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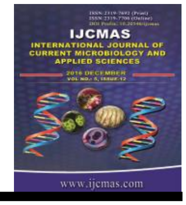

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Intercrops Influence Mycorrhizal Symbiosis Development, Growth and Nutrient Uptake of Banana

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A B S T R A C T

A greenhouse study was conducted to investigate the impact of intercrops on the mycorrhizal symbiosis development, growth and nutrient uptake of banana. Groundnut and sweet potato were raised as intercrops with banana. The banana in the intercropping system showed higher root colonization in terms of hyphae, Arbuscules and vesicules whatever the crop associated. Intercropping banana with groundnut did not affect banana growth. Contrarily, intercropping banana with sweet potato showed negative effects on banana growth. The concentration of minerals in banana shoot was affected by intercrops species. There was no interspecific competition between banana and groundnut for N and K uptake. However, P was negatively affected in banana shoot. N and P concentration was reduced in banana co-cultivated with sweet potato. These results show that intercropping can be used to improve mycorrhizal symbiosis activity in banana plantations. Moreover the choice of companion crops is the key for successful application of the system.

Introduction

Intercropping is the simultaneous cultivation of more than one crop species on the same piece of land. The system is based on the management of plant interactions to maximize growth and productivity (Willey, 1979a). Scientific investigations to evaluate this system have reported many advantages associated with intercropping such as risk minimization, effective use of available resources, efficient use of labour, food security, pest control (Ouma and Jeruto, 2010; Pelzer *et al.*, 2014 ; Nasri *et al.*, 2014). Intercropping may specifically facilitate the propagation of some beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) (Cruz *et al.*, 2000).

Arbuscular mycorrhizal fungi (AMF) belong to the Phylum Glomeromycota (Schüssler, 2001). They form symbiotic associations with more than 80 % of terrestrial plants (Fortin *et al*., 2008). Benefits of AMF to plants include promoting water and nutrient uptake especially P (Hodge *et al*., 2010). The fungi in return benefit from the supply of carbohydrates derived from photosynthesis. In addition these symbiotic fungi increase plant resistance to biotic and abiotic stresses (Gianinazzi *et al*., 2010; Vos *et al*., 2012 b). For full benefits of AM fungi to be realized, there is need to maintain a high and viable natural populations of AM fungi in the soil to increase the chances of root colonization. Hence two strategies may be adopted to increase the density of AMF in the soil: indirectly by favoring the effective indigenous AMF population through adequate cultural practices, and directly through soil or plant inoculation with selected strains of AMF. In both cases the determination of cultural practices beneficial to mycorrhizal association is of high importance (Sieverding and Leihner, 1984).

Intercropping has been shown to have the potential to maintain high and viable natural population of AMF in soils due to higher plant diversity thus optimum root colonization. Harinikumar *et al*., (1990) observed that intercropping maize and soybean stimulated the proliferation of AMF in both plant species as compared to monocropping system. Ishii *et al*., (1996) demonstrated that intercropping of *Citrus reticulate* (satsuma mandarin) and sod culture using *Paspalum notatum* Flugge (Bahiagrass) improved mycorrhizal colonization in the roots of satsuma mandarin compared to monoculture of this crop. The apparent low specificity observed in fungus – plant pairs (Smith and Read, 2008) indicated that the AMF hyphae distributed in the soil may connect several plant species and then promote a network system among plants. The interconnecting networks may act as conduits for interplant transfer of resources (Bücking and Kafle, 2015).

Banana is a major staple food for millions of people in the sub-Saharan Africa. Most of its production (80%) is conducted by small scale farmers. Primarily practiced by small holders for food security and additive income, intercropping is actually common due to increasing land pressure in most of the production regions (Lemeilleur *et al*. 2003). Also the usual spacing of 3m by 3m facilitated this system over monocropping (Ouma, 2009). Hence bananas are usually intercropped with food crops (bean, groundnut, sweet potato or vegetables), fruit trees and other cash crops (Cocoa, coffee, palm tree). Several studies have been conducted to investigate the effect of various intercrops on the performance of bananas with respect to yield, growth and pest incidences (McIntyre *et al*., 2001; De Waele *et al*., 2006). However, little attention was paid on the effect of intercropping on the mycorrhizal symbiosis of banana. Banana has been shown to be mycorrhizal-responsive. The growth and development of banana was improved in the presence of several AMF strains (Declerck *et al*., 1994). Van der Veken *et al*., (2008) demonstrated that the majority of banana intercrops were more or less compatible to AMF and hence could increase or maintain AMF rhizophere population. Groundnut (*Arachis hypogeae* L.) and sweet potato (*Ipomoea batatas* (L) Lam.) were regularly found as intercrops in most of the banana-based cropping systems in Cameroon.

The aim of the present study was to investigate the influence of intercropping banana with groundnut or sweet potato on the development of mycorrhizal symbiosis, growth and nutrient uptake of banana plantlets under greenhouse conditions. The study is based on the hypothesis that intercropping enhances mycorrhizal root colonization and subsequently growth and nutrient uptake of banana plantlets.

Materials and Methods

Biological material

Micropropagated banana plantlets *(Musa accuminata*. cv. Grande Naine) were obtained from Vitropic (Montpellier, France).

An unknown variety of sweet potato (*Ipomea batatas* (L) Lam) was obtained from the market in Belgium. Tubers were washed with tap water and surface-sterilized in 1% sodium hypochlorite and rinsed with several changes of sterile (121 C for 15 min) distilled water. They were then germinated on volcanic ash (DCM, DE Ceuster Meststoffen N.V/S.A Belgium). Plantlets of about 10 cm were obtained after 8 weeks of culture.

Groundnut seeds (*Arachis hypogeae* L.) cv A26 was provided by the National institute of agronomic research for development of Cameroun, IRAD, Njombé, Cameroun. Seeds were surface-sterilized by immersion in sodium hypochlorite (5 %) for 5 min, rinsed in sterile (121 C for 15 min) distilled water before sowing in boxes.

The AMF strain used to produce inoculum was *Rhizophagus rregulariss* MUCL 41883, supplied by GINCO (http://www. mycorrhiza.be/ginco-bel/index.php). *R. irregularis* was previously classified as Glomus intraradices N.C. Schenck & G.S.Sm. The inoculum was produced on leek plants *(Allium porrum)* for four months. Mixture of spores, hyphae and colonized leek roots (0.5 cm length) were used. The concentration was about 120 propagules per g of inoculum.

Inoculation

Banana plantlets were weaned in 125 ml pots two weeks before the inoculation with AMF. They were washed with tap water to remove residues of agar from the roots and were transferred to pots containing sterilized (2x1hour at 121°C) volcanic ash. Twelve grams of the AMF inoculum were spread as a layer between two layers of sterilized volcanic ash in each box before transferring plantlets into the box.

Experimental design

The experiment was conducted in a greenhouse (photoperiod 12 h, average temperature 24 - 28°C, and 70 - 80% relative humidity) in boxes (20 x 10 x 10 cm).

The design was completely randomized with ten replicates. Three treatments were considered: banana monoculture, banana-groundnut and banana-sweet potato combinations. The number of banana plants was 2 in monoculture. The ration of banana: intercrop was 1:1 in the intercropping treatment. Monoculture of groundnut and sweet potato were also considered.

Two weeks old weaned banana plantlets were transferred to the boxes. The plants were separated with a distance of 10 cm. Groundnut seeds were sown at the transfer time. Sweet potato plantlets were separated from the growing tubers, and their height was homogenized for about 10 cm by cutting the plantlet apex. The plants were fertilized at planting by 2g of osmocote ((NPK 10-11-18), Scott Benelux bvba/sprl, Belgium) per box and were watered when needed.

Variables assessment

All the plants were harvested 6 weeks after planting. Roots were washed with tap water before the determination of biomasses. Shoot fresh and dry weight and root fresh weight were measured for each plant. The shoot dry weight was determined after drying the leaves, pseudostem and cormus for 72 hours in an oven at 60°C. The banana height was measured from the top of the rhizome to the crossing point of the two unfolded leaves. The leaf area was calculated with the measures of the length (L) and the width (W) of the last unfolded leaf (LxWx0, 7). The diameter of pseudostem was measured at 2cm from the base of the plant using the vernier calliper. Only root fresh weight, shoot fresh weight and shoot dry weight were considered in intercrops.

Mycorrhizal root colonization was determined on an aliquot root sample of all the plant species (banana, groundnut, sweet potato). Roots were clarified using 10% KOH overnight at room temperature. They were washed several times with deionized water and soaked in alkaline 3, 5 % H_2O_2 for 30 min (Koske and Gemma, 1989). The roots were subsequently stained at room temperature for 45 min with a solution of blue ink (Parker[P] Quink®) diluted in 1 % HCl at 1:50 proportions (Vierheilig *et al*., 1998a). Root colonization was assessed by the method of McGonigle *et al*., (1990) to determine the number of arbuscules, vesicles and hyphae.

The mineral nutrients were assessed only for banana. The plant shoots were ground to < 1mm particle size and were analysed for N, P, K, Ca, Mg, Mn. Mineralization was done according to the method described by Chao and Sanzolone (1992). P, K, Ca, Mg and Mn concentrations were determined by atomic absorption spectrophotometer and N with an analyzer CN Thermo Finnigan.

Statistical analysis

Statistical analysis was performed with the statistical software Statistica. Data were analyzed by ANOVA and means were separated by HDS Tukey test ($P \leq 0.05$). Prior to analysis, data for percentage mycorrhizal root colonization was arcsin (x/100) transformed.

Results and Discussion

Mycorrhizal root colonization response in species combination

Intercropping significantly affected the root colonization of banana by *Rhizophagus intraradices* irrespective of the plant species combination. The percentage of hyphae was 75 % in intercropped banana as compared to monoculture with 58%. There was no significant difference between hyphae root colonization of banana plantlets under groundnut and sweet potato intercrops. The proportion of arbuscules was also higher in banana intercropping system 60 % and 46, 8 % respectively for groundnut and sweet potato combinations) compared to mono-system (30, 3 %) although non significative in the case of sweet potato combination (Table 1).

The plant combinations increase the proportion of vesicles in banana root, although non significative in comparison with monoculture.

Groundnut and sweet potato showed significant higher root colonization in the combination with banana as compared to that obtained in monoculture. The hyphae colonization was 52, 7 % and 46, 2 % respectively for groundnut and sweet potato in an intercropping system in comparison to monoculture (38, 7 % and 34 % respectively for groundnut and sweet potato) (Table 1). Arbuscules and vesicles showed similar trends. No significant differences were noted in root colonization of groundnut and sweet potato in respect on the hyphae, arbuscules and vesicules.

Plant growth response in species combination

The effect of cropping banana with other plant species on the plant growth parameters was variable according to the combination of crop species. Sweet potato significantly reduced the growth of banana. The relative reduction of growth was about 23%, 26% and 44% respectively for plant height, diameter of pseudostem and leaf area (Table 2). Root fresh weight, shoot fresh weight and shoot dry weight showed similar trends with a decrease of approximately 40 %, 55 % and 45 % respectively. In contrast, groundnut has shown to be more compatible in combination with banana. Although root fresh weight of banana plantlets significantly decreased in banana-groundnut combination, no significant differences were observed in shoot fresh and dry weight for both banana monoculture and banana - groundnut treatment (Table 2).

Groundnut and sweet potato were not significantly affected by crop combination. No significant difference was observed in root fresh weight, shoot fresh and dry weight when they were cropped with banana as compared to monoculture (Table 3).

Shoot nutrient content

Considering the nutrient content in banana shoots, the effect of intercropping is variable, depending on the intercrop and nutrient needs of each plant species (Table 4). Ca and Mg concentrations were not affected by crop combination, irrespective to species. The banana-groundnut pairs showed no difference in N and K concentration in banana shoot as compared to that obtained in monoculture. However the P content in the banana shoot significantly decreased in the presence of groundnut in comparison to monoculture. On the other hand, by combining sweet potato and banana, the N and K concentration in banana were significantly reduced over monoculture. However no effects were observed for P due to this combination. The Mn content in the banana shoots was significantly reduced by species combination.

In this study, we showed that growing banana with food crop species stimulated the colonization of roots by AMF. Plant root colonization was higher in the banana – intercrops - pairs than in monoculture treatment probably due to the development of the mycelium network between banana and intercrops (Mandou *et al*., 2015). Similar results were previously obtained by Harinikumar *et al*., (1990); Ishii *et al*., (1996) and Li *et al*., (2009). Lekberg *et al*., (2010) suggested that when two different species were grown together, they may provide more carbohydrates to the common formed extraradical mycelium and hence increase growth of intraradical mycelium and formation of arbuscules. Arbuscules are the preferential site of exchange between plant and AMF (Smith and Read, 2008) and can be used as an index of mycorrhizal symbiosis functioning. The arbuscule colonization was higher in banana – groundnut pairs in comparison to banana - sweet potato intercropping system. Van der Leken *et al*., (2008) demonstrated that although most of the banana intercrop species are mycotrophic, legumes have a higher AMF dependency than non leguminous crops. The root architecture of groundnut revealed the absence of root hairs, suggesting that this species is dependant to AMF for nutrient absorption in the soil (Krishna and Bagyaraj, 1984). However, increase in root colonization was not correlated with increase growth probably because of the early interspecific competition between groundnut and banana or sweet potato and banana.

In the presence of sweet potato, the growth of banana plantlets was markedly affected. This was probably due to competition for space and nutrients. A number of sweet potato roots reach the maximum 10 to 15 days after planting and root length is greater at 30 days (Hannsuk and Hozyo, 1983). At the end of the experiment root fresh weight of sweet potato was about two times that of banana. When intercropping between two species is established, early interference may involve competitive root effect (Hauggaard and Jensen, 2005) and the intercrop is able to exploit a large total soil volume if the component crops differ in rooting pattern (Hauggaard *et al*., 2001 b). In our case, the experiment was performed in boxes which limited volume for root exploration and hence facilitated root interaction competition.

The incorporation of legumes crops in an intercropping system was far considered as a productive combination particularly under conditions of limited nitrogen. This is probably because they provide nitrogen (N) to the system and the soil for succeeding crops via symbiotic N-fixation. Previous studies showed beneficial effects of legumes in an intercropping system in terms of growth, yields and nutrient uptake (Revees, 1992; Jensen, 1996 a; Inal *et al*., 2007; Rahman *et al*., 2006; Li *et al*., 2009). In our experiment, no growth increase of banana plantlets was observed in groundnut – banana combination in comparison to banana monoculture. One reason may be the lack of symbiotic N- fixation since groundnut was not inoculated with rhizobia strains.

The concentration of minerals in banana shoot was also affected by intercrops species. There was no interspecific root interaction between banana and groundnut for N and K uptake. Plants were fertilized with osmocote (N –P – K) which may provide adequate supply need for these elements as groundnut is less require. However, P was negatively affected in banana shoot. Groundnut as legume has high phosphorus requirements as it helps in developing better quality seed with high oil content (Mirvat *et al.*, 2006). Also, groundnut needs additional P for signal transduction and membrane biosynthesis (Ribet and Drevon, 1996). This suggested that there was a competitive root interaction between banana and groundnut for this element. It has also been reported that small amounts of P were required for bananas growth (Phirke and Mahorkar, 2010). However growth of banana plantlets was not affected in banana – groundnut combination.

As for growth, sweet potato has negative effect on mineral content in banana shoot. N and K were significantly reduced in banana shoot in the presence of sweet potato. These elements are very important for potato yield. N stimulates the growth of aboveground parts. K influenced the proportion of dry matter diverted to the tuber, so improved tuber number per plant (Bourke, 1985). Hannsuk and Hozyo (1983) observed the differentiation of tuberous roots 20 days after planting. In our case, six weeks after planting, many roots started to be differentiated into tubers, suggesting a great need of K for cambial activity in tuberous roots.

Table.1 Influence of intercropping on mycorrhizal root colonization of banana, groundnut and sweet potato.

Treatment	Hyphae (%)	Arbuscules (%)	Vesicles (%)
Banana			
Banana monocrop	58,33 ± 4,06b	30,33 ± 2,43b	23,55 ± 3,52a
Banana + groundnut	75,47 ± 3,49a	51,99 ± 5,74a	32,32 ± 2,94a
Banana + sweet potato	75,33 ± 3,79a	46,77 ± 5,77ab	30,10 ± 3,90a
Intercrops			
Groundnut	38,66 ±3,67b	20 ± 2,30b	13,55 ± 1,55b
Intercropping groundnut	52,66 ± 2,64a	33,66 ± 1,99a	26,66 ± 1,47a
Sweet potato	33,99 ± 5,20 b	19,33 ± 4,05b	12,88 ± 1,82b
Intercropping sweet potato	46,22 ± 1,54a	30,10 ± 2,08a	22,88 ± 2,70a

Data are means of 6 replicates. Means in the same columns followed by the same letter do not differ according to HDS Tukey test ($P \leq 0.05$)

Table.2 Growth of banana influenced by intercropping six weeks after planting (RFW: root fresh weight; SFW: shoot fresh weight; SDW: shoot dry weight)

Treatment	Height (cm)	Pseudostem diameter (cm)	Leaf area (cm^2)	RFW (g)	SFW (g)	SDW (g)
Banana monocrop	10,95 ± 0,57a	1,14 ± 0,04a	138,17 ±12,66 a	7,77 ±1,01a	24,45 ± 2,57a	1,78 ± 0,16a
Banana + groundnut	10,30 ± 0,37a	1,05 ± 0,06a	131,64 ± 9,00a	5,62 ± 0,66b	21,64 ± 2,42a	1,81 ± 0,21a
Banana + sweet potato	8,39 ± 0,27b	0,84 ± 0,03b	76,30 ± 5,24 b	4,43 ± 0,46b	11,87 ±1,37b	0,99 ± 0,12b

Data are means of 10 replicates. Means in the same columns followed by the same letter do not differ according to HDS Tukey test ($P \leq 0.05$)

Table.3 Growth of groundnut and sweet potato influenced by intercropping six weeks after planting (RFW: root fresh weight; SFW: shoot fresh weight; SDW: shoot dry weight). Data are means of 10 replicates. Means in the same columns followed by the same letter do not differ according to HDS Tukey test (P ≤ 0.05)

	Growth parameters (g)		
Treatment	**RFW**	**SFW**	**SDW**
Intercrops			
Groundnut monocrop	2,61 ± 0,21a	20,17 ± 1,67a	2,92 ± 0,12a
Intercropped groundnut	2,31 ± 0,23a	16,90 ± 1,60a	2,80 ± 0,30a
Sweet potato monocrop	9,71 ± 1,29a	60,31 ± 3,06a	6,58 ± 0,24a
Intercropped sweet potato	9,50 ± 1,06a	56,09 ± 3,11a	6,04 ± 0,30a

Data are means of 10 replicates. Means in the same columns followed by the same letter do not differ according to HDS Tukey test (P ≤ 0.05)

Table.4 Nutrient content of shoot of banana influenced by intercropping six weeks after planting.

Treatment	N	P	K	Ca	Mg	Mn
			g/kg DW			
Banana monocrop	30,10 ± 1,57a	2,25 ± 0,04a	85,56 ± 1,32a	12,46 ± 1,53a	5,66 ± 0,33 a	0,16 ± 0,019a
Banana + groundnut	32,56 ± 1,29a	1,89 ± 0,07b	84,86 ± 1,79a	11,64 ± 0,74a	5,90 ± 0,37a	0,11 ± 0;003b
Banana + sweet potato	24,34 ± 1,51b	2,56 ± 1,36a	76,21 ± 2,13b	10,35 ± 0,63a	6,17 ± 0,24a	0,09 ± 0,002b

Data are means of 5 replicates. Means in the same columns followed by the same letter do not differ according to HDS Tukey test (P ≤ 0.05)

It has also been reported that banana requires high quantity of K and N for its growth and yield (Phirke and Mahorkar, 2010). The combination of banana and sweet potato in an intercropping system may allow strong root competitive interactions and subsequently growth decrease. For intercropping to be more productive, component crops may differ greatly in growth duration so that their maximum requirements for growth resources occur at different times (Fukai and Trenbath, 1993).

In conclusion, intercropping banana with other crops species improve root colonization by arbuscular mycorrhizal fungi. This could be beneficial to banana since it is a perennial crop and may not pass his whole life cycle with companion crops. A long time period for further growth after the harvest of sweet potato should ensure good recovery and full use of available resources. However, the choice of components crops is the key for successful application of the system. These results should be considered with caution since the growth environment was limiting. In natural conditions, minerals are more available because of the constant renewal of the soil solution. Also the soil volume to be explored is infinitely larger. Further studies are thus required to evaluate under field conditions the benefits of increased mycorrhizal root colonization on growth and yield of banana.

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