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Chemical property of soil and mycorrhizal status *in Allanblackia floribunda* Oliver (Clusiaceae)

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ABSTRACT

The objective of this study was to describe the type of mycorrhizal fungus that is associated with *A. floribunda* and evaluate the effect of soil chemical properties on its rhizosphere, its mycorrhizal dependency in its natural range in four populations of the species (Mouanko, Yalpenda, Nkenlikok and Mbalmayo), two land use systems (disturbed and undisturbed), two age classes (circumference less than 50cm and up to 50cm). Results obtained so far demonstrated that *A. floribunda* is colonized by arbuscular mycorrhizas. Root colonization was significantly ($P < 0.01$) different in the targeted populations with the highest observed in Yalpenda (53.63 ± 1.33%). Land disturbance significantly ($P < 0.01$) affected root colonization. Unperturbed soils displayed the highest root colonization (35.29 ± 0.70%). Age classes had significant ($P < 0.001$) influence on root colonization and trees with circumferences up to 50 cm had the highest percentage (35.45 ± 0.72%). There was no significant correlation between root colonization and phosphorus ($R^2 = 0.437$, $p = 0.563$), in contrary to root colonization and copper ($R^2 = -0.934$, $p = 0.046$). These results constitute a prerequisite for the inoculation phase and molecular analysis of roots already begun.

Keywords: *Allanblackia floribunda*, arbuscular mycorrhizal fungi, root colonization,

INTRODUCTION

Allanblackia floribunda is a tree of tropical forest ecosystems. It is a multipurpose species, the root bark and leaves have medicinal value. *A. floribunda* also provides fodder, firewood or lumber (Anegbeh *et al.*, 2006). The oil from its seeds is rich in fatty acids and valued for its oleic and stearic known to lower cholesterol levels in human plasma while reducing the risk of heart attack (Bonanome and Grundy, 1988). Furthermore this oil is used in cosmetics and food industries. Today the seed supply chain is developed by Unilever promises an even greater value of the species. More the novella partnership (public-private) subsidizes the supply of seed but the amounts collected remain inadequate, yet this forest product is experiencing a renewed interest due to the increasing demand on the world market. Currently more than 100 000 tonnes of oil *Allanblackia SPP* is requested each year, but only about 200 tons are supplied on average, due to low amounts of harvested seed (ICRAF, 2011). However, the combined effects of random climatic factors and human action, this forest species has experienced a deterioration jeopardizing oil production and the income of farmers in Cameroon. Since then, many initiatives have been undertaken to ensure sustainable access of local populations and large international firms such UNILEVER, with multiple services and products of this kind, like the participatory domestication. The domestication of plants that generate non-timber forest products (NTFPs) is a recommended approach to meet their growing demand while ensuring the protection of the resource in the sampling locations (Leakey, 2001). Several studies have been made in the process of domestication of *A. floribunda* as genetic diversity (Russell *et al.*, 2009; Atangana, 2010), cuttings fragments of leafy stems (Atangana *et al.*, 2006) and grafting (Asaah *et al.*, 2011; World Agroforestry Centre, 2011). But only little information on the biological factors that support the growth of *A. floribunda* and determine its natural environment regeneration.

In the current state of knowledge, the physiological importance of mycorrhizae is increasingly recognized. Several authors have shown the efficiency of symbiotic microorganisms on plant growth (Diop *et al.*, 2003). These fungi in soils of most ecosystems, form symbiotic associations with the

roots of many terrestrial plants (about 80%) (Strullu, 1991; van der Heijden *et al.*, 1998a). In exchange for carbon resources received from the host plant, these fungi improve the collection and transport to the soles of very few mobile nutrients (mainly phosphorus) (Bolan, 1991), increase drought tolerance (Hardie and Leyton, 1981; Strullu, 1991) and reduce the effects of pathogenic infections (Abdalla and Abdel-Fattah, 2000). Mycorrhizal symbiosis also gives the plant tolerance to heavy metals (Leyval and Joner, 2001) and organic pollutants (Joner and Leyval, 2003). This symbiosis is expressed best in soils low in phosphorus, characteristics of tropical countries.

Incidentally, it is recognized that the soil in the wetlands of Cameroon is acid deficient in Ca, Mo, Mg, K and P, and toxicity of Al, Fe and Mn (Ambassa-Kiki, 2002). According to the latter author, acid soil covers approximately 75-100% of this part of Cameroon. Therefore, plants with low root development as *A. floribunda* should there grow very slowly (Swift, 1998).

In Cameroon the mycorrhizal status of several species is known, including *Prunus Africana* (Tchietchoua, 2012) and Aucoumea klaineana (Onguene, 2002), but the status of mycorrhizal *A. floribunda* has not been studied. To remedy this lack, this discussion will describe the type of mycorrhizal fungus that is associated with *A. floribunda* and evaluate the effect of soil chemical properties on its rhizosphere, its mycorrhizal dependency in its natural range.

MATERIAL AND METHOD

Study site

In this study four sites (Mouanko, Yalpenda, Ngoumou, Nkenglikok) were chosen because of the strong representation in this plant species and their belonging to different agro-ecological zones namely: the humid forest zone with a bimodal rainfall (Ngoumou and Nkenglikok) and the humid forest zone in unimodal rainfall (Mouanko and Yalpenda).

Methodology

Once in an agro ecological zone, we chose trees based on the factors studied to know the system status (forest and food crop fields and / or fallow more than 3 years), age group (below 50 cm and greater than or equal to 50 cm). In the middle, humidity, temperature, GPS coordinates were also considered. Although not being taken here as a factor, we thought that a correlation with the rate of colonization could help us in interpreting our results. Because according to Alexander (1989), both entities have influences on the rate of colonization and spore density.

Sampling and Harvesting of Soil Samples and Root

Samples of soil and roots were collected under each tree of rhizosphere areas. About 500 fragments of 2 cm fine roots (diameter less than or equal to 2 mm depending Brundret (1996) were harvested and kept in a bottle containing 90 ° ethyl alcohol diluted with water to 50%.

The samples of soil and roots were performed as in forests and fields at the foot of saplings and adults. The soil sampling method used was that of Sieverding (1991). Indeed, for each site, the trees *A. floribunda* were selected randomly. In the root zone of each tree, removal of soil and roots were made to the four cardinal points of the shaft between 0 and 30 cm in the soil. On each of these points, 550 g of soil in which are mixed the fragments of roots of A. floribunda were taken by a total of 2.5 kg for each tree. This soil once removed was introduced into different plastic bags marked, stored and transported at room temperature (250 °C) to the laboratory. The study was conducted according to the following sampling scheme:

Agro-ecological zone (2)	Population (4)	System (2)	Class circumference (2)	Number of tree
monomodal	Yalpenda	Disturbed	C < 50 cm	10
			C ≥ 50 cm	10
		undisturbed	C < 50 cm	10
			C ≥ 50 cm	10
	Mouanko	Disturbed	C < 50 cm	10
			C ≥ 50 cm	10
		undisturbed	C < 50 cm	10
			C ≥ 50 cm	10
bimodal	Nkenglikok	Disturbed	C < 50 cm	10
			C ≥ 50 cm	10

		undisturbed	C < 50 cm	10
			C ≥ 50 cm	10
	Ngoumou	Disturbed	C < 50 cm	10
			C ≥ 50 cm	10
		undisturbed	C < 50 cm	10
			C ≥ 50 cm	10

Chemical Analysis of Soil

Soil chemical analyses were conducted to better understand the ecology of arbuscular mycorrhizal fungi in the different study sites. These analyses relevant pH (potential hydrogen), CEC (cation exchange capacity), the determination of the exchangeable cations, the determination of total nitrogen, available phosphorus, organic carbon, exchangeable aluminum microelements (Zn, Cu, Fe and Mn).

pH H_2O was measured for a brine / water solution in a ratio 1 / 2.5 (ie 1g of soil on 2.5 ml of water) with a pH meter with a glass electrode. The pH value is directly read on the screen.

The cation exchange capacity (K, Mg and Ca) represents the sum of exchangeable cations at pH 7. It was determined in extracts by spectrometry (Benton and Vernon 1990).

Exchangeable cations were extracted with an ammonium acetate solution (CHOONH) at pH 7. The concentrations of cations were measured by the absorption of light from a hollow cathode lamp. The atomic absorption spectrometer was converted to the absorption at a given concentration (Mehlich, 1984).

The next total nitrogen was determined the method of Buondonno *et al.*, (1995). It was based on ammonification of nitrogen contained in the organic material by digestion in the presence of a strong acid salt, of a catalyst and ammonium levels measured by calorimetry (Anderson and Ingram, 1993).

Available phosphorus was determined by calorimetry water ammonium molybdate and ascorbic acid as reducing agent. The latter was extracted with combination of HCl and NaF (Murphy and Riley 1962).

The organic carbon was obtained according to the method described by Heanes (1984). This method is based on oxidation of organic carbon of the acid medium by potassium dichromate. The organic carbon contents were measured by calorimetry.

The exchangeable aluminum, Al3 + ions are extracted using a KCl solution. The Al dosage is made by complexing with aluminum fluoride neutralized by soda as Al (OH) 3, and titration of hydroxyl (OH-) released with the HCl solution (Mehlich, 1984).

Micro elements Zn, Cu, Fe, Mn are removed by the Mehlich (1984) procedure and are determined by the spectrophotometric method of atomic absorption.

Identification of Infections Intra Root

Washing

Experience shows that most samples are clean more evaluation is fast and accurate. For that roots were cleaned of soil particles by thorough rinsing under running water in a colander. When he left clods of earth around the roots, these were soaked in a solution of sodium hexametaphosphate (Calgon) for a few minutes that caused the dispersion of particles of earth (Brundrett *et al.*, 1994). Then, only small relatively clear and little Sclerotized roots were selected.

Clarification and Root Coloring

The roots were systematically lighted coloured before microscopic observation. The lightening technique and Phillips staining and Haymann (1970) was used. The roots were placed in tubes containing a solution of 10% KOH, in an oven at 90 ° C for 1 hour. They were then thoroughly rinsed with running water, drained and returned to the tubes where they were coated with a trypan blue solution 0.05% in lactophenol. The tubes were placed in an oven at 90 ° C for 15 minutes. The roots were then thoroughly rinsed and stored in distilled water before mounting.

Installation and observation

Root fragments of only a few centimeters (2-3) selected at random were collected and mounted in parallel in groups of 10 roots on a blade with three replicates (Toth *et al.*, 1990). The slides were observed under the microscope, each fragment being thoroughly checked throughout its length, the magnifications of 100x and 400x.

Evaluation Parameters

The importance of mycorrhizae was apprehended with the roots of mycorrhizal percentage parameter also called frequency mycorrhization and was determined according to Marx *et al.*, (1977).

F (%) = (nb fragments MYCOR / total fragments nb) x 100

Statistical Analyses

Statistical analysis between the different treatments applied during experiments conducted during these works were conducted with several models. The collected data (root colonization and chemical properties of soil) were coded and grouped by topic using the Microsoft Excel version 2013. The software and aggregated data were imported and analysed in SPSS software version 18. Normality tests were conducted. As distributions were not normal for cases of root colonization and chemical properties of soil, arcsinx and log transformations (X + 1) were applied on the two parameters. The data were then subjected to analysis of variance (ANOVA) multivariate. Means were separated using LSD. The general linear model was applied to the analysis of variance with P < 5%. Pearson correlations between colonization and (the contents of soil chemical elements, humidity, longitude, altitude, latitude and temperature) were performed.

RESULTS

Type of Mycorrhizal Fungi

Watching colourful roots, includes mycorrhizal structures: hyphae, vesicles and accessory cells. This indicates an active operation of the symbiosis. *A. floribunda* is colonized by the mycorrhizal fungi or endomycorrhizae.

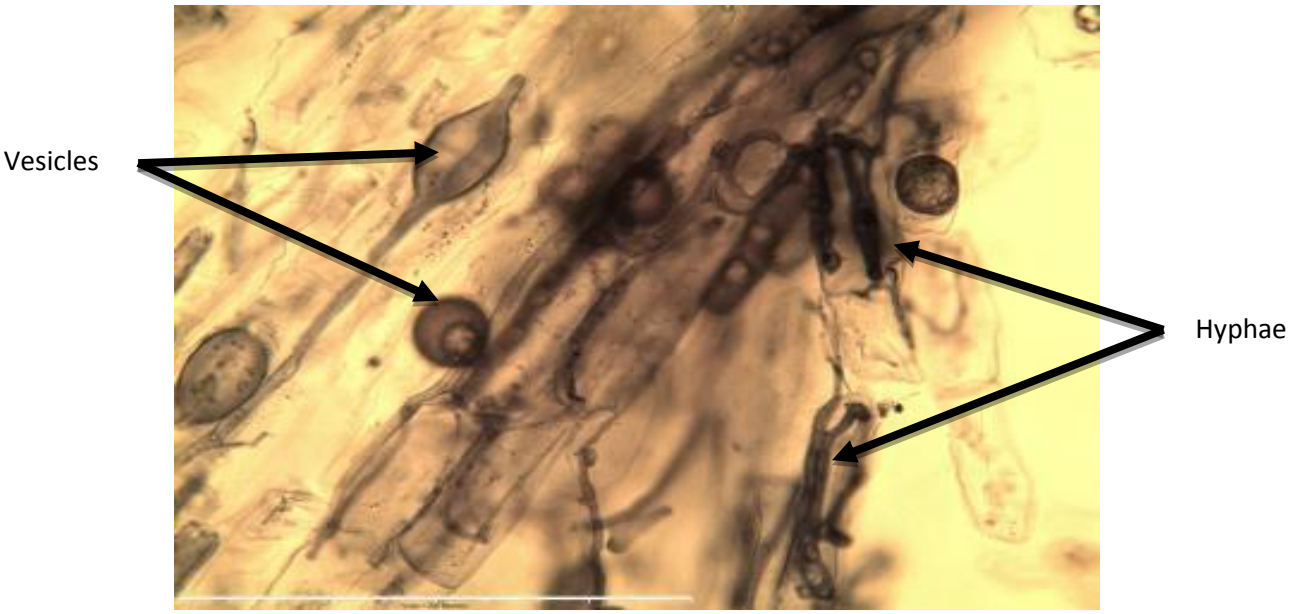

Figure1. *Structure CMA characteristics in the roots of A. floribunda (photoTchinmegni, 2015)*

Root Colonization

Effect of Agro-Ecological Zone on Root Colonization Rates

The highly significant difference (P < 0.001) was observed between the agro-ecological zones. The largest percentage of root colonization was observed in the agro-ecological zone unimodal (35.72 ± 0.77%) compared to (29.21 ± 0.77%) of the agro-ecological zone bimodal.

Table1. *Variation of root colonization rates by population*

Populations	Colonization rate (%)
Ngoumou	23.23 ± 0.7 a
Yalpenda	53.63 ± 1.33 b
Nkenglikok	24.35 ± 0.9 a
Mouanko	33.17 ± 0.8 c

Means followed by the same letter are statistically identical to the probability threshold of 5%

Effect of the Circumference of Class on the Colonization Rate

The age groups have had a significant influence ($P < 0.001$) on the rate of colonization of roots of the species. Adult trees or circumferences capacity higher than 50 cm showed the percent colonization of the roots higher (35.45 ± 0.72%) relative to those of class circumference less than 50 cm (30.05 ± 0.75%).

Effect of System Status on the Rate of Colonization

The system status has also had a significant influence ($P < 0.001$) on root colonization of the species. Analyses show a higher colonization rate in the unperturbed system (35.29 ± 0.70%) compared with the perturbed system (29.96 ± 0.77%).

Correlation between Chemical Element Content of the Soil and the Root Colonization Rate

Table 2 shows the effect of geographical coordinates, the circumferences of trees, and the contents of chemical elements in the soil of the rhizosphere *A. floribunda* on the rate of root colonization. The correlation between the rate of root colonization and soil chemical elements varies in the rhizosphere of *A. floribunda.* Indeed, a strong and negative correlation ($r = -0.93$; $P = 0.04$) was found between the rate of colonization and copper. A weak positive correlation was obtained between the rate of colonization and longitude ($r = 0.25$; $P = 0.02$). The correlation between the rate of colonization and the temperature was low and negative ($r = -0.30P = 0.003$). The correlation between moisture and root colonization was low and positive ($r = 0.38P = 0.03$).

Table2. *Correlation of the effect of chemical elements content of the soil, the circumferences and the geographical coordinates of the colonization rate*

dependent variables	colonization rates	
	Coefficient de Pearson	P
CHP	0.068	0.389
Latitude	-0.128	0.251
Longitude	0.251	0.023
Altitude	0.094	0.398
Temperature	-0.300	0.003
Humidity	0.383	0.037
CO %	-0.254	0.746
N %	-0.316	0.684
C/N	0.131	0.869
P_ppm	0.437	0.563
Al_ Cmol(+)/kg	-0.745	0.255
pH_H_2O	0.424	0.576
Ca_ Cmol(+)/kg	-0.053	0.947
K_ Cmol(+)/kg	-0.425	0.575
Mg_ Cmol(+)/kg	-0.362	0.638
Zn_ppm	-0.18	0.82
Cu_ppm	-0.934	0.046
Mn_ppm	-0.607	0.393
Fe_ppm	-0.624	0.376

DISCUSSION

Root Colonization

The observation of this colourful roots all mycorrhizal structures (hyphae, vesicles, and arbuscules helper cells) which implies that *A. floribunda* maintains the arbuscular mycorrhizal symbiosis. This statement confirms the fact that in the tropics most trees (about 80%) are associated with mycorrhizal fungi (Fortin *et al.*, 2008). The presence of the CMA on all trees *A. floribunda* and all sites show that there is no specificity of infection that is all mycorrhizal structures were observed on all trees colonized roots. In addition, the absence of hairs on the roots of *A. floribunda* justify colonization rate of its roots.

The highly significant difference was observed between the two agro-ecological zones with the highest percentage of root colonization observed in the agro-ecological zone in unimodal rainfall compared to the agro-ecological zone with a bimodal rainfall. Which joined the work of Onguene,

(2000) which showed that the infectious potential of mycorrhizal fungi was higher in wetlands in unimodal rainfall. On the other hand, this root colonization of *A. floribunda* was also significant in the various target populations with the highest rates of colonization observed Yalpenda while the lowest was observed in Ngoumou. This could be explained by the fact that Yalpenda belongs to the unimodal rainfall areas and therefore the climate is more favorable to mycorrhizal that Ngoumou located in the bimodal part.

In addition, highly significant differences were observed in the systems. The undisturbed soil (forest) have a colonization rate of higher roots compared to disturbed soils. These results corroborate those of Onguene *et al.,* (2000) on the diversity and dynamics of mycorrhizal associations in different system in southern Cameroon. Therefore, we could explain that soil disturbance has a negative influence on root colonization due to the destruction of propagules and certain mycorrhizal strains with chemicals such as pesticides and / or the bush and ploughing lights. Since the presence of arbuscular mycorrhizae in agricultural soils depends on the formation and survival of propagules (hyphae and spores colonized roots). Their persistence in soils is affected by tillage because mycorrhizae are concentrated in the upper soil layers. This argument corroborates that of Kabir (2005), confirming that the tillage breaks the hyphae and diluted. Moreover, inclusion in the cover crop rotations could be colonized by mycorrhizal inoculation increases the potential and density of hyphae. A soil disturbance reduction is accompanied by an increase in root colonization by mycorrhizal fungi.

Subsequently, the age groups have had a significant influence on root colonization of the species. Adult trees with circumferences above 50 cm showed the percentage of root colonization highest relative to minors and young plants trees with smaller circumferences than or equal to 50 cm. The low rate of colonization among young trees could be due to their very low requirements in minerals and also by adverse environmental conditions during the growing season. Druva-Lusite and Levinsh, (2010) reported that the environmental stress can affect the morphology of the symbiosis. This argument may be valid by ricochet to the adult trees that have their high mycorrhization to their high demand in minerals, because of the anatomy and morphology of the roots can affect the intensity of colonization because the thick roots tend to have high rates of mycorrhiza (Collier *et al.,* 2003). That would justify that the greatest amount of carbohydrates is released by mature trees, resulting attract the fungus able to establish symbiotic relationship (Mekahlia *et al.,* 2013).

Correlation between the Chemical Content of the Soil and Root Colonization

Mycorrhizal frequency depends on several factors such as the age of the host species, the physicochemical properties of the soil (Escudero and Mendoza 2005; Kessler *et al.,* 2010); Pande and Tarafdar 2004), biogeographic location, seasonal and climatic variations (Bradai *et al.,* 2015; Mekahlia *et al.,* 2013; Pimienta-Barrios *et al.,* 2002), the number of propagules in the soil, and sporulation (Bohrer *et al.,* 2004; Collier *et al.,* 2003).

The chemical characteristic of the soil indicates that *A. floribunda* adapts to soil very acidic pH according to the interpretation of standards (Handbook of Agronomy, 1993). Previous studies (Ba *et al.,* 2001; Brundrett, 2004; Calvente *et al.,* 2004) showed that the one factor that could influence the root colonization by mycorhziens fungi in the soil was the soil pH. However, in our analyses differences in the pH observed between study sites do not have a significant impact on root colonization. Yet the pH differences have been documented to have an impact on the viability of the spores and not on mycorrhizal symbiosis (Wang *et al.,* 2008). Our results corroborate those of earlier studies that do not show an influence of soil pH on mycorrhizal symbiosis (Wang *et al.,* 2008).

There was no significant difference on the correlation between root colonization and organic matter (organic carbon, total nitrogen). The contents of organic carbon and total nitrogen are very low in the soils studied. These values coincide with those obtained by Voundi (1998) on the floors of the Centre in Cameroon. In addition, the results also corroborate with those found on vitroplants banana in Cameroon (Tsane *et al.,* 2005). These plantlets of banana were more mycorrhizal (root colonization rate of 25%) on poor soils (low carbon in the soil) as opposed to a rate of 7% on soil amended with organic matter. This could be explained by adaptation of plants to face stress suffered several kinds (water deficit by low water retention of the soil, due to low soil fertility) in a humid environment (Déziel 2000). The C / N calculated different soils gave values that reflect a rapid mineralization of organic matter. This organic matter deficiency could be perceived here as a limitation to sustainable production.

The studied soils have low levels of available phosphorus relative to the normal value for acid soils which is between 18 and 20 mg / kg (Diary of Agronomy, 2006). There is no significant correlation between root colonization and phosphorus. This could be explained by the fact that under the conditions of acidity, phosphorus is present in the form of sparingly soluble compounds such as iron phosphate, aluminium phosphate and the occluded Phosphorus (Hodge *et al.*, 2010). These low phosphorus content available could also be explained by the fact that these soils have a high phosphorus fixing power thus reducing the proportion available to plants. Reducing the colonization of the fungus result from available phosphorus concentration too high or too low contrast (Fernando *et al.*, 2010). Moreover, the presence of a phosphorus source induce a general decline in the absorption of micronutrients, Fe, Mn, Cu ... This decrease is partially offset when the plant is mycorrhizal.

Trace elements are necessary for plant roots but in reasonable quantities. In soils of the tropics these deficiencies are rare (Anonymous, 2008). The contents of Fe, Zn, Cu, Mn are very variable in the different sites. In general, Fe and Mn are very abundant in soils. By cons, only the correlation between root colonization and copper was significant. With a high value of correlation coefficient Cu, whatever the soil considered, the percentage of colonization decreases linearly with increasing the proportion of copper. Which negatively affect root colonization. This could be explained by the fact that symbiosis is established and develops gradually with the depletion of nutrients readily available to plant roots in the soil (Strullu 1991). The low levels of Zn and Cu could be due to leaching of soluble forms.

CONCLUSION

The results of this study allowed to mount that the roots of *A. floribunda* are colonized by arbuscular mycorrhizae. The species established mycorrhizal Association throughout its life cycle. The result of the correlation between the copper content and root colonization in this study, suggesting a reduction in the copper substrate, which not only increase the potential of mycorrhizal association and thus improve the growth of cuttings. This study is an important and necessary step prior to inoculation phase and molecular analysis of the roots.

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