Arbuscular Mycorrhization and Growth Promotion of Peanut (*Arachis hypogaea* L.) After Inoculation with PGPR

4

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Abstract

Because of their potential to increase plant nutrition and yield, the use of some microorganisms in low-input agriculture and forestry has been addressed for successful agroecological investigations. A pot experiment was conducted to study the effects of some *plant growth-promoting rhizobacteria* (PGPR) on arbuscular mycorrhizal fungi (AMF) and growth of KP29's peanut variety grown in the northwest of Morocco. Seeds were inoculated with three *Pseudomonas* (PP22, GP70, and GR1) and two *Aeromonas* strains (PR29 and GR70). Then, they were grown in two unsterilized soils collected from subsistence farmers' fields of Laaouamra and Moulay Bousselham. Plant harvesting was made after 60 days of cultivation under growth chamber conditions, and the roots were removed and rinsed carefully. Results showed positive and negative effects of these rhizobacteria on growth and mycorrhization of peanut. Pseudomonad strains gave the greatest plant nutrient content (N, P, and K) and growth parameters. Also, bacterial inoculation had a positive impact on peanut mycorrhization by enhancing arbuscular abundance. Highest stimulation was noticed with pseudomonad strains on both soils. In addition, PR29 exhibited maximum values of mycorrhizal colonization on the soil of Laaouamra. However, the magnitude effect of inoculation on plant growth and mycorrhizal infection varied according to the origin of soils. On the other hand, only PP22 stimulated nodules formation on the

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soil of Laaouamra. In conclusion, this study reveals that GP70, GR1, and PP22 can enhance growth, yield, and nutrient uptake of peanut. They can also enhance biological nitrogen fixation and mineral uptake in combination with AMF.

4.1 Introduction

An intensive farming practice that warrants high yield and quality requires extensive use of chemical fertilizers, which are costly and can create serious environmental problems. Large amounts of chemical fertilizers are used to replace soil nitrogen and phosphorus. Despite the deleterious environmental effects, the total amount of inorganic fertilizers used worldwide is expected to produce more food via intensive agriculture for the increasing world population (Adesemoye et al. 2009). The challenge therefore is to continue more agricultural productivity in a way that minimizes harmful environmental effects of fertilizers. Current efforts have been focused on the decreased use of inorganic fertilizers in agriculture, prompting the search for alternative ways to improve soil fertility and crop production. Beneficial plant-microbe interactions in the rhizosphere are the determinants of plant health and soil fertility (Jeffries et al. 2003). Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality (Jeffries et al. 2003). The growth-promoting activities of some microbiota on plants can be explained in various ways, including through biocontrol and induction of disease resistance in the inoculated plant, biological N_2 fixation, phosphorus solubilization, and/or production of phytohormones (Mia et al. 2012). The symbiotic association between arbuscular mycorrhizae (AM) fungi and root provides a significant contribution to plant nutrition and growth; they are frequently associated with species from around 90% of plant families. AMF provide many benefits to plants and the environmental stability which includes nutrient uptake enhancement, drought tolerance, root pathogens, and soil aggregation improvement (Smith and Read 2008). In the last decades, the interest concerning elucidation of the mechanisms involved in establishing the complex interactions of microorganisms in the rhizosphere and their role in protecting and stimulating plant development had increased. The present study is designed to evaluate the effect of some PGPR inoculations on mycorrhization, yield, and nutrient uptake of peanut grown in the northwest region of Morocco.

4.2 Materials and Methods

4.2.1 Plant Material and Soils Used

The commercial KP29's variety of peanut (*Arachis hypogaea* L.), currently cultivated by farmers in various parts of Moulay Bousselham and Laaouamra, was used

as plant material. This peanut variety is a legume that belongs to the botanical group of "Valencia."

Sampling of soils, cultivated previously by peanut, is performed in the first 20 cm deep on both sites of Laaouamra (clay 10.10%, silt 6.11%, sand 80.81%, pH (H_2O) 6.1, total organic matter 1.1%, P_2O_5 97.47 ppm, total nitrogen 50 ppm) and Moulay Bousselham (clay 5.03%, silt 8%, sand 85.43%, pH (H_2O) 6.5, total organic matter 0.71%, P_2O_5 62.91 ppm, total nitrogen 35 ppm). The soil was air dried, sieved on 2 mm mesh sieves, and placed in favorable conditions throughout the duration of the study.

4.2.2 Inoculation of Seedlings with PGPR

The bacterial strains used as inocula are isolated from the rhizosphere of three varieties of rice (Puntal, Elio, and Guadiamar). There are three *Pseudomonas* (PP22, GP70, and GR1) and two *Aeromonas* (PR29 and GR70) (Aarab et al. 2015a, b). These bacterial strains were chosen because of their ability to solubilize the tricalcium phosphate and to secrete indole acetic acid (IAA). The peanut's seeds were surface sterilized with 0.5% sodium hypochlorite for 5 min, rinsed, and left to soak for 6 h in sterile distilled water. Seeds were then transferred aseptically to Petri dishes filled with 1% (w:v) water agar medium, and plates were incubated at 28 °C in the dark for 72 h. After germination, seedlings were planted in both soils of Laaouamra and Moulay Bousselham. In fact, two 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 (10^8 cfu ml^{-1}) grown in TSB. All pots were placed in a growth chamber with a 16:8 h light:dark photoperiod at 28 ± 2 °C and a photosynthetically active radiation (PAR) of 400 μE m^{-2} s^{-1}. Four replications were maintained for each treatment.

4.2.3 Mycorrhizal and Growth Parameters

Sixty days after sowing, plants were harvested. The leaf area was calculated by using the equation described by Ahmed and Morsy (1999): Leaf area (cm^2) = 0.70 (length × width) – 1.06. Then, plants were uprooted carefully from the soil and washed with water. The presence of root nodules was checked visually on each plant replicate. Plant growth was evaluated by measuring the dry mass of shoots (62 °C for 72 h) for each of the four plant replicates per treatment. A part of the root of each plant was collected, cleared, and stained as described by Phillips and Hayman (1970) and finally mounted on slides. Quantification of arbuscular mycorrhizal infection and colonization was evaluated 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.

4.2.4 Plant Mineral Analysis (N, P, K)

Shoot samples were oven-dried at 62 °C for 72 h, 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 determined using the method "ICP: inductively coupled plasma spectrophotometer" at the National Center for Scientific and Technical Research (CNRST) in Rabat, Morocco.

4.2.5 Statistical Analysis

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

4.3 Results

Most published works on study of PGPR and AMF have been focused as alternative fertilizers to improve yield and productivity of legume plants. The soil microorganisms may play a decisive role on nutrient uptake and ecological growth of peanut in the northwest of Morocco. As plants are valuable sources of nutrients for many categories of soil microorganisms, they represent the center of different types of interrelations, competition, or cooperation, in order to gain access to mineral nutrient.

4.3.1 Nodulation and Mycorrhization of Peanut After Inoculation with PGPR

The results of nodules number and mycorrhizal root colonization are shown in Table 4.1. No nodules were observed on hairy roots of uninoculated control and inoculated plants with PGPR on both soils, except with PP22 (three nodules/plant)

Table 4.1 Effect of PGPR inoculations on nutrient uptake (N, P, K) of peanut plants

Parameters*	Soils	Control	GP70	GT1	PR29	GR70	PP22
N (mg plant^{-1})	MB	7.59 ± 1.90^{bc}	9.50 ± 1.91^{ab}	9.86 ± 1.57^{ab}	7.89 ± 1.56^{bc}	5.76 ± 1.19^{c}	11.29 ± 2.40^{a}
	Laa	7.89 ± 2.09^{b}	8.87 ± 2.36^{ab}	9.52 ± 1.48^{ab}	8.63 ± 1.13^{ab}	7.66 ± 1.97^{b}	11.28 ± 3.65^{a}
P (mg plant^{-1})	MB	1.58 ± 0.51^{bc}	2.58 ± 0.41^{a}	2.43 ± 0.64^{a}	2.23 ± 0.66^{ab}	1.07 ± 0.31^{c}	2.19 ± 0.64^{ab}
	Laa	1.86 ± 0.12^{c}	2.07 ± 0.43^{bc}	2.48 ± 0.14^{ab}	1.99 ± 0.15^{c}	2.01 ± 0.38^{c}	2.66 ± 0.39^{a}
K (mg plant^{-1})	MB	12.10 ± 0.89^{ab}	14.39 ± 5.95^{a}	11.47 ± 1.99^{ab}	10.28 ± 3.40^{ab}	09.37 ± 0.90^{b}	12.04 ± 0.90^{ab}
	Laa	8.06 ± 0.83^{b}	10.37 ± 2.14^{ab}	12.51 ± 1.82^{a}	9.51 ± 1.50^{b}	8.77 ± 1.90^{b}	12.38 ± 2.57^{a}

*Values in lines followed by letter a, b and c differ significantly according to Fisher-protected LSD test ($p < 0.05$)

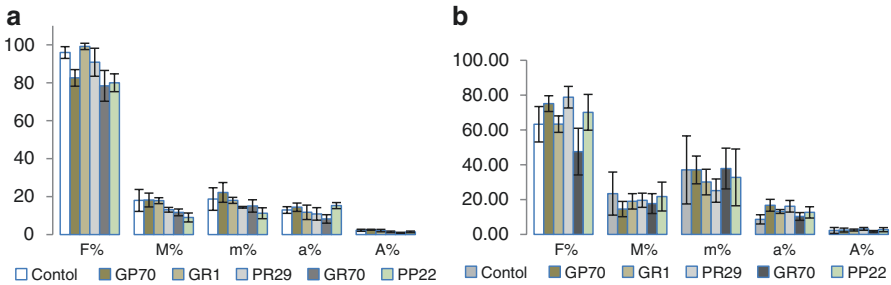

Fig. 4.1 Mycorrhizal parameters of peanut (KP29 variety) on the soil of Moulay Bousselham (**a**) and Laaouamra (**b**)

on the soil of Laaouamra. The promotion of mycorrhizal infection of peanut plants by native AM fungi after inoculation with PGPR was expressed by the frequency of root colonization, mycorrhizal intensity of the root cortex colonization, and abundance of arbuscules. On the soil of Moulay Bousselham, plants inoculation with GP70, GR70, and PP22 reduced frequency of root colonization (F%) compared to control (Fig. 4.1). Also, mycorrhizal intensity (M%) was decreased significantly by PR29, GR70, and PP22 strains. On the soil of Laaouamra, PR29 increased significantly the frequency of root colonization, and only GR70 had a negative impact on this mycorrhizal parameter. On the other hand, plant inoculation with GP70, GR1, PR29, and PP22 improved (sometimes not significantly) arbuscular abundance in the mycorrhizal root cortex (A% and a%), especially on the soil of Laaouamra.

4.3.2 Response of Peanut Plants to Inoculation with PGPR

In this experiment, inoculation with PGPR had a positive effect on yield and peanut growth, except with GR70 which presents sometimes negative effects. Generally, the significant improvements were registered after inoculation with *Pseudomonas* strains.

4.3.3 Shoot and Root Length

The results show significant differences due to bacterial inoculation. Significant increases in shoot length were observed after inoculation with GP70 and PP22 on the soil of Moulay Bousselham. On the soil of Laaouamra, all bacterial inoculants increased shoot length, but only PR29 had significant effect. On the other hand, a significant root shortening due to GR70 inoculation was detected on the soil of Moulay Bousselham. The roots of plants treated with GR1 and PR29 on the soil of Laaouamra and GP70 on the soil of Moulay Bousselham were significantly longer than roots of control plants.

Table 4.2 Effect of PGPR inoculations on growth and yield of peanut plants

Parameters*	Soils	Control	GP70	GT1	PR29	GR70	PP22
Root length (cm)	MB	17.00 ± 2.94^{ab}	20.85 ± 4.57^{a}	17.30 ± 4.42^{ab}	15.21 ± 1.29^{b}	7.83 ± 1.65^{c}	14.93 ± 1.65^{b}
	Laa	11.12 ± 2.65^{b}	13.25 ± 3.30^{ab}	18.66 ± 3.09^{a}	20.00 ± 8.64^{a}	14.42 ± 2.38^{ab}	15.00 ± 2.16^{ab}
Shoot height (cm)	MB	28.75 ± 3.20^{c}	40.38 ± 1.25^{a}	33.75 ± 3.77^{b}	33.50 ± 2.08^{b}	27.63 ± 2.13^{c}	38.25 ± 2.06^{a}
	Laa	34.50 ± 4.2^{b}	37.50 ± 5.50^{b}	43.25 ± 2.75^{a}	36.13 ± 3.42^{b}	37.63 ± 1.70^{b}	39.75 ± 3.30^{ab}
Leaf area (cm^2)	MB	1.01 ± 0.47^{b}	1.37 ± 0.17^{ab}	1.01 ± 0.22^{b}	1.51 ± 0.13^{a}	1.39 ± 0.17^{ab}	1.47 ± 0.23^{a}
	Laa	0.73 ± 0.2^{d}	1.07 ± 0.108^{c}	1.49 ± 0.342^{a}	1.12 ± 0.116^{bc}	1.36 ± 0.139^{ab}	1.45 ± 0.035^{a}
Nodules number	MB	00 ± 00^{a}	00 ± 00^{a}	00 ± 00^{a}	00 ± 00^{a}	00 ± 00^{a}	00 ± 00^{a}
	Laa	00 ± 00^{b}	00 ± 00^{b}	00 ± 00^{b}	00 ± 00^{b}	00 ± 00^{b}	03 ± 1.29^{a}
Fresh root weight (g)	MB	0.30 ± 0.02^{ab}	0.35 ± 0.05^{a}	0.21 ± 0.04^{c}	0.23 ± 0.06^{bc}	0.13 ± 0.03^{d}	0.25 ± 0.05^{bc}
	Laa	0.19 ± 0.09^{b}	0.20 ± 0.04^{b}	0.27 ± 0.05^{ab}	0.34 ± 0.09^{a}	0.22 ± 0.03^{b}	0.27 ± 0.04^{ab}
Fresh shoot weight (g)	MB	2.06 ± 0.69^{bc}	3.23 ± 0.71^{a}	2.80 ± 0.37^{ab}	2.24 ± 0.37^{bc}	1.78 ± 0.59^{c}	3.13 ± 0.55^{a}
	Laa	2.12 ± 0.36^{c}	2.71 ± 0.24^{abc}	3.24 ± 0.59^{a}	2.98 ± 0.48^{ab}	2.58 ± 0.35^{bc}	3.18 ± 0.41^{ab}
Dry root weight (g)	MB	0.05 ± 0.008^{ab}	0.07 ± 0.017^{a}	0.046 ± 0.012^{b}	0.048 ± 0.012^{b}	0.02 ± 0.008^{c}	0.045 ± 0.012^{b}
	Laa	0.042 ± 0.017^{a}	0.042 ± 0.012^{a}	0.045 ± 0.008^{a}	0.046 ± 0.015^{a}	0.042 ± 0.010^{a}	0.045 ± 0.023^{a}
Dry shoot weight (g)	MB	0.30 ± 0.089^{b}	0.46 ± 0.054^{a}	0.36 ± 0.064^{a}	0.30 ± 0.073^{b}	0.20 ± 0.041^{c}	0.37 ± 0.09^{a}
	Laa	0.30 ± 0.036^{c}	0.43 ± 0.055^{a}	0.45 ± 0.109^{a}	0.36 ± 0.091^{ab}	0.36 ± 0.051^{ab}	0.46 ± 0.084^{a}

*Values in lines followed by letter a, b, c and d differ significantly according to Fisher-protected LSD test ($p < 0.05$)

4.3.4 Leaf Area

On the soil of Laaouamra, all bacterial treatments increased significantly peanut's leaf area as compared to the control (Table 4.2), and maximum values were obtained with the GR1 and PP22 inoculants. However, only PR29 and PP22 increased this parameter growth on the soil of Moulay Bousselham.

4.3.5 Fresh and Dry Shoot Weight

Inoculation of plants with GP70 and PP22 increased significantly fresh shoot weight on the soil of Moulay Bousselham. In addition to these two bacterial strains, GR1 had the same effect on peanut's dry shoot weight. On the soil of Laaouamra, bacterial strains had a positive impact on fresh shoot weight of peanut's plants. Indeed, they all had a significant effect on dry shoot weight.

4.3.6 Macroelement Content

Concerning mineral content, the studied macroelements varied in a similar way depending on treatments. The P and K contents were significantly favored by GR1 and PP22 on the soil of Laaouamra. On the soil of Moulay Bousselham, inoculation had no significant effect on K content of plants. Nevertheless, GR1 had a positive significant impact on P content, compared with the control plants. Results showed also that only PP22 inoculants contributed to the significant increase of mineral N on both soils of Moulay Bousselham and Laaouamra.

4.4 Discussion

Our results demonstrate that soils origin influences magnitude and inoculum impact degree on plant growth and symbiosis establishment between peanut and microorganisms. In fact, the response of peanut yield and nutrient uptake to bacterial inoculation is of great relationship with soils' physicochemical characteristics. The inferred data suggest that inoculations might determine which bacterial strain can be exploited to increase such parameter of peanut growth on both soils of Moulay Bousselham and Laaouamra. Reduction of mycorrhizal frequency and intensity on the soil of Moulay Bousselham could be explained by negative impact of bacteria. They can establish biofilms on the surface of peanut root after inoculation. Thus, Frey-Klett et al. (2007) proposed that the bacteria exert two opposite effects on plant growth: the obvious beneficial ones independent of cell density and some detrimental ones toward the plant or fungus when at high densities. Then, bacteria may have physical effects on peanut mycorrhization through biofilm formation, as well as chemical effects through the release of compounds in the exudates. A harmful impact of bacterially derived volatiles (acids, alcohols, methane, ethyl acetate, acetaldehyde, acetoin, and diacetyl) on mycorrhiza formation has been previously demonstrated (Mackie and Wheatley 1999; Bruce et al. 2003). Moreover, significant negative effects on mycorrhiza formation were observed in the case of the spent broth experiment, particularly for spent broth produced during exponential phase growth (Aspray et al. 2005). On the other hand, GP70, GR1, PR29, and PP22 can positively influence the efficiency of mycorrhization by increasing arbuscular exchange area. The so-called mycorrhiza helper bacteria (MHB) have been shown to promote mycelial growth and mycorrhiza formation (Frey-Klett et al. 2007; Garbaye 1994). These bacteria can facilitate hyphal penetration through the soil, and when hyphae colonize plant tissues, they can continue their functions. The bacteria located on hyphae can be released to the intercellular spaces after hyphae penetration in roots. In the intercellular spaces, this bacteria cause dilatation of the cortical root cells and establish highly branched hyphae that develop between the fungal cell wall and the plasma membrane of plant cells.

In response to bacterial treatments, N, P, and K contents were favored by all bacterial inoculations. Among bacterial strains, which might be estimated as MHB, pseudomonads bacteria (PP22, GP70, and GR1) had higher positive impact on plant N and P content. Solubilizing phosphate is one of the most important actions of these three bacterial strains (Aarab et al. 2015b). Thus, they were also estimated as phosphate-solubilizing bacteria (PSB). Moreover, AMF provide more necessary mineral nutrients to plant in easily assimilated via improvement of arbuscular exchange area. Therefore, they could explain the increase of plant mineral content. Enhancement of leaf area, shoot height, and biomass might be attributed to P-solubilizing ability and the adequate uptake of other nutrients to roots. By increasing P availability and its uptake, PP22 stimulated nodule formation and nitrogen fixation on the soil of Laaouamra. Improvement of plant growth parameters could be also a result of the increased synthesis of IAA (Aarab et al. 2015b), which is a plant growth promoter. The production of IAA has been reported by many species, and *Pseudomonas spp.* has always been to produce

more auxin, thus increasing plant height, shoot weight, and more biomass production. Glick (1995) viewed that the mechanism most commonly invoked to explain the various effect of PGPR on plants is the production of phytohormones, and IAA may play the most role in growth promotion. Also, there is evidence that mycorrhizal plants contain higher concentration of growth hormones than their non-mycorrhizal equivalents. Effective nutrient acquisition by AMF is generally attributed to the extensive hyphal growth beyond the nutrient depletion zone surrounding the root and principal avoidance.

Conclusion

Our results confirm the suitability of PGPR inoculation to improve plant peanut growth. The management of the application of these rhizobacteria represents considerable extent especially with pseudomonads strains. The positive interactions with other beneficial microorganisms such as native AMF can also be taken into account as a method of enhancing peanut yield and growth. However, due to the high specificity involved in these types of bacterial inoculations, a previous screening to select the best microbe-host plant combination should be done in order to optimize results.

References

Aarab S, Ollero J, Megías M, Laglaoui A, Bakkali M, Arakrak A (2015a) Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities: an *in vitro* study. Int J Curr Microbiol App Sci 4(1):260–269

Aarab S, Ollero J, Megías M, Laglaoui A, Bakkali M, Arakrak A (2015b) Isolation and screening of inorganic phosphate solubilizing *Pseudomonas* strains from rice rhizosphere soil from Northwestern Morocco. Am J Res Commun 3(4):29–39

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Ahmed FF, Morsy MH (1999) A new methods for measuring leaf area in different fruit species. Minia J Agric Res Dev 19:97–105

Aspray TJ, Eirian Jones E, Whipps JM, Bending GD (2005) Importance of mycorrhization helper bacteria cell density and metabolite localization for the *Pinus sylvestris-Lactarius rufus* symbiosis. FEMS Microbiol Ecol 56:25–33

Bruce A, Stewart D, Verrall S, Wheatley RE (2003) Effect of volatiles from bacteria and yeast on the growth and pigmentation of sapstain fungi. Int Biodeter Biodegr 51:101–108

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210

Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:1376–1381

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16

Mackie AE, Wheatley RE (1999) Effects and incidence of volatile organic compound interactions between soil bacterial and fungal isolates. Soil Biol Biochem 31:375–385

Mia MAB, Shamsuddin ZH, Mahmood M (2012) Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. Afr J Biotechnol 11:3758–3765

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. London, UK: Academic Press

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. INRA edition, Paris