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

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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

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 endomycorhizal 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 hypha mycélien; < 10%, some hyphae presence and fungi arbuscules in the root; < 50%, a middle infection of the root with hyphae and especially of arbuscules; > 50%, a root infected with several bladders and or arbuscules and hyphae; > 90%, an overgrown root nearly completely by bladders and/or arbuscules 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	1.96ab	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.

Fig. 1 Fragment of Cocktail + PNT (100 mg) plant root (a) and fragment of IR-27 + PNT (100 mg) plant root (b).

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 Ndoeye *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 biomasses 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 hyphae 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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