Effect of Arbuscular Mycorrhizal Fungal Inoculation on *Sorghum bicolor* Growth at Different Phosphate Levels: A Greenhouse Study

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**Abstract:** In sub-Saharan Africa, Sorghum (*Sorghum bicolor*) is an important cereal for both human being and animals. Unfortunately, its production is confronted to soils with deficiency of phosphorus. Traditional use of mineral phosphate on this culture fertilization is expensive and may cause contamination. It is thus necessary to seek more efficient and economic reasonable techniques to improve sorghum growth. Arbuscular mycorrhizal fungi (AMF) constitute a reference for phosphorus improvement and plant nutrition. This study aimed to investigate the effects of AMF strains (*Rhizophagus irregularis*, *Glomus aggregatum*, *G. mosseae*) on growth of sorghum cultivated in greenhouse on Sangalkam soil (Senegal) sterilized with or without Tilemsi natural phosphate (PNT). The phosphorus can represent until 0.2% of the dry weight of the plant. Two fertilizers were used separately and together to doses of 20 g by strain, 100 mg and 200 mg of PNT. The experiment lasted for 120 d. Results showed that mycorrhizal colonization intensity varied between 40% and 80% for all treatments. AMF inoculation increased sorghum plant height and biomass, regardless of PNT amendment. The inoculation permits to bring strain of AMF that intervene efficiently in the transportation and the availability of phosphorus for the plant.

**Key words:** Arbuscular mycorrhizal fungi, Tilemsi natural phosphate, *Sorghum bicolor*.

1. Introduction

In world sorghum (*Sorghum bicolor*) is the fifth important cereal with a production estimated to 58 million tons in 2004. Its yield reaches 4 tons by hectare in United States of America, against 870 kg by hectare in Africa, where it is used as food for man and animals, the construction of dwellings and as fuel [1, 2].

In sub-Saharan Africa 80% of soils are nearly involved in assimilated phosphorus [3]. It constitutes plants growth limiting factor [4]. Phosphorus can be present in important quantity in soil, but largely inaccessible to plants because of its very weak solubility [5].

In Mali sorghum holds a primordial room in populations food, with a production estimated to 650 thousand tons in 2004 [6].

Nowadays cultures intensification is greatly dependent on mineral manures that enrich soils in necessary nourishing elements to the development of plants [7]. However, these products are expensive and inaccessible peasants to weak spending power [8]. An alternative to the use of these manures is the recourse to the microbial biofertilizers [9]. Among these biologic fertilizers, particular attention is
granted to arbuscular mycorrhizal fungi (AMF) [10], which encourage plants hydromineral nutrition [11]. Inoculation with these fungi strains permits to improve plants growth [12], especially when it is coupled to a phosphatic fertilization [13, 14]. Natural phosphates constitute a potential source of less dear phosphorus for peasants [8]. However, these phosphates used by plants are not always comfortable because of their weak solubility, from where the interest to apply them in combination with AMF strains. Under tropics characterized by a deficit of phosphorus [15], the AMF constitute a natural primordial source of hydromineral nutrition that assures plants growth and health [11, 16]. To this title the AMF are used to increase productions in agriculture, which would reduce the use of chemical fertilizing [17]. This work specifically aimed to value effect of fertilization with AMF strains and natural phosphate on height, biomasses production and mycorrhization (intensity, frequency) of sorghum plants cultivated on pots in greenhouse.

2. Materials and Methods

2.1 Materials

2.1.1 Culture Substratum

Culture soil has been appropriated to Sangalkam/Sénégal. It is a soil poor in assimilated P (2.1 ppm) [18]. This soil contains besides: 5.40% of clay, 5.80% of silt, 88.8% of sand, 0.60% of organic matters, 0.30% of total carbon, 0.02% of total nitrogen, 14% of C/N ratio, 333.5 ppm of total potassium, 41.4 ppm of total phosphorus, 2.1 ppm of assimilated phosphorus, 1.03 ppm of total calcium, and 0.3 ppm of total magnesium. The pH (H2O) and the pH (KCl) are respectively 6 and 4.6.

2.1.2 Plant Material

Sorghum seeds (cultivar CSM 63 Jakunbè) coming from FASO KABA/Mali were used. Its cycle is 100 d and it is cultivated in isohyet of 300-800 mm. Its middle output is 1.5 t/ha with the loose panicles to grains of white color.

2.1.3 Arbuscular Mycorrhizal Strains

Rhizophagus irregular (Ri), Glomus aggregatum (IR-27) and G. mosseae (Gm) were used. They come from the collection of the common laboratory of microbiology of Dakar/Senegal and gave convincing results in several former work [14, 18-23]. This inoculum is constituted of a mixture of spores, hyphes, fragments of root colonized by endomycorrhizal fungi and sands.

2.1.4 Natural Phosphate

The used natural phosphate is Tilemsi natural phosphate (PNT), Mali used under its pulverulente shape and content 30% of P2O5, 10% of elements lower to 40 µm, 60% of elements lower to 60 µm and present a solubility of 0.007% in water [24].

2.2 Methods

2.2.1 Culture Soil Sterilization and Pots Replenishment

Culture soil was autoclaved two times to 120 °C during 20 min. After autoclavage it has been divided on plastic pots of 5.23 cm³, at the rate of 2 kg by pot.

2.2.2 Seeds Scatting and Plants Thinning

Sorghum seeds were sown to three (3) by pot and germinated on 3 d. One week after seedling, the young plants were unmarried to two (2) by pot, then to one (1) at the 10th day after the seedling.

2.2.3 Setting Up and Conduct of Experimentation

Experimentation has been driven in greenhouse to the LCM. Two factors (inoculation with AMF strains to five modes; fertilization with PNT to three levels) have been studied. Fifteen (15) treatments have been considered: Wetness; Ri; IR-27; Gm; Cocktail; PNT (100 mg); Ri + PNT (100 mg); IR-27 + PNT (100 mg); Gm + PNT (100 mg); Cocktail + PNT (100 mg); PNT (200 mg); Ri + PNT (200 mg); IR-27 + PNT (200 mg); Gm + PNT (200 mg); Cocktail + PNT (200 mg). These treatments were arranged following an uncertain pull. Sixteen (16) pots have been used by treatment. The inoculum of AMF has been brought at the
Effect of Arbuscular Mycorrhizal Fungal Inoculation on *Sorghum bicolor* Growth at Different Phosphate Levels: A Greenhouse Study

A rate of 20 g/pot at the same time as the PNT during the seedling. Plants were watered regularly with the faucet water. Experimentation lasted four months (120 d).

2.2.4 Data Collection and Analysis

Plants height has been measured every 15 d during 60 d. Mycorrhization and biomasses were valued to the 60th and 120th days on eight (8) plants by treatment. Plants were withdrawn of pots. Some fine roots have been taken away, then plants aerial and root parts were separated. Roots were colored according to Philips and Hayman [25] method. The rate of endomycorhization corresponds to the frequency of infection (F%) that is equal to the number of time that the root is infected and the intensity of mycorrhization (I%) is correspondent to the degree of infection of the root by the fungi, which are valued to the optic microscope by the method of Trouvelot *et al.* [26]. The degree of endomycrhizal colonization of every fragment is estimated according to a scale constituted of six values corresponding to: 0%, a total absence of infection of the root; < 1%, a root infected with at least a hyphe mycélien; < 10%, some hyphes presence and fungi arbuscules in the root; < 50%, a middle infection of the root with hyphes and especially of arbusculeses; > 50%, a root infected with several bladders and or arbusculeses and hyphae; > 90%, an overgrown root nearly completely by bladders and/or arbusculeses and hyphae.

Analysis of variance (ANOVA) has been done with the software R (R version i386 3.2.2) and the test of Tukey contrasts to the doorstep of 5% has been used for the comparison of averages.

3. Results

3.1 AMF and PNT Effect on Plants Height and Biomasses Production

Results present in Table 1 show that to the course of the first three periods (15th, 30th and 45th days) of measure, IR-27 + PNT (100 mg) had a positive effect more marked on plants height with respectively 34.56, 66.96 and 86.62 cm. To these same periods the weakest values for this variable were 23.42, 40.07 and 55.32 cm, respectively, for witnesses, Cocktail + PNT (200 mg) and Cocktail.

At the 60th and 120th days of culture a meaningful effect has been noted between the different treatments for biomasses. So at the end of 60 d, Cocktail + PNT (100 mg) with 4.37 g and IR-27 + PNT (100 mg) with 1.56 g encouraged respectively production of dry aerial biomass (DAB) and dry root biomass (DRB). To this same period, the weak value for these variables was respectively 1.82 g and 0.68 g for IR-27 and witnesses.

At the 120th day, Ri + PNT (200 mg) with 3.71 g and IR-27 + PNT (100 mg) with 1.74 g had a positive effect more marked respectively on DAB and DRB production. While to the same period the weakest value for the DAB has been recorded at plants of IR-27 treatment with 1.58 g and the one of DRB at witnesses with 0.56 g.

3.2 Effect of Inoculation with AMF in Presence or/No PNT on Plants Mycorrhization

Results gotten to the 60th and 120th days (Table 2) for mycorrhization show a meaningful effect between the different treatments. Cocktail + PNT (100 mg) encouraged mycorrhization frequency and intensity for respectively 100% and 76.54% (Fig. 1a). On the other hand, the weak values of these same variables have been recorded respectively at plants dealt with Cocktail and Cocktail + PNT (200 mg) for respectively 90% and 47.72%. To the 120th day, IR-27 + PNT (100 mg), Gm + PNT (100 mg) and Cocktail + PNT (100 mg) with a value of 100% had a positive effect more marked on mycorrhization frequency, contrary to Cocktail that was less efficient with 93%. IR-27 + PNT (100 mg) with a value of 77.87% (Fig. 1b) was more efficient on mycorrhization intensity in opposition to Cocktail + PNT (200 mg) that was less efficient on this variable with a value of 50.32%.
Table 1  Middle value of height (cm) and biomasses (g) of sorghum plants inoculated or/no with arbuscular mycorrhizal fungi (AMF) strains in presence or/no Tilemsi natural phosphate (PNT), measured in different periods.

| Treatments            | Periods of measure and measured variables | 15 d | 30 d | 45 d | 60 d | 60 d | 120 d |
|-----------------------|-------------------------------------------|------|------|------|------|------|-------|
|                       | Height (cm) | Height (cm) | Height (cm) | Height (cm) | DAB (g) | DRB (g) | DAB (g) | DRB (g) |
| T0                    | 23.42a      | 44.50ab     | 64.00ab     | 87.06ab     | 1.82a   | 0.73absa | 1.90ab   | 0.56a   |
| Ri                    | 27.28acd    | 55.56bd     | 71.90ad     | 115.56cd    | 2.06a   | 0.70ab    | 2.56ac   | 0.62ab  |
| IR-27                 | 27.30acd    | 54.17bc     | 75.58bcd    | 74.43a      | 2.85ac  | 0.76ab    | 1.58a    | 1.33de  |
| Gm                    | 32.12de     | 60.20cd     | 76.05bcd    | 105.60bcd   | 3.08ae  | 0.94abc   | 3.01bc   | 0.86acd |
| Cocktail              | 27.00acd    | 53.58bc     | 55.32a      | 115.20cd    | 3.27ae  | 1.55d     | 2.66ac   | 0.81acd |
| PNT (100 mg)          | 31.01ce     | 59.75cd     | 76.38bcd    | 104.71bcd   | 2.88acd | 1.41cd    | 2.65ac   | 1.71ace |
| Ri + PNT (100 mg)     | 31.53ce     | 59.43cd     | 84.01cd     | 119.37d     | 3.82bce | 1.25bd    | 2.71ac   | 0.70ac  |
| IR-27 + PNT (100 mg)  | 34.56e      | 66.96d      | 86.62cd     | 125.12d     | 4.18ce  | 1.56d     | 3.15bc   | 1.74e   |
| Gm + PNT (100 mg)     | 29.91bce    | 53.36bc     | 80.67bcd    | 112.53cd    | 4.05ce  | 1.41cd    | 3.29c    | 1.25ce  |
| Cocktail + PNT (100 mg)| 28.38acd    | 55.06bd     | 80.36bcd    | 120.17d     | 4.37e   | 1.17ad    | 2.73ac   | 1.21bce |
| PNT (200 mg)          | 26.27ac     | 52.88bc     | 67.65ac     | 92.47ac     | 3.10ae  | 1.05ad    | 2.66ac   | 0.72acd |
| Ri + PNT (200 mg)     | 29.13ace    | 57.17bd     | 88.83d      | 116.06cd    | 3.72bce | 1.14bd    | 3.71c    | 1.11acd |
| IR-27 + PNT (200 mg)  | 32.17de     | 60.12cd     | 78.43bcd    | 111.50cd    | 3.07ae  | 1.02ad    | 2.52ac   | 1.09acd |
| Gm + PNT (200 mg)     | 29.22bce    | 58.02cd     | 78.08bcd    | 127.43d     | 4.31de  | 1.40cd    | 2.86bc   | 0.69ac  |
| Cocktail + PNT (200 mg)| 24.53ab     | 40.07a      | 75.9bcd     | 105.45bcd   | 2.43ab  | 0.68a     | 2.06ab   | 0.68ac  |
| Probability           | 0.001       | 0.001       | 0.001       | 0.001       | 0.001   | 0.001     | 0.001    | 0.001   |
| Significance          | HS          | HS          | HS          | HS          | HS      | HS        | HS       | HS      |

DAB: dry aerial biomass; DRB: dry root biomass; HS: highly significant.
Each value represents average for eight plants. In the same column values followed with the same letter are not statistically different between them to the doorstep of 5% according to Tukey contrasts test.

Table 2  Middle value of mycorrhization frequency F (%) and intensity I (%) of sorghum plants inoculated or/no with AMF strains in presence or/no PNT, at 60th and 120th days.

| Treatments            | Periods of growth |
|-----------------------|-------------------|
|                       | 60 d | 120 d |
|                       | F (%) | I (%) | F (%) | I (%) |
| Ri                    | 97.75e | 58.94c | 98d   | 61.33c |
| IR-27                 | 98.5fg | 75.23g | 98.75e | 77.50i |
| Gm                    | 98.25f | 68e   | 98.75e | 70.40f |
| Cocktail              | 90a   | 65.34d | 93a   | 66.51d |
| Ri + PNT (100 mg)     | 98.75g | 58.01c | 98.75e | 60.52c |
| IR-27 + PNT (100 mg)  | 98.75g | 73.15f | 100f  | 77.87i |
| Gm + PNT (100 mg)     | 98.75g | 58.05c | 100f  | 72.33g |
| Cocktail + PNT (100 mg)| 100h  | 76.54g | 100f  | 77.50i |
| Ri + PNT (200 mg)     | 95c   | 71.89f | 96.87c | 74.31h |
| IR-27 + PNT (200 mg)  | 96.25d | 66.26d | 98d   | 68.79e |
| Gm + PNT (200 mg)     | 96d   | 51.50b | 98.5de| 54.02b |
| Cocktail + PNT (200 mg)| 92.5b | 47.72a | 95b   | 50.32a |
| Probability           | 0.001 | 0.001 | 0.001 | 0.001 |
| Significance          | HS    | HS    | HS    | HS    |

Each value represents the average for eight plantations.
In the same column values followed by the same letter are not statistically different between them to the doorstep of 5% according to Tukey contrasts test.
4. Discussion

The inoculation with AMF permitted to get some elevated mycorrhization intensity (40% and 80%) according to strains. These results agree with those of Diop et al. [18] that found some elevated mycorrhization intensity (40%-65%) while inoculating with the three strains. The *G. mosseae* strain proved to be more efficient as for root colonization delay. This result is in harmony with those of Ndoye et al. [27] gotten at *Acacia senegal* inoculated with this strain and *G. aggregatum*. However, these results do not corroborate with those of Jansa et al. [28] that found that *R. irregularis* occupied an intermediate position with the middle mycorrhization rates. The variation of the mycorrhization intensity would be bound very well to differences observed between the used AMF strains, the used plant, to conditions of the middle, at the level of phosphorus in soil and/or to other environmental factories signaled by Boddington and Dodd [29] and Alkan [30]. Values gotten for parameters of growth seem to confirm performances of the used AMF strains. This positive effect of the inoculation gotten with these strains has been demonstrated by several authors [13, 14, 18, 27] that associated mycorrhizal inoculation to phosphatic fertilization to heighten the availability of the phosphorus. These results indicate that the three AMF strains (Ri, Gm and IR-27) stimulated significantly, in presence or/no PNT, height and biomass sorghum plants in relation to the witness without inoculation. Differences of answer have been noted between strains AMF and have been owed to a functional diversity of these last [31]. These differences are observed among others: in the degree of the root infection [32], the density of the hyphes network to form meetings in complex networks [33], the metabolism and the transfer of the phosphorus toward the plant host by AMF [34] and in short to needs in carbon of the plant host [35].

5. Conclusions

Of these results, it comes out again that inoculation with AMF strains in presence of PNT permitted to improve sorghum plants growth. Of this fact, the biologic fertilization to basis of AMF in natural phosphate presence could be counseled in order to improve deficient soil nutrition phosphatic in phosphorus. It is evident from these results that the IR-27 treatment + PNT (100 mg) can be recommended to improve the culture of the sorghum in the field.
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Effect of Arbuscular Mycorrhizal Fungal Inoculation on *Sorghum bicolor* Growth at Different Phosphate Levels: A Greenhouse Study

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