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Characterization test of endomycorrhiza strains inoculated to two plantain cultivars (Musa sp.) derived from in vitro culture in the Kisangani region (RDC), case of Libanga Likale and Tala Lola

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Abstract

The production of bananas and plantains is currently practiced on a large scale due to the high demand on the market, as a result of the strong demographic pressure in large cities. There is currently a lot of research being done on the use of symbiotic systems associating plant species with mycorrhizal fungi. Mycorrhizae give host plants the ability to develop in mineral-poor soils. As mycorrhizae are not well known in our environments and few studies have been carried out on their biodiversity, macroscopic and microscopic counts and characterizations, this study seeks to identify and characterize these mycorrhizal strains in symbiosis with the plantains of the Simi-simi experimental site at the University of Kisangani and then inoculate them with vitro plants from the plantains of two cultivars from in vitro culture. The root staining technique after four months of cultivation detected mycorrhizae in the roots of these two plantain cultivars. The results of this study revealed that the overall degree of mycorrhization is 80% on the total observed roots and phenotypic characterization has grouped the spores into four genera which are: *Glomus, Acaulospora, Scutellospora, Gigaspora* and a group of uncharacterized spores.

Keywords: Banana tree, mycorrhizal fungus, spores and Vitro plants.

INTRODUCTION

The production of bananas and plantains has been a long-standing agricultural activity. As a result, it is now being practiced on a large scale as a result of strong market demand, as a result of strong demographic pressure in cities and rising prices. In recent decades, agricultural systems in Africa in general and the Democratic Republic of Congo in particular have been subjected to enormous climate disruptions [1].

In terms of climate, this results in a significant decrease in precipitation and an extension of the dry seasons. Thus, farmers are confronted with a persistent drought characterized either by a decrease in the duration of the rainy season or by the intervention of more or less long dry periods during a rainy season [2].

The agricultural consequences are lower crop yields and soil fertility. This decrease in fertility is explained, in part, by a low return of organic matter of animal or plant origin to the soil and by the harmful effects of insufficient rainfall on the microflora and microfauna of the rhizosphere.

To remedy this situation, research is being carried out in the use of symbiotic systems associating plant species with mycorrhizal fungi. Mycorrhizal fungi belonging to the class Zygomycetes and the order Glomales are soil microorganisms found in most plant taxa and in 67% of plant families [2]. Their importance lies in the fact that they give host plants the ability to develop in mineral-poor soils.

Mycorrhizal fungi with vesicles and arbuscles are microorganisms that naturally form a symbiotic

association with the roots of many plants [3]. This symbiosis is reflected in the appearance of mixed mycelium-root organs called mycorrhizae [4] whose main role is to collect and transport very little mobile nutrients from the soil, mainly phosphorus [5], to the plant to gain a little carbon. Many studies have shown that mycorrhizal associations can play a significant role in:

The decomposition and mineralization of plant organic matter and the mobilization of nutrients for the benefit of the host plant[6], which results in often significant biomass gains[7] as well as the plant-plant transfer of nitrogen from symbiotic fixation[8]; improvement of plant water nutrition via mycorrhizal hyphae[9].

- The selective pressure exerted on saprophytic soil microorganisms both in terms of their genetic and functional diversity to form a trophic complex combining the symbiote, the mycorrhizospheric microflora and the plant [4];
- Plant protection by acting directly on stress factors (pathogens and herbivores in particular) or by stimulating plant defences [10].

These protective symbioses determine the ecological success of plants; they modify plant communities and food webs [11]. It has recently been shown that exudates from the roots of mycorrhized banana trees conferred resistance to Radopholus similis attacks on the plant [12]. It should also be noted that fungi produce storage vesicles in roots and asexual spores are differentiated in the soil and sometimes in roots; they serve as organs of propagation and survival [4].

It is interesting to note in this regard that the presence of mycorrhizae spores in a given soil is an indicator of the potential fertility as well as the health of that soil. Let us now look at another indicator of our tool, "productivity", which is crucial for analysing the sustainability of plantain-based cropping systems.

Mycorrhizae remain little known in our environments and few studies have been done on their biodiversity, their counts, their macroscopic and microscopic characterizations, the performance of their strains for the crops under consideration and their mass production. We will try to generate better knowledge on the detection and enumeration of mycorrhizal strains inoculated to vitro plants of two cultivars of the most consumed plantains in the city of Kisangani, those of Libanga Likale and Tala Lola.

MATERIAL AND METHODS

The soil is collected at a depth of 25 cm and at points between 10 cm and the stem of the plantains. After analysis, soil containing more than 120 spores per 100g of sample is retained for inoculum, the seedlings have been weaned and placed in ween streps containing sterile soils and inoculated. These seedlings had an average size of 12 cm. Our inoculum was made up of mixtures of soil and roots taken from the feet of plantain banana trees.

200 ml pots are filled with a sterile substrate composed of a mixture of unfertile soil 75% of relatively poor soil with 25% sand (by volume) on which a seedling is placed to promote the development of secondary roots. 100g of soil containing the mycorrhizal spores are deposited directly on the roots of the seedling which are then covered with substrate. The pots containing the inocula and controls are placed in the screen house for regular watering twice a day (morning and evening).

For the study of mycorrhizae, the fine roots of plantains are collected for trypan blue staining after 4 months of cultivation. Soil and root samples collected are transported in sterile bags and labelled and then sent to the laboratory for working on fresh soil or to be stored in the refrigerator (4°C). Drying the samples kills part of the microflora and makes it impossible to determine the microbial biomass. Water stress can also disrupt biological measurements [13].

Mycorrhizae detection is performed using the root staining technique described in [14]. The roots are washed with water. The finer ones are cut into fragments of about 1 cm and heated in the water bath for 30 minutes with a 10% potash solution (KOH).

After rinsing with distilled water, the roots that have become clear are acidified with 1% HCl for 10 minutes. They are rinsed again with distilled water, then coloured with trypan blue solution and finally rinsed again with distilled water. Trypan blue colors the structures of mycorrhizae that have colonized the roots and non-mycorrhized roots are not colored.

Root fragments are placed between slides and lamellae, in the presence of glycerol for microscopic observations.

For each sample, 10 fragments are mounted between slides and lamellae for 40X objective observations and the mycorrhization rate is calculated based on these ten observed fragments.

Spore observation

The technique consists in taking the soil from a depth of 15 to 20 cm at the base of the plant. In the laboratory, the principle consists of weighing 100g of soil in a beaker, adding tap water and shaking vigorously then letting it rest for 20 seconds, filtering through a 1 mm sieve and discharging the waste. Shake the mixture again, let it stand for 20 minutes and filter

through a 45µm sieve. Collect the debris with the water jet in a 100 ml beaker and return it to a tube. Centrifuge the tubes for 2 minutes at 2000 rpm (4°C). The supernatant is rejected and the tubes are filled with the sugar solution (30%) and then centrifuged again for 20 seconds at 2000 rpm. The supernatant is filtered through a 45 µm sieve and with a water jet, collect the debris in a Petri dish for microscopic observation [15]. Smaller spores are lost, but they are often very few in number [16].

Morphological description of spores

For this study, the morphological description allowed us to classify the mycorrhizae according to the following characteristics:

- Spore arrangement: isolated or sporocarped in the soil;
- The shape of the spore: ovoid, elongated or without a specific shape;
- The size of the spore: large or small;
- The colour of the spore: reddish, yellowish, blackish or purple;
- The sporal wall: spores can also have one or more sporal layers that vary in thickness, structure and appearance.
- The content of the spore: the spores contain lipids and other compounds
- The germination of the spore: this mechanism can be used to distinguish spores.
- The determination of spore families and genera was made using an identification key that classifies them according to spore germination [17].
- The particularity of this study is to keep the spores for 24 hours in physiological water to allow the spores to start germinating, which allowed us to group them according to the development of their hyphae.

RESULTS

Root infection with mycorrhizal fungi

Mycorrhizal fungi have colonized the young roots of plantains. The fungal partner's penetration into the root of the host plant always begins with the formation of an appressorium (a meeting place between the root and the fungus). It continues with the penetration of the hyphae into the root and may sometimes have H-shaped connections that penetrate from one cell to another but also pass through the intercellular meats. These hyphae often form dichotomously branched endocellular endocular arbuscles or intra-racinary spores in the innermost cortical cells. The fungus mycelium also colonizes the rhizosphere of the host plant to produce extra-root spores. Intra- and extracellular vesicles (Figure 1.) in the infill position on the hyphae are observed in the superficial cortex layers of infected roots.

The overall mycorrhization intensity observed is 80%.

The degree of colonization of plantain roots by mycorrhizal fungi is shown in Figure 1 below.

Fig-1: A root colonized by mycorrhizal fungi and having vesicles

It should be noted that during our research, among the samples collected, some roots were not mycorrhized [18] found the calculated overall mycorrhization intensity of 28.8% with 29% of its non-mycorrhized samples, which is lower than the result of this study.

The genera of the identified mycorrhizal spores. This study made it possible to distribute mycorrhizal spores in 5 groups divided into the following genera: Glomus, Acaulospora, Scutellospora, Gigaspora and a group of unidentified spores. These spores are presented in Figure 2 below:

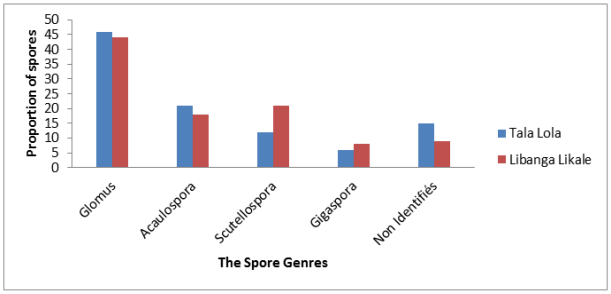

Fig-2: Above shows the proportions of the genera of the spores found in 100g of soil samples for both cultivars

It can be seen from Figure 2 that for both cultivars the genus Glomus dominates all other genera, this is justified by the fact that this genus is the most abundant in several crops and the most represented genus in different parts of the world.

Genus Glomus

It is the most abundant type of spore that has a great diversification and a wide distribution in many habitats in nature. Their colours range from yellow to

dark red. They have the attachment hyphae which are straight, curved or bifurcated, a specific characteristic of this group of spores. Spores are often gathered around a sterile hyphal plexus to form uncompacted clusters or sporocarps. Spore germination begins after 24 hours in physiological water with the emergence of new hyphae through the light of the suspensory hyphae.

The Acaulospora Genus

They are sessile as soon as they are formed, hence the name Acaulospora (Acaulo: tailless, and spora: spore). Very rare, this type of spores is individually formed in the soil and is large in size with a globular or sub-globular shape. The spores have a high internal consistency showing a thick wall.

They do not have attachment hyphae. The spores are spherical to ovoid, hyaline to yellow. They have a shiny appearance due to the presence of hyaline globular bodies and are laterally connected to sporiferous saccles in the terminal position.

The sporal wall has two layers: a hyaline and thin inner layer and a pale yellow laminated layer.

The Scutellospora Genus

This genus, distinguished from that of Gigaspora by [19], has been found in small numbers. The spores are solitary, creamy white to pale yellow. They have two walls: an outer sporal wall with two joined layers and a hyaline inner wall with two membrane layers. The outer parietal layer is decorated with warts. The laminated layer of the outer wall is brownish. On spores from direct extraction, the flexible layers of the sporal wall are strongly attached and therefore difficult to distinguish. The ovoid and hyaline germination shield shows serrated margins and swollen convolutions at the ends. The bulbous suspensory hyphae are large. The spores are individually formed (no sporocarps) with a globular shape, pale yellow to brownish yellow in colour and large in size, with a very high content of lipid reserves in the form of droplets. The attachment hyphae is enlarged at the exit of the spore and then tapered.

The Gigaspora Genus

The spores are solitary, spherical to ovoid in shape, yellow to light orange in colour. Only one spore forms in the terminal position on the suspensory bulb. The outer layer varies from yellowish to brownish in colour. It is characterized by spores that have non-flexible inner walls. The germination tubes come from a warty layer in contact with the cytoplasm of the spore. This genus has been encountered in our samples and is poorly represented. –

Unidentified Genus

These have various shapes and do not show any signs of germination as shown in Figure 3 below.

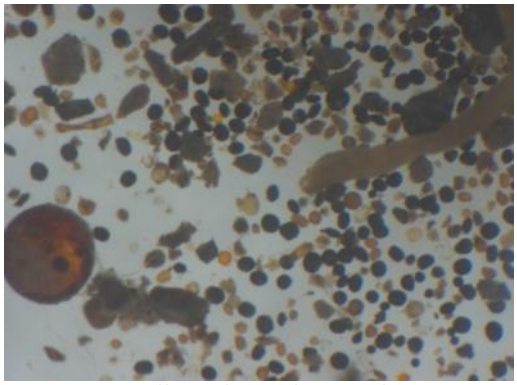

Fig-3: The different forms of unidentified spores.

DISCUSSION

The results observed by [18] revealed three genera and an unidentified group in the different cultivation systems of Kisangani plantains. These are the genera of Glomus, Gigaspora, Acaulospora and an unidentified genus [20] also showed that the genus Glomus alone gave 77.5%, the genus Gigaspora intervened with 20.4% and the two unidentified groups NI1 with 1.02% and NI2 with 1.04% [21]. As for them, they argue that banana cultivars are highly dependent on the rate of mycorrhizal colonization.

CONCLUSION

The exploration of the mycorrhizal biodiversity of the Kisangani region through the morphological characteristics of their spores has led to the identification of four genera of the region's native strains. The spore samples collected are morphologically comparable to the genera described in the literature. However, some morphological differences are observed in the same genera in terms of shape, size and colour.

The recognition and interpretation of subcellular structures of spores is not always easy because of the polyphyletic nature of this genus.

The description of spores from soil samples collected from plantain banana plants was made possible through wet sieve extraction by sucrose.

It is important to know the characteristics of the spores in our environments and to establish an appropriate and reliable diagnosis, then carry out a comparative study between the spores collected in the field and the spores obtained after trapping on host plants.

The morphological description compared to the original description of the listed species did not allow us to classify some spores. Hence, a thorough molecular study is required.

Based on the results obtained, it seems interesting to identify mycorrhizal fungi in order to involve them in various fields, namely agriculture through the bio-fertilization of soils that are poor in nutrients. These mycorrhizal associations are involved in the protection of crops against pathogens such as nematodes.

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