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Response of *Acacia senegal* (L.) Willd. seedlings and soil bio-functioning to inoculation with arbuscular mycorrhizal fungi, rhizobia and *Pseudomonas fluorescens*

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*(L.) Willd. Acacia senegal* is a multipurpose legume that is economically and ecologically important in Sahelian areas. The effects of arbuscular mycorrhizal fungal (AMF) inoculum (M) individually and in combination with rhizobia (R) and *Pseudomonas fluorescens* (Pf) were investigated on the growth and nutrition of *A. senegal* seedlings. In glasshouse conditions, all the inocula except Pf significantly enhanced *A. senegal* plant growth after 6 months in a non-sterile soil from Dahra (Senegal). However, no significant increase in shoot N content was recorded, whereas the application of MR, MP, and MRP significantly improved shoot P content, and that of MR and MP, shoot K content. The nodule number was significantly augmented by rhizobial inoculation and the root mycorrhizal colonization rate by MR, MP, and MRP treatments. Soil spore density was increased by all inoculants except MP, and soil AMF hyphal length by M, R and MRP, treatments. The inoculation with *Pseudomonas* and MP significantly stimulated soil acid phosphatase activity, but no significant effect was observed on soil fluorescein diacetate (FDA) activity. Thus, the dual inoculation with AMF and R can be beneficial to *A. senegal* growth under non-sterile soil, where nutrients particularly P and N are often deficient.

Key words: *Acacia senegal*, arbuscular mycorrhizal fungi, rhizobia, *Pseudomonas fluorescens*, plant growth, soil bio-functioning.

INTRODUCTION

The rhizosphere is a complex habitat with interferences between plants and their environment in relation to biotic and abiotic factors. Soil microorganisms play a key role in biogeochemical processes impacting plant nutrition and leading to plant productivity and soil fertility improvement (Lynch, 1990). Introduced microorganisms can affect plant growth and health, and also soil quality. Thus, any
change in microbial communities might affect plant-microorganism symbiosis (Abbott and Robson, 1991). Moreover, the effects of interactions between plant and symbiotic or other beneficial rhizosphere bacteria and fungi, such as nitrogen-fixing bacteria, plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) depend on the type of microorganisms, and on soil and environmental conditions (Qiu et al., 2008). AMF and rhizobia (R) are the most frequent soil symbiotic microorganisms interconnecting the roots and the soil system (Koide and Mosse, 2004). The AMF modify plant growth and alter rhizosphere processes by increasing plant nutrient uptake specifically Phosphorus and influencing rhizode position by the plant (Richardson et al., 2009). R influences plant nutrition, growth and productivity, and also plant resistance to biotic and abiotic factors by increasing atmospheric nitrogen fixation (Vessey, 2003). Pseudomonas fluorescens (P; PGPR) are microorganisms which rigorously colonize roots and provide beneficial effects to plant development (Lucy et al., 2004). These bacteria are known to stimulate plant growth directly by solubilization of minerals and production of plant growth regulators, or indirectly by transformation of toxic metabolites and stimulation of legume nodulation (Roest et al., 2006). Several studies have demonstrated the synergistic effect of the interaction between AMF and R (Diouf et al., 2005; Lalitha et al., 2011). But the literature reveals that it can be also negative (Aysan and Demir, 2009). Synergistic (Sanchez et al., 2004) and antagonistic (Requena et al., 1997) effects of interactions between AMF and PGPR have also been reported in several reviews.

In the Sahelian areas of Africa, several decades of drought and over exploitation of natural resources have led to dramatic deforestation. To reverse the trend, numerous leguminous trees like Acacia senegal have been planted on large-scale for soil fertility replenishment and biodiversity preservation, sustainable commercial supply of 'gum-arabic' and wood for various end uses. This legume is able to form symbiotic associations with microorganisms such as R for improving atmospheric nitrogen fixation; and also with AMF, therefore affecting soil mycorrhizal infectivity.

Many studies have shown that the use of symbiotic microorganisms can enhance plant productivity, re-establish degraded habitats, and phytoremediate polluted soils (Khan, 2005). However, little is known on the contribution of each soil microorganism alone or in combination on the development of forest trees and on soil quality, especially in tropical regions of Africa. To fully understand the contribution of AMF on plant growth, it is important to consider their interactions with other soil microorganisms (Purin and Rillig, 2008). The objective of our study was to evaluate the effectiveness of triple inoculation (arbuscular mycorrhizal fungi, rhizobia and Pseudomonas fluorescens) on Acacia senegal seedlings growth and nutrition, and on soil mycorrhizal potential and enzyme activities.

### MATERIALS AND METHODS

#### Production of AMF inoculum

The AMF inoculum (M) used in this study contained four species: Glomus intraradices, Glomus globiferum, Glomus sp. and Gigaspora gigantea. They were isolated from the rhizospheric soil of an A. senegal plantation in Goudiry (South-East of Senegal, 800 mm / year of rainfall). These AMF species were identified by morphological and molecular tools. For AMF propagation, soil samples collected from the rhizosphere of A. senegal trees were pooled and 250 g of the composite soil sample were placed into containers filled with 1 kg of sterilized (121°C, 2 h) sand. Three replicates were performed and seedlings of Zea mays (L.) served as host plant. Before sowing, maize seeds were surface disinfected by immersion in a 0.5% sodium hypochlorite stirred solution for 15 min and washed 3 times with sterile distilled water. Five seeds were subsequently sown at 2 cm depth in each container and covered with sterilized sand. Seedlings were watered every 2 days with demineralized water and, once a month they were fertilized with a 100 ml Long Ashton’s nutritive solution per container. After 3 months, the plants were uprooted, and the roots cut into 0.5 cm pieces were mixed with the soil from trap culture. The M consisting of a mixture of sand with an average of 20 spores/g of soil and 80% colonized mycorrhizal roots, was conserved at 4°C.

#### Production of bacterial inocula

**Rhizobial inoculum**

The four rhizobial strains (ORS 3574, ORS 3593, ORS 3607 and ORS 3628) used in this experiment were isolated from rhizospheric soils of Dahra and Goudiry either by trapping or in situ. They were selected for their symbiotic infectivity and efficiency in controlled conditions. Each strain was grown in YEM liquid medium (Vincent, 1970) for 5 days at 28 ± 2°C with rotator shaking at 150 rpm. The four individual grown cultures were mixed in equal proportions to obtain the rhizobial inoculum (approximately 10^8 cells/ml).

**Pseudomonas fluorescens inoculum**

Five strains of (P; PGP 21, PGP 22, PGP 25, PGP 28 and PGP 31) were isolated from rhizospheric soil of an A. senegal plantation in Dahra. They were then characterized for their capacity to promote phosphate solubilization and siderophore production (Ponmurugan and Gopi, 2006). Each strain was grown in King’s B liquid medium (King et al., 1954) on a rotary shaker for 3 days at 28 ± 2°C. Equal proportions of cultures were mixed to obtain the PGPR inoculum (approximately 10^8 cells/ml).

#### Characterization of soil substrate

The soil substrate was collected from Dahra (in Senegal) from 0 to 25 cm depth and sieved through a 4 mm mesh and homogenized. The physicochemical characteristics of the soil sample were determined at Laboratoire des Moyens Analytiques, IRD, Dakar, Senegal, LAMA; certified ISO 9001, version 2000): pH (H2O) 5.71, clay 2.6%, fine silt 2.74%, coarse silt 7.56%, fine sand 51.27%, coarse sand 35.83%, carbon 0.41%, nitrogen 0.05%, total phosphorus 33.12 mg/kg, available phosphorus 12.66 mg/kg, Calcium 0.56%, Magnesium 0.29%, Sodium 0.09% and Potassium 0.19%.

#### Experimental design

The experiment was set up in a glasshouse (30/25°C day/night, 16 h photoperiod) in the experimental station of IRD Institute in Dakar-
Senegal (Latitude 14°44'N - Longitude 17°30'W). Seeds of A. senegal (originating from Dahra, Latitude 15° 21' N - Longitude 15° 29' W), were chemically scarified with concentrated sulfuric acid (95%) for 14 min, rinsed off 6 times with sterile distilled water and pre-germinated overnight in agar gel at 37°C. Two pre-germinated seeds were sown into each polyethylene bag (23.5 × 9.5 cm) containing 800 g of non-sterile soil substrate slightly moistened to 50% water-holding capacity (for example, 5 g H₂O per 100 g soil). 20 g of the M were applied at 5 cm below the seedlings at sowing. One (1) week after planting, thinning was done and each plant was inoculated with 5 ml of rhizobial and/or P₃ inoculum. Seven treatments were performed: control, inoculation with M, R, P₃ and the following combinations MR, MP₃ and MRP₃. Plants were arranged in a completely randomized block design with 10 replicates per treatment and watered every 2 days during 6 months experimentation.

**Assessment**

After 6 months of culture, plants were harvested and the root nodules per plant were counted. The dry weights of shoots and roots (oven-dried at 70°C for 48 h) were determined. For each plant, the nutrient contents (total N, total P and total K) were quantified in shoot dry matter at LAMA. After drying, leaf tissues of each plant were ground, ashed (500°C), digested in 2 ml HCL (6N) and 10 ml HNO₃ (15N), then analyzed by colorimetry for P and by flame emission for K. Plant tissues were digested in 15 ml H₂SO₄ (36 N) containing 50 g l⁻¹ salicylic acid for N (Kjeldahl) determination.

For each plant, fine roots of A. senegal plants were collected and gently washed. Roots were then cleaned in KOH (10%) and stained with 0.05% Trypan blue following the method described by Phillips and Hayman (1970). Roots were then cut into 1 cm pieces, mixed and placed on slides for microscopic observation at 250X magnification. The intensity of mycorrhization (corresponding to the proportion of cortex colonized by the AMF) was assessed on about 50 root pieces as described by Trouvelot et al. (1986).

Soil mycorrhizal potential and enzyme activities were evaluated in soils at the end of the experiment. For each treatment, a composite soil sample obtained by pooling the rhizospheric soil of the 10 plants was stored at 4°C in plastic bag. Three replicates of soil samples were performed for each treatment.

AMF spores were extracted from a 50 g soil sample by wet sieving, decanting and sucrose centrifugation (Gerdemann and Nicolson, 1963). Then, the supernatant was poured successively through 50, 100, 200 and 400 μm pore-size sieves, and rinsed with running tap water. Spore density (total number of spores in 50 g dry soil) was determined by counting spores with a normal appearance under a compound microscope (40X).

Hyphae were extracted from 0.5 g of soil sample by aqueous membrane-filtration, and subsequent microscopic examination. The AMF hyphae were recognized following the morphological criteria described by Nicolson (1959). The total AMF hyphal length was estimated using the Gridline intersect method (Hanssen et al., 1974).

Soil microbial functioning was determined by fluorescein diacetate (FDA) and acid phosphatase activities. FDA (3.6'-diacetoyfluorescein) hydrolysis was evaluated as described by Alef (1998) in which fluorescein liberated was assayed after 1 h of soil incubation at A = 490 nm. The total microbial activity was expressed as μg of product corrected for background fluorescence g⁻¹ h⁻¹ soil. Potential acid phosphatase activity was measured in soil samples from all treatments with 5 mM disodium p-nitrophenyl phosphate tetrahydrate solution (Tabatabai, 1994) at 37°C and expressed as μg p-nitrophenol g⁻¹ h⁻¹.

**Statistical analysis**

All data were subjected to an analysis of variance (ANOVA) using the XLSTAT software version 2010 in order to assess the effects of microbial inoculation on plant growth and nutrition, soil mycorrhizal potential and enzyme activities. Means values of these parameters were compared using the Student-Newman-Keuls multiple range test (P < 0.05). Percentage data of root mycorrhizal colonization were arcsine transformed prior to analysis.

A principal component analysis (PCA) was carried out on the basis of the inoculation treatments and measured parameters using the same software. The parameters with the treatments were projected on the first two axes of the factorial plane in order to group the treatments with their similar characters.

**RESULTS**

**Growth and shoot nutrient contents of Acacia senegal seedlings**

After 6 months, all the inoculants, except *Pseudomonas* alone (P₃) significantly increased the shoot, root and total dry weight of A. senegal seedlings. For instance, the inoculations enhanced the total dry weight of plants by 64.55% (M), 81.02% (R), 82.91% (MR), 22.78% (MP₃) and 46.20% (MRP₃). The highest values were obtained with the treatments MR, R, M and MRP₃ and the lowest values with the treatment consisting of inoculation with *Pseudomonas* which decreased the total dry weight of plants by 25.95%. However, the shoot height was not significantly augmented, whatever the inoculation treatment (Table 1).

Although, there were no significant differences for the shoot N content between inoculated and control seedlings, an increasing trend was observed with the MR treatment. The shoot P content was significantly improved with the treatments MR, MRP₃ and MP₃ with augmentations of 32.48, 32.56 and 45.83%, respectively. The MR treatment was the most effective inoculum in raising the content of shoot K (+25.11%). On the other hand, the seedlings inoculated with *Pseudomonas* alone showed lower N and P contents than the controls (Figure 1).

**Nodulation and mycorrhization of Acacia senegal seedlings**

Nodule number and mycorrhizal parameters are shown in Table 2. The number of nodules per plant was significantly increased after inoculation with the rhizobial strains. The best response on AMF root colonization was obtained with the treatment MR and the lowest with the treatment P₃ (52.1 and 9.88%, respectively). The inoculation with MR, MP₃ and MRP₃ significantly improved the rate of root mycorrhizal colonization.

The application of all inocula except MP₃ significantly augmented the soil spore density as compared to the control. The soil AMF hyphal length was significantly enhanced after inoculation with M, R and MRP₃ treatments.

**Soil enzyme activities**

Although significant differences were observed between
Table 1. Effects of microbial inoculation on growth of *A. senegal* seedlings cultivated under non-sterile soil in glasshouse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot height (cm)</th>
<th>Shoot dry weight (g/plant)</th>
<th>Root dry weight (g/plant)</th>
<th>Total dry weight (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.00 ± 2.00</td>
<td>0.66 ± 0.03</td>
<td>0.92 ± 0.09</td>
<td>1.58 ± 0.11</td>
</tr>
<tr>
<td>M</td>
<td>35.23 ± 1.17</td>
<td>0.88 ± 0.08</td>
<td>1.73 ± 0.11</td>
<td>2.60 ± 0.18</td>
</tr>
<tr>
<td>R</td>
<td>32.92 ± 1.49</td>
<td>0.93 ± 0.05</td>
<td>1.90 ± 0.13</td>
<td>2.83 ± 0.16</td>
</tr>
<tr>
<td>P&lt;sub&gt;t&lt;/sub&gt;</td>
<td>32.66 ± 1.25</td>
<td>0.52 ± 0.02</td>
<td>0.63 ± 0.06</td>
<td>1.17 ± 0.08</td>
</tr>
<tr>
<td>MR</td>
<td>37.02 ± 1.20</td>
<td>0.97 ± 0.03</td>
<td>1.92 ± 0.12</td>
<td>2.89 ± 0.12</td>
</tr>
<tr>
<td>MP&lt;sub&gt;t&lt;/sub&gt;</td>
<td>35.74 ± 1.33</td>
<td>0.74 ± 0.04</td>
<td>1.21 ± 0.14</td>
<td>1.94 ± 0.16</td>
</tr>
<tr>
<td>MRP&lt;sub&gt;t&lt;/sub&gt;</td>
<td>37.97 ± 1.10</td>
<td>0.84 ± 0.05</td>
<td>1.47 ± 0.10</td>
<td>2.31 ± 0.14</td>
</tr>
</tbody>
</table>

Means (n = 10) ± standard errors are given. In columns, values followed by the same letter are not significantly different according to the Newman-Keuls test (P < 0.05). M, AMF inoculum; R, rhizobia; P<sub>t</sub>, *P. fluorescens*.

Table 2. Effects of microbial inoculation on nodule and mycorrhizal parameters of *A. senegal* seedlings grown under non-sterile soil in glasshouse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodule number/plant</th>
<th>Intensity of mycorrhization (%)</th>
<th>Spore number (50 g of soil)</th>
<th>Hyphal length (cm g&lt;sup&gt;−1&lt;/sup&gt; soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.40 ± 0.16</td>
<td>16.61 ± 2.06</td>
<td>262.67 ± 7.62</td>
<td>4.00 ± 1.15</td>
</tr>
<tr>
<td>M</td>
<td>1.60 ± 0.72</td>
<td>21.14 ± 1.94</td>
<td>405.67 ± 7.62</td>
<td>16.77 ± 1.76</td>
</tr>
<tr>
<td>R</td>
<td>4.60 ± 0.76</td>
<td>17.56 ± 1.34</td>
<td>404.33 ± 4.10</td>
<td>18.00 ± 1.15</td>
</tr>
<tr>
<td>P&lt;sub&gt;t&lt;/sub&gt;</td>
<td>0.30 ± 0.15</td>
<td>9.88 ± 0.76</td>
<td>283.67 ± 2.65</td>
<td>9.33 ± 0.67</td>
</tr>
<tr>
<td>MR</td>
<td>2.40 ± 0.83</td>
<td>52.10 ± 2.83</td>
<td>549.00 ± 6.39</td>
<td>11.33 ± 1.76</td>
</tr>
<tr>
<td>MP&lt;sub&gt;t&lt;/sub&gt;</td>
<td>2.20 ± 0.88</td>
<td>48.01 ± 4.38</td>
<td>257.00 ± 3.79</td>
<td>6.00 ± 2.00</td>
</tr>
<tr>
<td>MRP&lt;sub&gt;t&lt;/sub&gt;</td>
<td>0.50 ± 0.31</td>
<td>33.05 ± 2.28</td>
<td>352.00 ± 6.66</td>
<td>20.67 ± 3.71</td>
</tr>
</tbody>
</table>

Means (n = 10) ± standard errors are given. For spore number and hyphal length, n = 3. In columns, values followed by the same letter are not significantly different according to the Newman-Keuls test (P < 0.05). M, AMF inoculum; R, rhizobia; P<sub>t</sub>, *P. fluorescens*.

Table 3. Effects of microbial inoculation on soil enzyme activities under non-sterile conditions in glasshouse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FDA activity (µg FDA h&lt;sup&gt;−1&lt;/sup&gt; g&lt;sup&gt;−1&lt;/sup&gt; of soil)</th>
<th>Acid phosphatase activity (µg pNPP h&lt;sup&gt;−1&lt;/sup&gt; g&lt;sup&gt;−1&lt;/sup&gt; of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.55 ± 0.07</td>
<td>21.59 ± 1.59</td>
</tr>
<tr>
<td>M</td>
<td>1.31 ± 0.05</td>
<td>42.22 ± 4.99</td>
</tr>
<tr>
<td>R</td>
<td>1.37 ± 0.05</td>
<td>58.41 ± 2.57</td>
</tr>
<tr>
<td>P&lt;sub&gt;t&lt;/sub&gt;</td>
<td>1.52 ± 0.03</td>
<td>258.73 ± 2.18</td>
</tr>
<tr>
<td>MR</td>
<td>1.58 ± 0.01</td>
<td>93.96 ± 1.93</td>
</tr>
<tr>
<td>MP&lt;sub&gt;t&lt;/sub&gt;</td>
<td>1.61 ± 0.03</td>
<td>317.14 ± 4.15</td>
</tr>
<tr>
<td>MRP&lt;sub&gt;t&lt;/sub&gt;</td>
<td>1.62 ± 0.03</td>
<td>27.62 ± 1.00</td>
</tr>
</tbody>
</table>

Means (n = 3) ± standard errors are given. In columns, values followed by the same letter are not significantly different according to the Newman-Keuls test (P < 0.05). M, AMF inoculum; R, rhizobia; P<sub>t</sub>, *P. fluorescens*.

treatments, no significant increase in soil total microbial activity (FDA) was noticed after inoculation. In contrast, the activity of acid phosphatase in the control soil was 1.28, 1.95, 2.71, 4.35, 11.98 and 14.69 times lower than in the inoculated ones with MRP<sub>t</sub>, M, R, MR, P<sub>t</sub> and MP<sub>t</sub>, respectively. The inoculants improved the soil acid phosphatase activity as follows: C < MRP<sub>t</sub> < M < R < MR < P<sub>t</sub> < MP<sub>t</sub>. The significant rise was observed with the treatments P<sub>t</sub> and MP<sub>t</sub> (Table 3).

Correlations between inoculation treatments, plant and soil parameters

The PCA was performed on the correlation matrix of all data studied and treatments (Figure 2). Results showed that the first two axes of the PCA plane explained about 72.36% of the variability. Plant growth parameters (shoot height, total dry weight and shoot nutrient contents), root mycorrhizal colonization rate and soil spore density were
Figure 1. Shoot nutrient contents of *A. senegal* plants in response to microbial inoculation under non-sterile soil in glasshouse. Mean values (n = 10) and standard errors are shown. Box plots with the same letters are not significantly different according to the Newman-Keuls test (P < 0.05). A, Nitrogen; B, Phosphorus; C, Potassium.
positively correlated between them, and linked to the first axis which explained 44.34% of the variability. Soil AMF hyphal length and microbial activities (FDA and acid phosphatase activities) were linked to the second axis which explained 28.02% of the variability. Three main clusters can be identified: the Group 1 included the control plants and those inoculated with P_I and MP_I which were linked to soil enzyme activities. The Group 2 encompassed plants inoculated with the treatments M, MR and MRP_I and was correlated to plant height, total dry weight, nutrient contents and mycorrhizal parameters. The Group 3 composed of plants inoculated with rhizobial strains (R) was linked to plant nodule number.

**DISCUSSION**

This present study done in legume trees particularly in Sahelian regions clearly indicated that, under non-sterile soil, the inoculation of *A. senegal* with AMF alone and in combination with R and P_I provides various results. The effects of the treatments M, R, MR and MRP_I on plant growth, those of MR and MP_I on P and K nutrient contents of shoots and on root mycorrhizal colonization were positive. However, the effect of inoculation with *Pseudomonas* treatment alone on these above parameters was negative. Our results are in agreement with the findings of Roestri et al. (2006) who reported positive impact of inoculation with AMF on growth and nutrition of wheat plants. According to Richardson et al. (2009), the better exploration of the soil by hyphae and nutrients translocation from inaccessible soil zones to plant might explained the positive effect of AMF on plant growth. The positive correlation between plant growth, shoot nutrient contents, nodulation and mycorrhizal parameters observed in our experiment confirmed the beneficial roles of these symbiotic microorganisms.

Our results also showed a beneficial effect of rhizobial inoculation on plant growth, nodulation and mycorrhiza-
tion, but not on shoot nutrient contents. These results were supported by those of Belachew and Pant (2010) in pea and Hemissi et al. (2011) in chickpea. The non-significant effect of rhizobial inoculation on nutrient contents might be due either to the fact that nodulation requires additional energy and nutrient substances from the plant (Turk et al., 1993), or the higher plant biomass which can dilute the nutrient concentration in shoots.

Also, Hansen (1994) underlined that inorganic N is required by legume plants during the ‘nitrogen hunger period’ for their nodule development, shoot and root growth before the onset of N2-fixation process.

More interestingly, in the results reported here, the inoculation of A. senegal seedlings with the combined MR resulted in a significant enhancement in plant growth and nutrition, and in mycorrhizal parameters. As said by Gill and Singh (2002), increased N2-fixation due to increased mycorrhizal colonization and nodulation in roots may contribute to the growth of plants, suggesting that phosphorus is also essential, as is nitrogen for N2-fixation (Buchanan et al., 2000). The reports of Kim et al. (2010) and Xiao et al. (2010) showing a stimulatory effect of the dual inoculation that resulted in improvement of N and P contents in roots and shoots, were in agreement with our findings. Our results also showed a positive effect of single and dual inoculation with AMF and R on soil acid phosphatase activity. The better nutrient contents particularly P uptake in plants inoculated with MR might be attributed to extended AM hyphae which explore a larger volume of soil and to P solubilization by AMF root exudates and R from unavailable sources present in the soil. The significant augmentation in root colonization and spore density with the dual inoculation showed patterns similar to those observed by Subramanian et al. (2011) in soybean and, might be due to the fact that both AMF and R are active in root cortical cells. Thus, the synergistic effect of the interaction between AMF and R led to improved A. senegal seedlings growth and nutrition.

In this experiment, we observed a negative impact of inoculation with P. strains alone on plant growth, nutrient contents and root colonization by AMF. However, the application of these bacteria stimulates the activity of acid phosphatase in the soil. It is possible that these PGPR strains may not be effective or may compete for nutrient uptake with the indigenous microorganisms or the plant under non-sterile conditions (Raimam et al., 2007). These findings contradicted those of Dey et al. (2004) showing a positive effect of PGPR in improving nodulation, nitrogen uptake, growth and yield of plants. Our results also indicated that the application of the combined MP, led to an enhancement of plant growth, nutrition and root colonization, rate, and of the activity of soil acid phosphatase. It appears that PGPR influenced the symbiotic association between plant and other microorganisms including AMF and R (Vessey, 2003). AMF and PGPR are known to improve the bioavailability of essential nutrients, especially P through the solubilization and mineralization of nutrients from organic and inorganic sources (Idris et al., 2009). In this study, the interaction of Pseudomonas with AMF was more effective than its single application on plant growth and nutrition suggesting that both microorganisms are able to solubilize P in the soil. But, the negative correlation observed between soil acid phosphatase activity and shoot N and P contents in plants inoculated with Pseudomonas strains indicates a possibility that plants are not able to take-up nutrients especially P solubilized by these PGPR. This result might be attributed to the negative effect of these bacterial strains on the growth of plant roots. The decrease in root biomass does not facilitate nutrient uptake and translocation to plants. In fact, one mechanism by which PGPR affect nutrient uptake is by enhancing growth and development of plant roots, which are then able to access more nutrients (Adesemoye et al., 2008). The P. strains in combination with AMF might help to a better uptake and translocation of nutrients as it was confirmed by the positive effect of the dual inoculation MP. Artursson et al. (2006) suggested the existence of a high degree of specificity between these two microorganisms. This indicated that the success of co-inoculation depends not only on the symbiotic efficiency of microbes but also on the compatibility of the interactions between the participants (Puppi et al., 1994).

Our results showed a better colonization of A. senegal roots by AMF when plants were inoculated with the combinations MR, MP, and MRP. According to Boer et al. (2005), in the presence of mycorrhiza-helping bacteria, AMF root colonization is improved, which promotes plant growth and nutrient uptake. Also, the decrease in root colonization by AMF after inoculation with P alone might be attributed to a higher P level in the soil as demonstrated by the higher acid phosphatase activity. Thus, the availability of nutrients, especially P and N in soil might be the factors influencing the mycorrhization.

Further, our results revealed a significant positive effect of all the inoculants except MP on AMF spore density. These are comparable to reports of Meghvansi and Mahna (2009) on soybean inoculated with G. intraradices and Bradyrhizobium japonicum. Moreover, the combined MR inoculation enhanced soil AMF spore density, whereas that of MP, decreased it. Similar results were reported by Bisht et al. (2009) and might be due to an inhibition of spore germination by Pseudomonas that can produce nonvolatile, diffusible substances. A competition for inorganic nutrients might also explain the antagonistic effect of the interaction between Pseudomonas and AMF on spore germination.

Soil enzyme activities have been used as an indicator of soil quality. So, their measurement would be crucial for a better understanding of the inoculation impact with any microorganism on soil quality and functioning, leading to a better plant growth and protection (Lalitha et al., 2011). In our study, the combination of AMF and R stimulated the soil acid phosphatase activity. Similar results were
reported by Vasquez et al. (2000) in the rhizosphere of maize plants inoculated with *Glomus deserticola*. In fact, some PGPR are able to secrete enzyme phosphatases for solubilizing insoluble inorganic and organic P in soil (Hayat et al., 2010). The interactions between AMF, R and P_i can be synergistic or antagonistic suggesting that the microorganisms used here might compete for nutrients (Johansen and Jensen, 1996).

**Conclusion**

The present study clearly demonstrated that the effect of single, double or triple microbial inoculations on plant growth and nutrition depends on the compatibility of the different components of the association. Our results have shown beneficial effects of inoculation with all the treatments except P_i alone on *A. senegal* plant growth, and on soil mycorrhizal potential and enzyme activities. The dual inoculation with AMF and R exhibit the higher significant response on growth performance of *A. senegal* seedlings than the other treatments. Our findings also indicated that microbial inoculation induced significant changes in soil properties. Thus, the combination of AMF and R must be considered as potential inoculum candidates to improve *A. senegal* growth and nutrition under non-sterile conditions. Further research in field conditions are needed to determine the best response of *A. senegal* to the dual inoculation.

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**ABBREVIATIONS**

AMF, Arbuscular mycorrhizal fungi; PGPR, plant growth promoting rhizobacteria; M, AMF inoculum; R, rhizobia; P_h, *Pseudomonas fluorescens*; M+R, MR; M+P_h, MP; M+R+P_h, MRP.

**REFERENCES**


