



Original Research Article

Evaluation of Symbiosis Effect of Some *Arbuscular mycorrhizal* Fungi on Growth of Yams (*Dioscorea Alata*) on Experimental Conditions

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ABSTRACT

The yam cultivation in Ivory Coast is facing several problems including the low level of soil fertility. From the perspective of effective inocula selection of arbuscular mycorrhizal fungi (AMF) to improve productivity, an experiment was conducted in a greenhouse to test the effectiveness of three complexes of AMF on the growth of *Dioscorea alata* var. bètè bètè. These complexes were collected from yam fields of three villages (Logbakro, Zambakro and Seman) in the District of Yamoussoukro. A completely randomized design with four treatments and six replications (doublings) was adopted. One month after inoculation by complexes AMF all inoculated plants have mycorrhizal structures ($F > 15\%$) in the roots. However, the intensities of mycorrhiza are low ($< 10\%$). The growth parameters (gain in height, number of internodes and leaves, leaf area) were not significantly varied according to treatments. Inocula used AMF did not stimulate growth of *Dioscorea alata* var. bètè bètè. An extension of time for experimentation would help better appreciate the efficiency of complexes AMF used during this study on the growth of *Dioscorea alata* var. bètè bètè.

Keyword: *Dioscorea alata* var. bètè bètè; AMF; efficiency test; Yamoussoukro

INTRODUCTION

Ivory Coast is the world third ranked producer of Yams after Nigeria and Ghana. Yam is the top food crops in the country, with an annual production of 5.8 million of tons in 2013 [1]. Yam is mainly produced in four zones (Centre, Centre-west, north and, North-east) for personal consumption

and sale [2]. It also generates financial incomes for an important part of the minor producers [3]. From 1999 to 2000, the annual consumption per inhabitant has grown from 105 kg to 120kg [4]. The increase in the demand of yam has brought about the expansion of farming lands in order to increase the production. From 2000 to 2013, the total cultivated fields increased 65%, growing from 505408 ha to 835000 ha. But the incomes decreased the same year, while the ploughed fields were getting larger. The productions decreased from 8.82 to 6.95 tons per hectare and per year, meaning a drop of 21 % [1].

Various difficulties related to the production can account for this low-yield. Among the difficulties, there are: the quality of farming material, the diseases, and the destructions by nematodes and insects [5]. In addition to these constraints, there is the low level of soil fertility [6]. The use of chemical fertilizers and pesticides is envisaged to overcome this aspect. However, in some cases, the use of chemical fertilizers and pesticides has proved to be highly impoverishing the basis of natural resources for agriculture, and threatening the future productivity [7]. Also, [8, 9] have indicated that it is not easy for minor producers to afford the necessary inputs. Today, considering problems of land, water and labor force resources, in addition to an increase in the demand of yam [7], actions must be taken for the intensification of these food crops' production. That will help reducing the negative impacts on the environment and ensure sustainable production.

One of the possibilities for agriculture is the use of the potentialities of Arbuscular Mycorrhiza Fungi (AMF) in order to develop some sustainable technologies of production. Indeed, different studies have permitted to show the effectiveness of the AMF's potentialities for the growing of plants [10, 11]. The AMF are symbiotic organisms of the plants' roots that favour a better watering, thanks to a network of hypha [12] and provide mineral elements [13] to their host. Besides, they

are able to lower the impact of the phytoparasites nematodes [14].

As part of Programme of Agricultural Productivity in West Africa (PPAAO), the IVO-RHIZE, funded by FIRCA, with the support of the World Bank, has decided to evaluate the impact of a technology based on the use of AMF in order to improve the productivity of food crops, namely yam, in Ivory Coast. The objective is to use the positive aspects of the AMF to improve the yield of this culture.

The general objective to be reached is, first, the physicochemical characterization of the soils and the AMF, ploughed with yam. The second phase concerns the production of inoculum from efficient AMF, bole, and finally their popularization among the peasants.

The present study is intended to test the efficiency of different AMF complexes in the growth of yams in experimental condition in Ivory Coast. This will permit the selection of efficient complexes. To reach this general objective, the effect of AMF on few agronomic parameters of yam will be specifically evaluated: the length of the stems, number of leaves, number of internodes and the leaf area of *Dioscorea alata* yam plants.

MATERIAL AND METHODS

Presentation of the zone and the research sites

The experimentation has been conducted in Yamoussoukro, a town of the Centre in Ivory Coast. The town is located between 6° and 8° latitude North and, between 4° and 6° longitude West. The climate of the District of Yamoussoukro is a subequatorial one, with a bimodal pluviometer comprising four seasons (2 rainy seasons and 2 dry seasons). The average pluviometers vary between 1100mm and 1600mm and the average annual temperatures fluctuate between 24.6° C and 27.9° C. the rainiest month is May and the least one is December. The relative humidity rate of the air varies from 40% in December to 85% in rainy season.

The vegetation of the District of Yamoussoukro is a mosaic of Guinean savannah and semi-deciduous humid dense forest of *Aubrevillea kerstingii* kind [15]. The area is very less hilly, with an average altitude of 200m, but we can notice some undulations with hollows and irregular plains. The types of the soils are lateritic, gravel like, moderately desaturated and having a medium level of fertility. The

subsoil of the region is composed of vast granitic massifs, and long ranges of schistose metamorphic rocks oriented North-Northeast and South-Southwest [15]. To conduct the research, three villages of the District of Yamoussoukro are selected for the sampling of the soils. These sites are Séman, Zambakro, and Logbakro. The coordinates (WGS 84 system) of the sampling points have been noted (Table 1).

Table 1: The coordinates (WGS 84 system) of the sampling points

Samples points	Latitude	Longitude	Altitude (m)
Se 1/1	N 06° 52mn 48,3s	W 005° 18mn 35,5s	208
Se 1/2	N 06° 52mn 48,1s	W 005° 18mn 36,3s	211
Se 1/3	N 06° 52mn 48,9s	W 005° 18mn 37,1s	207
Zb 2/1	N 06° 44mn 09,6s	W 005° 23mn 56,8s	172
Zb 2/2	N 06° 44mn 09,2s	W 005° 23mn 55,2s	172
Zb 2/3	N 06° 44mn 09s	W 005° 23mn 53,3s	170
Lo 3/1	N 06° 43mn 42,1s	W 005° 12mn 29,4s	206
Lo 3/2	N 06° 43mn 42,4s	W 005° 12mn 27,8s	206
Lo 3/3	N 06° 43mn 43,3s	W 005° 12mn 27,6s	207

METHODOLOGY

The soils sampling of the rhizosphere of yam was done in July. For each site of experiment (village), three samples from three different fields had been mixed up in order to obtain a composite sample. Soil samples have been taken from mounds with a drill in the stratum of 0-20cm depth. The material used for samples collection is thoroughly sterilized with 10% diluted bleach 12°, before taking the next sample. In sum, 3 samples of soil are brought to the laboratory for analysis. The sampled fields were all of tree month, with a surface covered of about 0.5 ha.

The soils taken to the laboratory are used to make trap formation for the propagules of AMF. This trap formation consists in sowing maize grains, disinfected with 10% diluted bleach 12o, in 2 liter-pots, each containing 1 liter of soil from the different sampled fields.

After two months, a sample of soil containing some AMF propagules and mycorrhiza like maize roots is taken from each pot to serve as inoculum of mycorrhiza. Then three inoculums of mycorrhiza from the soil sampling sites (Logbakro, Séman, Zambakro) were prepared. The characterization of mycorrhizal inoculum used in the present study comprises several steps:

Extraction, counting and identification of the spores contained in the inoculum

The extraction of AMF spores is operated with the technique of humid riddling, described by [16], from 50g of inoculum soil followed by saccharose centrifugation by gradient. The counting of the spores is done as recommended by the method of International Culture Collection of Vesicular Arbuscular Mycorrhizal fungi: [17] is intended to determine the density of the spores in the inoculum. It represents the numbers of spores in 1g of dry soil. The viable

spores are isolated with forceps and a nozzle of eppendorf tube, then mounted between plate and lamella in a mixture of (Polyvinyl Lactoglycérol) and reagent of Melzer (1 :1, v/v).

After this arranging, the elements are observed with an optical microscope then photographed with a digital camera, in order to identify the morphology of spores. The observed and described spores are identified through collections of species and items of reference such as those of [17, 18 and 19]. Some identification key points are also used, in particular, those suggested by [20].

Estimation of the mycorrhizal colonization of the roots in the inoculum

The fine roots of maize, are taken, cleaned with water then cut into fragments of 1cm, and finally coloured in blue trypan, according to the method of [21]. These fragments are heated at 80°C: first in KOH at 10% for 1h, followed by a thorough washing with tap water. Then, it is heated in HC1 at 1% for 1h and finally in trypan blue at 0.05 % for 30 mn. After the coloring in trypan blue, the 10 fragments of root are mounted between plate and lamella, in 50% glycerol [22], so as to estimate the rate of colonization of the roots with 100 and 400 magnifying optical microscope. The estimation of the level of colonization of the roots is done, according to the method of [23]. In this method, the percentages of infection are rated from 0 to 5. Each rate corresponds to a class of infection percentage. The frequency F (%) and the intensity I (%) of mycorrhization are calculated according to the following formulas:

$$F (\%) = (\text{number of mycorrhized fragments} / \text{total number of observed fragments}) \times 100$$

$$I (\%) = (95N_5 + 70N_4 + 30N_3 + 5N_2 + N_1) / \text{total number of observed fragments},$$
 with: Ni = number of fragments corresponding to the rate.

Plant Material

The vegetal material used is composed of a variety of *Dioscorea alata* yam, commonly called *Bètè bètè* in Baoulé (language). The seed yam tubers were collected in the markets of the town of Yamoussoukro. This variety has been selected because of its availability during the period of the present study. The plants are obtained through the technique of modified mini-cuttings. It consisted in the preparation of the seed yam, their planting in the seed trays, and the follow up of the growing of the seed yams into yam plants. The preparation of the seeds consisted, in the selection of the intact and sound tubers which dormancy process is started.

These tubers go through nematicide treatment via a thermotherapy by soaking it for 10 mn in water boiled at 50°C hot water. The tubers are then divided transversally and longitudinally so as to obtain fragments of 100 to 150g, necessarily having a piece of epidermis. These fragments are soaked in a fungicide solution (Mancozèbe 80 %), and coated with sterile ash, then left dry for 24 hours in the shade. The seed tray is composed of basin containing some sterile sawdust. The basins are placed in a shadowed area. The seeds are spread in the basins and finally covered with a fine layer of sterile sawdust. A regular watering was done during two to four weeks, avoiding any excess of water [24, 25].

Experimental Dispositive

The experimental dispositive is a Device Completely Random (DCR). It comprises a factor, origin of the AMF, four treatments (T; Lo; Se and Zb) and six doublings, totalizing 24 plants. Each repetition is a 10 L bucket (Figure 1). T is the non-inoculated reference. Lo, Se, Zb correspond to the inoculums taken respectively from Logbakro, Seman, and Zambakro.

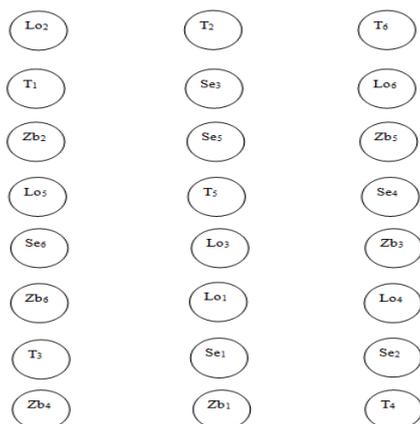


Fig. 1: Scheme of the experimental unit

T: Reference / **Lo:** Inoculum from the field of Logbakro/ **Se:** Inoculum from the field of Seman/ **Zb:** Inoculum from the field of Zambakro

Inoculation

After the pre-germination, the germs of yam are first transplanted individually in some 10 L strays containing 8 liters of sterile substratum composed of a mixture of sand and compost (1:3, v/v). And the plant is then inoculated with 40 g of inoculum. The inoculation of plants consists in putting the inoculum close to each system of root. Finally, after the inoculation, 1 liter of sterile growing substratum is used to cover the inoculum. [26]

Data Collection

A destructive sampling has been done one month after the inoculation of the plants with the AMF. Three plants selected randomly by treatment are picked up. The collected data concerns the length of the plants, the number of leaves, number of internodes, the leaf area, the frequency and intensity of the mycorrhization.

- **Length of the stalks:** The length of the plant stalks is measured with a decameter.
- **Number of leaves and number of internodes:** These parameters are obtained by counting.

- **Total leaf area:** For each plant sampled by treatment, the leaves have been classified in lots of «great» (G) and «little» (L) leaves, taking into account the fact that they have reached or not their maximum size. For each lot of leaves, a sample of 28 leaves is considered for the determining of the medium leaf area with the software MESURIM version 3.4.4.0.

The total leaf area is obtained by multiplying the number of leaves per surface by the corresponding average surface. Then the total leaf area is calculated with the following formula: $SFT = STG + STL$ avec $STG = \text{Leaf area of the lot of «great» leaves}$ and $STL = \text{Leaf area of the lot of «little» leaves}$ [27].

Frequency and intensity of mycorrhization: they are calculated according to the methods used for the characterization of the inoculum.

STATISTICAL ANALYSIS OF THE DATA

The obtained data are subjected to analysis of variance (ANOVA) with the software STATISTICA 7.1. The test of effectiveness of differences by Fisher in the threshold of 5 % is used for the comparison of the average values of the parameters measured on the yam plants subjected to the different treatments.

RESULTS

Relative diversity and abundance of the types of Arbuscular mycorrhizal fungi

The inoculum contains various kinds of AMF: *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora*, *Septoglomus*, *Sclerocystis*, *Claroideoglomus* (Figure 2) and some non-identified ones. The types *Glomus* and *Acaulospora* are the most abundant ones and they can be found in the different inoculums (Figure 3).

Mycorrhization of the fragments of roots contained in the inoculum

The frequency of mycorrhization is rated 100 % for the root fragments contained in the inoculums taken from Logbakro and Zambakro

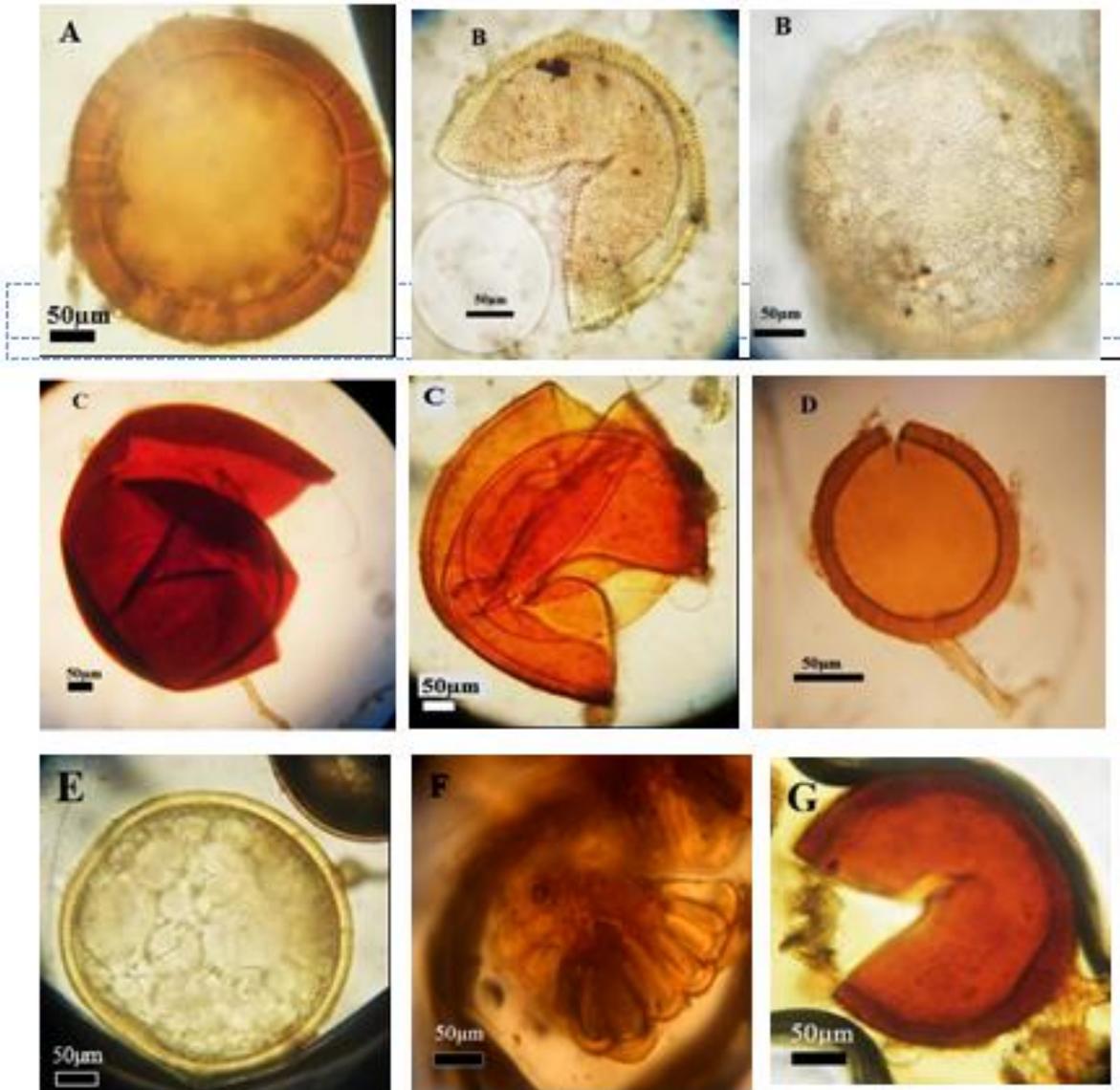


Fig. 2: Spores of arbuscular mycorrhizal fungi observed in the soil samples taken in Seman, Logbakro and Zambakro

A: Spore of *Glomus* genus; **B:** Spore of *Acaulospora* genus dont les ornements sont caractéristiques; **C:** Spore of *Scutellospora* genus; **D:** Spore of *Claroideoglomus* genus; **E:** Spore of *Gigaspora* genus; **F:** Sporocarp of *Sclerocystis* genus; **G:** Spore of *Septoglomus* genus.

71.4 % for the root fragments contained in the inoculum from Seman. The intensity of mycorrhization of the roots varies from 11.6 % to 30 % respectively in Seman and Zambakro. It is 20.6 % in Logbakro.

Frequency of mycorrhization

After one month of culture, the observation of the roots indicated the presence of the features of structures of mycorrhization (vesicles, hypha). The ANOVA did not reveal any

significant difference among the treatments at the threshold of 5 %. The frequency of mycorrhisation of the non-inoculated plants (Reference) is nil. Those of the plants cultivated with the inocula taken from Seman, Logbakro and Zambakro are respectively 47.40; 15 and 40 % (Table 2).

Intensity of mycorrhization

The intensities of mycorrhization of the yam plant roots inoculated with the inocula from Logbakro, Seman, and Zambakro are respectively 0.25; 4.93 and 4.90 %. The intensity of mycorrhization of non-inoculated plants (Reference) is nil. All these values are not statistically different at the threshold of 5 % (Table 3).

Parameters of growth

Lengths of the stalks of plants

The lengths of the stalks of yam plants subjected to different treatments do not indicate significant differences at the threshold of 5%. Nevertheless, the values obtained from the plants treated with the inoculums from Logbakro, Seman and Zambakro are respectively 165.3 cm, 144 cm and 128.1 cm.

The non-inoculated plants have an average length of 110.3cm (Table 3).

Number of internodes and leaves

The yam plants inoculated with AMF taken from Logbakro, Seman and Zambakro presented respectively 17; 17 and 21 internodes a month after the inoculation. The non-inoculated plants had an average of 15.5 internodes. These numbers of internodes are not significantly different at the threshold of 5 % (table 3). Also, the numbers of leaves of the yam plants are statistically identical in treatments at the threshold of 5 %. But, the plants inoculated with the inoculums taken from Logbakro, Seman,

Leaf area

There is no significant difference between the leaf area of inoculated plants and those of non-inoculated. The average leaf area vary between 1399.17 cm² for the non-inoculated plants (reference treatment), and 1822.96 cm² for the plants inoculated with inoculum from Séman. The leaf areas of the plants inoculated with inoculums taken from Logbakro and Zambakro have the respective values of 1629 cm² and 1740.83 cm² (table 3).

Table 2: Frequencies and intensities of mycorrhization of the roots of yam plants *Dioscorea alata* 1 month after inoculation with different inoculums of arbuscular mycorrhizal fungi

Treatments	Frequencies of mycorrhization F (%)	Intensities of mycorrhization I (%)
Logbakro	15 ± 4.08	0.25 ± 0.12
Seman	47.40 ± 26.92	4.93 ± 2.8
Zambakro	40 ± 31.88	4.90 ± 4.74
Reference	0	0

ns = non significatif au seuil de 5%. *= significatif au seuil de 5%.

Table 3: Parameters of growth of yam plants, *Dioscorea alata*, non-inoculated (references) and a month after inoculation with different complexes of arbuscular mycorrhizal fungi.

Treatments	Lengths of the plant stalks (cm)	Number of internodes	Number of new leaves	Leaf area (cm ²)
Logbakro	65.3 ± 35.03	17 ± 2.45	37 ± 6.38	1629.63 ± 1069.98
Séman	144 ± 32.66	17 ± 1.63	30 ± 3.27	1822.97 ± 173.46
reference	110.3 ± 13.31	15,5 ± 1.22	24,5 ± 3.67	1399.17 ± 208.93
Zambakro	128.1 ± 44.17	21 ± 4.97	27.5 ± 7.76	1740.83 ± 541.55

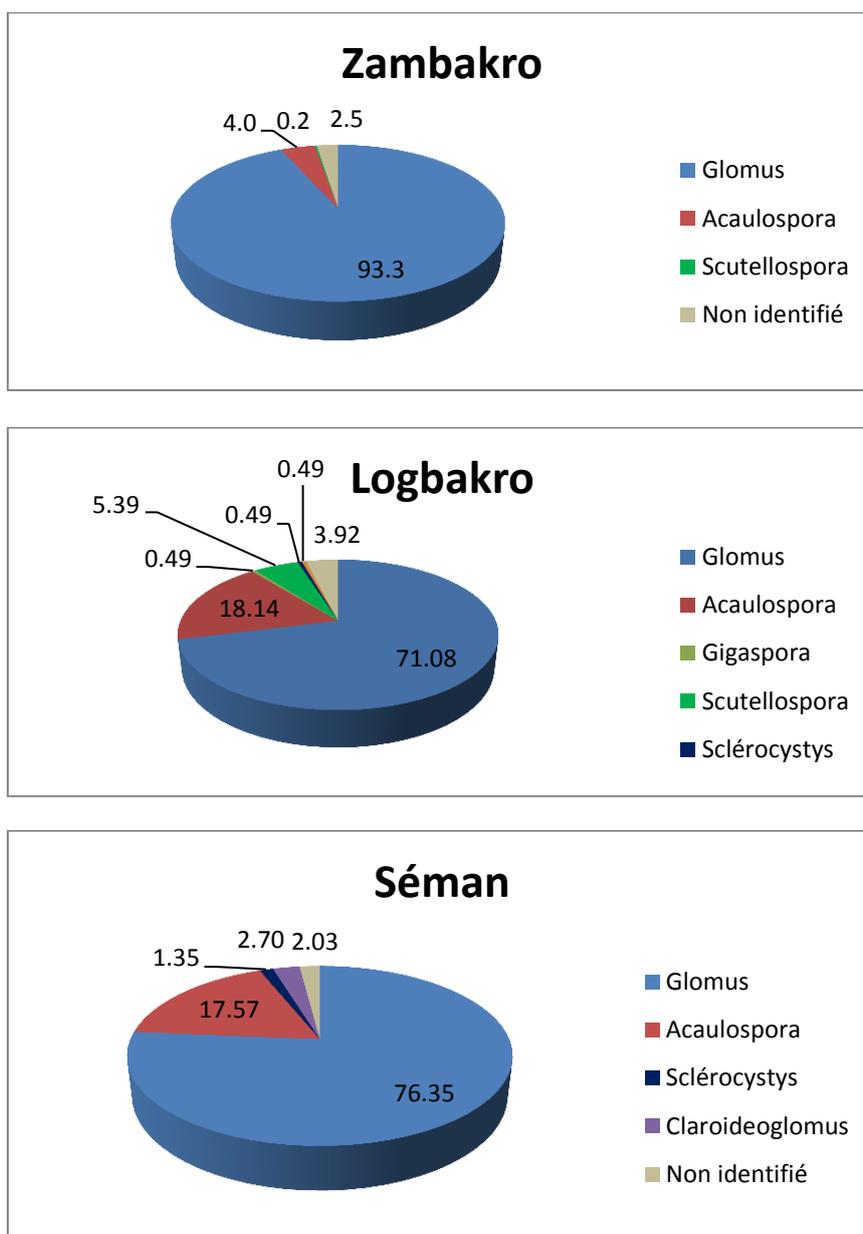


Fig.3: Diagram representing the percentages of the types of Arbuscular Mycorrhizal Fungi in the different inoculums

DISCUSSION

The observation of the tissues of the roots, a month after, revealed the presence of typical structures of the endomycorrhizal symbiosis of all the inoculated plants. Yet, the intensities of mycorrhization observed, for the inoculated plants are low (< 10 %) compared to those obtained by [28] which vary from 37.02 to 55.6 %. This difference can be justified by the relative shortness of the experimentation (1month), while those of [28] were determined three months after the inoculation. Also, [29], showed, during their works on the effect of arbuscular mycorrhiza on the growth of the clover, that the intensity of mycorrhization grows progressively as the clover develops until eight weeks. The frequency of mycorrhization of the reference plants is nil. This is a proof of the good condition of sterilization of the substratum used.

There are no significant differences between the effects of the treatments (inocula from Logbakro, Seman, Zambakro and Reference) on the parameters of mycorrhization of the yam variety «bètè bètè». This result is different from that of [30] who showed that the inoculated plants and the non-inoculated references give different statistical values of mycorrhization. There is also, no significant difference for the parameters of growth. The results obtained in this experiment are different from those of [31], which indicate that mycorrhization stimulates the growth of the plants of olive tree var. sigoise. Indeed, Sidhoum obtained his

results after three months of culture, while the present study lasted for only one month. The results of [32] also revealed that the mycorrhizal inoculation stimulates significantly the growth of sorghum (*Sorghum bicolor* L. Moench), and cowpea (*Vigna unguiculata* (L.) Walp). This could be accounted for by the duration of the experimental process of [32] (5 months). These plants are known for their high

level of mycotrophy and their capacity to keep the maximum spores of AMF [33, 34 and 35]. The results concerning the growth of the yam *Dioscorea alata* are similar to those obtained by [36] showing that the mycorrhization of millet by *Glomus aggregatum* did not stimulate its growth. These authors indicated that the exotic origin of the inoculum could justify its ineffectiveness. [28], also argued that not any significant differences were noticed on the growth in height of the plants of *Acacia Senegal*, inoculated during the first four-weeks of culture after inoculation. This period is similar to the duration of the present experimentation. The mycorrhizal symbiosis did not stimulate the growth of the yam significantly because of the shortness of the duration of the experimentation. The low intensity of mycorrhization could indicate the beginning of the setting of the mycorrhizal symbiosis, which will be done progressively with the vegetative development of the plant. Furthermore, out of all the plants that have been measured, the non-inoculated reference plants have the smallest values. That can be the foreshadowing of some significant differences among treatments, as the duration of the experimentation is extended.

CONCLUSION

The complexes of AMF taken from Logbakro, Zambakro and Seman, inoculated into yam plants *Dioscorea alata* in order to test the effect of the mycorrhization on the vegetative growth, have identic values of parameters statistically measured one month after inoculation. The plants inoculated with these AMF complexes have some statistic efficiencies like those of non-inoculated plants, as far as the length of the stalks, the number of internodes, number of leaves and leaf area, are concerned. The duration of the experimentation did not allow having results different from the plants with inoculums; for the selection of the best

complexes of AMF. The selection of effective arbuscula mycorrhizal fungi for the enhancement of the growth of yam in Ivory Coast requires that, the experimentation should be conducted for two months at least. This duration can permit a more efficient observation of the effect of mycorrhization.

For upcoming studies:

- a molecular characterization of the AMF types contained in the different inoculums and an efficiency test of these AMF types taken separately, not only on the studied variety, but also on other local varieties should be undertaken;
- the evaluation of the effectiveness of AMF on the resistance to diseases, the hydric and mineral nutrition of many local varieties of yam should also be studied.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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