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Effect of Rock Phosphate and Arbuscular Mycorrhizal Fungi on Maize (Zea Mays L.) Growth in Greenhouse Conditions

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Abstract

The objective of this study is to evaluate effect of arbuscular mycorrhizal fungi (AMF) and Tilemsi rock phosphate (PNT) on maize growth.

To do this, an experiment was carried out in a greenhouse with sterilized soil from Sangalkam (Senegal), considered to be poor in available phosphorus and sown with maize (3 seeds / pot). The inoculum used was 20 g per pot at the time of sowing, along with the

PNT tested at 0, 100 and 200 mg per pot. The AMF strains, taken separately or mixed and associated or not with PNT, made it possible to consider fifteen treatments.

Height measurements were taken every two weeks until 60th day. Two months after sowing, corn plants were sampled. Then, shoot and root biomasses were evaluated as well as mycorrhization parameters.

The results showed that for inoculated treatments, intensities of mycorrhization are very high and exceed 30%. The lowest intensities of mycorrhization (30.88%) were noted in plants inoculated with Glomus aggregatum (IR-27) in presence of 200 mg of PNT per kg of soil while all other treatments showed intensities of mycorrhization ranging from 39.24 to 79.64%. In addition, three strains of AMF, R. irregularis, G. mosseae, G. aggregatum significantly stimulated height and biomass of shoots and roots of maize plants in presence of 100 mg of PNT.

Under our experimental conditions, inoculation with AMF strains in presence of PNT improved maize growth from 16.22 to 26.41%.

Fertilization based on AMF and natural phosphates could therefore be an alternative to the use of chemical fertilizers.

Keywords: arbuscular mycorrhizal fungi, rock phosphate, maize, growth.

Introduction

In Sub-Saharan Africa, the soil poverty in organic matter and mineral elements and the rapid demographic growth compromise the crop production and agricultural sustainability. Indeed, nearly 80% of the soils are poor in nutrients especially in nitrogen (N) and phosphorus (P), two essential elements for plant growth (Bationo et *al*., 1998; Bâ *et al*., 2001; Sacko, 2014; Smith et *al*., 2011).

On the other hand, cereals contribute largely to food security in many Sub-Saharan countries. In Mali for instance, maize accounted for 4.71% of total agricultural production and thus constituted the 7th agricultural production in relative value over the period 2005-2009 (Criado, 2002; FAOSTAT, 2010). Maize is mainly used for the human consumption, through various local culinary preparations (tô, couscous, ears fresh or roasted). Its use in animal feed (poultry especially) is increasing in large urban centers such as Bamako, Segou and Sikasso.

Nowadays, the agricultural production, in particular maize production largely depends on chemical fertilizers which are inaccessible for some famers because of their high costs. To reduce the dependence to the chemical fertilizers, the use of local fertilizing resources such as rock phosphates (PNT) constitute an alternative option. In fact, it has been reported that rock phosphate (PNT), constitutes a potential source of phosphorus which is less expensive and might be more profitable compared to mineral phosphate and manures (Bationo *et al*., 1997; Piéri, 1989). However the low solubility of the PNT, limits the availability of phosphorus for plants (Strullu, 1991). In this context, inoculation with arbuscular mycorrhizal fungi (AMF) has been proposed to improve the solubilization of the PNT (Babana, 2003). Arbuscular mycorrhizal fungi are heterotrophic microorganisms that form symbiosis with 90% of terrestrial plants (Smith & Read, 2008). With their hyphal networks, they are able to improve the hydromineral nutrition of their host plant. Consequently, the AMF are an essential asset for crop plants to increase their productivity (Bâ *et al*., 2000; Al-Karaki *et al*., 2004; Schroeder and Janos, 2004; Oehl *et al*., 2004).

The objective of this study was to evaluate effect of arbuscular mycorrhizal fungi inoculation on growth of maize in presence or not of rock phosphate of Tilemsi under greenhouse conditions.

Material and Methods

Culture Substrate

Soil from Sangalkam (Dakar, Senegal) was used as culture substrate. According to (Diop *et al*., 2013). It has the following characteristics: clay 5.40%, silt 5.80%, sand 88.8%, organic matter 0.60%, total carbon 0.30%, total nitrogen 0.02%, C/N ratio 14%, total potassium 333.5 ppm, total phosphorus 41.4 ppm, available phosphorus 2.1 ppm, total calcium 1.03 ppm, total magnesium 0,3 ppm, pH (H2 O) and pH (KCL) are respectively 6 and 4.6. The soil was autoclaved twice at 120°C during 2 hours. The autoclaved soil was then placed in horticultural plastic pots (2 kg per pot) with a capacity of 5.23 Cm^3.

Maize Seeds

Seeds of variety Brico (given by FASOKABA/Mali) were used in this study. The cycle of Brico variety is 70 days, and this maize variety is cultivated in Mali in areas located between the 400 and 600 mm isohyets. Its average yield is 2-3 T/ha.

AMF Inoculum

AMF strains (*Rhizophagus irregularis* (Ri), *Glomus aggregatum* (IR-27) and *Glomus mosseae* (Gm)) used in this study were obtained from the collection of the Common laboratory of Microbiology (LCM, IRD/ISRA, Dakar- Senegal). The choice of these AMF strains was based on their positive effects on plant growth reported in several previous studies (Bâ *et al..*, 2001; Asimi, 2009; Haro *et al*., 2012; Diop *et al*., 2013; Sacko, 2014; Leye *and al*., 2015). The inoculum prepared from each AMF strain was constituted of a mixture of spores, hyphae, colonized root fragments and sands.

Rock Natural Phosphate of Tilemsi (PNT)

The rock phosphate used in our experimentation was collected from the valley of Tilemsi (PNT) in Mali. It was used in powder form and contained almost 30% of $P_2 O_5$ (Truong *et al*., 1977).

Experimental Design

The experiment was carried out in greenhouse at the Common laboratory of Microbiology LCM (Dakar-Senegal). Five inoculants (*Rhizophagus irregulars* (Ri), *Glomus aggregatum* (IR-27), *Glomus mosseae* (Gm), a mixture of the three AMF (Ri+IR-27+Gm), and sand without AMF (T0)) and three doses (0, 100 and 200 mg/Kg of soil) of PNT were used alone or in combination. Hence 15 treatments composed of T0, Ri, IR-27, Gm, Ri+IR-27+Gm, PNT (100 mg), PNT (200 mg), Ri+PNT (100 mg), IR-27+PNT (100 mg), Gm+PNT (100 mg); Ri+IR-27+Gm+PNT (100 mg), Ri+PNT (200 mg), IR-27+PNT (200 mg), Gm+PNT (200 mg) and Ri+IR-27+Gm+PNT (200 mg). Each treatment was replicated 8 times. The AMF inoculums (20 g per pot) and PNT were brought at sowing.

Three pre-germinated seeds of maize were shown per pot. The singling was performed 10 days after sowing and plants were watered regularly with tap water during 70 days of experimentation in greenhouse.

Determination of Plant Growth Parameters

The plant growth parameters measured are height, shoot and root biomass. Every 15 days, the height of 8 plants per treatment was measured up to 60 days after sowing. Shoot and root biomass were sampled at 60 days after sowing. In each treatment, 8 plants were randomly sampled. The shoot and root biomass of each treatment were dried at 60 °C during 72 hours, and then weighed.

Determination of Mycorrhizal Parameters

To determine the mycorrhizal parameters, roots were sampled at 60 days after sowing. Eight plants per treatment were sampled and roots were stained according to the method of Philips and Hayman (Phillips and Hayman, 1970) to assess the rates of mycorrhization. Hence, 10 slides of 10 fragments were prepared representing a total of 100 pieces of roots per treatment. Using a standard binocular microscope we classified the stained root fragments on a scale of 6 classes ranging from 0 to 5 (Trouvelot *et al*., 1986) which allowed assessing the degree of infection of each fragment. The frequencies (F) and intensities of mycorhization (I) were determines according to the method of Trouvelot et al. (Trouvelot *et al*., 1986).

Statistical Analysis of Data

The data were subjected to an analysis of variance (ANOVA) using R software (R version i386 3.2.2) and significant differences in means were determined at $p<0.05$.

Percentage data of root colonization were arcsine transformed prior to analysis.

Results

Effect of AMF Inoculation Alone or with PNT on Plant Height

60 days after sowing, individual AMF inoculation and / or mixed inoculation (cocktail) improved growth of corn plants. However, compared to control treatment, this increase was not significant.

When corn plants were inoculated with AMF and fertilized with 100 mg PNT per kg soil, only *Rhizophagus irregularis* (Ri) and *Glomus aggregatum* (IR-27) significantly stimulated height growth of corn plants with a gain of 26.41% and 16.22% respectively, 60 days after sowing. On other hand, when corn plants are fertilized with a dose of 200 mg of PNT per kg of soil, inoculation of AMF did not significantly improve height of plants. However, cocktail does not improve height of plant when it is used with a dose of 200 mg of PNT per kg of soil (Table 1).

Effect of AMF Inoculation Alone on Plant Biomass

Inoculation with AMF alone, without PNT, significantly increased shoot biomass with a benefit of 176.19%, 130.61% and 200% respectively for *Rhizophagus irregularis*(Ri), *Glomus aggregatum* (IR-27) and *Glomus mosseae* (Gm). However, only *Rhizophagus irregularis* (Ri) increased root biomass with a surplus of 144.28%.

In addition, inoculation of AMF with 100 mg of PNT per Kg of soil, considerably improved shoot biomass with respectively 199.31%, 282.99% and 238.09% for *Rhizophagus irregularis* (Ri), *Glomus aggregatum* , (IR-27) and *Glomus mosseae*(Gm). This inoculation of AMF also allowed respectively a very significant profit of root biomass with respectively 125.7%, 122.85% and 108.57%.

With 200 mg of PNT per Kg of soil, a significant improvement in shoot biomass was only observed with *Glomus mosseae* (Gm) with a benefit of 140.13%. However, its effect is not significant on root biomass despite a benefit of 57.14% and 71.42% respectively for *Rhizophagus irregularis* (Ri) and *Glomus mosseae* (Gm) (Table 1).

Effect of AMF Inoculation on Plant Root Mycorhization

Statistical analysis (ANOVA) showed a very significant difference ($p\leq0.001$) between different treatments for frequencies and intensities of mycorrhization 60 days after sowing (Table 2).

Regardless of treatment, mycorrhization frequencies were greater than or equal to 90%. The association with PNT 100 mg per Kg of soil improved frequency of mycorrhization except with *Rhizophagus irregularis* (Ri). However, a significant decrease in frequency was noted with combination of 200 mg PNT, except in presence of *Rhizophagus irregularis*. The highest mycorrhizal frequency (100%) was observed with *Rhizophagus irregularis* (Ri) only, *Rhizophagus irregularis* with 200 mg PNT per kg soil, *Glomus mosseae* (Gm) only, *Glomus mosseae* (Gm) with 100 mg PNT per kg of soil and *Glomus aggregatum* (IR-27) in presence of 100 mg of PNT per kg of soil.

The intensity of mycorrhization is high regardless of treatment of AMF, varying from 39.24 to 79.46%. The intensity of mycorrhization is high for all treatments, except with *Glomus aggregatum* (IR-27) in presence of 200 mg of PNT (30.88%). The contribution of 100 mg of PNT per kg of soil could only significantly improve mycorrhization intensity of plants inoculated with mixture of AMF at 56.83%.

In addition, with dose of 200 mg of PNT per kg of soil, decrease in mycorrhizal intensity was more significant with three strains of AMF used. However, mycorrhizal intensity of plants inoculated with AMF mixture was improved (44.55%).

Discussion

Effect of AMF Inoculation and Natural Phosphate on the Plant Growth

This study showed an increase in growth parameters of maize plants (height and biomass) in presence of phosphate fertilizers and AMF. Our results seem to confirm levels of symbiotic performance observed with different AMF strains used. The results also indicate that three (03) strains of AMF (*R. irregularis*, *G mosseae*, *G aggregatum*) significantly stimulated growth of corn plant (height and biomass) only or especially in presence of PNT, for report to witness. In fact, 60 days after sowing, 3 strains of AMF, in presence of 100 mg of PNT per kg of soil, significantly improved the biomass (root and shoot), excluded root biomass of plants inoculated with *Rhizophagus irregularis* (Ri). In fact, inoculation with AMF alone improved biomass, because AMF are better expressed in soils deficient in phosphorus P. After 60 days of cultivation, highest value is 81.98 cm, i.e. an increase of 26.41%; this record value was obtained with *Rhizophagus irregularis* (Ri) in presence of 100 mg of PNT per kg of soil. However, highest value of shoot biomass (5.63g, i.e. a benefit of 283%) is obtained with *Glomus aggregatum* (IR-27) in presence of 100 mg of PNT per Kg of soil while root biomass highest (1.71g, either a surplus of 125.7%) is obtained with *Rhizophagus irregularis* (Ri) at same dose. This is because *Rhizophagus irregularis* strain of fungi colonizes interior of roots.

The beneficial effect observed following contribution of PNT alone or in presence of AMF allowed an increase in shoot biomass varying between 37.41% and 283%. These results are similar to those of (Smith *et al*., 2011) who reported that phosphorus from PNT stimulates plant growth. These results also corroborate those of other studies carried out in Senegal and Burkina Faso (Diop *et al*., 2013 ; Haro *et al*., 2012) which showed good development and a significant stimulation of biomass of cowpea (*Vigna unguiculata*) shoots following inoculation with *R Irregularis*. These positive effects of inoculation on plant growth reveal role of AMF biofertilizers. The optimum growth observed when AMF are combined with 100 mg of PNT per kg of soil shows that these fungi play an important role in solubilization of rock phosphates due to phosphatases (enzymes) that they release (Joner and Johansen, 2000). In addition, thanks to their hyphae, AMF form a good soil mesh and improve hydromineral nutrition of plant (Smith *et al*., 2000; Drew *et al*., 2003).

Effect of the AMF Inoculation and Natural Phosphate on the Mycorrhizal Parameters

The greenhouse inoculation of maize plants with strains of *Glomus aggregatum* (IR-27), *Glomus mosseae* (Gm) and *Rhizophagus irregularis* (Ri) and the mixture of AMF in presence or not of PNT made it possible to obtain intensities mycorrhization ranging from 30.88 to 79.46%. These values are significantly higher in treatments with AMF strains taken separately or in partnership with the PNT except treatment with IR-27 in presence of 200 mg of PNT per kg of soil (30.88%). In fact, these treatments showed strong intensities of mycorrhization ranging from 39.24 to79.46%. Our results corroborate those of several authors (Diop *et al*., 2013; Alkan *et al*., 2006; Smith *et al*., 2004; Drew *et* *al*., 2003; Graham *et al*., 2000; Jansa *et al*., 2007) who obtained mycorrhization intensities ranging from 40 to 65%.

The AMF IR-27, *R. irregularis* and *G. mosseae* used were found to be more effective in colonizing roots of corn. Indeed, intensities of mycorrhization due to these strains are high and vary between 40.61% and 79.46%. Our results are similar to those of Jansa *et al*., 2007 with *G mosseae*, Alkan *et al*., 2006 with *R. irregularis*, Ndoye *et al*., 2012 with *G mosseae* and *G aggregatum* and Diop *et al*., 2013 with 3 strains of AMF, as in present study. The effects of inoculation with *G. mosseae* and *R. irregularis* are more significant than those obtained with IR-27, as evidenced by intensity (30.88%) of mycorrhization at 200 mg of PNT per Kg of ground.

In addition, intensity of mycorrhization has been reported to vary depending on strains of AMF, plant inoculated, environmental conditions and level of phosphorus in soil. (Boddington and Dodd, 2000; Alkan *et al*., 2006).

Conclusion

The greenhouse inoculation with AMF strains (*Rhizophagus irregulare and Glomus aggregatum; IR-27*) and the amendment with rock phosphate (100 mg rock phosphate of Tilemsi per Kg of soil) can improve the growth of maize on the soil of Sangalkam deficient in assimilable phosphorus (2,1ppm of P). Frequencies of mycorhization of maize plants have been raised whatever is the considered treatment and intensities have especially been raised with treatments of AMF taken insulation. Consequently, these bio-fertilizers in partnership with rock phosphates could be recommended to improve the phosphate nutrition of plants instead of chemical fertilizers in sub-Saharan Africa and particularly in Mali.

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Table 1: Height (cm) and biomasses (g) of maize plants at 15, 30, 45 and 60 days, inoculated with AMF strains or no in presence of PNT or no

Treatments	H (cm) 15 jours	H (cm) 30 jours	H (cm) 45 jours	H (cm) 60 jours	BA (g) 60 jours	BR (g) 60 jours
T0	35.03 acd	45.65 ac	61 ab	64.85 ab	1.47 a	0.70 a
Ri	38.65 bcef	60.82 def	72.07 bc	73.36 ac	**4.06 cdf**	**1.71 d**
IR-27	40.76 bcef	62.05 def	70.27 bc	70.62 ac	**3.39 bde**	1.21 ad
Gm	44.60 def	62.10 def	71.28 bc	69.95 ac	**4.41 df**	1.17 ad
Cocktail	42.23 cf	58.57 bcef	67.51 ac	68.83 ac	3.15 ade	1.11 ad
PNT (100 mg)	35.76 ace	54.03 ade	61 ab	61.00 ab	2.18 abc	0.86 ab
Ri + PNT (100 mg)	46.52 f	72.11 f	80.86 c	**81.98 c**	**4.40 df**	**1.58 cd**
IR-27 + PNT (100 mg)	44.12 def	66.23 ef	73.38 bc	**75.37 bc**	**5.63 f**	**1.56 cd**
Gm + PNT (100 mg)	45.13 ef	66.75 ef	74.41bc	74.50 ac	**4.97 ef**	**1.46 bcd**
Cocktail + PNT (100 mg)	42.08 cf	60.22 cef	66.68 ac	67.43 ac	3.22 ade	1.16 ad
PNT (200 mg)	31.36 ab	42.53 a	52.41 a	59.24 a	2.02 ab	0.98 ac
Ri + PNT (200 mg)	39.11 bcef	57.78 bcef	63.86 ab	64.18 ab	2.86 ad	**1.10 ad**
IR-27 + PNT (200 mg)	33.38 ac	50.60 acd	62.86 ab	66.91 ac	2.27 abc	0.77 a
Gm + PNT (200 mg)	36.96 acef	55.83 ade	65.50 ab	68.81 ac	**3.53 bde**	**1.20 ad**
Cocktail + PNT (200 mg)	28.67 a	43.85 ab	54.16 a	60.12 ab	2.13 ab	0.68 a
Probability	0.001	0.001	0.001	0.001	0.001	0.001
Significance	HS	HS	HS	HS	HS	HS

Every value represents the average for 8 plantations. In a same column values followed of a same letter are not statistically different between them by test of Tukey (t <5%) H: Height; BA: Shoot biomass; BR: Root biomass, HS: Highly Significant.

Table 2: Frequency and intensity of maize mycorhization inoculated with AMF strains in presence or not of PNT 60 days after sowing

Treatments	F	I
Ri	**100 e**	**79.46 j**
IR-27	98.5 d	**71.27 g**
Gm	**100 e**	**74.60 h**
Cocktail	90.00 a	39.24 b
Ri + PNT (100 mg)	**96.25 c**	77.32 i
IR-27 + PNT (100 mg)	**100 e**	51.64 d
Gm + PNT (100 mg)	**100 e**	62.01 f
Cocktail + PNT (100 mg)	97.5 d	**56.83 e**
Ri + PNT (200 mg)	**100 e**	57.90 e
IR-27 + PNT (200 mg)	93.75 b	**30.88 a**
Gm + PNT (200 mg)	96.25 c	40.61 b
Cocktail + PNT (200 mg)	**93.75 b**	**44.50 c**
Probabilité	0.001	0.001
Signification	HS	HS

Every value represents the average of 8 replicates. In a same column values followed by the same letter were not statistically different between according to the test of Tukey (t< 5%). F%: Frequency in percentage; I%: Intensity in percentage

Face 1: Root of maize inoculated with Ri (A) with IR-27 (B) (Koné, 2015)