Effect of PGPR on growth and mycorrhization of KT22's peanut variety (*Arachis hypogaea* L.) grown in the northwest of Morocco

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Abstract

The plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) represent the two main beneficial biofertilizers for agroecological plant growth. Our work is to assess the impact of some bacteria, estimated as PGPR, in interaction with native mycorrhizal fungi on development and growth of peanuts cultivated in the northwest of Morocco. In this framework, the diversity and richness of soil by AMF was assessed by direct counting and morphological identification of spores. The seeds germinated peanuts are inoculated by four bacterial strains: GP70, PT66, ET76 and GT70. After one month of growth, the plant biomass and mycorrhizal parameters were evaluated. The number of AM fungal spores detected in field-collected soils was relatively high. The morphological characters indicated that the spore populations consisted of six morphotypes, of which five main genus have been identified: *Acaulospora*, *Scutellospora*, *Rhizophagus*, *Septoglomus* and *Glomus*. Microscopic observations of stained roots showed a significant increase in mycorrhizal and arbuscular intensity for plants inoculated with GP70, PT66 and ET76. However, only inoculation with GP70 promoted a significant increase in aerial dry biomass (ADB) of peanut. Inoculation of plants by GT70 has favored the development of nematodes in root. This phytoparasitic infection is accompanied by an increase in the dry root biomass and a significant reduction in the ADB. The improvement in ADB for plants inoculated with GP70, can be explained by a good synergy between this bacterial strain, native AMF and host plant.

Keywords: Peanut, AMF, PGPR, growth, nematodes.

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Introduction

Peanut is one of the most cultivated legume plants in the north west of Morocco. The peanut farming is conducted in irrigated sandy soils in the region bordering the Atlantic between Assilah and Kenitra. Agriculture in this region is based on an excessive use of pesticides and chemical fertilizers in order to ensure a high performance and to compensate for productivity losses. However, these farming practices have a very negative impact on both human health and the ecosystem balance. Indeed, large amounts of chemicals used to reconstitute the soil in N and P are much more expensive, causing more severe contamination to the environment. Thus, nitrate lixiviation (Dong et al., 2005) led to the degradation of water quality at several levels and particularly at water table levels.

On the basis of this situation, the exploitation of the bio-fertilizers, containing beneficial microorganisms to the plant, offers a promising agroecological alternative to chemical fertilizers by providing a healthy supply of mineral material needed to growth and development of plant. Thus, the rhizospheric microflora contributes to mineral water and plant nutrition in symbiotic relationship with the root system. it's particularly the case of AM fungi, which occupies a special position in these relationships. They develop a vast mycelium, in the soil up to 20 m/cm^3, modifying the root architecture (Gamalero et al., 2002), and increasing the volume of soil prospected by plant. AMF increases the ability of plants to uptake phosphorus and trace elements present in less mobile form and limited quantities in soil through specific transporters (Gianinazzi Pearson et al., 2000; Harrison, 2005). However, the presence of AMF in the rhizosphere is driven by profound changes in the structure of the microrhizospheric community.

It was shown that some bacteria are able to promote plant growth by improving the establishment or functionning of the symbiosis between the roots, nitrogen-fixing bacteria (Remans et al., 2007) and mycorrhizal fungi (Frey-Klett et al., 2007; Garbaye 1994; Pivato et al., 2009). Bacteria which have the ability to promote the establishment of mycorrhiza, by

enhancing contact and colonization of fungus with roots of host plants, are called MHB (for Mycorrhiza Helper Bacteria; Garbaye 1994).

The aim of this study is to assess the effect of inoculation of some bacteria estimated as PGPR on growth and arbuscular mycorrhizal of plants, shoot length and peanut's plant biomass.

Materials and Methods

Plant Material

Sampling of soil, cultivated previously by peanut, are performed in the first 20 centimeters deep in the Laaouamra region. The mixture of samples gave rise to the composite sample. The soil was air dried, sieved (mesh size, < 2 mm) and placed in favorable conditions throughout the duration of the study, for a better conservation of the microflora. The physicochemical analysis are given in Table 1. The plant material used in this study is the KT22 variety of peanut (*Arachis hypogaea* L.) which belongs to the botanical group Virginia commonly known in the region of Larache "Jumbo".

Extraction of spores and identification of AM fungi

One hundred grams of dry soil was wet sieved on 500 to 50 µm mesh sieves and extracted by wet sieving and decanting, followed by sucrose centrifugation for 2 min at 1000 rpm (Gerdeman & Nicolson, 1963). Spores were counted under a stereomicroscope and grouped according to their morphological characteristics. The richness of AM fungal spores was calculated per 100 g of dry soil.

Morphological characters (spore size and color) were assessed in water under a stereomicroscope and photographed (an average of 20 spores). Spore wall structures and other specific attributes were observed under a photonic microscope (connected to a computer with digital image analysis software) on permanent slides prepared according to Azcon-Aguilar et al. (2003). Spore identification was mainly based on morphological features, e.g. colour, size, wall structure and hyphal attachment. Morphotypes were classified to the genus level. The original descriptions of species and descriptions provided on the website of the INVAM (According to the latest update in July 2014) served as a reference for the identification exercise.

Inoculation of seedlings with PGPR

The bacterial strains used as PGPR are isolated from the rhizosphere of three varieties of rice (Puntal, Elio, and Guadiamar). There are two *Pseudomonas* (GP70 and ET76) and two *Aeromonas* (GT70 and PR70). All these bacterial strains have the ability to solubilize the inorganic phosphate and to secret indole acetic acid (IAA), siderophores and hydrocyanic acid (Aarab,. 2013). The peanut's seedlings, previously disinfected and germinated, are inoculated with 1.5 ml of each bacterial strain.

Mycorrhizal parameters and plant growth

Plants were harvested one month after sowing in the light chambre under controlled conditions with day/night temperatures of 28/22°C and a 16/8 h photoperiod. Lengths of shoot and root portion are measured. Root and aerial dry biomass are measured after drying at 62°C for 72 hours. A part of 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.

Statistical analysis

Four replicates were analyzed for each treatment and all results were statistically compared by ANOVA test. A p-value ≤0.05 was considered to be statistically significant.

Results

Properties of soil

The physico-chemical analysis of soil, summarized in table 1, show a sandy texture with a slightly acidic pH. The soil is generally very poor in available phosphorus and potassium.

Table 1 : physicochemical characteristics of soils

Texture %					pH (water)	pH (KCl)	OM (%)	P_2O_5 (ppm)	K_2O (ppm)	N Total (ppm)
C*	FS*	CS*	FS *	CS *						
10,10	5,05	0,61	52,22	28,59	6,1	6,3	1,1	42	103	50

(*) C : **Clay** ; FS : **Fine silts**; CS: **Coarse silts**; FS: **Fine sand**; CS: **Coarse sand**

Richness, diversity and identification of AMF spores

The evaluation of mycorrhizal spores potential in the rhizosphere of peanut shows a density of 5834 spores/100 g of soil; spores extracted have generally a spherical shape (Fig.1).

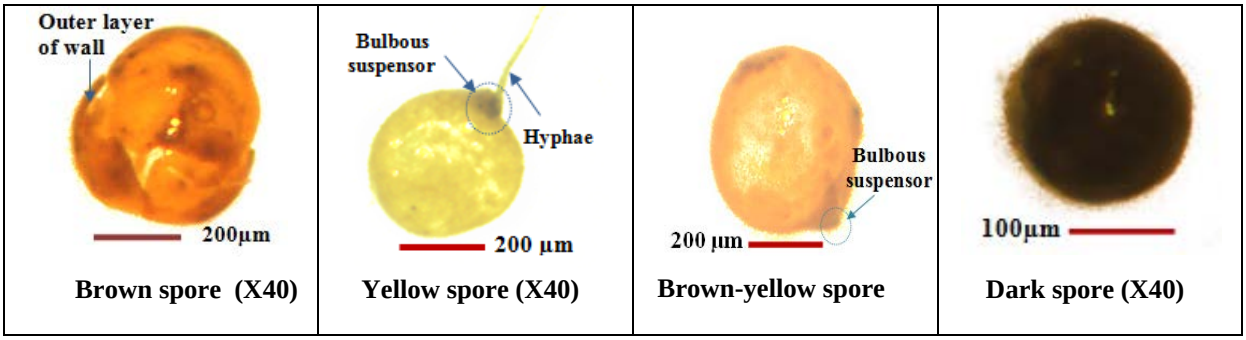
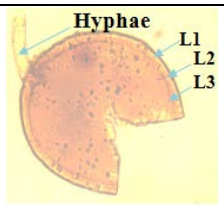
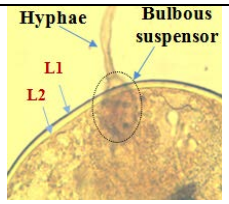
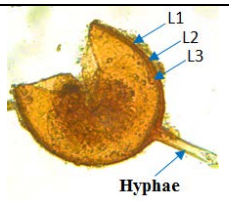
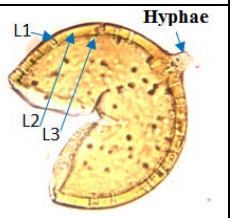


Figure 1 : Various types of AMF spores identified.

A detailed analysis of the morphological characteristics of this spore community revealed the presence of five genera (Table 2).

Table 2 : morphological characteristic of isolated AMF spores

Morpho-types	Shape/Color	Diameter (µm)	Spore wall structure	Genra	Coloration in PVLG (X400)
1	Globose/ Brown	200-350	Three layers	*Acaulospora*	
2	Globose, subglobose/Yellow	300-400	Two layers	*Scutellospora*	
3	Globose and regular /Brown	150-400	Three layers	*Glomus*	

4	Globose, subglobose /Dark	60-280	---	*Septoglomus*	
5	Globose / Brown- yellow	200-300	Three layers	*Rhizophagus*	

Endomycorrhizal infection in the rhizosphere of peanuts

One month after sowing, root examination showed that all plants were mycorrhized (Fig.2) and mycorrhizal colonisation rate varies between 78.75 and 96.08%, depending on bacterial inoculation (Fig. 3). Plants inoculated with GP70, ET76 and PT66 are densely colonized. They have significantly enhanced the intensity of colonization of the root cortex (M% and m%) with typical mycorrhizal structures (Fig. 2).

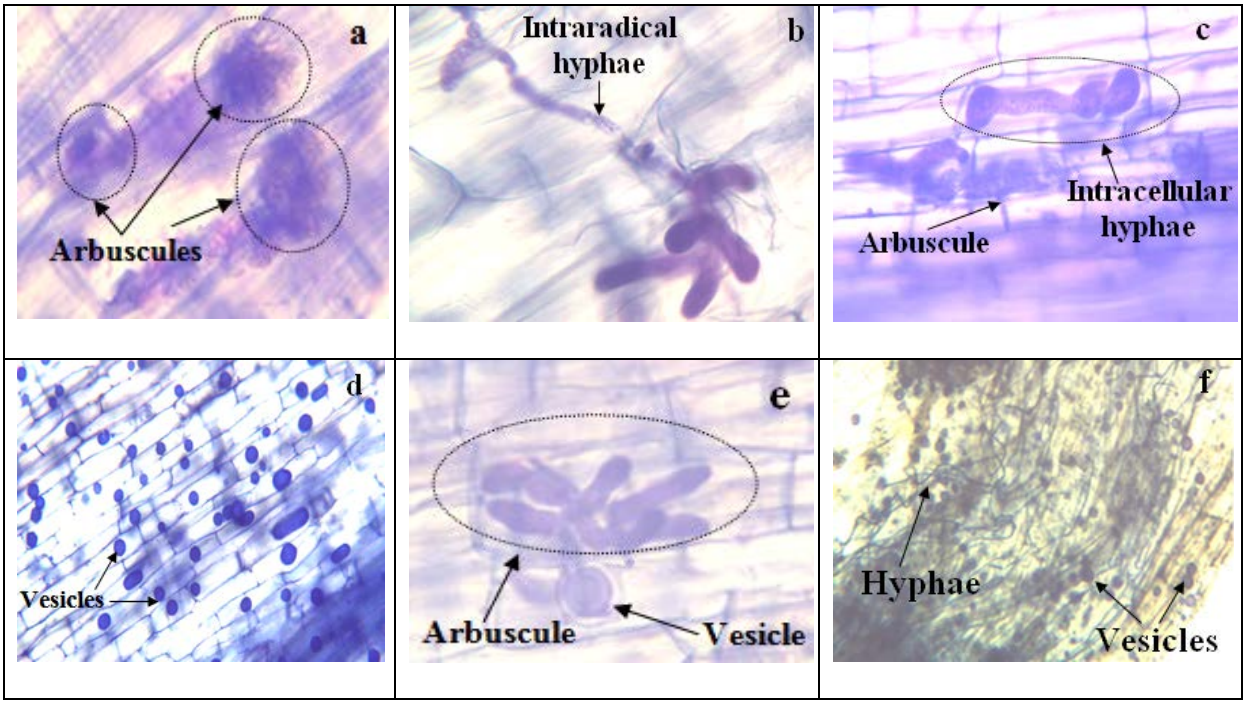


Figure 2 : Mycorrhizal infection of peanut in response to bacterial inoculation:
a : GP70 (X400) ; b : ET76 (X400) ; c : PT66 (X400) ; d : Control (X100) ; e : ET76 (X400) ; f : GP70 (X100)

Inoculation with GP70 promoted a significant increase in arbuscular abundance in the mycorrhized root cortex (A%). Analysis showed a significant reduction of all mycorrhization parameters of plants inoculated with GT70. Also, microscopic observations proved that inoculation with ET76 and PT66 (Fig. 2) privileges distinguished hyphae and arbuscules, compared to the control and other inoculations . Difference is marked by a development of hyphae diameter and a remarkable reduction of arbuscular ramifications.

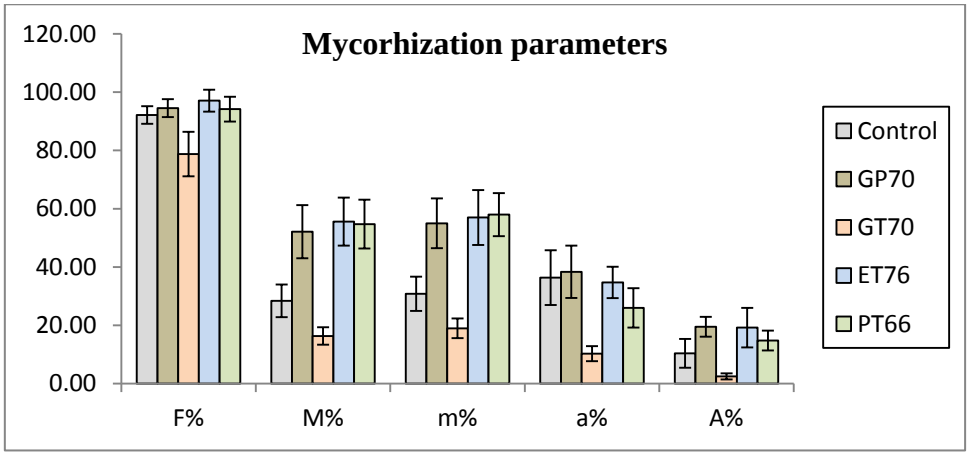


Figure 3 : Graphical presentation of mycorrhization parameters.

Plant biomass

The analysis showed that peanut growth responds differently to bacterial inoculum. Indeed, inoculation with PT66 and GT70 has significantly reduced shoot length compared to control. Furthermore, a modification of root architecture is observed for plants inoculated with GP70 and GT70. This modification is characterized by the increasing of lateral root branching and the development of root hairs for plants inoculated with GP70. On the contrary, the shortening of the root system for plants inoculated with GT70 is accompanied by the formation of galls and an increase of root drying. Thus, the observation under a stereomicroscope showed an abundance of microscopic nematodes on roots of plants inoculated with this bacterial strain; nematodes have been detected also at intracellular level during microscopic observation of root fragments, mounted between slide and cover slip (Fig.4).

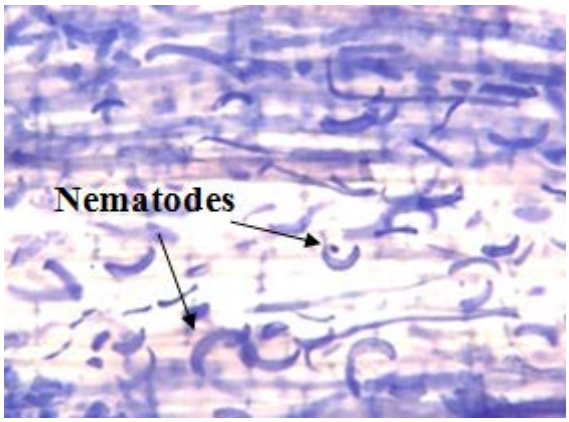


Figure 4: Intracellular nematodes at plant root level inoculated by GT70.

The peanut's aerial dry biomass (ADB) is significantly favored by GP70 bacterial strain (Fig.5). However, inoculation of plants with ET76 and GT70 has negatively influenced this parameter. As for root dry biomass (RDB), the only difference is the significant increase of this parameter after inoculation with GT70.

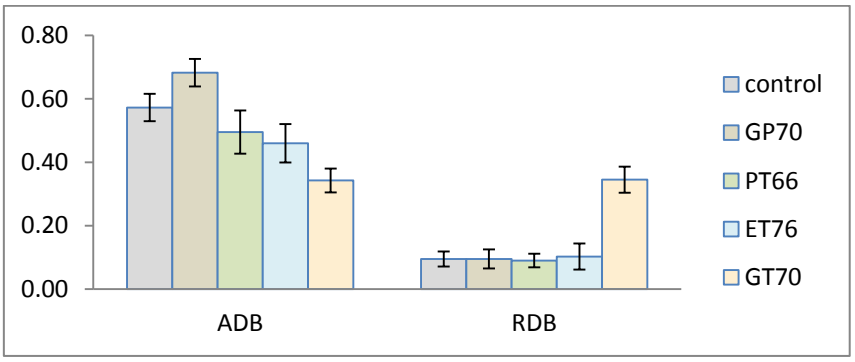


Figure 5 : Aerial and root dry biomass (g).

Discussion

This study showed that the density of spores in the sandy soil studied is very important in comparison with other bibliographical data. It indicates a high mycorrhizogenic potential since the number of spores per 100 g of soil is 5834. This is to say, the presence of various spores in soil, the diameter of which is between 40 and 500 µm (black, yellow, brown, brown-yellow), confirms with Li et al. (2007) that the soil collected just after culture gives

more density and diversity of AM spores. The identified genera belong to three different families, the largest proportion of which belongs to the families of Gloméraceae, when it comes to *Glomus sp*, *Septoglomus sp* and *Rhizophagus sp*. The two families of Acaulosporaceae and Gigasporaceae are represented by *Acaulosora sp* and *Scutellospora sp*.

The number of spores and mycorrhizal colonisation rate are typically higher in sandy soils, compared to clay soils (Kabir et al., 1997). Moreover, the content of the soil studied in organic matter (1.1%) can promote: (1) the modification of its physicochemical characteristics by increasing porosity and water holding capacity, Labidi (2007); (2) improving the availability of nutrients for the AMF (Shrestha Vaidya et al., 2008; Hammer et al, 2011); (3) the introduction of bacteria that facilitate proliferation of AMF (Gryndler et al, 2009); and (4) incorporation of compounds released during the decomposition of organic matter, some of which are produced by other microorganisms (Gryndler et al, 2009). Furthermore, low levels of available phosphorus in soil (P2O5 = 42 ppm) often explains its richness in AMF spores (Chelius and Triplett, 1999; Troech and Loynachan, 2003). Arbuscule abundance and intensity of mycorrhizal colonisation reach significantly higher levels for plants inoculated with GP70, PT66 and ET76. These inocula have a large capacity to promote mycorrhizal symbiosis by improving the contact and root colonization of plants. We can say that these bacteria belong to "mycorrhiza helper bacteria" (MHB) (Frey-Klett, P. et al, 2007;. Garbaye 1994). These rhizobacteria can enhance the germination of spores and mycelium growth by producing growth factors (Garbaye 1994). Indeed, they have the ability to stimulate the expansion of mycelium and to increase the chance of fungus-root contact. They are capable of facilitating the root colonization by a possible hydrolytic enzymes secretion which causes dilatation of the cortical cells by offering a greater intercellular surface. Thus, the AM fungi can easily penetrate through this space to colonize the roots, procuring more nutrients to the plant (Mamatha et al., 2002).

Both bacteria ET66 and PT66 develop thicker hyphae (runner hyphae (RH)) and arbuscules that can make a limited exchange surface with the host plant. In contrast, inoculation with GP70 has promoted the development of fine hyphae (fine branching (FB)) and arbuscles to thin and numerous ramifications. These extra and intraradicular structures can develop a very large exchange surface with the outside environment and cortical cells by ensuring a good mineral nutrition to the plant (Judge et al., 2009). The solubilizing activity of phosphate by GP70 and the strong exploration of the surrounding environment by fine branching of AMF may explain the increase in aerial dry biomass (ADB) of plants inoculated with GP70.

However, reduction of the ADB of plants inoculated with ET66 and PT66 is strongly linked to the nature of AMF implemented for the symbiotic establishment with plants. Thus, we can say that these inocula may foster and promote certain kinds of mycorrhizal fungi than some others. These results confirm the work which has shown that *Pseudomonas fluorescens* is able to promote root colonization by *Glomus mosseae* (Pivato et al., 2009). ADB reduction of plants inoculated with PT66 and ET76 can be explained also by the inhibitory action of native plant growth beneficial bacteria by this inoculum (Bakker et al., 2007). Root length reduction is compensated for plants inoculated with GP70 by a significant development of the root hairs via secretion of indole acetic acid (IAA). It has been assumed that the production of bacterial auxin leads to the proliferation of lateral roots, which results in better root surface area allowing the plant to absorb more nutrients and water from soil (Lambrecht et al . 2000; Bertrand et al., 2001; Vessey and Buss 2002; Taiz & Zeiger, 2010), which explains the improvement of ADB for peanut plants treated with GP70. The significant decrease in root length for plants inoculated with GT70 could be explained by the beneficial effect of mycorrhization. However, this reduction has been accompanied by a remarkable reduction in ADB and a net increase in RDB. Significant development of microscopic nematodes, either at the intracellular level or on the roots, as well as the formation of galls, have caused the disappearance of root hairs and principal root drying up. As a consequence, There was a disruption of water and mineral absorption of plant (Kerry and Bourne, 2002). Indeed, low mineral absorption and the mobilization of photosynthates towards roots, offsetting the nutritional needs of nematodes, may explain the reduction of air dry biomass of the plant. So, GT70 bacteria do not have a nematicidal activity against this type of phytoparasite. In addition, they can act directly on the abundance of nematodes in soil by secreting enzymes facilitating hatching their eggs.

Conclusion

This study shows that the KT22 variety of peanut is much more mycotrophic legume. Nevertheless, the growth of peanut in symbiotic relationship with AMF depends on the type of rhizobacteria used as inoculum. In fact, rhizobacteria can be beneficial, neutral, or can affect negatively the functionality of mycorrhiza and plant growth. Indeed, the inoculation of peanut with GT70 promotes the appearance of plant parasitic nematodes, which have a

negative effect on plant growth. On the other side, GP70 could be used as a main component of biofertilizers able to improve mycorrhization and yield of peanut.

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