Temporal variation in arbuscular mycorrhizal fungi colonization of *Bactris gasipaes* Kunth in Buenaventura, Colombia

Variación temporal en la colonización de hongos micorrízicos arbusculares de *Bactris gasipaes* Kunth en Buenaventura, Colombia.

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Abstract

The peach palm *Bactris gasipaes* is an important tropical agricultural crop cultivated for the fruits and heart of palm. We evaluated the levels of root colonization, soil spore count and morphotypes of arbuscular mycorrhizal fungi (AMF) associated with cultivated *B. gasipaes* in the rural areas of Citronela and Zabaletas, municipality of Buenaventura, Department of Valle del Cauca, Colombia, during three evaluation periods 2006 – 2007, to determine the influence of rainfall on the AMF colonization. The percentages of root colonization in Citronela varied between 58% and 90% while in Zabaletas root colonization varied between 63% and 79%. The average spore number in 50 g of wet soil per sample was higher in Citronela (244.6 ± 116.0 SD) compared with that in Zabaletas: 50.3 ± 24.1 SD). Twenty two morphotypes of AMF were identified from soil spores. *Glomus* was the most abundant mycorrhizal fungi genus in both localities, but *Scutelospora* was also detected. This study showed both geographic and temporal variation in mycorrhizal parameters in an important crop for the wet tropical agriculture.

Key words: Arbuscular mycorrhizal fungi (AMF), *Bactris gasipaes,* Buenaventura, *Glomus,* morphotypes

Resumen

El chontaduro *Bactris gasipaes* es un cultivo tropical importante del cual se cosechan los frutos o los palmitos. En el estudio se evaluaron los niveles de colonización de las raíces, se realizaron conteos de esporas en el suelo y la identificación de morfotipos de hongos micorrízicos arbusculares (HMA) asociados a cultivos de *B. gasipaes* en el áreas rural de Citronela y Zabaletas, municipio de Buenaventura, Departmento de Valle del Cauca, Colombia, durante tres periodos de entre 2006 y 2007, para determinar la influencia de la pluviosidad en la colonización de HMA. Los porcentajes de colonización en Citronela variaron entre 58% y 90% mientras que en Zabaletas la colonización de raíces varió entre 63% y 79%. El número promedio de esporas en 50 g de suelo húmedo fue mayor in Citronela (244.6 ± 116.0 SD) que en Zabaletas (50.3 (± 24.1 SD). Se identificaron 22 morfotipos de HMA del suelo asociado a las palmas de de *B. gasipaes. Glomus* fue el género de hongos micorrízico más abundante en ambas localidades, pero *Scutelospora* también fue detectado. Este estudio demuestra la existencia de variación geográfica y temporal en parámetros micorrízicos en un cultivo importante para la agricultura del trópico húmedo.

Palabras clave: *Bactris gasipaes*, Buenaventura, *Glomus*, hongos micorrízicos arbusculares (HMA), morfotipos

Introduction

The peach palm (*Bactris gasipaes* Kunth, Arecacea) is a widely domesticated palm in the Neotropics, and is considered to have an Amazonian origin (Clements, 1988). It is an important tropical agricultural crop cultivated for the fruits as well as heart of palm, or 'palmito'-the unexpanded leaves above the meristems. The fruits are a main component of subsistence diets in lowland humid regions, while the heart of palm has a considerable commercial value (Clements *et al.,* 1993; Clements and Mora-Urpi, 1987), particularly in Brazil, Ecuador and Costa Rica where cultivation is almost totally devoted to the extraction of palmito (Bovi et al., 1998). The fruit has a high nutritional value, and is an important component of subsistence farming in resource-poor communities in lowland humid regions. In Colombia, where *B. gasipaes* is known as 'chontaduro', the commercialization of the fruit is gaining traction in markets beyond the cultivation regions, such as in the big cities in the Andean interior.

The natural environmental conditions of peach palm crop are high precipitation environments with high temperatures, and acidic soils of tropical wet forests that are poor in nutrients such as nitrogen and phosphorus (Bowen, 1980; Sieverding, 1991; Gomez-Carabali *et al.*, 2011). The crop is frequent in poor wet and acid Oxisols and Ultisols (Clements y Habte, 1995). Little is known about arbuscular mycorrhizal fungi (AMF) interactions in tropical palms. The AMF are known to enhance growth in other palms including the African oil palm (*Elaeis guineensis* Jacq) (Blal *et al.*, 1990, Morel and Gianinazzi, 1990). Palms are thought to be dependent on mycorrhizal fungi having fungal infection throughout the year, and even during stress periods (Nuñez-Castillo and Alvarez –Sánchez, 2003). For example, *Desmoncus orthacanthos* Mart., a palm from Yucatan Peninsula, Mexico, was colonized all year including the dry season (Ramos-Zapata *et al.*, 2006a, b). The southeastern US palm *Serenoa repens* (Bartr.) Small had 130-1100 spores/50 g from the genus *Glomus* and *Gigaspora* (Fisher and Jayachandran, 1999). Similarly, *Phoenix dactylifera* L. in Morocco were highly colonized (72%) and 238 to 1840 spores per 10 g. of soil from *Glomus*, *Acaulospora* and *Scutellospora* (Bouamri *et al.*, 2006).

Bactris gasipaes has no root hairs indicating that AMF are important for nutrition, and the palm is highly dependent on AMF under cultivation (Janos 1977, Clements and Habte, 1995). AMF are known to promote a significant increase of productivity in the poor wet and acid Oxisols and Ultisols where Peach palm grows (Clements and Habte, 1995), mainly due to the transfer of phosphorus to plants in the tropics (Janos, 1984; Enríquez and Bernal, 2009). Several *Glomus* species have been reported associated with *B. gasipaes* in Peru (Ruiz, 1992) but little is known about AMF communities associated to peach palm in Colombia.

The pacific region of Colombia comprises one of the wettest ecosystems in the world. It is a region recognized for its extremely high biodiversity (Myers *et al.*, 2000).

The agro-forestry system associated with peach palm production in the pacific cost is high in biodiversity (eg. Otero and Sandino, 2003), and these agricultural systems or agroecosystems (Sieverding, 1991) are an important habitat of soil microorganisms (Torsvik *et al.*, 1990). The aim of this study was to describe the diversity of AMF associated with the root system in two *B. gasipaes* agroforestry systems in the Pacific Coastal region of Colombia, and to evaluate mycorrhizal activity changes over seasonal variation in precipitation.

Material and methods

Study site

This study was performed at sites in the districts of Citronela and Zabaletas in the rural area of Buenaventura Municipality (Valle del Cauca department) in the Pacific Coastal region of Colombia. This region is a tropical rain forest life zone, with precipitation between 6000 and 7000 mm (Eslava, 1994). The two sites were located 15 km apart and have similar agro-ecological conditions: less than 20 m in elevation, 26º C of average temperature, 3 h/day of solar bright and 87% average relative humidity (Eslava, 1994). The average annual rainfall is 6408 mm with two

peaks of higher precipitation in April and October and short drier periods in January and July, soils are Inceptisols correspondent to Fluvaquentic Epiaquepts in Citronela and Fluvaquentic Dystrudepts in Zabaletas.

Sampling procedure

During 2006 and 2007 three samplings were performed, two in the wet period (October 2006 and April 2007) and one in the drier period (January 2007). Sampling sites were selected randomly from the crop land. Roots were sampled randomly from peach palm plantations from at least 10 different trees. Tertiary roots and the surrounding rhizosphere soil were collected because these absorb nutrients and water (Trujillo, 1981). Five samples were collected per site composed by three subsamples of 500 g. Samples were processed at Pacific University laboratory and microbiology laboratory at National University of Colombia, Palmira Campus. When samples were not immediately processed they were preserved in sterile plastic bags in the fridge at 10 °C for no longer than 3 days before processing (García *et al.*, 2003).

Percentage of colonization

Five young roots per plant were stained to measure the percentage of colonization using a modified methodology of Sieverding (1991). The roots were washed with tap water and transferred to a falcon tube. A solution of 10% KOH was added and incubated at 90 °C for 3 minutes. The KOH was decanted and roots were rinsed in abundant water. A 10% solution of HCl was added and incubated at 90 °C for 1 min. The HCl was decanted and roots were rinsed in abundant water. Roots were stained with 0.1% Tripan blue and incubated at 90 °C for 2 min. and then rinsed in abundant water. Stained roots were stored in 50% glycerin. For observation, the roots were transferred to petri dishes and 1 cm sections were dissected transversally and mounted in microscope slides and observed in a light microscope NiKon©. The percentage colonization was estimated in ten 1 cm segments per root measuring colonized and non-colonized regions.

Spore counting and identification

To isolate the spores from the soil we follow the method proposed by Sieverding (1991). A sample of 50 g of moist soil was diluted in one liter of water. The soil solution was passed through a series of 120, 230, 325 µm sieves. The content of the 325 mesh was collected in Falcon tubes and a 50% sucrose solution was added. The samples were centrifuged for 3 min at 2800 rpm. The solid pellets were recovered and rinsed in water to eliminate the excess sucrose. All spores in the pellet were counted in a petri dish under a Leica dissection microscope. Spores were mounted in microscope slides for identification. Mycorrhizal spores were identified to morphotypes level by comparing the samples with the available literature (Sieverding, 1984; Peña *et al.*, 2006).

Statistical analysis

To evaluate the difference in mycorrhizal colonization and number of spores in 50 g of wet soil among sampling periods and study sites we used a split plot statistical design. Data were analyzed using two way ANOVA in the software SAS (SAS, 2006). A Duncan test was applied to detect differences among treatments. To determine the effect of precipitation on the mycorrhizal activity a correlation analysis was performed. Percentage data were normalized by calculating the square root. Precipitation data was obtained from the nearest climatic station to each farm.

Results and discussion

Mycorrhizal colonization

The peach palm root AMF colonization had the common mycorrhizal structures such as intra radical mycelium, vesicles, and arbuscules. The most common structures observed were internal root mycelium and vesicles, but arbuscles were rarely observed. The percentage of mycorrhizal colonization varied among sampling periods 58.3 - 90.7% at Citronela and 63.3 - 79.3% at Zabaletas (Figure 1). There were significant differences in mycorrhizal infection among sampling periods ($F_{1.16}$ = 10.28, P: 0.005; Figure 1), but not among sampling sites ($F_{1.16}$ = 1.075, P: 0.315). The

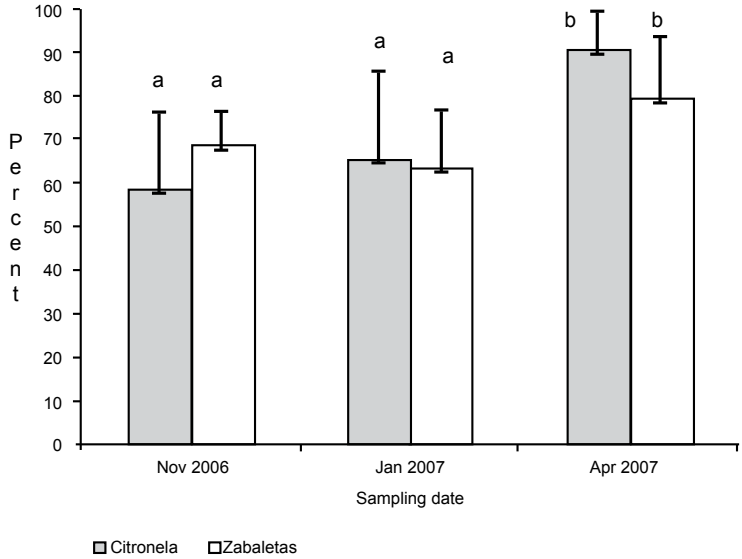

Figure 1. Percentage of arbuscular mycorrhizal fungi (AMF) colonization in peach palm (*Bactris gasipaes*) roots at two localities. Municipality of Buenaventura, Department of Valle del Cauca, Colombia.

highest average colonization of AMF was in April 2007 for both localities, corresponding to the period with highest precipitation at both sites.

Spores counts and identification

Significant differences were detected in the number of spores in the soil among sampling sites ($F_{1.16}$ = 45.027, P = 5.01E-06; Figure 2) and periods ($F_{1.16}$ = 27.198, P = 8.50E-05). The smallest number of spores present was recorded in April 2007 for Zabaletas and November 2006 for Citronela. A higher number of spores was detected in Citronela than in Zabaletas throughout the study. The number of spores over the different sampling periods

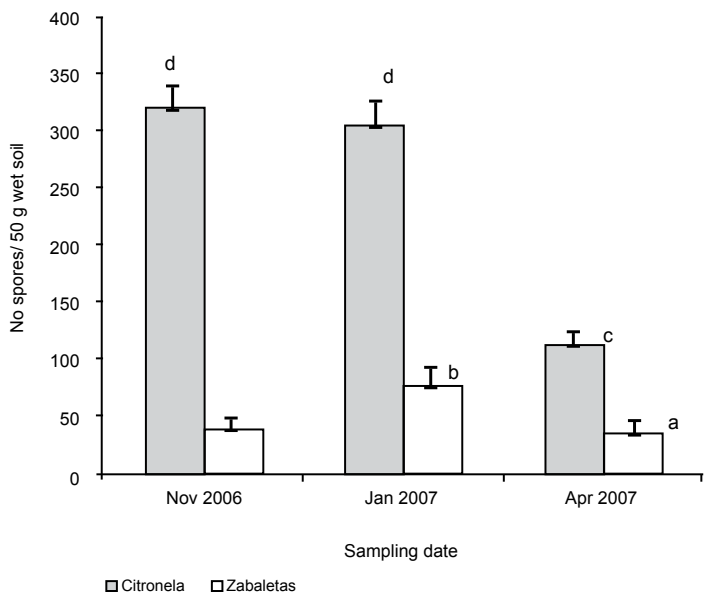

Figure 2. Average number of arbuscular mycorrhizal fungi (AMF) spores in peach palm (*Bactris gasipaes*) roots at both study sites. Municipality of Buenaventura, Department of Valle del Cauca, Colombia.

in 50 g of wet soil was 244.6 (±116.0 SD), in Citronela (Figure 2) and 50.3 (±24.1 SD) spores in 50 g of wet soil in Zabaletas (Figure 2). There were significant differences in the number of spores per sampling site ($F_{1.16}$ = 10.860, P= 0.004). Citronela had the higher number of spores during November 2006 but at Zabaletas there were no significantly differences among sampling periods (Figure 2).

Spores identity

We found 22 morphotypes of AMF, 14 corresponding to *Glomus* and 8 to *Acaulospora* (Figure 3). 19 morphotypes were shared between the two sampling sites; two *Glomus* morphotypes were exclusive to Citronela and one to Zabaletas. No significant influence of rainfall on mycorrhizal percentage or spore

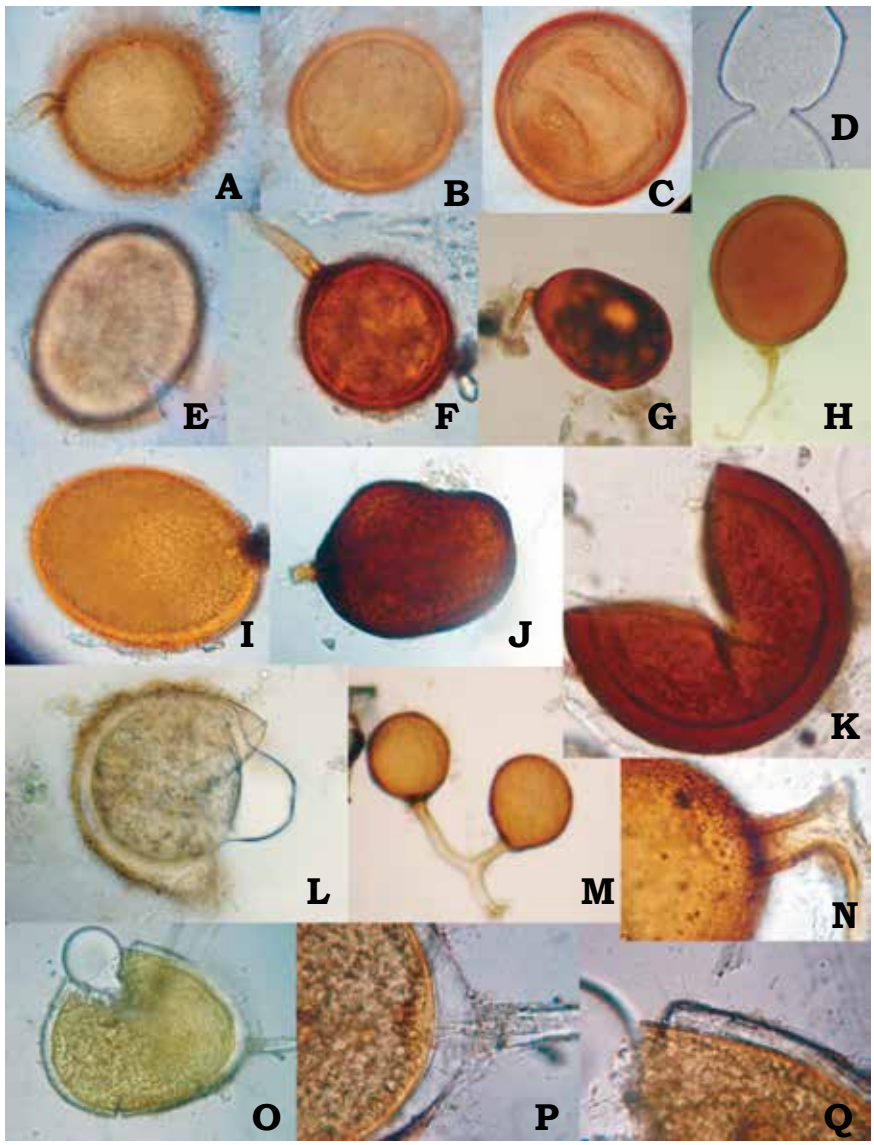

Figure 3. Structure of AMF spores observed on peach palm in Buenaventura, Colombia. A, B y C = globose spores with different, external and internal wall 40X; D = globose hyaline spore broken with lipid excretion 40X; E = sub-globose spore with external and internal wall; F = globose spore with differentiation of hyphal connection; internal, media and external wall, 100X; G y H = sub-globose spore with differentiation of hyphal connection, internal and external wall, 40X; I = sub-globose spore with differentiation of external and internal laminated wall 100X; J y K = globose spore with differentiation of external and internal wall 40X; L = broken hyaline globose spore with external, media and internal wall, and lipid excretion 40X; M) hyphal bifurcation 40X; N = hyphal connection 100X; O= broken hyaline globose spore with lipid excretion 40X; P = hyphal connection 100X; Q = external, media and intern wall, 100X.

numbers was detected. The square root of the mycorrhizal percentage followed a linear relationship with rainfall following the function *M(%) = 0.097 Rain + 13.036* (R^2 = 0.28, N = 6; P = 0.30) where *M%* is the square root of the average mycorrhizal percentage and *Rain* is the local rainfall in mm for the sampling month. The number of spores per 50 g of soil followed a linear relationship with rainfall following the function *NS = 12.06 Rain + 376.7* (R^2 = 0.38, N =6; P = 0.19) where *NS* is the average number of spores per 50 g of soil.

In this study we identified *Glomus* and *Acaulospora* as the main mycorrhizal fungi of peach palm, the same fungal genera reported previously for the Pacific Coast of Colombia (Ballesteros *et al.*, 2004) and Ecuador (Cedeño and Isaacc, 2010). In Tumaco, a southern site in the Colombian Pacific Coast there is a report of 20% mycorrhizal infection on peach palm (Ballesteros *et al.*, 2004). Studies of AMF associated with other domesticated palm species also show association with *Glomus* and other fungal species. In the southeastern US palm *Serenoa repens* was mainly associated with *Glomus* and *Gigaspora* (Fisher and Jayachandran, 1999). Similarly, *Phoenys dactylifera* were associated with *Glomus*, *Acaulospora* and *Scutellospora* (Bouamri *et al.*, 2006).

In this study we found temporal variation in Arbuscular mycorrhizal root infection and site and temporal variation in spore numbers in peach palm (Figures 1, 2). This variation in mycorrhizal colonization appears to be related with the rain. We also found a strong site effect on the number of mycorrhizal spores. The two sites differed in phosphorus (P) amount, Zabaletas had 26.55 $mg.kg^{-1}$ and Citronela had 6.54 $mg.kg^{-1}$ (unpublished data). The site with lower phosphorus availability (Zabaletas) had more spores per soil unit than the site richer en this element (Citronela); nevertheless, the P concentration was not related with mycorrhizal colonization. Palms are thought to be dependent on AMF for phosphorus uptake having fungal infection through the year, and even during stress periods (Nuñez-Castillo and Alvarez-Sánchez, 2003). *Desmoncus orthacanthus,* a palm from Yucatan Peninsula, Mexico, was found to be colonized all year including the dry season (Ramos-Zapata *et al.*, 2006a).

The information on AMF associated to palms is limited. The southeastern US palm *S. repens* were mainly associated with *Glomus*, and *Gigaspora* (Fisher and Jayachandran, 1999). Similarly, *P. dactylifera* was associated with *Glomus*, *Acaulospora* and *Scutellospora* (Bouamri *et al.*, 2006). We found that *B. gasipaes* varied both temporal and spatially in percentage of colonization and spore density in the soil. Similarly, in Brazil, Silva-Junior and Cardoso (2006) studied the mycorrhizal colonization in *B. gasipaes* under different cultural conditions (agro-forestry and monoculture). They found 43.95% mycorrhizal colonization in Peach palm during the dry season and 13.54% during the wet season and the spore number was 27 - 48 spores/50 cm^3 of soil. In Tsáchilas province, Ecuador, the mycorrhizal infection was up to 87% (Enríquez *et al.*, 2008). The nutrition with nitrogen increased the percentage of mycorrhization up to 70% in Brazil (Bovi *et al.*, 1998).

Here we reported a higher percentage of colonization and spore numbers than previous studies (Ballesteros *et al.*, 2004, Cedeño and Isaacc, 2010), suggesting that mycorrhizal interactions in the studied sites are microbiologically healthy. Nevertheless, higher values of spore count have been reported for other palms. The elevated colonization percentage and the high number of spores as well as the low nutrient values recorded in the soil analysis confirms the high dependence of *B. gasipaes* for AMF to provide nutrition requirements. In the US native palm *Serenoa repens* the number of spores in 50 g of soil varied between 130 - 1100 (Fisher and Jayachandran, 1999). Similarly, *Phoenix dactylifera* in Morocco was highly colonized (72%) and had 238 to 1840 spores per 10 g of wet soil (Bouamri *et al.*, 2006). The recognition of the importance of peach palm as local food for the native people and for their economic sustainability and the importance of mycorrhizal symbiosis for peach palm nutritional requirements justify the development of local technologies based on AMF in peach palm cultivation.

Conclusion

- The main mycorrhizal fungi associate with *Bactris gasipae* is *Glomus* sp. We found site and time variation in both percentage of mycorrhizal infection and spore numbers in peach palm agroecosystem, but this variation was not correlated with rainfall.

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