

Efficiency of different genera of arbuscular mycorrhiza fungi associated with cashew cultivars on maize growth and nutrient uptake

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Accepted 27th July, 2015

A pot experiment was carried out to assess the effect of different genera of arbuscular mycorrhiza fungi (AMF) associated with different cashew cultivars on maize growth parameters. The study was carried out at School of Crop Sciences, University of Abomey-Calavi in Benin during 45 days (from August 15 to September 30, 2013). The soil samples of 0-20 cm depth collected under different cashew cultivar trees were used for maize pot experiment. AMF density and diversity were previously studied on this soil. The four dominant genera of AMF isolated (*Glomus*; *Glomus+Scutelospora*; *Glomus+Scutelospora+Acaulospora* and *Glomus+Scutelospora+Acaulospora+Entrophospora*) from cashew cultivars were used solely or jointly as treatments. The experiment was laid out at a randomized completed bloc design with six replications. Plants' root, above ground and root biomass, number of AMF spores, rate of root infection, and N and P uptake were assessed. Soil dominated by *Glomus* had induced significantly ($P<0.05$) high and vigorous plants compared with the other AMF genera. Furthermore, in the soil drenched with *Glomus* and *Glomus+Scutelospora* spores, we noticed the highest aboveground biomass production (1.47 ± 0.13 to 1.81 ± 0.23 g DM/plant), root biomass (7.35 ± 0.33 to 8.56 ± 0.65 g DM/plant) and rate of root colonization by the AMF spores (52.44 ± 4.20 to $59.00 \pm 2.00\%$) compared with the other treatments. The results also showed that only *Glomus+Scutelospora* treatment induced the highest P and N uptake by maize plants (0.80 ± 0.03 and 1.55 ± 0.37 mg/plant respectively). *Glomus* and *Glomus+Scutelospora* association were effective in enhancing growth and development and nutrient uptake of maize. Soil from these cashew cultivars is an opportunity for production of inoculum to improve the growth and development of tree nursery.

Key words: *Anacardium occidentale*, soil fertility, plant nutrition, growth and development, Benin.

INTRODUCTION

The decline in soil fertility is a major agricultural constraint in sub-Saharan Africa (Erenstein, 2003). In

the traditional cropping systems, soil fertility restoration is often insured by long natural fallows

(Kolawolé et al., 2003). It was mentioned by farmers in the study carried out by Balogoun et al. (2014) that cashew tree improves soil fertility. This farmers' perception is relevant as in this ecosystem, cashew trees contribute to nutrient and organic carbon cycling. However, nutrients flow from the deeper layer and recycle in the litter. Furthermore, it was found that roots of cashew trees form efficient symbiotic association with arbuscular mycorrhiza fungi (AMF) (Ananthakrishnan et al., 2004; Ibiremo et al., 2012; Proborini et al., 2013). The potential of AMF in increasing plant productivity by helping them uptake nutrients and water from the soil have been widely recognized (Ananthakrishnan et al., 2004; Saïdou et al., 2009; 2012; Bhuiyan et al., 2014). AMF are essential for maximizing P and other soil nutrient use efficiency (Cardoso and Kuyper, 2006). Generally, in tropical region, P is considered as the main nutrient that is drawn from soil deep layer by mycorrhiza because the abiotic and biotic factors reduce their mobility (Cardoso and Kuyper, 2006; Saïdou et al., 2009; Ibiremo et al., 2012). Moreover, they are known to increase the rate of photosynthesis of the host plants (Syvertsen and Graham, 1999).

The present work is a follow up of the previous study carried out by Balogoun et al. (2015) on the diversity of AMF associated with cashew cultivars in central parts of Benin. Seven species of AMF belonging to four genera (*Scutelospora*; *Acaulospora*; *Enthrophospora* and *Glomus*) were identified on the basis of their morphological characteristics. Little is known about the effect of each of these AMF genera on the cashew (fruit and kernel) yields and the kernel quality as in the studied area farmers do not apply fertilizers (Balogoun et al., 2014). To address this issue, maize crop was used as a model crop in order to assess the performances of each of these genera of AMF on the growth and development and nutrient uptake for further valorization so as to improve cashew productivity. We hypothesized that, the effectiveness of each AMF genus on maize plant would be an opportunity for inoculum mass production to improve growth and development of cashew plant seedling. The purpose of this work was to study the efficiency of AMF associated with different cultivars of cashew to improve the trees' productivity in the areas where there is no fertilizer application in the cropping system. Therefore, it aims to study the contribution of AMF genera identified on the agronomic performance and nutrient uptake by maize as test crop in pots.

MATERIALS AND METHODS

Study area

The pot experiment was set up at the green house of the School of Crop Production of the Faculty of Agronomic Sciences, University of Abomey-Calavi in Benin (West Africa). The plant pots were filled with

soil samples used in the previous study on AMF biodiversity in the cashew cropping system of Benin (Balogoun et al., 2015).

Soil samples were collected in the cashew plantation from Adourékoman village, district of Glazoué in the central parts of Benin around 234 km from Cotonou. It is located between 7°91'58"N and 2°27'30"E and 152 masl. Maize were planted in pots experiment containing soil samples collected under cashew trees including each dominant genera or association of AMF genus that were checked off and identified in the study carried out by Balogoun et al. (2015). The soil is tropical ferruginous from Precambrian crystalline rocks (granite and gneiss), classified as Ferric Lixisol (FAO, 1990). Soils were sampled under each cashew tree identified during the agro-morphological characterization study (Chabi Sika et al., 2015) at 0-20 cm depth considering the North, South, West and East and then mixed up to obtain representative composite samples per tree. Table 1 shows soil chemical properties at 0-20 cm depth according to cultivars characteristics and DBH classes of cashew trees.

Plant material

Maize variety EVDT 97 STR, an early cultivar of 90 days, was used. The pots were polyethylene buckets with 28 cm high and 30 cm of upper diameter and 20 cm of lower diameter (17 liters of volume). Four seeds were sown per pot 24 h after watering the soil to field capacity (August 15, 2013).

Trial installation

Soil samples collected were crushed to pass through a 2 mm sieve. The field capacity was determined at the Laboratory of Plant Biology by putting 100 ml of water on 100 g of soil in four replications using a funnel and Whatman N°14 filter paper. The field capacity was obtained through the difference between the 100 ml of added water and the average quantity of water that pass through the soil. Thus, the portions of 10 kg of dried soil were weighed up in each trial pot. The soil of each pot was watered and homogenized at 2/9th of the field capacity (Saïdou et al., 2012). Pots were not perforated in order to avoid losses of AMF spores by percolation.

The extraction and counting of AMF spores were done according to the method described by Brundrett et al. (1996). The spores were grouped according to their morphological characteristics (spore size, color, and hyphal attachment). They were identified using information from the International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi website (<http://www.invam.caf.wdu.edu>) and named according to the current valid taxonomy (Johnson et al., 2013). From each cashew plant characteristic studied by

Table 1. Soil chemical properties at 0-20 cm depth according to cultivars characteristics and DBH classes of cashew trees.

Cultivars characteristics	Class of DBH (cm)	pH (water)	pH (KCl)	Organic carbon (g/kg)	Total N (g/kg)	P Bray1 (mg/kg)	K ⁺ (cmol/kg)
Big nuts and big apples	0 - 20	6.50±0.09a	5.61±0.18a	12.09±1.26a	1.25±0.18a	37.21±9.34a	0.80±0.03a
	20 - 40	6.23±0.07a	5.29±0.12a	8.72±0.36b	1.41±0.21a	37.94±12.94a	1.25±0.26a
	> 40	6.27±0.06a	5.38±0.12a	12.92±0.58a	0.99±0.21a	35.42±13.41a	0.74±0.07a
	Mean	6.33±0.05A	5.43±0.08A	11.24±0.63A	1.29±0.11A	36.86±6.53A	0.93±0.10A
Small nuts and small apples	0 - 20	6.39±0.09a	5.38±0.11a	10.41±1.53a	1.20±0.22a	38.69±14.06a	0.80±0.04a
	20 - 40	6.32±0.08a	5.28±0.13a	9.94±1.13a	1.38±0.21a	25.71±7.29 a	0.77±0.02a
	> 40	6.18±0.10a	5.43±0.12a	12.46±0.92a	1.28±0.19a	59.85±22.30a	0.81±0.14a
	Mean	6.30±0.05A	5.36±0.07A	10.93±0.71A	1.21±0.12A	41.42±9.22A	0.79±0.04A

Within column, means followed by letters with the same characters are not significantly different ($P > 0.05$) according to Student Newman-Keuls test. Source: Balogoun et al. (2015).

Table 2. Density per gramme of dry soil of the AMF spores (mean values ± standard errors) on the basis of cashew's cultivars characteristics.

Cultivars characteristics	<i>Glomus</i>			<i>Acaulospora</i>		<i>Scutelospora</i>	<i>Entrophospora</i>
	<i>Glomus sp</i>	<i>G. hoi</i>	<i>G. geosporum</i>	<i>A. colossica</i>	<i>A. lacunose</i>	<i>S. gregarina</i>	<i>E. infrequens</i>
Big nuts and big apples	3.39±0.25 a	0.60 ± 0.09 b	0.08 ± 0.01 b	0.008 ± 0.005 b	0.0028 ± 0.0028 a	0.22 ± 0.03 b	0.13 ± 0.04 a
Small nuts and small apples	3.52±0.32 a	0.63 ± 0.07 a	1.17 ± 0.14 a	0.036 ± 0.02 a	0 a	0.35 ± 0.08 a	0.12 ± 0.02 a

Within column, means followed by letters with the same characters are not significantly different ($P > 0.05$) according to Student Newman-Keuls test. Source: Balogoun et al. (2015).

Balogoun et al. (2015), the four dominant genera or combination of genus found in the soil samples (Table 2) were used as treatments for the pot experiment. They were:

- *Glomus*;
- *Glomus*+*Scutelospora*;
- *Glomus*+*Scutelospora*+*Acaulospora*;
-
- Glomus*+*Scutelospora*+*Acaulospora*+*Entrophospora*.

Experimental design was a completely randomized bloc design. The treatments were replicated six times with a total of 24 pots.

Data collected

Height and circumference at collar of maize plants were measured each week at 14th, 21st, 28th and 35th days after sowing (DAS). Maize plant heights were measured using a pentameter and circumference at collar with ribbon meter. The harvest was done after 35 days after sowing. Maize plants were cut with blade; fresh aboveground biomass was weighed per pot with scales. The dry matter was assessed after drying at 65°C during 72 h in the oven in the laboratory then weighed again. These samples were stored for nutrient content determination.

After the harvest of the aboveground biomass, maize fresh roots of each pot were also weighed after removing soil from the pot and cleaned with tap water. Some parts of the roots collected were stored in a freezer at 4°C temperature for the assessment of AMF infection rate. The remaining root samples were

weighed and dried in the oven at 65°C during 72 h to assess the dry matter content.

Plant analysis

Total N and P content of the aboveground biomass and the roots samples were determined in the Laboratory of Soil Sciences of the Faculty of Agronomic Sciences, University of Abomey-Calavi. Total N was quantified by wet digestion in a mixture of H₂SO₄-Selenium followed by distillation and titration. Determination of P consisted of two steps: dry ashing a plant sample in a muffle furnace at 550°C for 4 h and gathering the residues in 1 N HNO₃, involving a period of heating. P was subsequently measured colorimetrically by ammonium molybdate with ascorbic acid at a wavelength of 660 nm.

Assessment of spores number and root colonization by AMF

Maize fine roots of the root samples from each pot stored were coloured according to the method describes by Brundrett et al. (1996). Thus, about 1 g of fine root samples was placed in a bottle, cleared and bleached (successively with 10% KOH in a water bath at 90°C for 30 minutes, rinsed with water, and finally with 10% H₂O₂). According to Brundrett et al. (1996), the roots were rinsed again with tap water and after they were stained with 0.03% w/v Chlorazol black E in a lactoglycerol solution (1:1:1 lactic acid, glycerol and distilled water). At least, 7 days stayed in the Stain's solution favour an entire coloring of roots

and enable an observation of roots content. The counting was done with a stereomicroscope at x 40 (Stemi DRC Zeiss) and the results were expressed in percentage of root length colonized. The proportion of root length colonized by AMF of each sample was determined by the gridline intersection method (Giovannetti and Mosse, 1980).

Statistical analysis

Statistical analyses were performed using Statistical Analysis System (SAS v 9.2) package. Maize height, circumference at collar, aboveground and root biomass, maize aboveground nutrient content and fractional AMF infection of maize roots were subjected to one-way analysis of variance (ANOVA). The only factor was the dominant AMF genus found in the soil collected under the cashew's trees. Before running ANOVA, variance homogeneity was tested. Therefore, no data transformation especially AMF root infection rate were needed. The Student Newman-Keuls test was performed to compare differences in means among treatments. Pearson's correlation coefficients were calculated between fractional root infection by AMF spores, biomass and nutrient uptake. All significance levels were set at $P < 0.05$.

RESULTS

Effect of AMF genera on maize growth parameters

The analysis of variance revealed that the AMF genera had not significant effect ($P > 0.05$) on the maize height and collar circumference during the different development stages. Figure 1 shows the contribution of each AMF genus treatment on the plants' height. It was observed that, the AMF genus *Glomus* induced the shortest plants (6.45 ± 0.55 and 7.66 ± 0.56 cm at 14 and 21 days after planting – DAP-, respectively) and *Glomus+Scutelospora* induced the highest plants (7.43 ± 0.19 and 8.43 ± 0.23 cm at 14 and 21 DAP respectively).

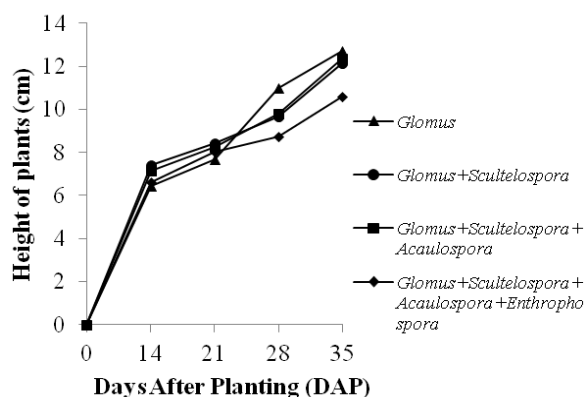


Figure 1. Effect of the AMF genus treatment on maize plant height in pot at different development periods.

On the 28th and 35th DAP, the genus *Glomus* had induced the highest plants (11.0 ± 1.03 and 12.71 ± 0.53 cm respectively) while, the shortest plants (8.73 ± 0.35 and 10.61 ± 0.72 cm respectively) were induced by *Glomus+Scutelospora+Acaulospora+Entrophospora* treatment. The results showed significant effect ($P < 0.05$) of different AMF on maize heights only at 21th DAP.

The effect of AMF genus on the circumference at collar of maize plants at different development periods is shown in Figure 2. It appears from the graph that, plants' collar circumference development follows almost the same pattern as plants' height. Thus, *Glomus+Scutelospora* treatments had induced the most vigorous maize plants in pot (1.41 ± 0.02 and 1.79 ± 0.03 cm respectively at 14th and at 21st DAP). Furthermore, at 35th DAP, *Glomus* had induced the most vigorous plants (2.63 ± 0.07 cm). The AMF identified had significantly ($P < 0.05$) affected maize collar circumferences only at 35th DAP.

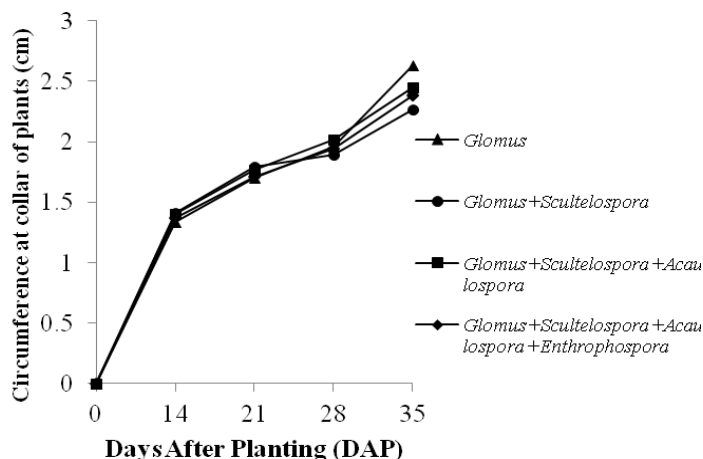


Figure 2. Effect of the AMF genus treatments on circumference at collar of maize plant in pot at different development periods.

Effect of AMF on maize aboveground biomass and roots' production

The analysis of variance shows that, AMF in the soil under cashew trees did not significantly ($P > 0.05$) affect maize aboveground and roots' biomass. Figure 3 presents the effect of AMF treatments on the aboveground and root biomass production of maize plants in pots. It was observed that, soil dominated by AMF *Glomus* induced the highest aboveground biomass (1.81 ± 0.23 g DM/plant) while *Glomus+Scutelospora* induced the highest roots' biomass production (8.56 ± 0.65 g DM/plant). The lowest root biomass production (7.35 ± 1.33 g DM/plant) was induced in the soil dominated by AMF genus *Glomus* and the lowest aboveground biomass production (1.03 ± 0.10 g DM/plant) in the soil dominated by *Glomus+ Scutelospora+ Acaulospora+ Entrophospora*.

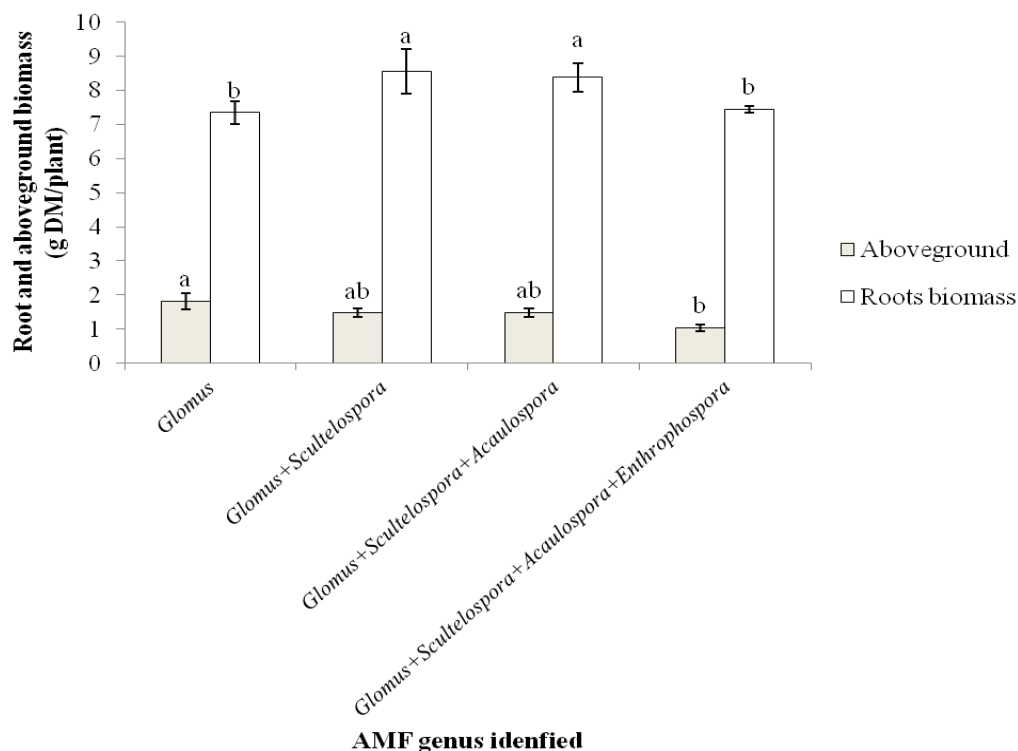


Figure 3. Effect of each AMF genus identified under the cashew trees on maize aboveground and roots biomass production in pot. Vertical bars denote standard errors. Bars of the same types labeled with the same letter are not significantly different ($P > 0.05$) according to Student Newman-Keuls test.

Effectiveness of the AMF genus on maize fine roots' colonization

Considering the rate of fine root colonization, there were no significant ($P > 0.05$) differences between the activities of the AMF genera identified from the different cashew's tree cultivars. However, the highest rate of maize fine roots' colonization was obtained in soil dominated by the *Glomus* and the lowest in soil dominated by *Glomus+Scutelospora+Acaulospora* (Table 3) even if the difference was not significant according to Student Newman-Keuls test. This result showed that *Glomus* is of great interest in the improvement of crop performance.

Contribution of AMF in N and P uptake by the maize plant

The AMF did not significantly ($P > 0.05$) affected N and P content and uptake by plant maize (Table 4). However, results of Table 4 show that soil sample dominated by AMF genus *Glomus* induced the highest N concentration (22.89 ± 2.17 g/kg) while *Glomus+Scutelospora* induced the highest P concentration (1.04 ± 0.14 g/kg) in maize tissue. Moreover, the highest N and P uptake by the maize plants was found only in soil dominated by AMF genus *Glomus+Scutelospora* (1.55 ± 0.37 and 0.80 ± 0.03 mg/plant respectively), even if the difference was

not significant ($P > 0.05$) according to Student Newman-Keuls test.

Pearson's correlation coefficients between fractional maize of root colonization by AMF, biomass and nutrient uptake

The results of the correlations between root colonization rate, nutrient uptakes and aboveground biomass are presented in Table 5. The fractional maize root colonization by the *Glomus* genus was significantly correlated ($P < 0.05$ or $P < 0.01$) with root biomass ($R = 0.80$), N ($R = 0.84$) and P ($R = 0.68$) content in the plant tissue. In the soil dominated by AMF genus *Glomus+Scutelospora*, only the rate of root colonization was significantly correlated ($P < 0.05$ and $R = 0.54$) with P content in the maize plant tissue.

DISCUSSION

Effect of AMF genus associated with cashew cultivars on maize plant performance

The effect of the four treatments on maize growth parameters was almost the same. This is due to the fact that, in the soil dominated by *Glomus* genus, abundant spores of the species were found in the 0-20 cm depth (Balogoun et al., 2015) which inhibited

Table 3. Maize root colonization rate (average value \pm standard errors) by AMF identified in the soil under cashew trees.

AMF genus	Colonization rate
<i>Glomus</i>	59.00 \pm 2.00 a
<i>Glomus+Scutelospora</i>	52.44 \pm 4.20 a
<i>Glomus+Scutelospora+Acaulospora</i>	47.26 \pm 1.66 a
<i>Glomus+Scutelospora+Acaulospora+Enthrophospora</i>	51.00 \pm 4.07 a

The average followed by the same alphabetic letter of the same character and for the same factor are not significantly ($P>0.05$) different according to Student Newman-Keuls test.

Table 4. Mean values (\pm standard error) of N and P concentration, N and P uptake by the maize plant on the basis of AMF identified in the soil under cashew trees.

Genus of AMF	P total (g/kg)	Uptake of P (mg/plant)	N total (g/kg)	Uptake of N (mg/plant)
<i>Glomus</i>	0.61 \pm 0.04 a	0.74 \pm 0.03 a	22.89 \pm 2.17 a	1.10 \pm 0.07 a
<i>Glomus + Scutelospora</i>	1.04 \pm 0.14 a	0.80 \pm 0.03 a	14.48 \pm 1.15 a	1.55 \pm 0.37 a
<i>Glomus + Scutelospora +Acaulospora</i>	0.88 \pm 0.09 a	0.70 \pm 0.03 a	18.96 \pm 1.23 a	1.33 \pm 0.24 a
<i>Glomus+Scutelospora+Acaulospora+Enthrophospora</i>	0.84 \pm 0.20 a	0.77 \pm 0.05 a	19.09 \pm 1.67 a	0.96 \pm 0.33 a

The average followed by the same alphabetic letter of the same character and for the same factor are not significantly ($P>0.05$) different according to Student Newman-Keuls test.

Table 5. Pearson's correlation coefficients between fractional maize of root colonization with AMF, biomass and nutrient uptake.

Genus of AMF	Aboveground (g DM/plant)	Root biomass (g DM/plant)	Uptake of P (mg/plant)	Uptake of N (mg/plant)
<i>Glomus</i>	0.49 ns	0.80 *	0.68 *	0.84 **
<i>Glomus + Scutelospora</i>	0.27 ns	0.21 ns	0.54 *	0.17 ns
<i>Glomus + Scutelospora +Acaulospora</i>	0.29 ns	0.12 ns	0.39 ns	0.30 ns
<i>Glomus+Scutelospora+Acaulospora+Enthrophospora</i>	0.47 ns	0.55 ns	0.56 ns	0.44 ns

*, $P<0.05$; **, $P<0.01$; ns, not significant.

activities on the remaining genera. This lack of efficiency of *Glomus* could be due to the fact that, symbiosis process involves the flow of photosynthates to the root system (Fernández et al., 2011). Therefore, an adequate mycorrhizal development due to the use of these substances involves a strong root system which is not the case with the root of 35 days old maize in our study. One could expect significant effect with mature plant. *Glomus* genus induced gradually the highest and more vigorous plants from 28th DAP compared with the other AMF treatments. Our results confirm those of Fernández et al. (2011) who observed a real response after 20 days of planting (DAP).

Effect of AMF genus associated with cashew cultivars on N and P uptake

Phosphorus and nitrogen content in the aboveground biomass whatever AMF effect are under the critical threshold (2.3 and 34 g/kg) respectively for phosphorus and nitrogen (Saïdou et al., 2009). Nevertheless, *Glomus+Scutelospora* treatment had induced the highest phosphorus uptake by the maize plants. This could be attributed to the quantity of

biomass that the treatment brought about. High mycorrhizal inoculum at the start of the growing season had major effects on the growth and phosphorus uptake of cereals and legumes (Miller, 2000). These results suggest therefore, that the build-up of AMF inoculum is beneficial to the nutrition and productivity of subsequent maize. Similar shoot and root weight increases were reported with cotton (Tian et al., 2004) and soybean (Sharifi et al., 2007).

Efficacy of AMF genus associated with cashew cultivars on root colonization rates

The maize root colonization rates (varying between 47.26 \pm 1.66% and 59.00 \pm 2.00%) corroborate those obtained by Sogansa (2003) on *Acacia mangium* roots (49%) at Ouèdo in Benin. But the root colonization rate caused by *Glomus* (59%) in the present study was slightly higher when compared with colonization rate (44.6 to 51%) obtained by Ananthakrishnan et al. (2004) in India after inoculation of cashew plant in nursery with three *Glomus* species: *G. aggregatum*, *G. fasciculatum* and *G. mosseae*. This difference is due to the spores density and the host plants. In contrast, these rates obtained in the

present study were inferior to the rate obtained by Houngnandan et al. (2009) with *Isoperlinia doka* in Benin (64%). This is due to the fact that, plants mycorrhizal infection varied widely from one plant to another within the same specie but, also according to the genotypes of the species, environmental conditions, plant age, nitrogen content etc. (Duponnois et al., 2001). Nevertheless, the genus *Glomus* produced the highest root colonization rate and this is due to their relative abundance (91.80%).

From a long-term perspective, the benefits provided by mycorrhizal associations to crops are based on their efficacy in mining nutrients in the soil. Therefore, the effect of mycorrhizas on crop yield improvement should not be taken as a claim that mycorrhizal fungi can be considered as bio-fertilizers. But in cropping systems where hyphae of AMF can enlarge the volume of soil explored for nutrients, AMF can then increase crop productivity (Saïdou et al., 2012).

However, from our results, one can conclude that, roots' infections observed would be very little caused by the spores of Glomales dominated in these soils under the different cultivars of cashew. These results confirm those of Neree (2000) and Houngnandan et al. (2009) in Cameroon and Benin respectively and would be due to the similarity of our experimental conditions.

The lack of correlation ($P > 0.05$) observed between the colonization rate and the aboveground and root biomass lead to the conclusion that roots infection by AMF cannot allow to predict biomass production.

Conclusion

This study shows that *Glomus* and *Glomus+Scutelospora* association induced an important effect on the plants' agronomic performance, on the phosphorus and nitrogen mobilization and finally on the roots colonization. The maize roots was infected (colonization rate varied between 47 and 59%) and would not have been caused by the Glomales spores that were abundant under cashew whatever the tree's cultivar. The genus *Glomus* induced the highest root colonization rate. *Glomus* and *Glomus+Scutelospora* association appeared to be effective in enhancing growth and development and nutrient uptake of maize. They could be an opportunity for production of inoculum to improve the growth and development of tree nursery.

ACKNOWLEDGEMENTS

The authors are grateful to the "Scientific Council of the University of Abomey-Calavi in Benin" for providing financial support to the present research in the framework of the project Biodiversity and valorization of cashew products in Benin (PROANAC). They also thank the anonymous reviewers for critically

reading the manuscript and providing valuable input.

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