

Greenhouse evaluation of the growth of *Zea mays* L. inoculated by arbuscular mycorrhizal fungi strains in native arbuscules on ferrous soil

Aguégué M. Ricardos¹, Ahoyo Adjovi Nestor R², Agbodjato Nadège A¹, Noumavo Pacôme A^{1,3}, Allagbé Marcellin², Chabi Nicodeme W⁴, Glele Kakaï Romain⁵, Ramón Rivera⁶, Adjanooun Adolphe² and Lamine Baba-Moussa^{1*}

¹Laboratory of Biology and Molecular Typing in Microbiology, Department of Biochemistry and Cell Biology, Faculty of Sciences and Techniques, University of Abomey-Calavi, 05 BP: 1604 Cotonou, Benin.

²National Institute of Agricultural Research of Benin, 01BP: 284, Cotonou, Bénin.

³Laboratory of Microbiology and Food Technologies, Department of Plant Biology, Faculty of Sciences and Techniques, University of Abomey-Calavi, 04 BP: 1107 Cotonou, Benin.

⁴University of Abomey-Calavi, Polytechnic School of Abomey-Calavi, Teaching and Research Laboratory in Food Microbiology, Benin.

⁵Biomathematics and Forest estimation laboratory, Faculty of Agronomic Sciences, University of Abomey-Calavi, 04BP:1525, Benin.

⁶Departamento de Biofertilizantes y Nutrición de las Plantas, Instituto Nacional de Ciencias Agrícolas, Cuba.

*Corresponding author. E-mail: laminesaid@yahoo.fr

Accepted 10th February, 2020.

Abstract. Maize production faces many constraints, including declining yields due to the steady decline in the fertility of cultivated soils and attacks by maize plants by pathogenic microorganisms, despite its importance and increasing demand for environmental pollution in Benin. The exploitation of new biological tools in agriculture opens up opportunities for innovation and improvement of crop systems to minimize the risks of environmental pollution and food contamination. This study aims to evaluate the growth of maize (*Zea mays* L.) in response to arbuscular mycorrhizal inoculation. Five (5) natives' strains (*Glomus caledonius*, *Rhizophagus intraradices*, *Funneliformis geosporum*, *Acaulospora capsicula*, *Acaulospora dilatata* and *Diversispora globifera*) were tested in greenhouse following a device in complete random blocks. Inoculated plants and chemically fertilized plants were used as controls. The results showed that co-inoculation of *Glomus caledonius*, *Rhizophagus intraradices* and *Funneliformis geosporum* improved the growth of corn plants (80.54%) and dry matter production (109.5%) compared to controls without chemical fertilizers and not inoculated. This study exhibited that native mycorrhizal fungi halve the recommended dose of 200kg/ha of NPK and 100 kg of Urea for corn fertilization. Results offered an alternative for the recovery of native mycorrhizal fungi in the form of organic fertilizer.

Keywords: Arbuscular Mycorrhizal Symbiosis, Biofertilizer, Growth Behavior, Benin, *Zea mays* L.

INTRODUCTION

World maize production in 2013 was 839 million tons, compared to 653 million tons for wheat (statistical planet scope, 2013). In most West African countries, maize forms

the basis of the diet of rural populations (N'DA et al., 2014). In Benin, maize plays an important role in agricultural production systems in all agro-ecological areas

(Adjanohoun et al., 2012). It remains Benin's first local cereal, with a national production of 1,012,630 tons in 2010, far ahead of sorghum (168,090 tons) and rice (124,975 tons) (Agro et al., 2014).

Despite the importance of this speculation and increasing demand, maize productivity is still below 0.5 t/ha against a potential yield of 3 to 5 t/ha depending on whether or not it has brought mineral fertilizers (Azontondé et al., 2005). Declining soil fertility is one of the main causes (Balogoun, 2012). Soil degradation is one of the crucial problems facing global agriculture (Igué et al., 2013). In Benin, 90% of cultivated land has symptoms of degradation (Amadji and Migan, 2001), with differences from region to region. To this end, a lot of research has led to the development of integrated soil fertility management technologies (INRAB, 2006). Unfortunately, very few of these technologies are adopted by producers because of the changes in producer behavior required by their use (Toleba, 2017).

Faced with consumer demands for healthy and environmentally friendly agriculture, farmers are increasingly turning to ecological intensification (Griffon, 2010) mainly based on the enhancement of agro-ecological practices. In particular, the introduction of symbiotic species such as Arbuscular Mycorrhizal Fungi (AMF) into nutrient optimization is well in line with ecological systems for sustainable intensification of agricultural production and therefore maize. These fungi form a symbiotic association with nearly 80% of plant species (Garbaye, 2013). This association results in an improvement in plant growth by making available mineral elements that are naturally inaccessible to the plant (Cardenas, 2010). Although mycorrhizal fungi are ubiquitous in natural soils, cropping practices most often disrupt their populations and diversity (Atama et al., 2018). Under these conditions, the many experiments conducted over decades most often demonstrate that the addition to soils of spores or propagules of these fungi results in faster plant development and increased yields. It is in this context that this study was initiated to assess the effect of the symbiotic relationship between the AMF native to the rhizospheric soils of Benin and the variety of maize EVDT- 97- STR- C1 on Ferrous soil in greenhouse.

MATERIALS AND METHODS

Plant material

The maize variety EVDT 97 STR C1 provided by the National Institute of Agricultural Research in Benin was used. It is an early 90-day variety, with a potential yield of grain ranging from 4 to 5 t/ha in a peasant environment. The seed of this corn are white color, toothed and their texture is mid-farinaceous and mid-vitreous. EVDT 97 STR C1 variety has a good resistance to American rust,

helminthosporiose, curvulariose and drought (Yallou et al., 2010).

Cultivation substrate and fungal inoculum

Three (3) different types of fungal inoculum were tested in this study. The first and second are co-inoculations respectively of 3 species of the genus *Glomeraceae* (*Glomus caledonius*, *Rhizophagus intraradices* and *Funnelliformis geosporum*), 2 species of the genus *Acaulosporaceae* (*Acaulospora capsicula* and *A. dilatata*) and the third is mono inoculation with *Diversispora globifera* isolated from the rhizospheric soils of maize in Benin. Inoculum was produced and multiplied by the association of spores of each genus of fungi with young sorghum plants. The sorghum seeds were disinfected in a bleach solution (5%), then rinsed and soaked in Sterile Distilled Water (SDW) for 24 hours. Sorghum plants were grown in greenhouses in pots containing sterilized substrate consisting of a mixture of clay and peat (2:1v/v) for four months to ensure good sporulation of the stumps. After four months of cultivation, the inoculum, consisting of a mixture of spores and root fragments, was collected for testing.

Cultivation substrate and seed inoculation

The culture substrate used was a ferrous soil, previously sifted (5 mm) and steam sterilized (120°C for 1 hour/day for 3 consecutive days) before use. The chemical characteristics of the soil were: pH (water) 5.34; organic matter 1.15%; Ratio C/N 6.43; Pass 153 ppm; CEC 6.72 cmol.kg⁻¹; Ca²⁺ 1.53 cmol.kg⁻¹; Mg²⁺ 0.773 cmol.kg⁻¹; K⁺ 0.355 cmol.kg⁻¹. Plastic jars of 3 kg, previously washed and disinfected with bleach (chlorine at 12 degrees), were filled at ¾ of the volume with the substrates of the disinfected soil. Each pot was moistened to 2/9th of the substrate maximum retention capacity of 1000 ml of SDW 24 hours before sowing (Eteka, 2005). The pots are watered at 1/9th of the RMC or 500 ml of EDS every 48 hours after germination of the seeds for 30 days. For each type of inoculum, the amount of inoculum applied was 20% of corn seeds weight. An amount of water equivalent to 1200 ml/kg of product was added to obtain the mixture. The seeds were coated in the resulting mixture. They were then dried in the ambient air in accordance with the recommendations of Fernandez et al. (2000).

Experimental device

The experimental device was a Complete Random Block of nine (9) three-repeat treatment. The different treatments are:

- Control = Ctrl (without AMF, nor NPK+ Urea);
- *G. caledonius* + *R. intraradices* + *Funneliformis geosporum* = Glom;
- *A. capsicula* + *A. Dilatata* = Acau ;
- *Diversispora globifera* = Divers ;
- 50% of the recommended dose of NPK + Urea = ½ NPK + Urea;
- Glom + 50% of the recommended dose of NPK + Urea = Glom ½ NPK + Urea;
- Acau + 50% of the recommended dose of NPK + Urea = Acau ½ NPK + Urea;
- Divers + 50% of the recommended dose of NPK + Urea = Divers ½ NPK + Urea;
- Recommended dose NPK + Urea = NPK + Urea.

Collecting growth parameters

Plant height and diameter data were measured every 96 hours from the 7th to the 35th day after sowing. The leaf surface was measured only on the 35th day after sowing. At each pot, the height of the corn plant was measured using a tape meter. The diameter of the plants was measured using a sliding foot and the leaf surface was estimated by the product of leaf length and width affected by coefficient 0.75 (Ruget and Chatier, 1996).

Dry yield

At harvest, 35 days after sowing, the aerial and root parts of the plant of each pot are harvested per treatment. Fresh air and underground masses were determined by direct weighing and air and underground dry masses were measured after drying at the seed at 65°C for 72 h until the constant weight (Yadav et al., 2010) for dry weight determination (DW). Dry matter (DM) will therefore correspond to the ratio of dry weight (DW) to the weight of fresh biomass (FB) (in %) ($DM = \frac{DW}{FB} \times 100$).

Determining mycorrhization parameters

Measurements of root colonization by the AMF were made 35 days after sowing according to the coloring method described by Phillips and Haymain (1970) and the mycorrhizal root infection estimation was made using the intersection method developed by Giovannetti and Mosse (1980) and the reading described by Trouvelot et al. (1986). Two hundred (200) minigames of corn roots previously washed and cut into small pieces were weighed into the test tubes. A 10% KOH solution has been added. The mixture was left for fifteen (15) minutes in the ambient air and put in the stub at 90°C for 1 hour. Roots were then thoroughly rinsed with running water, drained and put back into the test tubes and then covered with a 0.05% Trypan blue solution. The mixture was left

in the ambient air for 15 min and the test tubes were replaced in the stub at 70°C for 15 min. One hundred (100) root fragments were appreciated. A class score of between 0 and 5 corresponding to the estimate of the proportion of cortex colonized by the mycorrhizal symbiont was assigned to each of the root fragments observed. The root observation was made in gridded boxes using a stem DRC/ZEISS binocular magnifying glass (×180) equipped with an eye ×10/23 mm tilted at 45 degrees. The presence of shrubs, vesicles and hyphae was noted simultaneously. The rate of mycorrhization was apprehended by the parameters of shrub mycorrhizal infections. Two (2) parameters of shrub mycorrhizal infections were calculated:

- The **frequency of mycorrhization (F)** that reflects the degree of infection of the root system: $F(\%) = \frac{N-n_0}{N} \times 100$ where **N**: number of fragments observed and **n₀**: number of fragments without trace of mycorrhization.

- The **intensity of mycorrhization: m** (absolute mycorrhization intensity) that expresses the portion of the colonized cortex in relation to the entire root system:

$$m (\%) = \frac{95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1}{N - n_0}$$

In this formula, **n₅**, **n₄**, **n₃**, **n₂** and **n₁** are the numbers of fragments respectively recorded in the five infection classes marking the importance of mycorrhization: 5 = more than 95%, 4 = 50 to 95%, 3 = 30 to 50%, 2 = less than 30%, 1 = trace. It is this parameter that best reflects the degree of mycorrhization.

Statistical analysis

Data on growth, performance and mycorrhization parameters were encoded using the 2018 Microsoft Excel spreadsheet. The graphs were made using the GraphPad Prism 8.0.2. All analyses were carried out with the R Core Team 2018. The averages were combined for the different treatments and compared by Student-Newman-Keuls (SNK) to the 5% threshold.

RESULTS

Effect of CMA on corn plant growth parameters

The average height of maize plants between the 7th day after sowing (DAS) and the 15th DAS, did not show any difference between the different treatments (Figure 1). As early as 19th DAS, there was a difference ($P \leq 0.01$) between the average heights of corn plants. This difference had continued until the 35th DAS. Repeated custom variance analysis results and the 5% threshold SNK test show that the best height growth was obtained

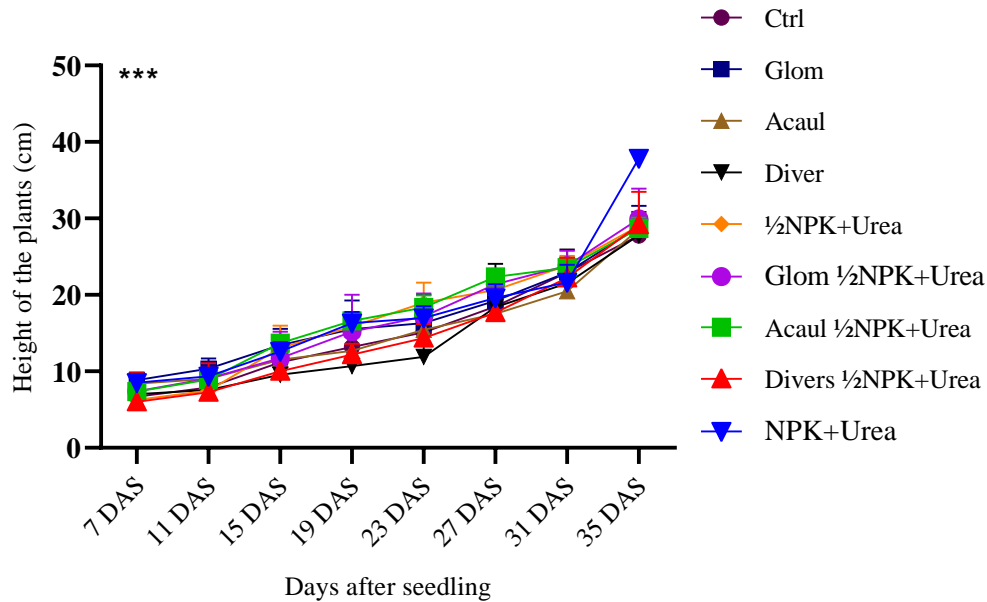


Figure 1. Changes in the average height of corn plants by time and by treatment.

Table 1. Arbuscular mycorrhizal fungal effects on corn plants height, diameter and leaf area.

Treatments	Height (cm)		Diameter (cm)		Leaf of area (cm ²)	
	m	σ	m	σ	m	σ
Control	27.77 ^b	3.09	1 ^{cd}	0.26	107.85 ^e	14.29
Glom	28.9 ^b	2.75	1.2 ^{bcd}	0.26	157.05 ^d	5.77
Acaul	28.6 ^b	1.22	1.17 ^{bcd}	0.12	121.58 ^e	5.12
Diver	27.8 ^b	1.25	1.2 ^{bcd}	0.26	151.43 ^d	3.18
1/2NPK + Urea	29 ^b	1.00	1.3 ^{bc}	0.10	207.65 ^c	3.99
Glom 1/2NPK + Urea	29.93 ^b	3.97	1.4 ^b	0.17	330.70 ^a	18.27
Acaul 1/2NPK + Urea	28.67 ^b	1.42	1.2 ^{bcd}	0.10	255.25 ^b	8.50
Divers 1/2NPK + Urea	29.2 ^b	4.28	0.88 ^d	0.29	159.66 ^d	21.02
NPK + Urea	37.87 ^a	0.55	1.93 ^a	0.12	272.38 ^b	14.24
Signification	**		***		***	

m = mean; σ = Standard deviation; ** = $P < 0.01$ (highly significant); *** = $P < 0.001$ (very highly significant). The means (m) followed of the same letter are not significantly different.

with plants treated with 100% NPK + Urea followed by plants treated with Glom 1/2 NPK + Urea which improved the height by 4% and 7% respectively compared to plants treated with 1/2 NPK + Urea and control plants (without AMF and NPK + Urea) (Table 1).

The average diameter at the collar of the corn plants between the 7th DAS and the 11th day after sowing did not show any difference between the different treatments (Figure 2). From the 15th day after sowing, there was a significant difference ($P \leq 0.01$) between the average diameter at the collar of corn plants. This difference had continued until the 35th DAS with a more noticeable gap from the 23rd DAS. Repeated custom variance analysis results and the 5% threshold SNK test show that the best growth in diameter at the collar was obtained with the

plants treated with 100% NPK + Urea followed by plants treated with Glom 1/2 NPK + Urea that improved the neck diameter by 10% and 28% respectively compared to plants treated with 1/2 NPK + Urea and control plants (without AMF and NPK + Urea) (Table 1).

Table 1 shows us the results of repeated custom variance analysis and the SNK test at the 5% threshold of the leaf surface of the leaves of corn plants at the 35th DAS. From the table, it appears that maize plants that received the intake of mycorrhizal shrub mushrooms in combination or not with 1/2 NPK + Urea et 100% of NPK + Urea presented the average values of the highest leaf surfaces, greater than or equal to 159.66 cm² ($P \leq 0.001$). On the other hand, the average leaf surface values of the

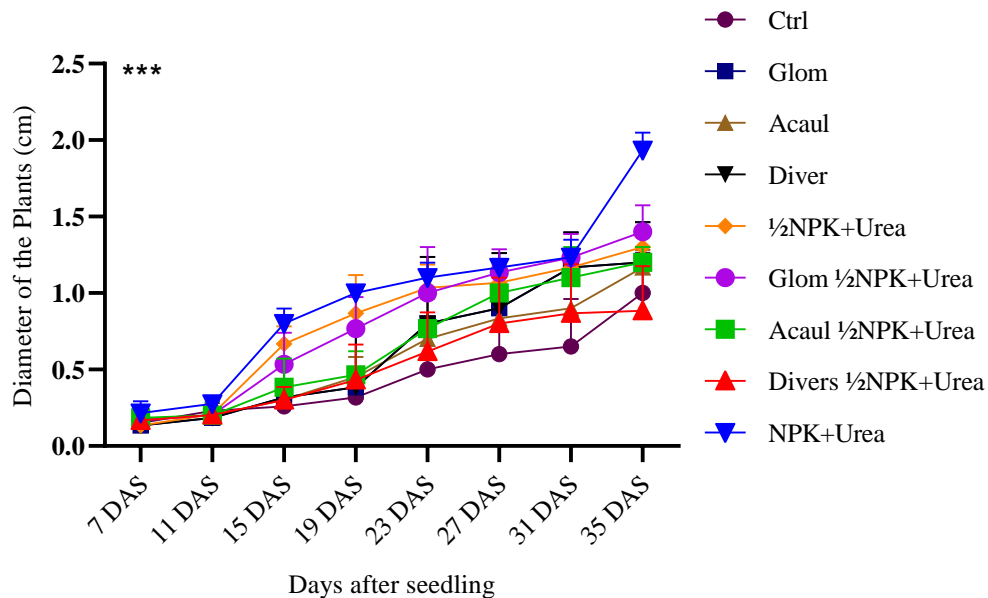


Figure 2. Changes in the diameter at the average collar of corn plants by time and by treatment.

Table 2. Effect of corn plant inoculation on aerial and underground dry matter production.

Treatments	Aerial dry matter		Underground dry matter	
	m	σ	m	σ
Control	12.03 ^d	1.80	26.89 ^e	3.57
Glom	12.93 ^{cd}	0.26	69.13 ^{bc}	5.11
Acaul	11.58 ^d	2.09	71.87 ^b	2.94
Diver	12.16 ^d	0.80	36.67 ^d	5.77
1/2NPK + Urea	11.88 ^d	0.68	62.63 ^c	3.73
Glom 1/2NPK + Urea	16.43 ^a	1.37	76.11 ^b	6.73
Acaul 1/2NPK + Urea	14.25 ^{bc}	0.95	62.22 ^c	4.59
Divers 1/2NPK + Urea	15.95 ^{ab}	1.61	68.4 ^{bc}	3.90
NPK + Urea	14.7 ^{abc}	2.34	92.86 ^a	6.49
Signification	***		***	

m = mean; σ = Standard deviation; ** = $P < 0.01$ (highly significant); *** = $P < 0.001$ (very highly significant). The means (m) followed of the same letter are not significantly different.

lowest corn plants, about 107.85 cm² were obtained with control plants (without AMF, nor NPK + Urea). Plants treated with Glom 1/2 NPK + Urea improved the leaf surface of corn plants by 59.25% and 206.65% respectively compared to plants treated with 1/2 NPK + Urea and control plants (without AMF and NPK + Urea).

Effect of AMF on aerial and underground dry matter

The results of the effect of AMF on air and underground dry matter production are shown in Table 2. Repeated measurement variance results show a significant difference ($P \leq 0.001$) of dry matter between different treatments. Plants treated with Glom and Divers strains in

combination or not with the intake of 1/2 NPK + Urea indicated the best air-dry matter of 36.92 and 32.92% on the one hand and underground of 200% and 167% on the other hand respectively compared to control plants (without AMF, without NPK + Urea).

Rate of colonization of corn plant roots

Observation of the roots of corn plants 35 days after sowing showed that the roots were well colonized by shrub-borne mycorrhizal fungi. The percentage of root colonization varied according to the different treatments ($P \leq 0.001$). The SNK test at the 5% threshold (Table 3) shows that the best frequency (44.36%) mycorrhization is

Table 3. Effect of corn plant inoculation on mycorrhization parameters.

Treatments	Frequency (%)		Intensity (%)	
	m	σ	m	σ
Control	0 ^d	0	0 ^c	0
Glom	22.33 ^c	3.06	23.24 ^b	6.03
Acaul	29.33 ^b	8.96	23.38 ^b	4.29
Diver	24.33 ^{bc}	3.51	20.35 ^b	0.72
½NPK + Urea	0 ^d	0	0 ^c	0
Glom ½NPK + Urea	44.37 ^a	2.10	28.98 ^a	2.96
Acaul ½NPK + Urea	27 ^{bc}	2.65	21.46 ^b	2.31
Divers ½NPK + Urea	29 ^{bc}	3.61	20.60 ^b	2.49
NPK + Urea	0 ^d	0	0 ^c	0
Signification	***		***	

m = mean; σ = Standard deviation; *** = $P < 0.001$ (very highly significant). The means (m) followed of the same letter are not significantly different.

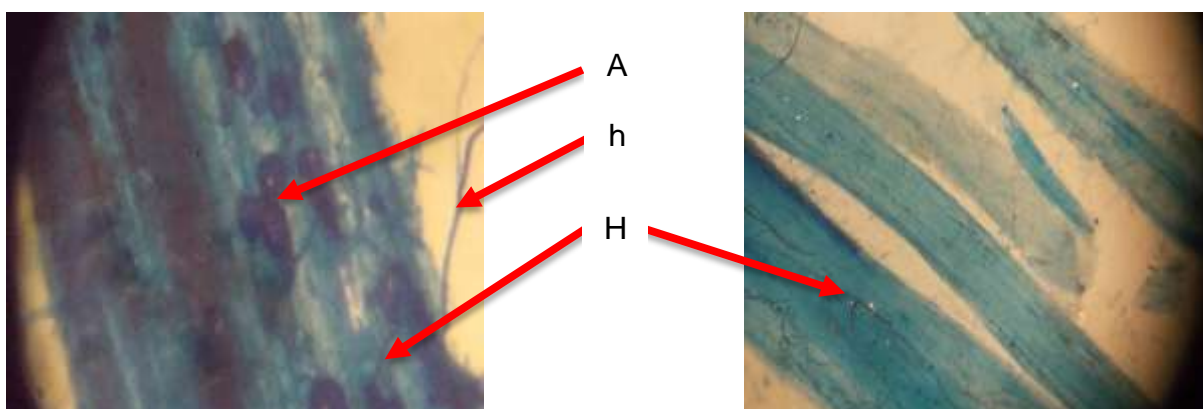


Figure 3. Infected corn roots colonized by shrub-shaped mycorrhizal fungi structures. (A: vesicular; h: intra-root hyphae; H: Extra-root hyphae (×180).

observed at the roots of the plants treated with strains of *G. caledonius* - *R. intraradices* - *F. geosporum* in combination with ½NPK + Urea followed by plant roots with strains of *A. capsicula* - *A. dilatata* (29.33%) and *D. globifera* (29%). As for the intensity of mycorrhization, the best is obtained by the same treatment (14.66%). In addition, the intensity of mycorrhization of the roots of other mycorrhizal plants ranges from 20.36 to 23.37%. It should be noted, however, that no mycorrhization was observed at the roots of control plants as well as plants treated with NPK + Urea and 100% NPK + Urea. The binocular magnifying glass observations of the roots of corn plants revealed the presence within them of hypes and vesicles (Figure 3).

Correlation between different parameters measured on corn plants

Table 4 shows the correlation between the different parameters measured on the maize plants. The

correlation is negative and non-significant ($r < -0.29$ ns) between mycorrhization parameters and growth parameters on the one hand, and a low positive correlation ($r > 0.34^*$) between mycorrhization parameters and dry matter on the other hand. On the other hand, there was a high significant positive correlation ($r > 0.6^*$) between growth parameters and dry matter parameters.

DISCUSSION

This study clearly shows that maize plants growth is improved by the simultaneous presence of strains of *G. caledonius* + *R. intraradices* + *F. geosporum* in combination with ½ NPK + Urea. The synergistic effect of its 3 strains of the genus *Glomeraceae* improved the growth of maize plants by 24.42 and 80.54% respectively compared to plants treated with ½ NPK + Urea and control plants (without AMF and NPK + Urea). Several authors reported that the synergistic effect of the

Table 4. "r" correlations between parameters measured on corn plants.

	Height	Diameter at collar	Leaf surface	Aerial dry matter	Underground dry matter	Frequency	Intensity
Diameter at collar	0.88**	1					
Leaf surface	0.12	0.13	1				
Aerial dry matter	0.38	0.20	0.18	1			
Underground dry matter	0.70*	0.63*	0.75*	0.53*	1		
Frequency	-0.35ns	-0.29ns	-0.31ns	0.47	0.19	1	
Intensity	-0.39ns	-0.32ns	-0.43ns	0.34*	0.16*	0.97***	1

* = $P < 0.05$ (significant); ** = $P < 0.01$ (highly significant); *** = $P < 0.001$ (very highly significant); ns= no significant

combination of *Glomeraceae* species on plant growth. According to Pellegrino et al. (2011) and Ortas (2014), the species richness of AMF and their interaction are known to have a positive impact on growth. Also, Jansa et al. (2008) stated that functional complementarity has been observed between species within a community of AMF colonizing the same root system. The soil of experiment showed a low fertility. However, the pH (5.34) and the Carbon-Nitrogen ratio (C/N=6.43) obtained are favorable for a better expression of *Glomeraceae*. In their work, Wang et al (1993) reported that the optimal values of pH for good AMF expression are between 5.5 and 6.5. This result shows the beneficial effect of AMF on plant growth and development as demonstrated by several research studies by El-yazeid et al. (2007), Laminou et al. (2009) and Leye et al. (2015). According to Hamza (2018), mycorrhizal, by associating with plant roots, facilitate better root development, allowing plants to feed better.

Plants treated with co-inoculation of *G. caledonius*, *R. intraradices* and *F. geosporum* in combination with ½ NPK + Urea and the *D. globifera* strain improved aerial dry matter production by 36.92 and 32.92%. On the other hand, plants treated with co-inoculation of *G. caledonius*, *R. intraradices* and *F. geosporum* in combination with ½ NPK + Urea and those treated with co-inoculation of *A. capsicula* and *A. dilatata* induced more the best underground dry matter by 183 and 163.64% respectively compared to control plants (without AMF, without NPK + Urea). Results showed that mycorrhizal plants increased production of aerial and underground dry matter. Results confirmed those of Atama et al. (2018) which revealed that the application of AMF on rice plants (IR841 variety) in greenhouses increased their root dry matter by more than 51% (*G. mosseae*) and 36.41% (*A. spinosa*) compared to non-inoculated plants. Moreover, results of Laminou et al. (2009) in Niger showed that inoculation of AMF (especially *G. intraradices*) to plants allowed plants to increase their yield in total biomass. Also, has been reported by several that rice roots plants association with several species of AMF (*G. mosseae*, *G. hoi*, *G. versiformae*, *G. diaphanum*, *G. geosporum*, *G. cladonius*, *G. clarum*, *Ascaulosporum* spp., *Archacospora* spp.,

Paragloms spp.) increased root area for better nutrient acquisition (Zhang et al., 2005, Gao et al., 2007; Raimam et al., 2007; Rajeshkannan et al., 2009; Fernandez et al., 2011). The inoculation of the maize plants in our study by AMF significantly improved the dry weight of the aerial and root parts. This beneficial role of AMF on development and dry biomass was also observed on cucumber (Chen et al., 2013), lettuce (Baslam et al., 2013), pepper (Kaya et al., 2009) and tomato (Mujica Perez et al., 2010; Copetta et al., 2011; Mujica Perez et al., 2012).

Indeed, a high level of diversity maintained in a low intensity farm management system (Adriano-Anaya et al., 2006) is defensible because each strain of AMF brings benefits to agricultural production under different stress situations (Sieverding, 1990), resulting in safer production, and is compatible with farms whose fragility and risk aversion are in conflict with high levels inputs use (Plenchette et al., 2005).

Best frequency (44.36%) mycorrhization is observed at the roots of plants treated with co-inoculation of *G. caledonius*, *R. intraradices* and *F. geosporum* in combination with ½ NPK + Urea. Pellegrino et al. (2011) reported that a native mycorrhizal inoculum was also effective on *Trifolium alexandrinum* L. However, the low frequency (29%) of mycorrhization observed in plants treated with the co-inoculation of *A. capsicula* + *A. dilatata* in combination with ½ NPK + Urea is explained by the fact that some native AMF species are not necessarily better adapted to specific soils in improving growth and nutrient uptake (Schreiner, 2007). As for the intensity of mycorrhization, the best performance is obtained the previous treatment (14.66%). The absence of mycorrhiza at control plants roots as well as those treated only with chemical fertilizers confirmed that the study soil has been well sterilized. However, these results were compared to those obtained by Wang et al. (2015) and Jeong et al. (2015) which reported mycorrhization rates of about 50% of rice seeds inoculated with AMF in nurseries. During this period, fungi most likely began to grow, causing their energy reserves to decrease (Douds et al., 1995), which may explain its lack of aggressiveness in the roots of corn plants. In addition,

the absence of shrubs and a strong dominance of intra- and extra-root hyphae are signs of early root colonization, which explains the low correlation between growth and mycorrhization parameters. Low root colonization may be due to the confinement of roots in the pots reducing oxygen concentration around the roots. On the other hand, the direct effect of mineral fertilizers on fungi in pots may influence root colonization. Graham and Syvertsen's (1984) work on Citrus plants showed that fertilization increased the amount of phosphorus in the leaves but decreased the rate of root colonization by *Glomus intraradices* from 64 to 23%. This low colonization could also be due to the maize seed (EVDT-97- STR C1) which is an improved variety. According to Harrier and Watson (2003), a decrease in mycorrhizal dependency has been observed in all cultivars developed since 1950. This results in a loss of plant potential to benefit from AMF inoculation or cultivation practices that increase soil ineffectiveness. Plenchette et al. (2005) exhibited that breeding maize varieties resistant to fungal diseases shows a greater inability to mycorrhizal colonization. However, these observations may be due to the short growing period, but also to non-optimal growing conditions for both the host plant and fungal symbiotes (Khasa et al., 1990).

It should also be remembered that the behavior of endo-mycorrhizal strains is highly variable and their effectiveness depends on their degree of ineffectiveness (Nouaïm et al., 1994). In fact, species that are not effective under normal conditions are not effective under stress conditions, for example. Sieverding and Toro (1988), by inoculating cassava plants with 7 species of AMF, found that under good water conditions 5 species are effective, whereas under water stress, only 3 species are able to increase growth compared to no mycorrhizal plants. However, for a first level of sorting, strains most effective for growth and mineral nutrition are those that colonized roots and produced the most extra-root mycelium, should be able to be used (Sanders et al., 1977). Because of the importance of endo mycorrhizal fungi, especially in poor soils and arid and semi-arid areas, it is in the best interest to manage this symbiosis through appropriate agronomic practices (Sieverding, 1991; Abbott and Robson, 1994).

CONCLUSION

Results showed that co-inoculation of *G. caledonius*, *R. intraradices* and *F. geosporum* natives of rhizospheric soils of maize in Benin reduced by half the recommended dose of 200 kg/ha of NPK and 100 kg of Urea for corn fertilization. This has resulted in an increase in size, diameter at the collar and leaf surface of corn plants, as well as higher dry matter yield compared to control plants. However, these results will need to be replicated in real-world environments until the full maturity of corn plants for an actual assessment of root symbiosis effects

in the study.

ACKNOWLEDGEMENTS

The authors thank the National Institute for Agricultural Research in Benin (INRAB in French), the Agricultural Productivity Project in West Africa (APWA), the National Maize Specialization Centre (NMS-Maize) for funding this work and the Biology and Molecular Typage in Microbiology of the Faculty of Science and Technology of the University of Abomey Calavi for technical support.

REFERENCES

- Abbott LK, Robson AD (1985).** Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 99:245-255.
- Adjanohoun A, Noumavo PA, Sikirou R, Allagbe M, Gotoechan-Hodonou H, Dossa KK, Baba-Moussa L (2012).** Effets des rhizobactéries PGPR sur le rendement et les teneurs en macroéléments du maïs sur sol ferrallitique non dégradé au Sud-Bénin. *Int. J. Biol. Chem. Sci.* 6(1):279-288.
- Adriano-Anaya M, Solis-Dominguez F, Gavito-Pardo ME, Salvador-Figueroa M (2006).** Agronomical and Environmental Factors Influence Root Colonization, Sporulation and Diversity of Arbuscular Mycorrhizal Fungi at a Specific Phenological Stage of Banana Trees. *J. Agron.* 5:11-15
- Agro A, Akissoé NH, Manful J, Mestres C, Hounhouigan J (2014).** Optimisation de la fermentation en milieu semi-solide pour la production d'ablo, pain cuit à la vapeur d'Afrique de l'ouest. *J. Appl. Biosci.* 82(1):7469-7480.
- Amadji GL, Migan DZ (2001).** Influence d'un amendement organique (compost) sur les propriétés physico-chimiques et la productivité d'un sol ferrugineux tropical. In *Annales des Sciences Agronomiques du Bénin* 2(2):123-139).
- Atama G, Kodjo TA, Manguilibè T, Atti T, Mawuko AAK, Komlan B (2018).** Evaluation au champ du potentiel de croissance et de la production du riz (*Oryza sativa* L.) variété IR841 inoculé en pépinière par quatre souches de champignons mycorrhiziens à arbuscules. *European Sci. J.* 14(12):452-481.
- Azontondé, A, Hazoumè FAG, Gnagassi C, Kpagbin G (2005).** Impact d'une plante de couverture (*Mucuna pruriens utilis*) sur la productivité du maïs et les propriétés d'un sol ferrallitique du Sud-Bénin. *Bulletin de la Recherche Agronomique du Bénin*, 50:47-56.
- Balogoun I (2012).** Essai de validation des formules d'engrais et des périodes de semis recommandées par le modèle DSSAT pour la production de maïs (*Zea mays* L.) au Sud et Centre Bénin. *Mémoire de Diplôme d'Etude Approfondie, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Bénin*, p. 78.
- Baslam M, Esteban R, Garcia-Plazaola JI, Goicoechea N (2013).** Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. *Appl. Microbiol. Biotechnol.* 97(7):3119-3128.
- Cárdenas R (2010).** *La mycorrhization favorise-t-elle l'accès à des formes d'azote complexes? Étude sur la nutrition du pin parasol Pinus pinea* (Master's thesis, FRANCIA/Université François Rabelais de Tours/2010).
- Chen S, Jin W, Liu A, Zhang S, Liu D, Wang F, He C (2013).** Arbuscular mycorrhizal fungi (AMF) increase growth and secondary metabolism in cucumber subjected to low temperature stress. *Scientia Horticulturae*, 160:222-229.
- Copetta A, Bardi L, Bertolone E, Berta G (2011).** Fruit production and quality of tomato plants (*Solanum lycopersicum* L.) are affected by green compost and arbuscular mycorrhizal fungi. *Plant biosystems*, 145(1):106-115.
- Douds Jr DD, Galvez L, Janke RR, Wagoner P (1995).** Effect of tillage

- and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. *Agriculture, ecosyst. Environ.* 52(2-3):111-118.
- El-Yazeid AA, Abou-Aly HA, Mady MA, Moussa SAM (2007).** Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. *Res. J. Agric. Biol. Sci.* 3(4) :274-286.
- Etèka AC (2005).** Contribution des 'Jachère' Manioc dans l'Amélioration du Rendement des Cultures et du Prélèvement des Nutriments : Cas de la Succession Culturelle Manioc-Maïs au Centre du Bénin. *Bénin : FSA/UAC.*
- Fernández F, Dellrsquo JM, Angoa MV, de la Providencia IE (2011).** Use of a liquid inoculum of the arbuscular mycorrhizal fungi *Glomus hoi* in-rice plants cultivated in a saline Gleysol: A new alternative to inoculate. *J. Plant Breed. Crop Sci.* 3(2):24-33.
- Fernández F, Gómez R, Vanegas LF, Martínez MA, de la Noval BM, Rivera R (2000).** Producto inoculante micorrizógeno. *Oficina Nacional de Propiedad Industrial. Cuba, Patente, (22641).*
- Gao X, Kuyper TW, Zou C, Zhang F, Hoffland E (2007).** Mycorrhizal responsiveness of aerobic rice genotypes is negatively correlated with their zinc uptake when nonmycorrhizal. *Plant and Soil*, 290(1-2):283-291.
- Garbaye J (2013).** La symbiose mycorrhizienne : une association entre les plantes et les champignons. *Editions Quae.*
- Graham JH, Syversten JP (1984).** Influence of vesicular-arbuscular mycorrhizae on the hydraulic conductivity of roots of two citrus rootstocks. *New Phytologist*, 97:277-284.
- Griffon M (2010).** Pour des agricultures écologiquement intensives, l'Aube. *La Tour d'Aigues*, p.112
- Hamza N (2018).** Application des mycorhizes arbusculaires en culture maraîchère cas de la pastèque (*Citrullus lanatus*) (Doctoral dissertation).
- Harrier L, Watson C (2003).** The role of arbuscular mycorrhizal fungi in sustainable cropping systems, *Adv. Agron.* 79:185-225.
- Igue AM, Saidou A, Adjanohoun A, Ezui G, Attiogbe P, Kpagbin G, Ouedraogo J (2013).** Evaluation de la fertilité des sols au sud et centre du Bénin. *Bull. Rech. Agron. Bénin, Spécial numéro, Fertilisation du maïs*, pp. 12-23.
- Jansa J, Smith FA, Smith SE (2008).** Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi?. *New Phytologist*, 177(3):779-789.
- Jeong K, Mattes N, Catausan S, Chin JH, Paszkowski U, Heuer S (2015).** Genetic diversity for mycorrhizal symbiosis and phosphate transporters in rice. *J. Integr. Plant Biol.* 57(11):969-979.
- Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL, Cullu MA (2009).** The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. *Scientia Horticulturae*, 121(1):1-6.
- Khasa P, Furlan V, Fortin JA (1990).** Effets de différentes espèces de champignons endomycorhiziens sur la croissance de dix espèces de plantes tropicales au Zaïre. *Tropicultura*, 8:159-164.
- Laminou Manzo O, Ibrahim D, Campanella B, Paul R (2009).** Effets de l'inoculation mycorrhizienne du substrat sur la croissance et la résistance au stress hydrique de cinq espèces fixatrices de dunes : *Acacia raddiana* Savi ; *Acacia nilotica* (L.) Willd. Ex Del. var. *adansonii* ; *Acacia senegal* (L.) Willd ; *Prosopis chilensis* Stunz. et *Bauhinia rufescens* Lam.. *Geo-Eco-Trop*, 33:115-124.
- Leye EHM, Ndiaye M, Diouf M, Diop T (2015).** Etude comparative de l'effet de souches de champignons mycorrhiziens arbusculaires sur la croissance et la nutrition minérale du sésame cultivé au Sénégal. *Afr. Crop Sci. J.* 23(3):211-219.
- Mujica Pérez Y, Fuentes Martínez AG (2012).** Efecto a la biofertilización con hongos micorrízicos arbusculares (HMA) en el cultivo del tomate en condiciones de estrés abiótico. *Cultivos Tropicales*, 33(4):40-46.
- Mujica Y, de la Noval B, Dell'Amico J (2010).** Respuesta del cultivo de tomate a la aplicación de dos inoculantes de hongos micorrízicos arbusculares por vías diferentes de inoculación. *Agronomía Trop*, 60(4):381-387.
- N'DA HA, Akanvou L, Kouakou CK, Zoro AIB (2014).** Diversité morphologique des variétés locales de maïs (*Zea mays* L.) collectées au Centre et Centre-Ouest de la Côte d'Ivoire. *Eur. Sci. J.* 10(12).
- Nouaim R, Chaussod R (1996).** Rôle des mycorhizes dans l'alimentation hydrique et minérale des plantes, notamment des ligneux de zones arides. La mycorrhization des plantes forestières en milieu aride et semi-aride et la lutte contre la désertification dans le bassin méditerranéen. *Zaragoza : CIHEAM, 1996. (Cahiers Options Méditerranéennes 20:9-26.*
- Ortas I, Ustuner O (2014).** The effects of single species, dual species and indigenous mycorrhiza inoculation on citrus growth and nutrient uptake. *Eur. J. Soil Biol.* 63:64-69.
- Pellegrino E, Bedini S, Avio L, Bonari E, Giovannetti M (2011).** Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. *Soil Biol. Biochem.* 43(2):367-376.
- Phillips JM, Hayman DS (1970).** Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transact. Brit. mycol. Soc.* 55(1):158-168.
- Planetoscope-Statistique (2013).** Statistiques mondiales en temps réel sur les céréales www.planetoscope.com/cereales/193-production-mondiale-de-mais. Consulté le 17/08/2015 à 21h01mn.
- Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA (2005).** Managing arbuscular mycorrhizal fungi in cropping systems. *Can. J. Plant Sci.* 85:31-40.
- Raiman MP, Albino U, Cruz MF, Lovato GM, Spago F, Ferracin TP, Nogueira MA (2007).** Interaction among free-living N-fixing bacteria isolated from *Drosera villosa* var. *villosa* and AM fungi (*Glomus clarum*) in rice (*Oryza sativa*). *Appl. Soil Ecol.* 35(1):25-34.
- Rajeshkannan V, Sumathi CS, Manian S (2009).** Arbuscular mycorrhizal fungi colonization in upland rice as influenced by agrochemical application. *Rice Sci.* 16(4) :307-313.
- Ruget F., Bonhomme R, Chartier M (1996).** Estimation simple de la surface foliaire de plantes de maïs en croissance. *Agronomie, EDP Sci.* 16(9):553-562. [hal-00885817](https://hal.archives-ouvertes.fr/hal-00885817).
- Sanders FE, Tinker PB, Black RLB, Palmerley SM (1977).** The development of endomycorrhizal root system. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. *New Phytologist*, 78:257-268.
- Schreiner RP (2007).** Effects of native and nonnative arbuscular mycorrhizal fungi on growth and nutrient uptake of 'Pinot noir' (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus. *Appl. Soil Ecol.* 36(2-3):205-215.
- Sieverding E (1990).** Ecology of VAM fungi in tropical agrosystems. *Agric. Ecosyst. Environ.* 29:369-390.
- Sieverding E (1991).** Vesicular-Arbuscular ycorrhizam management in tropical agrosystems. G.T.Z., Ed., *Eschborn*, RFA.
- Sieverding E, Toro (1988).** Influence of soil water regimes on V.A. performance of different VAM fungal species with Cassava. *J. Agron. Crops Sci.* 161:322-332
- Toléba MS, Biao G, Zannou A, Saïdou A (2017).** Caractérisation des systèmes de production à base de maïs dans les principales zones de culture au Bénin. *Annales des Sciences Agronomiques*, 21(1):53-75.
- Trouvelot A (1986).** Mesure du taux de mycorrhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. *Physiological and genetical aspects of mycorrhizae*, pp. 217-221.
- Wang GM, Stribley DP, Tinker PB, Walker C (1993).** Effects of pH on arbuscular mycorrhiza I. Field observations on the long-term liming experiments at Rothamsted and Woburn. *New Phytol.* 124(3):465-472.
- Wang Y, Li T, Li Y, Björn LO, Rosendahl S, Olsson PA, Fu X (2015).** Community dynamics of arbuscular mycorrhizal fungi in high-input and intensively irrigated rice cultivation systems. *Appl. Environ. Microbiol.* 81(8):2958-2965.
- Yadav J, Verma JP, Tiwari KN (2010).** Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (*Cicer arietinum* L.) under in vitro conditions. *In Biological Forum* 2(2):15-18).
- Yallou CG, Aïhou K, Adjanohoun A, Toukourou M, Sanni OA, Ali D (2010).** Itinéraires techniques de production de maïs au Bénin. Fiche technique. Dépôt légal N° 4922 du 3 Décembre, *Bibliothèque Nationale du Bénin*, p. 18.

Zhang XH, Zhu YG, Chen BD, Lin AJ, Smith SE, Smith FA (2005).
Arbuscular mycorrhizal fungi contribute to resistance of upland rice to
combined metal contamination of soil. *J. Plant Nutr.* 28(12):2065-
2077.

NOTE:

Kindly cited the references color red or permit us to delete