

Arbuscular Mycorrhizal Fertilization Of Corn (*Zea mays* L.) Cultivated on Ferrous Soil in Southern Benin

Mèvognon R. Aguegue

D épartement de Biochimie et de Biologie Cellulaire,

Faculté des Sciences et Techniques, Université d'Abomey-Calavi, B énin.

Pac ôme A. Noumavo D épartement de Biologie et de Biologie Cellulaire & D épartement de Biologie V ég étale, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, B énin.

Gustave Dagbenonbakin

Centre de Recherches Agricoles d'Agonkanmey,

Institut National des Recherches Agricoles du B énin, B énin.

Nadège A. Agbodjato, Sylvestre Assogba & Abdel D. Koda

D épartement de Biochimie et de Biologie Cellulaire,

Faculté des Sciences et Techniques, Université d'Abomey-Calavi, B énin.

Adolphe Adjanohoun

Centre de Recherches Agricoles Sud Niaouli,

Institut National des Recherches Agricoles du B énin, B énin.

Ram ón Rivera & Blanca M. de la Noval Pons

Departamento de Biofertilizantes y Nutrición de las Plantas,

Instituto Nacional de Ciencias Agr colas, Cuba.

Lamine Baba-Moussa (Corresponding author)

Département de Biochimie et de Biologie Cellulaire, Facult é des Sciences et Techniques,



Université d'Abomey-Calavi, B énin. E-mail: laminesaid@Yahoo.fr

Received: August 1, 2017Accepted: August 16, 2017Published: September 17, 2017doi:10.5296/jas.v5i3.11881URL: https://doi.org/10.5296/jas.v5i3.11881

Abstract

The attainment of the millennium for development objectives require to improve the productivity of major cultures like maize (Zea mays L.). The present study aimed to assess the potential of Glomus cubens, Rhizophagus intraradices and Funneliformis mosseae to improve the productivity of corn cultivated on reddish ferrous soil in Southern Benin. A block of nine treatments with four repetition completely randomized was installed. Plant height and diameter and leaf area are growth parameters measured. Grain yield and endomycorrhizal infection were also evaluated. The best growths and grain yield (2.33 t/ha and 2.12 t/ha) were obtained respectively with the plants treated with R. intraradices + 50% of NPK and G. cubens + 50% of NPK compared to the control plants (1.48 t/ha). Moreover, the great frequencies of mycorhization (44% and 32.25% were observed respectively on the plants treated with G. cubens + 50% of NPK and R. intraradices + 50% NPK. These results show that the respective combination of these two Arbuscular Mycorrhizal Fungi with 50% NPK can increase of 50% the seeds yield of corn compared to the seeds yield obtained with the control plants. In addition, G. Cubens and R. intraradices are active in the colonization of maize roots. Use of R. Intraradices or G. cubens in combination with 50% of NPK help to increase the corn productivity and to reduce of half the chemical fertilizer commonly used by corn farmers at Southern Benin.

Keywords: Inoculation, *Zea mays* L., productivity, biological fertilization, Arbuscular Mycorrhizal Fungi; ferrous soil, Benin.

1. Introduction

The problems of food safety are a very relevant question which worries all the countries. The food and nutritional safety of West African countries was made worse by the crisis of 2008 highlighting the painful consequences of these countries' dependency of the world food markets (Mendez et al., 2011). One of the convincing alternatives to ensure the self-sufficiency food production of the population is the intensive of the cereal cultures among the corn. In Benin, the maize represents the major crop in all agroecological zones (Adjanohoun et al., 2012). It remains the first cereal produced and consumed in Benin, far in front of the sorghum *(Sorghum bicolor)* and rice (*Oriza spp.*).

In spite of the importance of this crop and its gradually increased request, the maize productivity still remains low in Benin. However, some farmers continue to obtain a few grains yield (lower than 0.5 t/ha) against a potential of grains yield from 3 to 5 t/ha with or without to add or no mineral manures' (Azontond é et al., 2005). Thus, to increase the corn



yield, farmers use intensively the inputs such as fertilizers and pesticides from chemical synthesis which have negative consequences on environment, human and animals' health. It is advisable to search other realistic approaches capable of reducing the pesticides and mineral fertilizers use while improving the corn productivity. One of the current approaches preached by the ecologists is biological fertilization based on Arbuscular Mycorrhizal Fungal (AMF).

Indeed, the majority of the plants form a marbling symbiosis with the AMF. The AMFs are the obligatory symbiotes which use the sugars (hexoses) excreted by the plants to realize their carbonaceous metabolism (Read and Perez-Moreno, 2003). AMFs play an important part in plant phosphorus absorption, mainly in tropical areas where the soil content of available phosphorus is very weak (Matos et al., 1999). Also, the AMF facilitate the phosphorus mobilization through the "hyphae" system (Ndoye et al., 2016) and a better water absorption (Aug é et al., 2004) and in biogenic salts (Sharma et al., 2016). Moreover, they contribute to the restoration of damaged grounds (Zhang et al., 2016). Benjelloun et al. (2014) showed that the corn plants inoculated by indigenous mycorrhizical fungi present a better growth compared to the not inoculated plants; this tendency is maintained even in water stress condition. The present study aims to evaluate in field conditions the response of corn to inoculation of three AMFs (*Glomus cubens, Rhizophagus intraradices* and *Funneliformis mosseae*) on reddish ferrous soil in Southern Benin.

2. Materials and Methods

2.1 Seeds Variety

EVDT 97 STR C1 was the corn variety used. This variety is an early variety of 90 days of development cycle, developed by International Institute of Tropical Agriculture (IITA) and the National Institute of Agricultural Researches of Benin (INRAB). 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, striation, helminthosporiose, curvulariose and drought (Yallou et al., 2010).

2.2 Fungal Strains

The three species of Arbuscular Mycorrhizal Fungal evaluated in this study are *Glomus cubens* (Rodr guez et al., 2012), *Rhizophagus intraradices* (Walker and Schübler, 2004) and *Funneliformis mosseae* (Walker and Schübler, 2004). These fungi are obtained from the laboratory of mycorhize of Instituto Nacional de Ciencias Agricolas (INCA) of Cuba.

2.3. Site of Experimentation

The field of experimentation of Southern Benin Agricultural Research Center located at S & a (Atlantic Department, Benin) has to be used as framework for the setting of the test. The geographical coordinates of the experimental site is following: altitude 87 m; longitude 2 °15,183' E and latitude 6 °37,715' N (Figure 1).



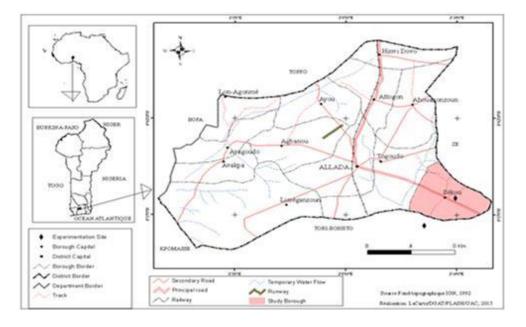


Figure 1. Geographical map showing the experimental site (lacarto/DGAT/FLASH/UAC, 2015)

2.4. Chemical Characterization of Field Soil

The soil samples were collected before sowing and after harvest to 0-20 cm of depth to each catch. The method of Boudoudou et al., (2009) was used for the determination of the pH of the field soil. The method of Bray and Kurtz (1945) was used for the determination of assimilable phosphorus and that of Thomas (1982) for the determination of the exchangeable cations (Ca, Mg and K). In addition, according to the method of Walkley and Black (1934), the organic matter and carbon were determined.

2.5. Experimental Design

The experimental design was composed of 36 elementary plot of land (complete random block). An elementary plot of land had a surface of 12.8 m² and contained four lines of 4 m length separated of 0.80 m. The seedlings were sown with a density of 31,250 plants/ha (Yallou et al., 2010). The data had been collected on 2 central lines of each field pot. The treatments assessed were:

Control = (without mineral manures, without AMF); Gc = Glomus cubens; Ri = Rhizophagus intraradices; Fm = Funneliformis mosseae; $\frac{1}{2}$ NPK = 50% of recommended N₁₅P₁₅K₁₅; Gc $\frac{1}{2}$ NPK = G. cubens + 50% of recommended N₁₅P₁₅K₁₅; Ri $\frac{1}{2}$ NPK = R. intraradices + 50% of recommended N₁₅P₁₅K₁₅; Fm $\frac{1}{2}$ NPK = F. mosseae + 50% of recommended N₁₅P₁₅K₁₅; NPK = 100% of recommended N₁₅P₁₅K₁₅. N₁₅P₁₅K₁₅ indicate the quantities of N (15 Kg/ha), P₂O₅ (15 Kg/ha) and K₂O (15 Kg/ha)



used.

2.6. Seed Inoculation with AMF

The seeds inoculation was made the just before sowing. The corn seed were coated with the AMF (*G. cubens*, *R. intraradices* and *F. mosseae*) for the different treatments. Indeed, the spores of each arbuscular mycorrhizal fungal were trapped in a sandy support to constitute the inoculum as afertilizing product. A quantity corresponding to 10% of the weight of corn seed was applied as bio fertilizers. A quantity of water equivalent to 600 ml/kg of inoculum was added to obtain the mixture. The seed were coated in this mixture and dried with the ambient air (Fern ández et al., 2000).

2.7. Assessment of the Growth Parameters

The 15th, 30th, 45th and 60th Days After Sowing (DAS), were measured the diameter collet and the height of the plants. The leaf area data of plants were measured at 60th DAS. On the level of each elementary piece, the height of 10 seedlings selected out of the 2 central lines was measured using a meter ribbon. The diameter of the seedlings was measured using a slide caliper and the leaf area was estimated by the affected product length and width of the sheets of coefficient 0.75 according to Ruget and Chatier, (1996).

2.8. Determination of Maize Yield

The maize cobs were collected at 90^{th} DAS and their weight was given using a scale of precision (Highland HCB 3001, max: 3000 X 0.1 G). Their percentages of moisture were given using a humid meter (LDS-1F). The average maize yield was determined according to the described formulate (Ferro Vald \leq et al., 2013):

 $R = \frac{P \times 10.000}{S \times 1.000} X \frac{14}{H}$ in which:

- R= Average of seeds yield of the corn seedlings (t/ha);
- P = Seeds weight of the corn seedlings (kg);
- S = Harvest area (m);
- H = Percentage of grains moisture (%).
- 2.9. Estimation of Endomycorrhizal Infection Rates

The randomly sampled roots were collected at 60 DAS from each plant. It was washed with tap and deionized water. They are then cut of small pieces (0.5 cm length). These root fragments were soaked for 1 h into KOH solution (10%) at 90 °C. The cleared roots obtained were then stained with 10% of Trypan blue according to Phillips and Hayman (1970). The rate of roots infection was given according to the method of intersection developed by Giovannetti and Mosse (1980). This method consists of a calculation of the points of impact of mycorrhizal infection on the roots. A total of 100 root pieces (1 cm) per plant were randomly selected. Histological observations were achieved by means of a microscope (Stereo Microscope, 20x/40x XSP-BM-2CEAC). The frequency (F) and intensity (m) of



mycorrhization of the roots observed were obtained by the formulate described by Trouvelot et al. (1986) :

Frequency of mycorrhization (F): $F(\%) = \frac{N-n_0}{N} \times 100$ With, N: number of

observed fragments and n0: number of non-mycorrhizal fragments.

Intensity of mycorrhization: \mathbf{m} (%) = $\frac{95n_5+70n_4+30n_3+5n_2+n_1}{N-n_0}$. With, n = number of fragments assigned with the index 0, 1, 2, 3, 4 or 5. 0: no, 1: trace, 2: less than 10%, 3: 11 to 50%, 4: 51 to 90%, 5: more than 91%.

2.10. Statistical Analysis and Map Realization

The software R (version 2.15.3) was used for the Analysis of Variance of the data. A comparison of the averages data of each parameter was made thanks to the method of Student Newman and Keuls (SNK) at probability level 0.05. The geographical map of experimental field was realized using the ArcMap software version 9.2.

3. Results

3.1 Chemical Characteristics of Soil

The chemical characteristics of the used top soil are presented in the table 1. With a strongly acidic pH, experimental soil was poor in available phosphorus, organic carbon, calcium and potassium showing its low fertility.

рН		Organic carbon	Base	Exchangeables cations			Exchangeable	Phosphorus available	
Water	KCl -	Carbon	saturation	Ca	Mg	Κ	capacity Cations	available	
		[%]				$[mg kg^{-1}]$			
5.1	4.4	1.35	46.00	1.50	0.59	0.38	5.56	3.75	

Table 1. Chemical characteristics of experimental field soil

3.2 Effects of arbuscular mycorrhizal fungal on plant growth parameters

3.2.1 Plant Height

The figure 2a presents the height tendency of corn seedlings under influence of the various treatments in study. Fifty days after sowing, all plants have presented same heights. From 45^{th} days after sowing, a clear differentiation was observed and continued until 60^{th} days after sowing. Indeed, a heighly significant (p < 0.001) was difference between the treatments. Also, time highly effected (p < 0.001) the height of plants. At 60^{th} days after sowing, the corn seeds treated with the combination of *G. cubens* and 50% of NPK and those treated with 100% of NPK present the maximum heights respectively 169.48 cm and 169.25 cm. On the other hand, the corn seedlings inoculated with *F. mosseae* showed the weakest growths in height (134.02 cm) (Table 2).



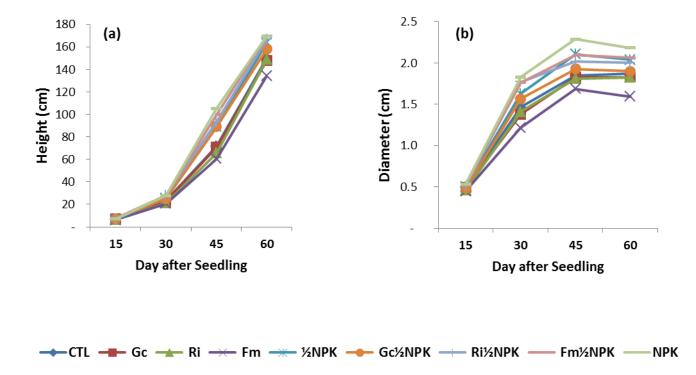


Figure 2. AMF effects on height (a) and diameter (b) growth of corn plants according to days of seedling.

CTL= Control without mineral manures and AMF); Gc = *Glomus cubens*; Ri = *Rhizophagus intraradices*; Fm = *Funneliformis mosseae*; $\frac{1}{2}$ NPK = 50% of recommended NPK; Gc $\frac{1}{2}$ NPK = *G. cubens* + 50% of recommended NPK; Ri $\frac{1}{2}$ NPK = *R. intraradices* + 50% of recommended NPK; Fm $\frac{1}{2}$ NPK = *F. mosseae* + 50% of recommended NPK; NPK = 100% of recommended NPK.

3.2.2 Diameter of the Plants

The average diameter of the plant between 15^{th} days after sowing and 30^{th} days after sowing did not present a difference between treatments (Figure 2b). From 30^{th} days after sowing, a difference was noted and maintained until 60^{th} days after sowing with avariability noticed starting from 35^{th} days after sowing. Indeed, the diameter with the collet of the seedlings was very different from a treatment another according to time (p < 0.001). The numerical classification applied to adjusted averages of the plant diameters under the effects of the 9 treatments for the 4 periods of measurement gave a classification presented on Table 2. Corn seedlings inoculated with NPK (50%), NPK (100%) the combination of NPK (50%) with *R. intraradices* and the combination of NPK (50%) with *G cubens* presented a relatively fast growth. Plant showed an average diameter of 2.16 cm compared to other treatments which average diameter of 1.78 cm.

3.2.3 Leaf Area of Plants

The results showed that leaf area was significantly affected (p < 0.01) by treatments. Thus,



Thus, NPK (100%), combination of *R. intradices* and NPK (50%) and combination of *G. cubens* and NPK (50%) induced the best leaf area respectively to 599.43 cm, 576.43 cm and 561.82 cm (Table 2).

Treatments	Height (c	Diameter (cm)		Leaf of area (cm ²)		
	m	σ	m	σ	m	σ
Control	149.81 ^{cd}	4.64	1.87 ^{cd}	0.06	497.16 ^{ab}	24.69
Glomus cubens	147.63 [°]	4.12	1.83 ^c	0.07	489.84 ^{ab}	14.26
Rhizophagus intraradices	148.97 ^{cd}	5.70	1.83 ^{cd}	0.15	511.66 ^{ab}	50.10
Funneliformis mosseae	134.02 ^{cd}	12.22	1.60 ^{cd}	0.16	450.35 ^b	69.63
50% NPK	163.74 ^{ab}	9.98	2.04 ^{ab}	0.13	548.94 ^{ab}	24.07
F. mosseae + 50% NPK	157.88 ^{ab}	22.80	1.90 ^{cd}	0.12	534.58 ^{ab}	29.33
R. intraradices + 50% NPK	166.54 ^{ab}	7.71	2.00^{ab}	0.07	576.43 ^a	27.65
G. cubens + 50% NPK	169.48 ^a	3.99	2.07 ^{ab}	0.09	561,82 ^a	43.05
100% NPK	169.25 ^a	4.58	2.18 ^a	0.13	599.43 ^a	17.95
Signification	***	***			**	

Table 2. Arbuscular mycorrhizal fungal effects on corn plants height, diameter and leaf area

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

3.2.4 Effects of Arbuscular Mycorrhizal Fungi on Corn Yield

The results of the variance analysis showed a very highly significant (p < 0.001) difference between treatments. Thus, seeds treated with mineral manures (50% and 100% NPK) and those received arbuscular mycorrhizal fungi in combination or/not with NPK (50%) recorded average on corn yield definitely higher than the control (Figure 3). The yield obtained with seeds only inoculated with arbuscular mycorrhizal fungi (1.82 t/ha) are lower than those obtained with the seeds that received the combination of arbuscular mycorrhizal fungi with 50% of NPK (2.18 t/ha). It should be announced that the two treatments 100% of NPK and 50% of NPK combined with *R. intraradices* induced the best yield of corn seeds (2.87 t/ha and 2.33 t/ha).



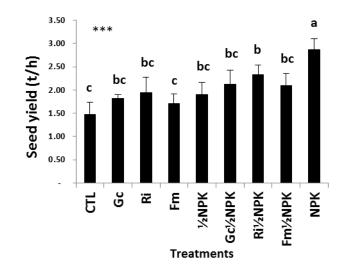


Figure 3. Effects of AMF on corn yield

CTL= Control without mineral manures and AMF); Gc = *Glomus cubens*; Ri = *Rhizophagus intraradices*; Fm = *Funneliformis mosseae*; ¹/₂NPK = 50% of recommended NPK; Gc¹/₂NPK = *G. cubens* + 50% of recommended NPK; Ri¹/₂NPK = *R. intraradices* + 50% of recommended NPK; Fm¹/₂NPK = *F. mosseae* + 50% of recommended NPK; NPK = 100% of recommended NPK. *** = p < 0.001 (very highly significant).

3.2.5 Endomycorrhizal Infection Parameters of Corn Plants

The figure 4 presents the evaluated parameters of mycorhization. The same intensity of mycorhization was observed on the level as of seeds of the various treatments (p > 0.05). On the other hand, the frequency of mycorhization was highly effected (p < 0.001) by treatments. The frequencies and intensities of mycorhization of the roots of the seeds treated with the combination of arbuscular mycorrhizal fungi and 50% of NPK were high compared to the seeds only treated with arbuscular mycorrhizal fungi (Figure 4 A and C). The frequencies of mycorhization vary to 20.25% (*R. intraradices*) with 53.5% (*G. cubens* combined with 50% of NPK). As to intensity of mycorhization, it varied from 5.19% (*G. cubens*) to 9.32% (*G. cubens* combined with 50% of NPK). It should be noted however, that roots of the control were not realized the mycorhization as well as the seeds treated with NPK (50% and 100%).

The quantity of spores of arbuscular mycorrhizal fungi per gram of soil was weak whatever the treatments. However, the highest quantities of spores (3 spores/g of soil) were counted on the corn seeds having received the inoculation of *G cubens* and *F. mosseae* without complement of mineral manures, like at the seeds inoculated with combination of *R. intraradices* and 50% NPK. The results of the variance analysis showed a very highly significant difference (p < 0.001) between treatments regarding to the quantity of spores (Figure 4B).



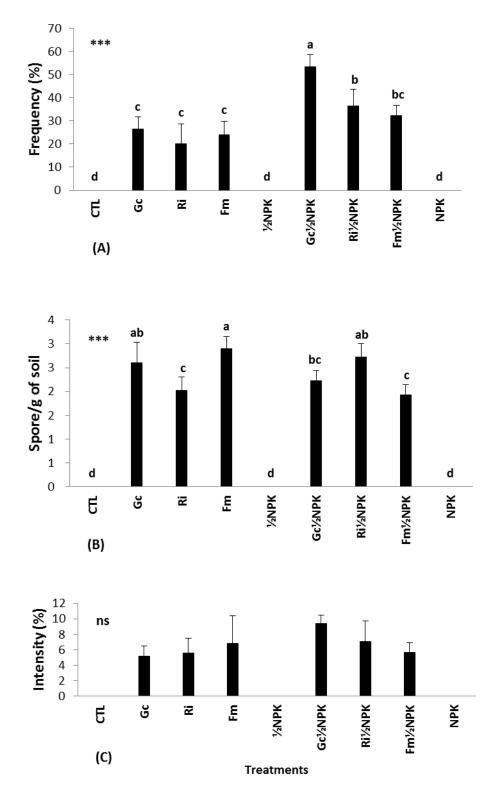


Figure 4. Endomycorhizal infection parameters of corn plants (A: frequency, B: spores, C: intensity).

CTL= Control without mineral manures and AMF); Gc = *Glomus cubens*; Ri = *Rhizophagus intraradices*; Fm = *Funneliformis mosseae*; ½NPK = 50% of recommended NPK; Gc½NPK



= *G. cubens* + 50% of recommended NPK; $Ri\frac{1}{2}NPK = R$. *intraradices* + 50% of recommended NPK; $Fm\frac{1}{2}NPK = F$. *mosseae* + 50% of recommended NPK; NPK = 100% of recommended NPK. ns = no significant; *** = p < 0.001 (very highly significant).

4. Discussion

The soil of experiment showed a low fertility. Same observations were done by (Adjanohoun et al., 2011). Thus, soil of was acid and the soil meets the required conditions of arbuscular mycorrhizal fungi to express their potentiality. Wang et al. (1993), reported that the optimal values of pH are between 5.5 and 6.5.

The effectiveness of the arbuscular mycorrhizal fungi was noticed more starting from 30^{th} days after sowing (Figure 2). This observation would be explained by the fact that at 30^{th} days after sowing, corn seed develop enough roots to support the process of mycorhization. Indeed, the presence of plant host root allows the development of vegetative mycelium of arbuscular mycorrhizal fungi spores.

This can colonize between 60-90% of the radicular system under favorable conditions (Bonfante and Perotto, 1995). In addition, symbiosis is possible thanks to continuous signals of recognition and acceptance between the plant and arbuscular mycorrhizal fungi (Vierheilig and Pich é, 2002) using root exudates of the plant host (Akiyama, 2007). The contribution of the arbuscular mycorrhizal fungi had a significant effect on corn seedlings growth (Table 2). G. cubens combined with 50% of NPK induces the height (13%), diameter (11%) and leaf area (13%) increase of the corn seedlings compared to the control seedlings. The effectiveness of the arbuscular mycorrhizal fungi is more observed when they are combined with 50% of NPK. In addition, seeds treated with the combination of arbuscular mycorrhizal fungi and 50% of NPK present a better growth compared to the seeds only treated with arbuscular mycorrhizal fungi on the one hand, and the controls seedlings on the other hand. These results are explained by the fact that more than 40% of the photosynthesis products assimilated by the plant are transferred to arbuscular mycorrhizal fungi but our soil of study is low in minerals element like N, P and K (Diallo, 1998) or from 20% (Merryweather, 2001). The results obtained corroborate those reported by Roveda and Polo (2007), which observed an increase in the matter and leaf area in corn seeds inoculated with arbuscular mycorrhizal fungi cultivated on a the soil whose phosphorus content is weak. Subramanian et al. (1995) observed in water stress conditions, a better growth of the corn seeds inoculated with an indigenous arbuscular mycorrhizal fungi compare with control plants. Moreover, Usharani et al. (2014) showed the significant positive effect of the kind Glomus (G. fasciculatum) on the corn growth (+32.67%) in India. In station, the seedlings of fonio (Digitaria exilis Stapf) inoculated with arbuscular mycorrhizal fungi (G. manihotis) had an increase (+ 28.5%) compared to the seedlings controls (Ndoye et al., 2016). Augé et al. (2004) explained the growth improvement by a better water absorption, while Yamawaki et al. (2013) and Sharma et al. (2016) explain it by a better absorption of mineral manures.

The seeds yields of 2.33 t/ha and 2.12 t/ha are obtained with the contribution of 50% of NPK combined with *R. intraradices* and *G. cubens* respectively (Figure 3). These values of seeds yields exceed of those obtained with control seedlings (50%), with the seedlings only treated



with the arbuscular mycorrhizal fungi (15%) and with the seedlings treated with 50% of NPK. Nevertheless, these values are lower than 30% in comparison to seeds yield obtained with the seedlings treated with 100% of the amount of recommended NPK. The application of 50% of NPK combined with the arbuscular mycorrhizal fungi *R. intraradices* and *G. cubens* had a significant positive effect on the seeds yields of the corn seeds. These results are similar with those reported by Rivera et al. (2003) in Cuba who found an increasing from 15 to 50% of the plant yields of sorghum *(Sorghum bicolor)*, tomato (*Lycopersicum esculentum*) compared to the control treated by the application of the arbuscular mycorrhizal fungi in combination with the NPK. These results are higher than those brought back by Usharani et al. (2014) which indicates an increasing of corn seeds yield of 11.06%, under the effect of the application of *Glomus fasciculatum* in India.

The frequency of mycorhization of the corn seedlings root and the quantity of spore of arbuscular mycorrhizal fungi per gram of soil are weak whatever the various treatments (Figure 4). Indeed, the absence of spores on the level of the grounds of the control seedlings like on the level of plants treated with 50% NPK and 100% NPK show that it there not arbuscular mycorrhizal fungi natives in our soil of study. However, this small quantity of the spores would explain that the weak colonization of the roots of corn seedlings. The colonization of the corn seedlings root lies between 20 and 53%. Our results are conform to those obtained by Kumar et al. (2013) which observed frequencies of mycorhization varying from 20 to 70% at other species of Poac ées (Sporobolus indicus, Grevillea pteridofolia, Eragrostis tenella, Digitaria bicornis and Cynodon dactylon). However, this value is low for a good effectiveness of the arbuscular mycorrhizal fungi on the growth and the yield of corn seeds. Many experiments in greenhouse and field realized by Rivera et al. (2003) and by Tian et al. (2013) showed a colonization from 76 to 80% of the roots of corn following the combination of the arbuscular mycorrhizal fungi with the half-amount mineral manures recommended during and afterwards of the moderate states of water stress. The frequencies and highest intensities of mycorhization were obtained with G. cubens and R. intradices. Ndoye et al. (2016) found that the frequencies and highest intensities of mycorhization of the roots of fonio (Digitaria exilis) seedlings with G. aggregatum and R. irregularis. In addition, Incesu et al. (2015) observed rates of higher root colonizations of Diospyros virginiana with R. irregularis and G. caledonium compared with other species of arbuscular mycorrhizal fungi (G. etunicatum, F. mosseae and G. clarium). Smith et al. (2009) explained this by the capacity of a plant to develop a symbiotic association of preferential type with a species of arbuscular mycorrhizal fungi although it is colonized by various species of arbuscular mycorrhizal fungi, involving different rates of colonization on roots while McGonigle and Fitter (1990) explain these differences in effect by an ecological specificity.

The study proves the potential use of the arbuscular mycorrhizal fungi like organic fertilizers. However, it does not reveal the optimal amount of mineral manure to bring to obtain optimal roots colonization of corn by *R. intraradices* and *G. cubens*. Moreover, it does not show the influence of the other micro-organisms (pathogenic or not) on the effectiveness of the arbuscular mycorrhizal fungi tested.



6. Conclusion

The arbuscular mycorrhizal fungi (*G cubens, R. intraradices* and *F. mosseae*) does not present a significant effect on the growth of corn seedlings when there is no potassium, phosphorus and nitrogen complementary application. The combination of these three arbuscular mycorrhizal fungi with 50% of recommended NPK has a significant effect on the corn growth and yield. That resulted in the increase of heigh, diameter, leaf area and seeds yield of the corn seedlings compared to the control seedlings. The combination of the arbuscular mycorrhizal fungi *R. intraradices* and *G cubens* with 50% of NPK has a better performance compared to the combination of the arbuscular mycorrhizal fungi *R. intraradices* and *G cubens* with 50% of NPK has a better with 50% of NPK. The study proves the potential use of the arbuscular mycorrhizal fungi like organic fertilizers. However, it does not reveal the optimal amount of mineral manure to bring to obtain optimal roots colonization of corn by the two arbuscular mycorrhizal fungi *R. intraradices* and *G cubens*. The posterior studies on the determination of the optimal amounts of phosphoric mineral manures prove to be necessary.

Acknowledgements

The authors thank the National Centre of Maize Research and the West Africa Agricultural Productivity Programme and National Funds for Scientific Research and Technological Innovation to find this work.

Conflict of Interests

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

References

Adjanohoun, A., Allagbe, M., Noumavo, P. A., Gotoechan-Hodonou, H., Sikirou, R., Dossa, K. K., ... Baba-Moussa, L. (2011). Effects of plant growth promoting rhizobacteria on field grown maize. *Journal of Animal & Plant Sciences, 11*(3), 1457-1465.

Adjanohoun, A., Noumavo, P. A., Sikirou, R., Allagbe, M., Gotoechan-Hodonou, H., Dossa, K. K., ... Baba-Moussa. (2012). Effets des rhizobact éries PGPR sur le rendement et les teneurs en macro él éments du ma ïs sur sol ferralitique non d'égrad é au Sud-B énin. *Int. J. Biol. Chem. Sci.* 6(1), 279-288. http://dx.doi.org/10.4314/ijbcs.v6i1.24.

Akiyama, K. (2007). Chimical identification and functional analysis of apocarotenoids involved in the development of arbuscular mycorrhizal symbiosis. Bioscience Biotec.Biochem., 71 (6), 1405-1414. http://doi:10.1271/bbb.70023.

Aug é, R. M., Sylvia, D. M., Park, S., Buttery, B. R., Saxton, A. M., Moore, J. L., & Cho, K. (2004). Partitioning mycorrhizal influence on water relations of *Phaseolus vulgaris* into soil and plant components. *Can J. Bot.*, *82*, 503-514. https://doi.org/10.1139/b04-020.

Azontond é, H. A., F. A. G., Hazoume, C., Gnagassi, G., & Kpagbin. (2005). Impact d'une plante de couverture (*Mucuna pruriens utilis*) sur la productivit é du ma ïs et les propri ét és



d'un sol ferrallitique du Sud-Bénin. Bulletin de la Recherche Agronomique du Bénin n 50-Décembre, 47-56.

Benjelloun, S., El Harchli, E. H., Amrani Joutei, K., El Ghachtouli, N., Fikri Benbrahim, K., & El Yamani, J. (2014). Etude De L'importance De La Mycorhization Dans La Synthèse Des Compos és Phénoliques Chez Le Maïs (*Zea mays* L.) En condition de stress hydrique. Research Inventy, *International Journal of Engineering And Science*, 4(12), 43-49. Issn (e), 2278-4721, Issn (p) : 2319-6483.

Bonfante, P., & Perotto, S. (1995). Strategies of arbuscular mycorrhizal fungi when infecting host plants. *New Phytol, 130*, 3-21. https://doi.org/10.1111/j.1469-8137.1995.tb01810.x

Boudoudou, H., R., Hassikou, A., Ouazzani Touhami, A., Bado, A., & Douira. (2009). "Paramètres Physicochimiques et Flore Fongique des Sols de Rizières Marocaines," *Bulletin de la Soci ét é de Pharmacie de Bordeaux*, *148*(1-4), 17-44.

Bray, R. H, & Kurtz, L. T. (1945). Determination of Total, Organic and Available Forms of Phosphorus in Soils. *Soil Science*, *59*(2), 39-45. https://doi.org/10.1097/00010694-194501000-00006

Diallo, A. T. (1998). Contribution à l'étude taxonomique et écologique des Glomales et de l'influence de la mycorhization avec *Glomus mosseae* et *Glomus versiforme* sur la croissance et la productivité du ni ébé, *Vigna unguiculata* (L.) Walp. cultivé en condition de d'éticit hydrique. *Thèse de doctorat de 3 ène cyclede Biologie v ég étale, UCAD.*, 113.

Fern ández, F., Gómez, R., Vanegas, L. F., Mart nez, M. A., de la Noval, B. M., & Rivera, R. (2000). Producto inoculante micorrizógeno. *Oficina Nacional de Propiedad Industrial. Cuba, Patente*, (22641).

Ferro Valdés, E. M., Chirino González, E., Márquez Serrano, M., Mirabal Báez, E., Rós Labrada, H., Guevara Hernández, F., & Alfaro Hernández, F. (2013). Experiencias obtenidas en el desarrollo participativo de hbridos lineales simples de maíz (Zea mays, L.) en condiciones de bajos insumos agrícolas. *Cultivos Tropicales*, *34*(2), 61-69.

Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular infection in roots. *New Phytol.* 84, 489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x.

Igu é, A. M., Adjanohoun, A., A hou, C., & Mensah, G. A. (2015). Document Technique d'Information: Evaluation de l'état de la fertilit é des sols champs des producteurs dites de ma s du B énin. D ép ôt l égal N 8116 du 09/09/2015, 3 ème Trimestre, Biblioth èque Nationale (BN) du B énin - ISBN : 978-99919-0-707-9.

Incesu, M., Yesiloglu, T., Cimen, B., Yilmaz, B., Akpinar, C., & Ortas, I. (2015). Effects on growth of persimmon (Diospyros virginiana) rootstock of arbuscular mycorrhizal fungi species. *Turkish Journal of Agriculture and Forestry 39*, 117–122.

Kumar, A., Raghuwanshi, R., & Upadhyay, R. S. (2003). Vesicular-arbuscular mycorrhizal association in naturally revegetated coal mine spoil., 251-254. *Tropical Ecology*, 44(2).



Matos, R. M. B., da SILVA, E. M. R., & Lima, E. (1999). Fungos micorr zicos e nutrição de plantas. Embrapa Agrobiologia-Documentos (INFOTECA-E). *Documentos*, *98*, 36.

McGonigle, T. P., & Fitter, A. H. (1990). Ecological specificity of vesicular-arbuscular mycorrhizal associations. *Mycological research*, 94(1), 120-122.

https://doi.org/10.1016/S0953-7562(09)81272-0.

Mendez Del Villar, P., Bauer, J. M., Maiga, A., & Ibrahim, L. (2011). Crise rizicole, évolution des march és et s écurit é alimentaire en Afrique de l'Ouest. Paris. *MAEE.*, *61*. http://agritrop.cirad.fr/562204 (July 29, 2017).

Merryweather, J. (2001). MEET THE GLOMALES-the ecology of mycorrhiza. *British Wildlife*, 13(2), 86-93.

Ndoye, F., Diedhiou, A. G., Gueye, M., Fall, D., Barnaud, A., Sy, M. O., ..., & Kane, A. (2016). Réponse du fonio blanc (Digitaria exilis Stapf) à l'inoculation avec des champignons mycorhiziens à arbuscules en conditions semi-contrôl és. *Journal of Applied Biosciences*, *103*(1), 9784-9799.

Phillips, J. M., & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British mycological Society*, *55*(1), 158IN16-161IN18. https://doi.org/10.1016/S0007-1536(70)80110-3.

Read, D. J., & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance?. *New Phytologist*, *157*(3), 475-492. https://doi.org/10.1046/j.1469-8137.2003.00704.x.

Rivera, R., Fern ández, F., Hern ández-Jim énez, A., Mart n, J. R., & Fern ández, K. (2003). El manejo efectivo de la simbiosis micorr zica, una v a hacia la agricultura sostenible., 1-42. *Estudio de caso, el Caribe.(Rivera, R. y Fern ández, K., Eds. p. 166, 2003). Ediciones Instituto Nacional de Ciencias Agr colas, La Habana, Cuba.* ISBN: 959-7023-24-5. https://www.researchgate.net/publication/299979710.

Rodr guez, Y., Dalp é, Y., Seguin, S., Fern ández, K., Fern ández, F., & Rivera, R. A. (2012). Glomus cubense sp. nov., an arbuscular mycorrhizal fungus from Cuba. *Mycotaxon*, *118*(1), 337-347. https://doi.org/10.5248/118.337.

Roveda, G., & Polo, C. (2007). Mechanisms of maize adaptation associated with Glomus spp. in soils with low phosphorus availability. *Agron. colomb.*, *25*(2), 349-356. ISSN 0120-9965.

Ruget, F., Bonhomme, R., & Chartier, M. (1996). Estimation simple de la surface foliaire de plantes de ma s en croissance. *Agronomie*, *16*(9), 553-562.

Sharma, N., Yadav, K., & Aggarwal, A. (2016). Growth response of two Phaseolus mungo L. cultivars induced by arbuscular mycorrhizal fungi and Trichoderma viride. *International Journal of Agronomy*. http://dx.doi.org/10.1155/2016/1524304.

Smith, F. A., Grace, E. J., & Smith, S. E. (2009). More than a carbon economy: nutrient trade



and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytologist*, *182*(2), 347-358. https://doi.org/10.1111/j.1469-8137.2008.02753.x.

Subramanian, K. S., Charest, C., Dwyer, L. M., & Hamilton, R. I. (1995). Arbuscular mycorrhizas and water relations in maize under drought stress at tasselling. *New Phytologist*, *129*(4), 643-650. https://doi.org/10.1111/j.1469-8137.1995.tb03033.x.

Thomas, G. W. (1982). Exchangeable cations. *Methods of soil analysis*. *Part 2*. (159-165). *Chemical and microbiological properties*, (methods of soil an2).

Tian, H., Drijber, R. A., Li, X., Miller, D. N., & Wienhold, B. J. (2013). Arbuscular mycorrhizal fungi differ in their ability to regulate the expression of phosphate transporters in maize (Zea mays L.). *Mycorrhiza*, 23(6), 507-514.

Trouvelot, A. (1986). Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une significantion fonctionnelle. *Mycorrhizae: physiology and genetics*, 217-221.

Usharani, G., Sujitha, D., Sivasakthi, S., & Saranraj, P. (2014). Effect of Arbuscular Mycorrhizal (AM) Fungi (Glomus fasciculatum L.) for the improvement of growth and yield of Maize (Zea mays L.). *Central European Journal of Experimental Biology*, *3*(2), 19-25.

Vierheilig, H., & Piche, Y. (2002). Signalling in arbuscular mycorrhiza: facts and hypotheses. In *Flavonoids in cell function*, 23-39. Springer US.

Walker, C., & Schüßler, A. (2004). Nomenclatural clarifications and new taxa in the Glomeromycota Pacispora. *Mycological Research*, *108*(9), 981-982.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science*, *37*(1), 29-38. https://doi.org/10.1097/00010694-193401000-00003.

Wang, G. M., Stribley, D. P., Tinker, P. B., & Walker, C. (1993). Effects of pH on arbuscular mycorrhiza I. Field observations on the long-term liming experiments at Rothamsted and Woburn. *New Phytologist*, *124*(3), 465-472.

https://doi.org/10.1111/j.1469-8137.1993.tb03837.x

Yallou, C. G., A ħou, K., Adjanohoun, A., Toukourou, M., Sanni, O. A., & Ali, D. (2010). Itin éraires techniques de production de ma ïs au B énin. Fiche technique. D ép ôt l égal N 4922 du 3 D écembre, *Biblioth èque Nationale du B énin, 18*.

Yamawaki, K., Matsumura, A., Hattori, R., Tarui, A., Hossain, M. A., Ohashi, Y., & Daimon, H. (2013). Effect of inoculation with arbuscular mycorrhizal fungi on growth, nutrient uptake and curcumin production of turmeric (Curcuma longa L.). *Agricultural Sciences*, *4*(02), 66. http://dx.doi.org/10.4236/as.2013.42011.

Zhang, H., Liu, Z., Chen, H., & Tang, M. (2016). Symbiosis of arbuscular mycorrhizal fungi and Robinia pseudoacacia L. improves root tensile strength and soil aggregate stability. *PloS one*, *11*(4), e0153378. https://doi.org/10.1371/journal.pone.0153378.



Copyright Disclaimer

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).