

Influence of Arbuscular Mycorrhizal Fungi Native to Senegal on the Growth and Yield of Sesame (*Sesamum indicum* L.) Grown Under Glass in Central African Republic

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Abstract: In the natural environment, sesame (*Sesamum indicum* L.), like any other crop, is subject to biotic and abiotic stresses. The presence of symbiotic microorganisms in the soil is often beneficial to the crop. Among these, arbuscular mycorrhizal fungi (AMF) are particularly effective in stimulating plant growth and it has been well shown that their inoculation into the soil is one of the biological means by which the crop can resist certain stresses and increase productivity. The objective of the present work is to evaluate the comparative effect of mycorrhizal inoculation with strains indigenous to Senegal on the growth and yield of sesame grown under controlled conditions. In this study, sesame was grown under greenhouse conditions and inoculated with four AMF inocula. Growth and yield parameters were evaluated at 14, 28, 42, 56 and 70 days after sowing. Above-ground, root and total biomass were assessed at 70 days after sowing. The results show an improvement in height by 78.34%, crown diameter by 78.34%, above-ground biomass by 54.83%, root biomass by 54.16% and total biomass by 54.65% compared to the non-inoculated control. Regarding the number of leaves, the difference observed with the control treatment was not significant ($P = 0.0005$). The frequency and intensity of mycorrhization are quite high. Statistical analyses show significant differences ($P < 0.05$) between the different treatments. It is noted that the roots of the controls are not mycorrhized. Similarly, the yield of the inoculated sesame plants resulted in a better improvement in the number of pods (131.25%), average number of seeds per pod (137.03%), seed yield (448.14%) and 1000 seed weight was not improved (0%). From this study, it appears that AMF provide significant benefits to the plant, and can be used in the field to improve sesame growth and yield.

Keywords: Arbuscular Mycorrhizal Fungi, Growth, Yield, *Sesamum indicum*

1. Introduction

Sesame (*Sesamum indicum* L. Pedaliaceae) is a plant of warm regions generally located in tropical and intertropical areas. It is known for its modest water and manure requirements [1]. Its economic importance is mainly due to the fact that its seed is

rich in quality oil (50%) and a protein composition (25%) comparable to that of meat [2]. Sesame seeds are used in the sugar industry and in baking as ornaments. The oil extracted from the seeds is biochemically stable [3] and is used for human consumption, but also in the soap industry, perfumery, margarine, cosmetics and confectionery. In medicine, sesame oil has antioxidant, analgesic and anti-inflammatory properties. The

world production of sesame seeds increased by 42.7% between 2000 and 2009, from 2.78 to 3.97 million tons; with decreases observed in 2002 (-12.4%), in 2005 (-2.3%) and in 2007 (-0.8%). This increase is mainly due to an increase in cultivated areas and not to an improvement in average yield, which rarely exceeds 500 kg per hectare. Yields vary greatly depending on the level of inputs used and the care taken during harvesting [4]. The largest African producers are Sudan, Ethiopia, Uganda and Nigeria.

The Central African Republic (CAR) is the 9th largest African sesame producing country with an annual production of 5,008 tons [4]. In the tropics in general, and more particularly in CAR, where soils are relatively poor in certain nutrients, notably nitrogen and phosphorus [5; 6], as well as in trace elements such as copper and zinc [7], the presence of mycorrhizae is therefore an indispensable asset [8]. Especially since in this environment the majority of cultivated plants associate with AMF [9] while maintaining their productivity [10]. Arbuscular mycorrhizal fungi (AMF) are particularly effective in stimulating plant growth and their inoculation into the soil has been shown to be one of the biological means by which the crop can withstand stress and increase productivity [11].

Some authors have shown that sesame forms a symbiosis with AMF fungi and that the effect of mycorrhization on plant growth and production is no longer in question [12-18]. Exploiting this symbiosis would be a possibility to improve sesame productivity. The present study was conducted to evaluate the comparative effect of mycorrhizal inoculation with native Senegalese strains on the growth and yield of sesame (*Sesamum indicum* L.) grown under controlled conditions.

2. Material and Methods

2.1. Plant and Fungal Materials

Sesame seeds were used to evaluate the effect of mycorrhizal inoculation on sesame. The germination rate of

this variety is 96%. These seeds were obtained at the Higher Institute of Rural Development (HIRD) in Boukoko, Central African Republic.

The fungal material was composed of inocula of AMF strains and a mycorrhized root obtained from the Laboratory of Fungal Biotechnology of the Cheik Anta Diop University of Dakar (Senegal). These are the following strains: G1: *Glomus mosseae*, (Nicholson, Gerd, Trappe DAOM 227 131); G2: *Glomus aggregatum* (Schenke, Smith, Emend, Koske) (DAOM 227 128); G3: *Glomus fasciculatum* (Thaxter, Sensus, Gere-mann, gerd) (DAOM 227 130); G4: *Glomus intraradices* (Schenker, Smith) (DAOM 127 198).

2.2. Methods

2.2.1. Aseptization of Seeds

Corn seeds were sanitized by soaking in 70° ethanol for 2 min followed by soaking in 3% sodium hypochlorite for 5 min. This was followed by three successive rinses with sterile distilled water for 5, 10 and 15 min respectively. These rinses removed all traces of sodium hypochlorite. These seeds were then sown at a rate of 3 per pot.

2.2.2. Setting up of the Trial

The trial was conducted in a greenhouse for 4 months in the experimental field of the liaison office of the HIRD of Mbaïki located in the Faculty of Health Sciences (FAHS) of the University of Bangui (altitude: 436m, latitude: 4° 22'38" N and longitude: 18°33'37" E). The plants were grown under the following conditions: average temperature $30/25.9 \pm 2^\circ\text{C}$ and relative humidity above 50%. The trial consisted of growing sesame in 10-liter pots containing 7000 g of sterilized soil (1h at 120°C) from Bakéré in the sub-prefecture of Bossembélé (Ombella Mpoko), whose physico-chemical characteristics were recorded in Table 1 below.

Table 1. Physico-chemical characteristics of the soil of Bakéré in the sub-prefecture of Bossembélé (Ombella Mpoko).

Component elements	Sand (%)	Silt (%)	Clay (%)	Organic material (%)	Total Carbon (%)	Total nitrogen (%)	C/N ratio (%)	Total phosphorus g/kg	Potassium (g/kg)	pH (p/v: 1/2,5)
Sand content (100 g)	87,20	4,50	8,75	19,12	11,1	0,79	14,1	0	0,057	5,01

Source: [19]

2.2.3. Experimental Setup

The experimental design used was that described by [20] modified. It is a completely randomized design with two factors: inoculum and variety. The inoculum factor included uninoculated controls and plants inoculated with G1, G2, G3 and G4. The variety factor had only one level: variety 1 and each treatment was repeated 4 times.

2.2.4. Inoculation of Sesame Plants

Inoculation was done 10 days after sesame planting. Each plant received 10g of inoculum per packet, with an average of 40 spores/g, in accordance with the dose used in sterilized media [21]. The inoculum was buried in the growing medium at a depth of 2 to 3 cm in contact with the root system of the sesame

plant. The mycorrhizal inoculum was composed of a mixture of spores, fungal propagules, mycorrhizal root fragments and soil.

2.2.5. Parameter Measurements

To evaluate the effect of MCA on sesame growth, height, crown diameter, average number of leaves, average number of branches were measured and counted every 14 DAS. Above-ground and root biomass were measured on the 70th DAS.

Plant height measurements were taken in each experimental unit at 14, 28, 42, 56 and 70 days after transplanting (DAS) using a tape measure.

Diameter at the crown was measured with an electronic caliper at the separation zone between the root system and the aerial part at 14, 28, 42.56 and 70 days after transplanting.

The number of leaves (NL) was counted on each plant of the experimental units every 14, 28, 42, 56 and 70 days after transplanting, the leaves of the main stem as well as the leaves of the branching stem were all counted.

The method used for measuring the biomass of aerial, root and total parts was that described by Hadou *et al.* [12] modified. At 70 days after transplanting, each plant was carefully stripped to recover the aerial part and all roots of the plants. All these parts were oven-dried at 70°C for 72 hours until a constant weight was obtained for the measurement of above-ground, root, and total biomass.

Some yield parameters were determined, namely: weight of 1000 seeds, average number of seeds per plant, average number of seeds per pod and average seed yield per hectare.

2.2.6. Calculation of the Rate of Improvement

The method used to calculate the rate of improvement is that described by Hadou *et al.* [12]. The rate of improvement of mycorrhizal inoculation was calculated using the following formula:

$$A = (V_m - V_t) / V_t \times 100$$

with A: rate of improvement (%), V_m : value of the inoculated treatment that obtained the maximum value and statistically different from the control, V_t : value of the control treatment.

2.2.7. Assessment of Mycorrhization Rate

The rate of mycorrhization was assessed by observation

under a light microscope using the method of Philips and Hayman [22]. The fine roots of the plants were cut into 1 cm fragments and stained according to the method described by Philips and Hayman [22]. The stained fragments were mounted between slides and coverslips in glycerol. The frequency (L) and intensity (I) of mycorrhization were determined by the gridline intersect method [23].

2.2.8. Statistical Analysis

All data obtained were subjected to analysis of variance (ANOVA) using SPSS 20 software for Windows. Tukey's test was used to determine any significant differences between the different varieties at the $p < 0.05$ threshold. Results were expressed as means \pm standard deviation.

3. Results

3.1. Effect of AMF on Sesame Height Measurements

Table 2 presents the results of height measurements of sesame inoculated with arbuscular mycorrhizal fungi (AMF) native to Senegal. Plant height was found to vary with the inoculum used and time. At 14, 28, 42, 56 and 60 days after sowing, statistical analysis showed no significant difference ($P > 0.005$) between the different treatments. The highest values were obtained with the G3 inoculum (179.75 \pm 4.93 cm). This inoculum improved the height growth of sesame by 78.34% compared to the non-inoculated controls.

Table 2. Evolution of the average height of plants during the cycle.

Treatments	Average height of plants (cm)				
	D14	D28	D42	D56	D70
Control	38.00 \pm 8.29 ^d	71.75 \pm 6.65 ^c	101.75 \pm 4.35 ^d	109.50 \pm 4.82 ^c	112.00 \pm 4.02 ^c
G1	51.25 \pm 7.35 ^b	88.00 \pm 9.38 ^b	106.25 \pm 9.32 ^c	140.75 \pm 1.06 ^b	159.25 \pm 5.16 ^b
G2	46.5 \pm 6.61 ^c	73.25 \pm 7.80 ^c	118.75 \pm 9.60 ^c	133.50 \pm 9.98 ^b	156.25 \pm 7.42 ^b
G3	45.25 \pm 6.80 ^c	78.50 \pm 8.19 ^b	136.00 \pm 7.79 ^a	163.75 \pm 1.09 ^a	199.75 \pm 4.93 ^a
G4	60.50 \pm 7.94 ^a	96.75 \pm 5.12 ^a	124.50 \pm 9.68 ^b	137.75 \pm 5.56 ^b	161.75 \pm 4.99 ^b

Control = non inoculé; G1: *Glomus mosseae*; G2: *Glomus aggregatum*; G3: *Glomus fasciculatum*; G4: *Glomus intraradices*. D14 = 14 DAS; D28 = 28 DAS; D56 = 56 DAS; D70 = 70 DAS.

For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

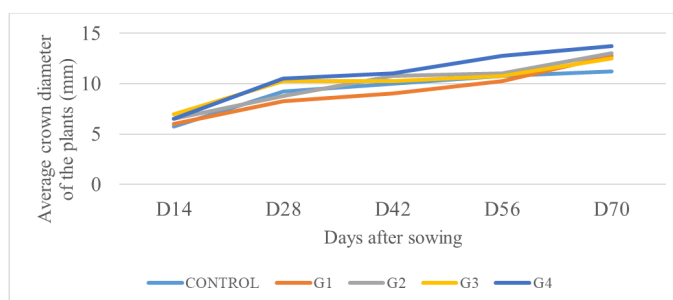


Figure 1. Evolution of the average of crown diameter of plants during the cycle. Control = non inoculé; G1: *Glomus mosseae*; G2: *Glomus aggregatum*; G3: *Glomus fasciculatum*; G4: *Glomus intraradices*. D14 = 14 DAS; D28 = 28 DAS; D56 = 56 DAS; D70 = 70 DAS.

3.2. Effect of AMF on Crown Diameter Measurements of Sesame

Figure 1 shows the results of crown diameter measurements

of sesame inoculated with AMF. Sesame growth varied with treatment. Statistical analyses show significant differences ($P < 0.05$) between inoculated and uninoculated treatments. Sesame growth was improved by mycorrhizal inoculation

compared to the control and the best improvement was obtained with G4 inoculum (13.75 ± 0.43 mm) followed by G2 (13.00 ± 0.83 mm) and G3 (12.50 ± 1.12 mm). The G4 inoculum improved the growth of sesame crown diameter by 22.22% compared to the uninoculated controls.

3.3. Effect of Mycorrhizal Inoculation on Average Leaf Number of Sesame Plants

The number of leaves over the cycle did not differ

significantly between inoculated plants and non-inoculated controls ($p < 0.005$) (Table 3). Compared to controls, inoculated strains did not affect plant development more. Inoculated plants had similar mean number of leaves at 70 DAS (23.00 ± 5.16 , 23.50 ± 1.00 and 19.50 ± 3.00) with G1, G2, G3 and G4 respectively to that of controls (18.50 ± 1.00 leaves) (Table 3). The rate of improvement in diameter growth at the sesame crown was 20.02% compared to the non-inoculated controls.

Table 3. Evolution of the average leaf number production of sesame plants inoculated with four strains of arbuscular mycorrhizal fungi.

Treatments	Average leaf number by plant				
	D14	D28	D42	D56	D70
Control	13.50 ± 3.79^c	15.00 ± 2.58^b	17.00 ± 2.59^c	18.00 ± 1.63^c	18.50 ± 1.00^c
G1	15.00 ± 1.15^a	16.00 ± 1.00^a	20.50 ± 4.12^a	23.00 ± 2.58^a	23.00 ± 5.16^a
G2	13.50 ± 1.00^c	15.50 ± 2.31^b	18.00 ± 2.31^b	19.50 ± 3.00^b	22.00 ± 4.32^a
G3	14.50 ± 1.00^b	16.50 ± 1.91^a	18.00 ± 1.63^b	22.00 ± 0.00^a	23.50 ± 1.00^a
G4	15.00 ± 2.00^a	16.50 ± 1.00^a	18.00 ± 4.32^b	19.50 ± 3.00^b	22.50 ± 2.52^a

Control = non inoculé; G1: *Glomus mosseae*; G2: *Glomus aggregatum*; G3: *Glomus fasciculatum*; G4: *Glomus intraradices*. D14 = 14 DAS; D28 = 28 DAS; D56 = 56 DAS; D70 = 70 DAS.

For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

3.4. Effect of Mycorrhizal Inoculation on Above-Ground, Root and Total Biomass Yield Per Plant

The results indicate a satisfactory vegetative development throughout the crop. No signs of necrosis or leaf loss were observed. For each treatment, the dry weight of aerial parts varied according to the inoculated strain. The dry weight of inoculated plants was generally higher than that of control plants

for all treatments. Inoculations generally induced an improvement in biomass compared to the control, and all inoculated treatments had significantly higher biomasses than the control. The best improvement was obtained with the G2 inoculum for aboveground biomass (120g), root biomass (74g) and total biomass (194g) (Table 4). The inocula used improved the growth of aboveground (54.83%), root (54.16%) and total (54.65%) biomass compared to the non-inoculated controls.

Table 4. Effect of arbuscular mycorrhizal fungi on above-ground, root and total biomass yield per plant at 70 DAS.

Treatments	Above-ground biomass (g)	Roots biomass (g)	Total biomass (g)
Control	62.02 ± 0.25^d	24.05 ± 0.25^c	86.07 ± 0.50^d
G1	87.05 ± 0.06^c	32.15 ± 1.00^b	11.20 ± 1.06^c
G2	120.25 ± 0.00^a	74.15 ± 0.05^a	194.40 ± 0.05^a
G3	66.05 ± 0.12^d	39.02 ± 0.06^c	105.07 ± 0.18^c
G4	96.01 ± 0.96^b	37.00 ± 0.00^b	133.01 ± 0.96^b

Control = non inoculé; G1: *Glomus mosseae*; G2: *Glomus aggregatum*; G3: *Glomus fasciculatum*; G4: *Glomus intraradices*. D14 = 14 DAS; D28 = 28 DAS; D56 = 56 DAS; D70 = 70 DAS.

For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

3.5. Evaluation of Mycorrhization Frequency and Intensity

Table 5 shows the mycorrhization frequency and intensity of sesame. Mycorrhization frequency is quite high than

mycorrhization intensity (ranging from 70.33% to 97.25% and 21.10% to 30.25%) respectively. Statistical analyses show significant differences ($P < 0.005$) between the different treatments. It is noted that the roots of the controls are not mycorrhized, this indicates that the treatments are free of any mycorrhizal contamination.

Table 5. Evaluation of mycorrhization frequency and intensity of sesame inoculated with four mycorrhiza strains.

Treatments	Frequency of mycorrhization (%)	Intensity of mycorrhization (%)
Control	0	0
G1	70.33 ± 1.00^d	28.00 ± 0.01^b
G2	97.25 ± 2.10^a	32.33 ± 3.00^a
G3	86.66 ± 0.00^c	21.10 ± 0.00^c
G4	90.10 ± 1.10^b	30.23 ± 0.15^a

Control = non inoculé; G1: *Glomus mosseae*; G2: *Glomus aggregatum*; G3: *Glomus fasciculatum*; G4: *Glomus intraradices*. D14 = 14 DAS; D28 = 28 DAS; D56 = 56 DAS; D70 = 70 DAS. For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

3.6. Determination of the Average Number of Seeds Per Plant and Seeds Per Pod, the Average Seed Yield Per Hectare and the 1000 Seed Weight

The average number of pods per plant over the cycle differed significantly between inoculated plants and non-inoculated controls ($p < 0.001$) Table 6. The rate of improvement in the number of pods per plant was 131.25%. Regarding the average number of seeds per pod, the analysis of variance shows a significant difference between the inoculated plants and their controls ($p < 0.0235$). The inoculated plants had an improvement of 137.03% compared to the control (Table 6).

The analysis of variance revealed significant differences ($P < 0.0001$) between the average seed yields (kg/ha) obtained. Mycorrhizal inoculation significantly affected seed production in inoculated plants and their non-inoculated controls. The best yield was obtained with plants inoculated with G2 ($1231.36 \pm 0.8 \text{ kg} \cdot \text{ha}^{-1}$) while in G4 and G1 the yields were similar ($726.18 \pm 0.4 \text{ kg} \cdot \text{ha}^{-1}$, $698.88 \pm 0.0 \text{ kg} \cdot \text{ha}^{-1}$) respectively followed by G3 ($635.44 \pm \text{kg} \cdot \text{ha}^{-1}$) and in the non-inoculated controls ($224.64 \pm 0.3 \text{ kg} \cdot \text{ha}^{-1}$) (Table 6).

Inoculation had no significant effect on the average 1000-seed weight ($p = 0.0003$). It remained approximately equal to 2.6 g in both inoculated plants and non-inoculated controls (Table 6).

Table 6. Effect of AMF on average number of pods per plant, average number of seeds per pod, average seeds yield and 1000-seed weight.

Treatments	Average number of pods per plant	Average number of seeds per pod	Average seeds yield ($\text{kg} \cdot \text{ha}^{-1}$)	1000-seed weight (g)
Control	32 ± 0.2^d	54 ± 0.2^d	224.64 ± 0.3^d	2.613 ± 0.5^a
G1	56 ± 0.4^b	96 ± 1.1^b	698.88 ± 0.4^b	2.520 ± 0.7^a
G2	74 ± 0.0^a	128 ± 1.3^a	1231.36 ± 0.8^a	2.643 ± 0.2^a
G3	52 ± 0.5^c	94 ± 0.3^c	635.44 ± 0.1^c	2.618 ± 0.1^a
G4	57 ± 0.1^b	98 ± 0.4^b	726.18 ± 0.0^b	2.562 ± 0.0^a

Control = non inoculé; G1: *Glomus mosseae*; G2: *Glomus aggregatum*; G3: *Glomus fasciculatum*; G4: *Glomus intraradices*. D14 = 14 DAS; D28 = 28 DAS; D56 = 56 DAS; D70 = 70 DAS. For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

4. Discussion

The general objective of this study was to evaluate the effect of mycorrhizal inoculation on the growth and yield of sesame with strains imported from Senegal. The results obtained on the growth of sesame showed that mycorrhizal inoculation improves the growth of this plant. Thus, the G3 inoculum (*Glomus fasciculatum*) improved the growth in height by 78.34%, the G4 inocula improved the diameter at the collar by 22.22 % and the biomass (aerial biomass (54.83 %), root biomass (54.16 %) and total biomass (54.65 %) compared to the non-inoculated control. It can be seen that the rate of improvement in this study is low compared to previous work by Haro and Sanon [20] under the same conditions. It can also be considered that the dose of inoculum provided would be insufficient (10 g) to induce a sufficient rate of mycorrhization that could lead to yield increases. Whereas sesame does not show specificity in the choice of fungus to form a symbiosis [24]. These results could be explained by the improvement of mineral nutrition of sesame by the inoculated symbiotic strains. This improvement by arbuscular mycorrhizal fungi (AMF) is manifested first by mycorrhizal infection and then by improved growth and biomass production. These results are in agreement with the work of some authors [20] who showed that mycorrhizal inoculation improves growth height, relative height growth rate, crown diameter, above-ground biomass, root biomass and total biomass compared to the non-inoculated control. Similar results were found by Diouf *et al.* [18] who showed that mycorrhizal inoculation increased leaf area, leaf number and root biomass of sesame.

In addition, the work of Haro *et al.* [25] who showed that mycorrhizal inoculation with undesirable strains improved cowpea growth better and Haro *et al.* [14] showed that inoculation with indigenous microorganisms improved above-ground biomass production and yield of cowpea to the same extent as fertilization with chemical fertilizers (NPK), at the rate of 100 kg/ha. On the other hand, the work of Diatta *et al.* [16] shows that there was no significant difference between the inoculated plants and the controls for all the variables considered.

The results of this work show that the frequency and intensity of mycorrhization of sesame seedlings vary significantly among treatments, these results are similar to the work of some authors [14] on sesame (*Sesamum indicum* L.) who indicated that mycorrhization frequency is quite high while mycorrhization intensity remains low and Diatta *et al.* [16] who showed that mycorrhization intensity of sesame seedlings is low in both inoculated seedlings and their non-inoculated controls. Statistical analyses showed a significant difference on the average number of pods per plant ($p < 0.035$), average number of seeds per pod ($p < 0.005$), average seed yield per hectare, and average 1000 seed weight was not significant ($p = 0.0001$). These significant differences could be explained by the improved mineral nutrition of inoculated plants due to AMF [12]. Mycorrhizae improve sesame nutrition, which promotes increased growth, productivity [14; 20; 26], and thus yield [27]. It can increase yields of sesame (*Sesamum indicum* L.) in Senegal [18]. Arbuscular mycorrhization can be used by farmers to reduce the use of chemical fertilizers, improve soil fertility, and increase their yields [28]. Mycorrhization results show that sesame roots are highly mycorrhized by the mycorrhizal strains used. The

mycorrhization frequency rate is quite high than the intensity raised. These results corroborate the work of Leye et al. [15].

5. Conclusion

The use of chemical fertilizers in agriculture allows satisfactory yields, however, it can have harmful effects on the environment and human health. The reduction of the quantities of fertilizers used then becomes a necessity. To this end, arbuscular mycorrhizal fungi (AMF) are effective biological tools for optimizing their use. The present study confirms the dependence of sesame on arbuscular mycorrhizal fungi (AMF). Inoculation resulted in a significant improvement in the growth and yield of sesame plants. These results suggest that AMF can provide farmers with an additional tool to reduce chemical fertilizer inputs, improve soil fertility and increase yields. Thus, microbial biofertilizers based on AMF could be recommended to farmers on soils poor in mineral elements such as phosphorus.

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