MAIZE (Zea mays L.) RESPONSE TO MYCORRHIZAL FERTILIZATION ON FERRUGINOUS SOIL OF NORTHERN BENIN

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ABSTRACT

The use of Arbuscular Mycorrhizal Fungi (AMF) is considered one of the effective organic ways to increase the crops productivity. The aim of this study was to evaluate the growth promoting effect of three mycorrhizal fungi (Glomus cubense, Rhizophagus intraradices and Funneliformis mosseae) on maize crops in a ferruginous soil of Northern Benin. Maize seeds were inoculated with mycorrhizal fungi in combination with or without minerals fertilizer. Study was conducted in a completely randomized block design with nine treatments and four replicates. The endomycorrhizal infection was evaluated on 68th days of sowing while the crop was harvested after 90 days. Results of study revealed that application of AMF have significant effect (p < 0.01) on the growth attributes and performance of maize. Compared to the control, maximum height (increases of 29%) was recorded in the plants treated with a complete dose of NPK, followed by the plant treated by F. mosseae combined with 50% NPK.
1 Introduction

Agriculture occupies an important place in the economy of Sub-Saharan countries such as Benin. Maize cultivation accounts about 70% of total cereal production area and is the basis of food for Beninese populations (MAEP, 2010). Unfortunately, low productivity and unstable yields jeopardize food security and keep farmers in precarious financial conditions (Delphine et al., 2011). This situation became more serious by certain constraints, among these parasite complex (insects, nematodes and striga) and the chronic decline of soil fertility, which marked by deficiency of assailable nitrogen and phosphorous (Igué et al., 2015). Thus, for a production of maize that can cover people’s food needs and generating commercial income, producers are increasingly using mineral fertilizers and phyto-sanitary products obtained from chemical industries (Priou, 2013). Further, availability and high prices of these agricultural inputs in Sub-Saharan countries do not allow small producers to acquire them (Micaela, 2015). In addition, prolonged use of these mineral fertilizers leads to acidification of the soil and contamination of ground water. Along with these access used of these chemicals may stimulated the accumulation of heavy metals in harvest products (Dalpé, 2005).

One of the best alternatives of these chemical fertilizers is the use of soil microorganisms that can colonized in plant roots and enhance the soil fertility and crop productivity. Among the currently used soil microorganisms, use of mycorrhizal fungi is most common one (Johnson, 2008). In fact, many researchers from all over the world have been using native or exotic endomycorrhizal symbiosis to improve the productivity of various agricultural crops (Johnson, 2008; Megueni et al., 2011). Further, this association not only improving the plant productivity but also have significant effect on microbial diversity and soil quality (Wardia, 2011). Exploitation of these endogenous endomycorrhizal potential of soils can optimize growth, hydro mineral nutrition and nitrogen fixation.

In fact, in most of the cases, beneficial effect of mycorrhiza is due to an improvement in the mineral nutrition of the host plant, especially with regard to the low mobility elements in the soil such as phosphorus, zinc and copper (Javot et al., 2007) through extensive exploration of the absorption surface and the volume of soil prospected by fungal hyphae. The aim of our study was to evaluate the effect of three selected mycorrhizae fungi (*Glomus cubense*, *Rhizophagus intraradices* and *Funneliformis mosseae*) strains inoculation on the growth parameters and yield of maize on ferruginous soil in North Benin.

2 Materials and methods

2.1. Variety of maize

Maize variety EVDT 97 STR C1 obtained from the National Institute of Agricultural Research of Benin (INRAB, 1995). This variety has good resistance to lodging, breakage and diseases with a grain yield between 2 and 3 t/ha (Yallou et al., 2010). The vegetative period is 140 to 169 days.

2.2 Microorganisms

Three mycorrhizal fungi strains named *Glomus cubense*, *Rhizophagus intraradices* and *Funneliformis mosseae* were used in this study. These strains were provided by the mycorrhiza laboratory of the “Instituto Nacional de Siencias Agricolas (INCA)” of Cuba.

2.3 Characteristics of the experimental site

Present study was conducted at the north experimental station of the Agricultural Research Center of the National Institute of Agricultural Research of Benin, Ina (Borgou) located at 358 meters altitude and between 9°58’N latitude and longitude 2° 44’E (Figure 1). The experimental site is characterized by a tropical ferruginous soil with variable characteristics and sensitive to leaching. The soil of Ina has high content of organic matter (1.92%), nitrogen (0.092% at pH 5.7), phosphorus (58 ppm) and potassium (0.49 meq/100g of soil). The sum of the bases and the CEC (Cation Exchange Capacity) are average on surface horizon (Igué et al., 2015). The saturation in bases is average and the pH is moderately acidic. The cumulative rainfall recorded at the experimental site during the trial period is 743.3 mm, distributed over the months of August (493.5 mm), September (144.7 mm) and October (105.1 mm).

2.4. Experimental design

The experimental design used for this study was completely randomized block design with nine treatments and four replicates. Each treatment covered a plot of 16 m² contained 4 lines of 4 m. Seeding was done by keeping a distance of 0.80 m x 0.40 m, a...
density of 31.25 plants/ha. The paths between plots and between repetitions were 1.8 m and 2 m respectively. The growth and yield data were collected from the representative 2 central lines of each useful plot (6.4 m²). The different treatments formulated were Absolute control (no fungi or mineral fertilizers - CTL), *Glomus cubense* (*Gc*), *Rhizophagus intraradices* (*Ri*), *Funneliformis mosseae* (*Fm*), ½ dose of N₁₅P₁₅K₁₅ recommended (½ NPK), *G. cubense* + ½ dose recommended N₁₅P₁₅K₁₅ (*Gc+½ NPK*), *R. intraradices* + ½ dose of N₁₅P₁₅K₁₅ recommended (Ri+½ NPK), *F. mosseae* + ½ dose of N₁₅P₁₅K₁₅ recommended (Fm+½ NPK) and full recommended dose (150 kg / ha) of N₁₅P₁₅K₁₅ (NPK) (Akpinfa et al., 2017).

2.5. Maize seeds inoculation with Arbuscultive Mycorrhiza Fungi

The inoculation of maize seed with selected mycorrhiza fungi (*G. cubense*, *R. intraradices* and *F. mosseae*) was carried out as per the method given by Fernández et al. (2000). The mycorrhiza fungi applied to the 10% of the maize seed’s weight mixed with sterile distilled water (600 ml kg⁻¹). After mixing, seeds have been coated and dried on ambient temperature during 12 hours. The same process was repeated for all three AMF strains.

2.6. Seedlings and plants maintenance

Two maize seeds was deposited in a hole of about 5 cm depth and immediately closed. Three weeding was carried out; the first weeding was coupled with one planting per plant two weeks after sowing, the least vigorous plant was removed. The second and third weeding were respectively realized six and eight weeks after sowing.

2.7. Growth and yield data collection

The data related to leaf area of the plants was taken sixty days after sowing. The product of leaf length and width affected by the coefficient 0.75 estimated the leaf area (Ruget et al., 1996). In each elementary parcel, the plant height was measured by using the ruler and stem diameter with the help of caliper. The measurements were taken at each 15 days of intervals and carried out upto 60th days after Seedling (DAS). To avoid all contamination, the two lateral lines on each side were eliminated.

Figure 1: Geographical location of the experimental station
the first two feet on each side were removed, and other two central lines have been eliminated. Twelve plants were selected from the two central lines at the rate of six plants per line. In addition, the leaf area of the plants was taken 60 DAS. The leaf area of the plants has been taken sixty (60) days after seedling.

### 2.8 Evaluation of the yield parameters

At 103th days after seeding, 12 plants from the two central lines of each elementary plot were harvested. After destalking corncobs, the total weight of the corncobs in each plot was taken using an
The influence of various treatments on growth, yield, mycorrhizal parameters and nutritional status of plants compared with control and examined using analysis of variance model based to two factors (Block and treatment). After that, the Dunnett test was used to assess the relative performance of each treatment compared to controls. The influence of the AMF on the mycorrhizal parameters has been analyzed with a stationary model of analysis of the variance followed of the t test of Student Newman Keuls for the treatments structuring. The regression of Fisher has been used to model build the production of spore number and the intensity of mycorrhization. Categorization and discrimination of treatment was made with hierarchical digital classification followed by Principal Component Analysis (ACP) with using the R software 3.3.2 with FactoMineR, car, MASS, nnet, heplots et candisc package. Presentation of the segregation of treatment groups in the axis plan (can1, can2).

3 Results

3.1. Effects of various treatments on plant growth and maize grain yield

Effect of different mycorrhizal treatments on maize plant growth and grain yield was found highly significant compared to the control (p <0.01). Thus, the results of Dunnett’s test showed that all treatments induced a significant difference in maize height, maize leaf area, and maize grain yield compared to the control (Table 1). The induced effects of mycorrhizal fungi are very high (R² >50%). Among the various texted combinations, full-dose of NPK resulted a significant difference in collar diameter of plants. For the aerial part, higher value was reported for the treatment Fm+½ NPK (F. mosseae + ½ dose of NPK) and NPK (full dose of NPK). While in case of underground biomass, highest value was reported for the treatment NPK (full dose of NPK) and ½ NPK (½ dose of NPK) compared to controls. The effects induced for the latter are relatively low (R² <50%).

3.2. Determination of treatments with better performance on maize growth and yield parameters

3.2.1 Growth parameters

The results obtained after analysis of variance showed highly significant difference (p <0.001) between all treatments for plant height and leaf area. Highly significant difference (p <0.01) was reported in case of plants diameter between all tested treatments (Table 2). Plants treated with the full dose of NPK showed superiority over the all other treatments. However, plants treated with F. mosseae + ½ dose of NPK (Fm+½ NPK) also showed good growth in length (24.78%), thickness (14.28%) and leaves width (14.75%) compared to control plants (Table 2).

3.2.2 Yield parameters

The Analysis of variance indicated a significant difference (p <0.05) between treatments for plant biomass and maize grain yield (p <0.001) (Table 3). The highest values of aboveground and
belowground biomass were obtained with plants treated with *Funneliformis mosseae* + ½ NPK dose (Fm+½ NPK) followed by those treated with the full NPK dose. In terms of grain yield, the treatment having Fm+½ NPK (F. mosseae + ½ NPK dose) and NPK (full-dose NPK) yielded highest yields, with respective increases of 46.57% and 53.42% yield compared to control plants (CTL).

### 3.3. Effects of endomycorrhizal infection on mycorrhization parameters

In case of mycorrhizal frequency, mycorrhizal intensity and spore number, the analysis of variance revealed non significant difference between the different AMFs (Table 4). However, the plants receiving only *Rhizophagus intraradices* induced the best roots.
frequency of mycorrhization (38.50%) and a high number of spores (1.73 spores/g of soil). While those receiving only F. mosseae induced the best roots intensity of mycorrhization (11%). Globally, plants that received AMFs in combination with mineral fertilizers had the lowest values for all mycorrhizal parameters.

3.4. Categorization and discrimination of treatments according to their potential for action on maize plant growth and grain yield

The results of the hierarchical classification based on the performances of mycorrhizal fungi on maize plant growth and grain yield showed four groups of treatments (Figure 2). The cluster 1 is composed only of treatment Fm+½ NPK and the cluster 2 is consists of treatment NPK. The cluster 3 is composed of the ½ NPK, Gc+½ NPK and Ri+½ NPK treatments whereas the cluster 4 is composed of the Gc, Ri and Fm treatments. The canonical discriminative analysis carried out on the clusters in order to differentiate them with respect to the growth and yield parameters showed that the Cluster 1 treatment induced a high production of the aboveground and underground biomass and a good yield (Figure 3). Cluster 2 treatments, as it concern, caused mainly high growth in neck height and diameter and large leaf area of plants with high maize grain yields. Cluster 3 treatments induced mean aboveground and belowground biomass and opposed Cluster 4 treatment, which yielded seedlings of height, leaf area and average neck diameter (Figure 3).

4 Discussion

This increase in growth may be due to the fineness of the mycorrhizal hyphae (diameter <3 μm) which allows them to penetrate all the interstices of soil particles to facilitate the transport of non-mobile mineral elements particularly phosphorus in favor of the plant (Lambers et al., 2008). Laminou (2010) reported that mycorrhizal inoculation stimulates the growth of sorghum (Sorghum bicolor L.), these results are in agreement with the findings of present study. Similarly, Ouahmane et al. (2007) also reported the beneficial effects of mycorrhizal symbiosis on the growth of Gaussen (Cupressus atlantica). Moreover, Diouf et al. (2009) and Bourou (2012) observed that F. mosseae induced an improvement in the foliar growth of Sesame
(Sesamum indicum L.) plants and the neck diameter of tamarind (Tamarindus indica L.) plants. Balzergue (2012) suggested that mycorrhizae establish symbiotic associations with the roots of plants, which allows them to provide plants water and minerals which help in establishing good yield.

The highest yields of aerial and underground biomass production were obtained with plants treated with *F. mosseae* combined with half-dose of NPK. These results confirm by the work of Diouf et al. (2009), who observed an increase in aerial and underground biomass of *Sesamum indicum* plants inoculated with *F. mosseae*. This stimulation is the result of an improvement in the nutritional and especially phosphate status of plants (Benjelloun et al., 2004) as well as an improvement in plants photosynthesis (Jesús et al., 2004).

In case of maize grain yield, plants treated with the full-dose of NPK followed by those treated with *F. mosseae* and half-dose of NPK significantly improved maize grain yield with increases of 53.42% and 46.57%, respectively compared to control plants. These results confirmed with the findings of Haro (2016) in Burkina Faso that showed that *Scutellospora* ssp. and *Gigaspora* ssp. have great potential to increase cowpea yield from 28.78% to 60.16% respectively. Similarly, Labidi et al. (2014) reported the beneficial role of mycorrhizal inoculation in Tunisia on the yield of durum wheat (*Triticum durum* Desf.). On the other hand, although CMAs positively influenced grain yield, these results would be even better if plants had received the normal amount of rain. Indeed, during the years 1990 to 2012, the rainfall ranged from 873 to 1700 mm in North Benin. In the period of our study, the rainfall recorded during the three test months was 743.3 mm, with a decrease in rainfall especially in the months of September (144.7 mm) and October (105.1 mm). Similar results were observed by Diouf & Lambin (2001) who have shown through their work that maize plants that have undergone water stress (465 mm and 430 mm) have a low yield (2.28 t / ha and 1.44 t / ha) compared to those which have been irrigated normally.

The results obtained on endomycorrhizal infection revealed that there is no significant difference between treatments. However, plants treated with *R. intraradices* had a better frequency of mycorrhization (38.50%). Results of present study are in agreement with the findings of Meddad et al. (2011), who observed that *R. intraradices* have high rate of mycorrhization (69.96%). Some differences were observed between these two studies which can be explained by the use of different soil. However, in some studies level of infection is very high and it is more beneficial to the plant (Diagne & Ingleby, 2003) than the result of present study but it might be due to difference in climatic conditions. Hetrick et al. (1992) also observe that plant growth is not necessarily related to the degree of colonization of their roots by AMFs.

Among the three treatments with half-dose of NPK, only treatment with *F. mosseae* gave the lowest frequency of mycorrhization (32%). Our results evolved in the same direction as those obtained by Meddad et al. (2011). Indeed, these authors have shown in their work that the inoculation of the olive tree (*Olea europea* L.) with *F. mosseae* induces a mycorrhization frequency of 51.06% and it was lower than that of *R. intraradices* (69.96%). This difference in value might be difference in the soils and nature of the plant used for experimentation. In addition, Diatta et al. (2013) worked on sesame (*Sesamum indicum*) and reported that *F. mosseae* have no significant difference on the intensity of mycorrhization between treatments. These results are in agreement with the findings of present study. Similarly, Haro (2016) have shown that the mycorrhization frequency of inoculated plants is high (82%) whereas the intensity of mycorrhization remains low (27.89%).

In addition, the results obtained in present study showed that plants received only *R. intraradices* induced a high number of spores (1.73 spores / g of soil) compared to the other two strains. However, after adding the NPK, the number of spores is one per g of soil. Other studies have shown that the addition of inorganic phosphorus decreases the colonization rate of *R. intraradices* (Nouaim & Chaussod, 1996). Similarly, Mäder et al. (2000) and Goalbaye et al. (2013) have reported that the increase in mineral phosphate fertilizer in conventional agriculture decrease the AMFs colonization in root.

**Conclusion**

The results of the present study showed the beneficial role of mycorrhizae inoculation on the growth and seeds yield of maize plant. The assessment in station showed that the combination of these three arbuscular mycorrhizal fungi with 50% of recommended NPK had a meaningful effect on the growth and the yield of the maize on ferruginous soil in the Northern Benin. The most important result was the one with *F. mosseae* combined to half dose of recommended NPK. This bio product can be recommended in the future to improve the productivity and the durability of agricultural system. This study is a complementary contribution of the fertilizing organic on ferruginous soil to the Northern Benin. It will be interesting to identify and to characterize the native species of arbuscular mycorrhizal fungi in Northern Benin, and evaluate their effects on the growth and seeds yield of maize.

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Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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