2.44 b	3.87 a
Nodules number	M.B	09.00 b	02.00 c	00.00 c	00.00 c	00.00 c	22.00 a
	Lmra	03.00 c	09.00 ab	08.00 b	00.00 d	00.00 d	11.00 a
Dry shoot Weight (g)	M.B	0.94 bc	1.05 b	0.77 cd	1.02 bc	0.62 d	1.58 a
	Lmra	0.69 c	1.36 a	0.86 bc	0.85 bc	0.71 c	1.04 b

Values in lines followed by different letter differ significantly

Table.2 Effect of PGPR inoculations on nutrient uptake (N,P,K) of peanut plants

N (mg plant ⁻¹)	M.B	26.49 c	33.05 b	22.86 c	33.44 b	15.98 d	52.07 a
	Lmra	18.65 de	40.85 a	23.08 cd	26.75 c	17.98 e	32.32 a
P (mg plant ⁻¹)	M.B	6.18 c	6.88 bc	5.08 d	7.49 ab	5.05 d	8.31 a
	Lmra	3.48 c	7.02 a	4.83 b	7.72 a	3.78 bc	4.11 bc
K (mg plant ⁻¹)	M.B	29.19 bc	32.82 ab	28.42 cd	35.32 a	24.87 d	37.07 a
	Lmra	19.55 c	37.71 a	27.90 b	35.71 a	20.47 c	28.66 b

Values in lines followed by different letter differ significantly

Figure.1 Mycorrhizal parameters of peanut (KT22 variety) on the soil of Moulay Bouselaham (a) and Laaoumra (b)

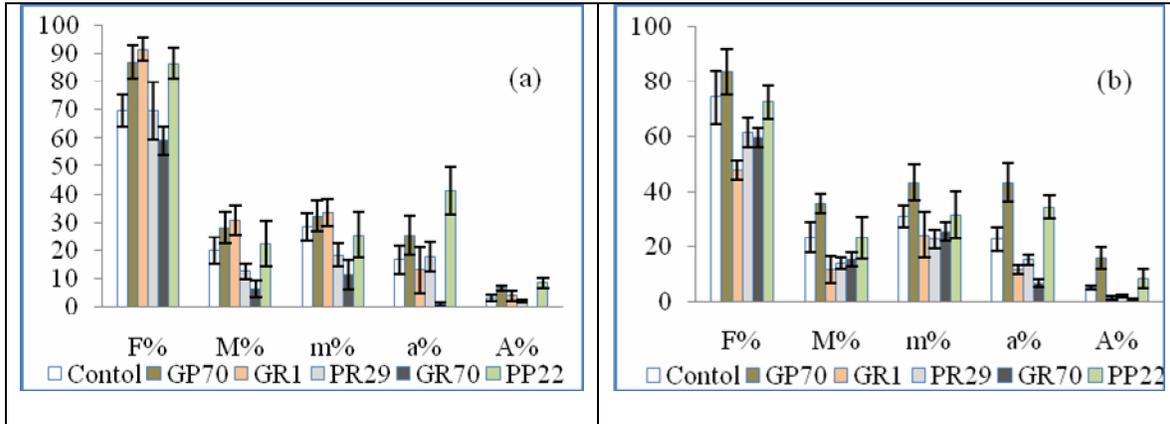
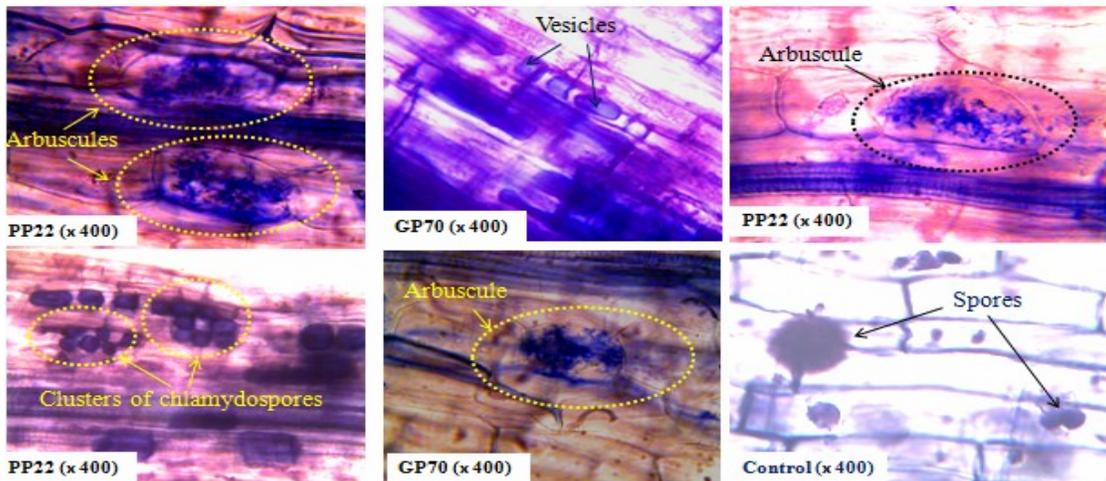


Figure.2 Mycorrhizal infection of peanut in response to bacterial inoculation



An important supply of phosphate for plants inoculated with PP22 and GP70 explains also the increase in leaf area and shoot height. Studies have shown that mycorrhization had a significant effect on leaf area and shoot height of date palm (Radi et al., 2014). In addition, Ben Brahim et al. (1996) and Ben Brahim (1996) showed, according to their studies of maritime pine seedlings, that growth is affected by a phosphorus deficiency and reduction in leaf area. Indeed, the rate of leaf elongation (or the rate of leaf expansion) is the determining factor in reducing the final size of the leaves in case of P deficiency (Chiera et al., 2002).

In conclusion, we report that PP22 and GP70 bacterial strains, in interaction with indigenous micro-organisms, have great benefits on mineral nutrition and growth of peanut. Thus, interactions between peanut, AMF and rhizobia can be strongly influenced by these bacteria estimated as MHB and PSB. Because of their potential to increase plant nutrition and growth, PP22 and GP70 seem therefore to be the key biofertilizers able to promote the agroecological yield of KT22's peanut variety cultivated in the Northwest of Morocco. However, further investigations are needed to explore if these

rhizobacteria behave similarly in field studies.

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