

# Phosphate Solubilizing Bacteria Efficiency on Mycorrhization and Growth of Peanut in the Northwest of Morocco

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**Abstract** The present study aims to assess the effect of phosphate solubilizing bacteria (PSB) in interaction with native arbuscular mycorrhizal fungi (AMF) and rhizobia on peanut growth cultivated in the Northwest of Morocco. To perform this aim, seeds were inoculated with 5 PSB strains: PP22, GP70, GR1, PR29, and GR70, and then grown in unsterilized soil collected from peanut fields. Plant harvesting was made after 8 weeks of growth under chamber conditions. The results demonstrated that PSB treatments showed positive effect on mycorrhizal colonization, nodulation and plant growth parameters. The best results were found with *Pseudomonas* strains, GP70, PP22 and GR1. This study indicates the great potential of some PSB to improve yield and nutrient uptake of peanut plants in the presence of native rhizobia and AMF. They could serve as biofertilizers, minimizing chemical fertilization that is currently used to obtain high yields for peanut agriculture.

**Keywords:** AMF, growth, peanut, PSB, rhizobia

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## 1. Introduction

Modern agriculture, which is characterized by intensive cultivation methods, is totally dependent on regular input of numerous types of inorganic fertilizers. Phosphorous (P) is an essential macronutrient required for plant growth and development. However, it is often a limiting mineral nutrient for many agricultural crops, because it is abundant in many soils but in poorly assimilable forms [1]. In the context of increasing international concern for agricultural yields and environmental quality improvement, the use of AMF and beneficial bacteria for reducing chemical inputs in agriculture is of great importance. In fact, AMF are known of a major importance on plant growth promotion. They provide many benefits to plants and the environmental stability which includes nutrient uptake enhancement, drought tolerance, root pathogens, and soil aggregation improvement [2]. On the other hand, many categories of rhizospheric bacteria have the capacity of stimulating the plant development beyond the presence of the AMF. Moreover, mycorrhizal fungi interact and present complementary functions with beneficial soil organisms, including both free and nitrogen fixing bacteria, soil phosphate solubilizing bacteria (PSB), as well as the Plant Growth Promoting Rhizobacteria (PGPR) [3]. Furthermore, phosphate-solubilizing fungi and PSB could enhance P nutrition of plants through P solubilization and hence sustainable P recycling within soils. Also, many soil bacteria mobilize phosphate ions from organic and inorganic phosphorous sources such as

tricalcium phosphate, hydroxyapatite and rock phosphate [4,5]. Through the enhanced soil contact area by the arbuscular mycorrhizal hyphae, the plant has also greater access to orthophosphate and inorganic phosphate in the soil solution [2]. Phosphate solubilized by AMF and bacteria are taken up more efficiently by the plant through a mycorrhizal-mediated channel between the plant roots and surrounding soil. It has been reported that AMF and phyto-beneficial rhizobacteria could interact synergically to stimulate plant growth through a range of mechanisms that include improvement of nutrient acquisition and inhibition of fungal plant pathogens [6]. Thus, such microorganisms have been used as biofertilizers.

The property of peanut as a legume is its ability to develop root nodules and to fix atmospheric nitrogen (N<sub>2</sub>) in symbiosis with compatible rhizobia [7]. Since the peanut rhizosphere harbors a high diversity of phyto-beneficial bacteria with great potential to be used as inoculants [8,9], the objective of present study is to elucidate the effect of some bacteria estimated as PSB, in interaction with native AMF and rhizobia present in unsterilized soil, on nutrient uptake and plant growth of local variety of peanut cultivated in the Northwest of Morocco.

## 2. Materials And Methods

### 2.1. Soil Samples and Plant Material

The soil samples were collected from peanut field soil (20 cm of depth) in the site of Sidi-Elyamani (clay 15.23%,

silt 6.0%, sand 80.81%, pH (H<sub>2</sub>O) 5.00, 1.1%, P<sub>2</sub>O<sub>5</sub> 2.71 ppm, total nitrogen 8.5 ppm). This site contains important fields used for peanut agriculture in Northwestern Morocco. The soil was air dried, sieved on 2 mm mesh sieves and placed in favorable conditions throughout the duration of the study. The vegetal material used in this study was the local variety of peanut (*Arachis hypogaea* L.).

## 2.2. Inoculation of Seeds with PGPR

The bacterial strains used as inocula were 3 *Pseudomonas* (PP22, GP70 and GR1) and 2 *Aeromonas* (PR29 and GR70) selected for their multiple plant growth promoting (PGP) activities *in vitro* and their positive effect on rice growth [10,11]. 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. One germinated seed was sown in each plastic pot (18 cm diameter, 20 cm height) filled with 3 Kg of non-sterilized soil of Sidi-Elyamani site, and inoculated directly with 1.5 ml of fresh bacterial culture (10<sup>8</sup>cfu ml<sup>-1</sup>) grown in TSB (Tryptic Soil Broth). All pots were maintained at 28±2°C with 16 hours photoperiod and a light intensity of 400 µE m<sup>-2</sup> s<sup>-1</sup>. Four replications were prepared for each treatment.

## 2.3. Mycorrhizal and Plant Growth Parameters Study

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 [12] (Leaf area (cm<sup>2</sup>) = 0.70 (length x width) – 1.06). Then, plants were up rooted carefully from the soil and washed with water. The shoot weight was measured after oven drying at 62 °C for 72 hours. A part of the root of each plant was collected, cleared, stained [13] and finally mounted on slides. Quantification of arbuscular mycorrhizal infection and colonization was performed, using the notation scale of Trouvelot [14]. Parameters of mycorrhization were calculated with MYCOCALC

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

## 2.4. Shoot Mineral Analysis (N, P, K)

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

## 2.5. Statistical Analysis

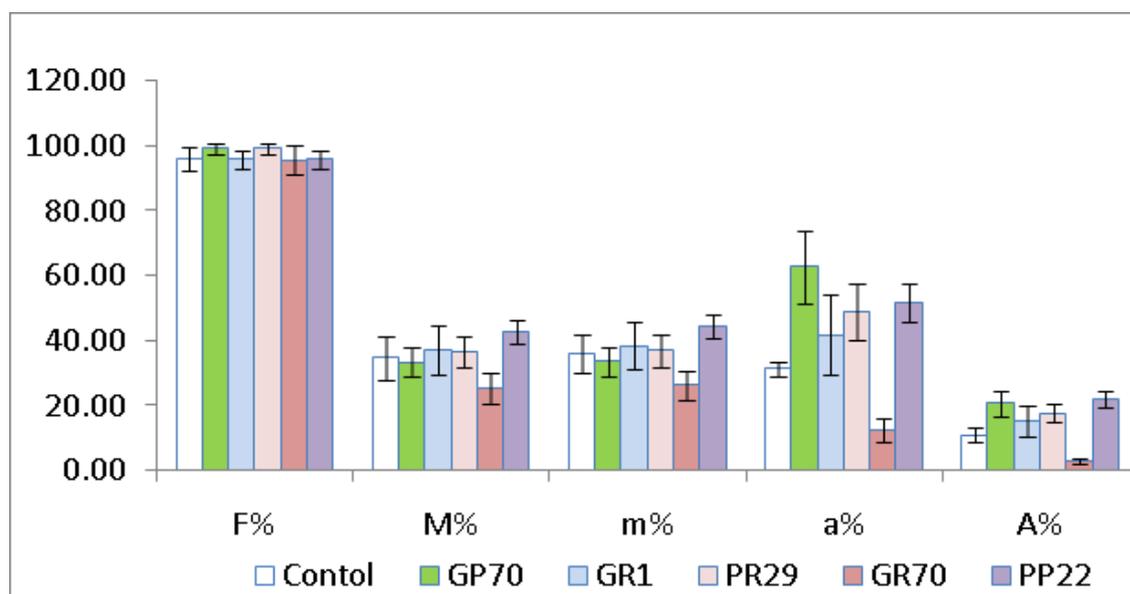
Statistical analysis of data were performed using ANOVA test. A p-value ≤0.05 was considered statistically significant. Data analysis was performed on mycorrhizal infection, vegetative growth and mineral nutrition.

## 3. Results and Discussion

Many works interested in the effects of PSB and symbiotic microorganisms on growth and yield of legume plants were performed. Present study leads to carry out the efficiency of 5 PSB in interaction with indigenous microorganism on nutrients uptake and growth of peanut in the Northwest of Morocco. Based on previous work [10,11], these PSB have different plant growth promoting activities (Table 1).

**Table 1. Some PGP activities of bacterial strains used in inoculation test**

PGP Activities	GP70	GR1	PR29	GR70	PP22
Mineral phosphate solubilization	+	+	+	+	+
indole acetic acid (IAA) production	+	+	+	+	+
Hydrogen cyanide (HCN) production	+	+	-	-	-
Siderophores production	+	+	+	+	+



**Figure 1.** Mycorrhizal parameters of peanut after PSB inoculations

### 3.1. Evaluation of Plant Mycorrhization

Plants inoculation with GP70, GR1, PR29 and PP22 had no significant effect on mycorrhizal frequency (F%) and intensity (M%) compared to control (Figure 1). However, these bacterial strains increased arbuscular abundance in the mycorrhizal root cortex (A% and a%), especially GP70, PP22 and PR29 which had a significant effect (Figure 1). Also, these 3 bacteria have a positive impact on root mycorrhization by increasing exchanging area between AMF and host plant (Figure 2). In this context, several types of interactions between bacteria and

AMF have been described [15]. The so-called mycorrhiza helper bacteria (MHB) have been shown to promote mycelial growth and mycorrhiza formation [16,17]. They can facilitate the arbuscular formations by a possible hydrolytic enzymes secretion, which causes 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. Thus, the AMF provide necessary mineral nutrients to plant in easily assimilated form via increasing arbuscular exchange area and mutualistic symbiotic relationships [18].

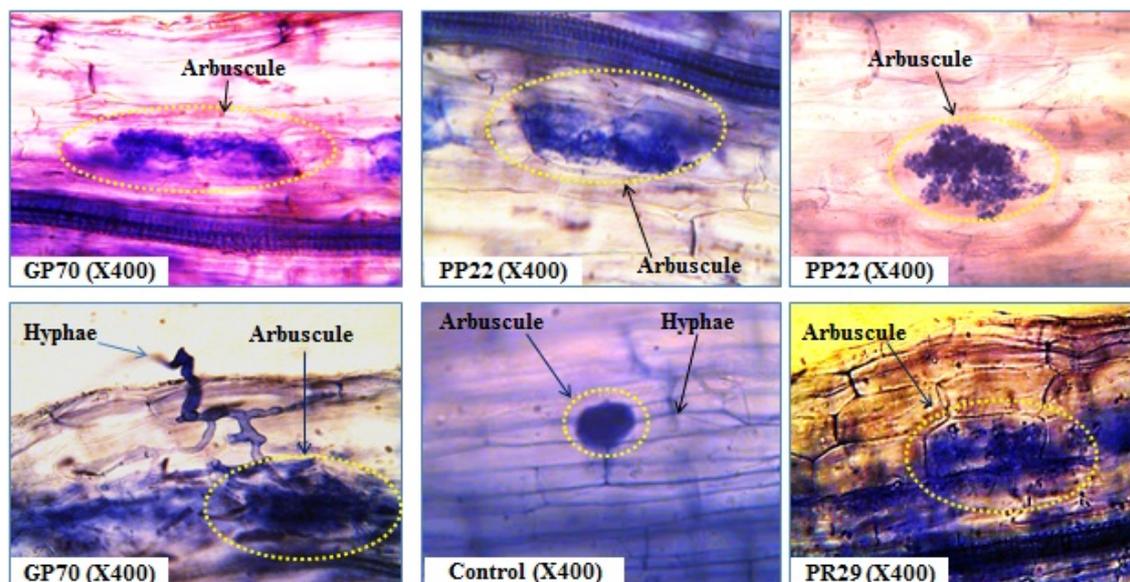


Figure 2. Mycorrhizal infection (Arbuscules) of peanut in response to PSB inoculation

### 3.2. Inoculation Effect on Nutrients Uptake and Peanut Growth

In response to PSB inoculation, the amounts of macronutrients in peanut shoots are shown in Table 2. All bacterial inoculations favored significantly the N, P and K contents, except GR70 that had no effect on N content. Plants treated with GP70 had the highest N content

(170.64% over control). Moreover, the maximum P and K contents were recorded for plants inoculated with GR1 (191.79% and 187.38% respectively) and PP22 with 172.26% over control for K uptake. This N, P and K uptake improvement obtained after PSB inoculation could lead to enhance multiple parameters including leaf area, shoot height as well as shoot and root weight of inoculated peanut plants.

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

Parameters	Control	GP70	GR1	PR29	GR70	PP22
N (mg plant <sup>-1</sup> )	14.53 <sup>c</sup> ±1.06	39.65 <sup>a</sup> ±0.63	28.79 <sup>c</sup> ±4.53	25.34 <sup>d</sup> ±2.72	14.30 <sup>c</sup> ± 1.02	32.72 <sup>b</sup> ±0.76
P (mg plant <sup>-1</sup> )	1.34 <sup>c</sup> ±0.14	2.49 <sup>b</sup> ±0.40	3.91 <sup>a</sup> ±0.92	2.72 <sup>b</sup> ±0.27	2.62 <sup>b</sup> ±0.77	2.58 <sup>b</sup> ±0.35
K (mg plant <sup>-1</sup> )	9.59 <sup>c</sup> ±0.48	21.58 <sup>bc</sup> ±6.38	27.76 <sup>a</sup> ±2.96	19.66 <sup>cd</sup> ±0.70	15.76 <sup>d</sup> ±4.33	26.11 <sup>ab</sup> ±4.16

Values in same line followed by different letter differ significantly.

Table 3. Effect of PSB inoculations on growth and yield of peanut plants

Parameters	Control	GP70	GR1	PR29	GR70	PP22
Nodules number	00 <sup>b</sup> ±00	14 <sup>a</sup> ±1.82	17 <sup>a</sup> ±5.68	05 <sup>b</sup> ± 1.63	00 <sup>b</sup> ±00	15 <sup>a</sup> ±6.21
Leaf area (cm <sup>2</sup> )	0.86 <sup>d</sup> ±0.16	4.53 <sup>a</sup> ±0.47	4.67 <sup>a</sup> ±0.19	2.40 <sup>b</sup> ±0.11	1.58 <sup>c</sup> ±0.08	4.41 <sup>a</sup> ±0.25
Root length (cm)	22.68 <sup>ab</sup> ±3.63	25.31 <sup>ab</sup> ±4.26	28.68 <sup>ab</sup> ±7.58	24.93 <sup>ab</sup> ±3.27	22.12 <sup>b</sup> ±4.97	30.50 <sup>a</sup> ±5.31
Shoot height (cm)	46.63 <sup>b</sup> ±1.88	48.50 <sup>ab</sup> ±2.64	52.63 <sup>a</sup> ±4.64	52.38 <sup>a</sup> ±3.30	45.75 <sup>b</sup> ±1.55	49.63 <sup>ab</sup> ±2.28
Fresh root weight (g)	0.33 <sup>a</sup> ±0.06	0.38 <sup>a</sup> ±0.04	0.40 <sup>a</sup> ±0.10	0.31 <sup>a</sup> ±0.04	0.21 <sup>b</sup> ±0.02	0.35 <sup>a</sup> ±0.03
Fresh shoot weight (g)	3.98 <sup>bc</sup> ±0.53	6.41 <sup>a</sup> ±0.97	5.95 <sup>a</sup> ±1.11	5.04 <sup>ab</sup> ±0.69	3.34 <sup>c</sup> ±0.52	6.06 <sup>a</sup> ±0.81
Dry root weight (g)	0.055 <sup>c</sup> ±0.008	0.098 <sup>a</sup> ±0.008	0.087 <sup>ab</sup> ±0.018	0.07 <sup>b</sup> ± 0.007	0.045 <sup>c</sup> ±0.006	0.08 <sup>b</sup> ±0.008
Dry shoot weight (g)	0.41 <sup>d</sup> ±0.05	0.93 <sup>a</sup> ±0.06	0.79 <sup>b</sup> ±0.14	0.65 <sup>c</sup> ±0.08	0.41 <sup>d</sup> ±0.04	0.82 <sup>b</sup> ±0.06

Values in lines followed by different letter differ significantly.

The results of growth parameters evaluation showed an improvement in all treatments, especially with *Pseudomonas* treatment (Table 3). The GP70, PP22 and GR1 strains favored significantly plant nodulation (between 14 and 17 nodules/plant) (Table 3), while no nodule was found in the roots of uninoculated control and plants inoculated with GR70 (Table 3). Besides, the nodules number recorded in plants inoculated with PR29 was low (5 nodules/plant) and didn't differ significantly with the control. Enhancement of nodules number by the 3 PSB (GP70, PP22 and GR1), which belong to *Pseudomonas* genera [11], might be attributed to high P-solubilizing ability and the adequate uptake of other nutrients (N and K) to roots. By increasing P availability and its uptake, these 3 P-solubilizing *Pseudomonas* (PSP) and AMF stimulated nodule formation and nitrogen fixation, while GR70 and PR29 did not affect this process.

All bacterial treatments increased significantly peanut's leaf area as compared to the non-inoculated control (Table 3) and maximum values were obtained with the same 3 PSP inoculants. Also, length of root and shoot was enhanced particularly in presence of GR1 and PR29 (52.63 and 52.38 cm respectively). Moreover, all treatments enhanced fresh weight of shoot and root, except GR70 that gave the lowest values even less than the control. Dry shoot weight augmentation was higher in the presence of GP70 (0.93 g plant<sup>-1</sup>, 126.82%), followed by PP22 (0.82 g plant<sup>-1</sup>, 100%) and GR1 (0.79 g plant<sup>-1</sup>, 92.68%). Also, maximum values of dry root weight were observed with GP70 and GR1, compared with other bacteria.

The stimulation of peanut growth could be a result of an advantageous synergic interaction between PSB and other indigenous microorganisms present in non-sterilized soil (especially native AMF and rhizobia). Correspondingly, dual inoculation of fruit crops like papaya with *Azospirillum* and AMF enhanced the total dry matter and leaf area than non-colonized plants [19]. Moreover, a recent study reported significant interaction of *Azotobacter chroococcum* and *Glomus mosseae* in pomegranate leading to better leaf area, shoot dry weight, and uptake of N, P and K compared to either AMF or bacterial inoculation alone [20].

Plants growth improvement is the result of an increase of cell division and cell expansion parameters in response to enhancement of nutrients uptake. So, primary plant reaction to P addition is expressed by an enhancement in shoot growth caused by increases in leaf expansion [21], while deficiency of phosphorus [22,23] or nitrogen [24] causes leaf growth reduction. Consequently, the promotion of root and shoot growth obtained in this study was more important in response to inoculation with *Pseudomonas* strains that gave the highest amounts of N, P and K uptake, compared with *Aeromonas* strains inoculants and the non-inoculated control.

Phosphorus is an important element that is also required for plant growth through photosynthetic energy production and carbohydrate transport [25] and thus, phosphorus limitation may affect photosynthesis and plant growth by means of changes in the activity of Calvin enzymes, ribulose-1,5-bisphosphate (RuBP) regeneration or Rubisco activity [25]. So, all the 5 bacterial strains used as inoculants in the present study were capable to solubilize mineral P [10,11]. Thus, the significant improvement of parameters growth found particularly in

the presence of *Pseudomonas* strains might be due to their higher ability to solubilize mineral phosphate in comparison with *Aeromonas* strains. Besides, numerous studies showed that the stimulation of root hairs growth and lateral roots elongation by secreting IAA provides more active sites and access for symbiotic associations with rhizobia and AMF. Hence, it seems that auxin levels in the host legume plants are necessary for nodule formation [26,27]. Consequently, the ability of these 3 *Pseudomonas* inoculants to produce IAA [11] may be greater than *Aeromonas* strains for improving weight and root architecture by stimulating the elongation of root hairs. Previous studies showed that PGPR can influence root architecture and promote shoot and root growth of plant by modifying hormonal signaling [28,29]. It has been reported as well that auxins excreted by rhizobacteria can improve root growth, resulting in an increased uptake of essential nutrients [30].

## 4. Conclusion

Results of the present work demonstrated the beneficial effects of P-solubilizing *Pseudomonas* inoculants (GP70, PP22 and GR1) on peanut plants. Thus, the presence of synergic interaction between P-solubilizing bacteria, indigenous AMF and rhizobia was found to have great importance to choose PSB as biofertilizers. Furthermore, exploiting these beneficial soil microbial populations is required in order to improve peanut production and realize a sustainable agriculture of this legume in the Northwest of Morocco.

## List of Abbreviations

AMF: Arbuscular Mycorrhizal Fungi  
 CNRST: National Center of Scientific and Technical Research  
 MHB: Mycorrhiza Helper Bacteria  
 PGPR: Plant Growth-Promoting Rhizobacteria  
 PSB: Phosphate Solubilizing Bacteria

## Competing Interests

The authors have no competing interests.

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