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Why is there a tripartite symbiosis in banana crops?

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ABSTRACT

Dark septate endophytes (DSE) and arbuscular mycorrhizal fungi (AMF) can simultaneously colonize the roots of different plants; their function and association can range from symbiotic to parasitic. The type of interaction is believed to be dependent on environmental and soil nutritional conditions. We quantified banana root colonization by arbuscular mycorrhizal fungi and by dark septate endophyte, to evaluate the relationship between these and certain edaphic factors. Since banana is cultivated under high P it is unlikely the fungal symbionts help with P nutrition. Organic carbon had a positive and K+ content a negative relation with colonization by DSE and AMF, while the clay content had a negative correlation with the DSE/AMF relationship with values between 0.4 and 0.7 in banana crops in Colombia. Under these circumstances, organic carbon and K+ are nutritional influencers on the colonization by DSE and AMF but the ratio DSE/AMF colonisation did not indicate a significant influence of soil nutritional factors. Both the ecological function and benefit to the plant of both organisms in this three way symbiosis remain unknown.

1. Introduction

At the edaphic level, root colonization by arbuscular mycorrhizal fungi (AMF) is reported in this crop, with a positive effect shown on absorption of P (Usuga et al., 2008a, 2008b) and nutrients such as Ca, K, Zn and Cu (Oliveira et al., 2003). Arbuscular mycorrhiza represent a mutualistic association, formed between different groups of fungi of the Phylum *Glomeromycota* and the higher plants, that is present among 80–90% of the species on the Earth's surface (Brundrett et al., 1984; Elsen et al., 2003a, 2003b; Perez et al., 2011; Posada, 2001). In this relationship, the host plant receives various benefits, e.g., greater efficiency in the absorption of nutrients such as P (Smith and Read, 2008), resistance to conditions of hydric stress (Augé, 2001) and protection of the root system against pathogens (Elsen et al., 2003a, 2003b; Vaast et al., 1997). In the same sense, the dark septate endophyte (DSE) are a group of endophytic fungi, mutualistic or otherwise, that present dark pigmentation and septate hyphae and colonize a wide range of plants from the roots to the leaves (Jaison et al., 2012; Jumpponen and Trappe, 1998). While few studies have been conducted, it has been observed that they produce an increase in acquisition of P and N (Jumpponen and Trappe, 1998; Newsham, 2011) for the plant, and increased root and shoot biomass (Newsham, 1999) as well as having a possible function as biocontrolers (Narisawa et al., 2002; Tellenbach and Sieber, 2012). In *Musa paradisiaca* the root colonization by DSE has been reported without reference of the associative effects on this plant species (Jaison et al., 2012).

A widespread tripartite (AMF-DSE-Plant) relationship con means a co-evolutionary mechanism, a strategy that can helps to improve plant surviving or confers benefits compared to plants without these relationship. Simultaneous colonization by both groups of fungi (AMF and DSE) has been reported in different plant species (Fuchs and Haselwandter, 2004; Jaison et al., 2012; Mathew and Malathy, 2008) that often coexist in the same space and fulfill complimentary functions; in *M. paradisiaca* was reported by (Jaison et al., 2012) for India. Arbuscular mycorrhizal fungi (AMF) positively affect the growth, nutrition, competitive capacity and productivity of plants (Andrade-Linares et al., 2011; Melloni et al., 2000; van derHeijden et al., 1998), while DSE may appear as a possible alternative for the improvement of the development of harvests, nutrition from other mineral sources and pest control (Alberton et al., 2009; Jumpponen and Trappe, 1998; Narisawa et al., 2002; Newsham, 1999), for which reason understanding this tripartite relationship could be important to improving the growth and productivity of this crop, reducing the use of fertilizers and contributing to conserve the soil microbiota.

The objective of the present study is to evaluate, in different banana (*Musa paradisiaca* L.) production zones with high levels of fertilization in Colombia, 1) the variations in the relationship between root colonization by AMF and DSE, where we expect a relatively stable value (DSE/AMF) for this plant specie and simultaneously 2) to explore the association between edaphic parameters (pH, cation exchange capacity,

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concentration of calcium, magnesium, potassium, sodium, phosphorous, exchangeable aluminum, base saturation, organic carbon and texture) the levels of these two root colonizations, and the values of relation (DSE/AMF) where a direct relationship is expected with some of the edaphic parameters, as has been reported for AMF in different crops (Minggui et al., 2012), but still has not been reported for DSE nor for the DSE/AMF relation.

2. Materials and methods

2.1. Study and sampling zone

This study was conducted in three Colombian banana production zones: zone 1 (Z1), in the state of Cundinamarca, presents low productivity polycultures, soils of type inceptisol and texture sandy-loam and an average temperature of 22 °C; zone 2 (Z2), in the state of Magdalena, presents extensive monocultures, soils of type inceptisol and texture clay with an average temperature of 27°; and zone 3 (Z3), in the state of Antioquia, presents extensive monocultures, soils of type entisol and texture sandy-clay with an average temperature of 28 °C.

All samples were taken between the 21st of July and the 8th of August 2013, corresponding to the dry season (Z1 = 131.56 mm/month, Z2 = 193.02 mm/month, Z3 = 84.86 mm/month). In each of the zones, four plantations were selected, separated by at least 20 km. In each plantation, six plants were randomly chosen, separated by at least 100 m, at the fruit filling stage, with no symptoms of nutritional deficiencies or mechanical damage. Four subsamples of approximately one kilo of soil were taken in a cross pattern at 5–20 cm around the stem of the banana plant in quadrants of 20 × 20 cm where the tertiary and quaternary roots were carefully exposed and mixed to form a compound sample. Banana roots were collected along with the associated soil and used to evaluate colonization. All samples were stowed in labeled bags and refrigerated until subsequent processing.

2.2. Analysis of soil samples

The samples were processed in the laboratory of the Corporación Universitaria Minuto de Dios (Bogotá, Colombia) in order to obtain: 1) one kg subsample for physicochemical analysis 2) one 15 g subsample for determination of moisture content and 3) one subsample of roots to determine colonization of dark septate endophytic and AMF. All subsamples were refrigerated at 2–4 °C until analysis, while the remaining material was stored in the shade at ambient temperature.

Moisture content was determined with 15 g of soil through weight difference after oven drying at 85 °C for 72 h. The other physicochemical analyses were conducted by the soils laboratory of the Instituto Geográfico Agustín Codazzi (IGAC), where the following methods were conducted:

Cationic exchange capacity (CEC) and interchangeable basis Calcium (Ca), Magnesium (Mg), Potassium (K) and Sodium (Na) were determined by saturation with ammonium acetate (CH_3COONH_4) 1 N pH 7, and quantification by volumetry and atomic absorption-emission (Rhoades, 1982); pH by the potentiometric method, in distilled water 1:1 (Bates, 1954; Willard et al., 1974).

Total phosphorous (TP) by fusion in a mixture of potassium nitrate (KNO_3)/Sodium Nitrate ($NaNO_3$) and colorimetric quantification by Bray II; available phosphorous (AP) in citric acid at 1% and colorimetry with ammonium molybdate (Bray II). Bray II method follow to Bray and Kurtz (1945).

Organic carbon (OC), by wet oxidation of soil with Potassium dichromate in acid condition, and combustion in an elemental analyzer (Walkley and Black, 1934); texture was analyzed by soil fractioning to sand, silt, and clay portions (Bouyoucos, 1951).

2.3. Colonization

All of the roots were washed thoroughly with water in order to remove the adhering soil and the tertiary and quaternary roots were then selected. A minimum 20 roots per plant for a total of 120 per plantation were selected in order to determine the mycorrhizal colonization in a clearing process, covering completely the roots with KOH 10% and boiled by 45 min or until roots become transparent was performed; cleared roots were carefully washed with water, and dyed using black ink (Sheaffer) at 5% in vinegar at 4% (Vierheilig et al., 1998) in test tubes, and heated at 90 °C by 5 min in boiling chamber. Stained roots were carefully washed with water and immersed in 4% vinegar by 20 min, and placed in water for manipulation (Sánchez de Prager et al., 2010; Vierheilig et al., 1998). The described method served for the dyeing both dark septate endophytic (Alberton et al., 2009; Vohník and Albrechtová, 2011) and arbuscular mycorrizal fungi (Vierheilig et al., 1998). Five dyed roots were placed perpendicular to the main axis on microscope slides. These were then covered with Polyvinyl Glycerol (PVGL) for preservation, and the work conducted in four replicates per root. Quantification of colonization was conducted with the intercept method described by McGonigle et al. (1990). The diagnostic characteristic for the mycorrhiza was the presence of a coenocytic hyphae, dyed blue, with irregular internal edge and the presence of vesicles and/or arbuscules (Nicholson, 1959). For the dark septate endophytic, the presence of septate hyphae of a dark color and/or the presence of microsclerotia within the roots was determined (Peterson et al., 2004; Vohník and Albrechtová, 2011).

2.4. Statistical analyses

To determine the presence of trends in the distribution of the plantations according to the edaphic parameters, principal component analyses (PCA) were conducted with standardized data, using the program MVSP Version 3.11 h (Kovach Computing Services, 1985–2000). One-way ANOVAs were conducted in order to evaluate differences among plantations in terms of the physicochemical edaphic variables (cationic exchange capacity (CEC), pH, calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) content, total phosphorous (TP), available phosphorous (AP), organic carbon (OC) and texture) and the microbiological variables (AMF, DSE and DSE/AMF relationship). Variables that did not present normality were evaluated using the Kruskal-Wallis test and those that presented significantly different means were discriminated using Dunns or Tukey tests. These analyses were conducted with the program InfoStat 2014 (Di Rienzo et al., 2014).

To explore the relationship between the physicochemical and microbiological variables, Spearman correlation analysis of ranges were conducted, in which significant relationships were selected with $P < 0.05$ and $r > 0.5$. This analysis was conducted with the program SigmaStat 3.11 (Stat Software Inc). The ratio of colonizations (DSE/AMF) was conducted for data of colonization by DSE and AMF for each plantation using One-Way ANOVA and the means separated by plantation using the Tukey Test.

3. Results

The first axis explains 47.9% of the variability, represented mainly by loam, CEC, pH, OC, Ca, Mg and Na, the second axis explains 22.1% of the variability and is represented mainly by TP, sand and clay content and CEC. Two groups are clearly observed, corresponding to different zones; the first group to the left, corresponds to the plantations of Z1, the second group towards the lower part corresponds to the Z2 plantations and the remaining plantations belonging to Z3 do not form a cluster. This led to consider the convenience of working each zone separately (Fig. 1).

In general, colonization by AMF was greater than by DSE; the