

Original Research Article

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The Influence of Arbuscular Mycorrhizal Fungi Inoculation on Micro-Propagated Hybrid Yam (*Dioscorea spp.*) Growth and Root Knot Nematode (*Meloidogyne spp.*) Suppression

Atti Tchabi^{1*}, Fabien C.C. Hountondji², Bisola Ogunsola³, Louis Lawouin⁴,
Danny Coyne⁴, Andres Wiemken⁵ and Fritz Oehl⁶

¹Université de Lomé, Ecole Supérieure d'Agronomie, Département de la
Défense des Cultures, BP 1515 Lomé, Togo

²Université des Sciences, Arts et Techniques de Natitingou, Ecole Nationale Supérieure des
Sciences et techniques Agronomiques de Djougou

³International Institute of Tropical Agriculture (IITA), Oyo Road, Ibadan, Nigeria

⁴International Institute of Tropical Agriculture (IITA), IITA-Benin, Abomey Calavi

⁵Plant Science Center Zurich-Basel, Institute of Botany, University of Basel, Hebelstrasse 1,
CH-4056 Basel, Switzerland

⁶Agroscope Reckenholz-Tänikon Research Station ART, Ecological Farming Systems,
Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

*Corresponding author

ABSTRACT

The use of commercial inoculants containing non-indigenous arbuscular mycorrhizal fungi (AMF) is an emerging technology towards improving crop production in Africa. The present study aims at evaluating the influence of two strains of commercial arbuscular mycorrhizal fungi (AMF) products, based on *Funneliformis mosseae* and *Glomus dussii*, on yam growth and on root knot nematodes suppression. Using micropropagated plantlets two cultivars each of *Dioscorea alata* (TDA98-01183 and TDA98-165), and *D. rotundata* (TDR97-00551 and TDR 745) were inoculated with the *F. mosseae* and *G. dussii* products separately, at transplanting into 2L pots, and then inoculated one month later with 500 infective juveniles of *Meloidogyne* spp. and grown for further seven months in the greenhouse. Results demonstrated that even with low colonization rates (6%), AMF led to improved yam growth, especially for *D. alata*. When challenged with *Meloidogyne* spp., AMF inoculation significantly suppressed galling symptoms across the treatments and led to higher tuber yield. This study indicates the potential of AMF to sustainably improve yam quality and productivity, although further screening should be done in order to identify a suitable combination AMF species/strain x cultivar compatibility to optimise the results.

Keywords

Bio-control,
damage,
Meloidogyne spp.,
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Introduction

Botanically, yam (*Dioscorea* spp.) is a monocotyledon plant with more than 600 species in the genus. It is widely cultivated as a tuber crop in West and Central Africa, in Asia and in Central and South America (Ayensu and Coursey, 1972; Okwor, 1998). Globally, yam is the second most important root and tuber crop after cassava, in terms of production and in West and Central Africa production zone, where it is a particularly important staple food crop; its consumption contributes more than 200 calories per day for almost 60 million people (FAO, 2013). In West Africa, yam production is expanding annually but the realized yields are usually considerably lower than potential yields (IITA, 2014). The increasing human population and consequent land pressure result in shortened fallows or more consecutive cropping of land, leading to degradation in soil fertility and an increase in pest and disease levels (Sanchez, 2002). Among the key pests, the plant parasitic nematodes *Meloidogyne* spp. and *Scutellonema bradys* are a particular nuisance to productivity, tuber quality, storage and seed viability in West (Adegbite *et al.*, 2005). *Meloidogyne* spp. so far identified to be associated with yam in West Africa are *M. arenaria*, *M. enterolobii*, *M. incognita*, *M. javanica*, and *M. hapla* of which *M. incognita* is reported to be the most important (Bridge *et al.*, 2005; Kolombia *et al.*, 2014). Infected tubers become deformed and disfigured, affecting their quality and marketability, but also act to re-infect following crop cycles if infected tubers are used as seed material. The value of galled tubers is estimated to be between 39-52% lower than healthy ones [9] (Nwauzor and Fawole, 1981). The proportion of galled tubers collected from yam barns and markets in Nigeria can be as high as 90% for *Dioscorea alata* and 70% for *D. rotundata* (Adesiyun and Odihirin,

1978), although in general the proportion of affected yams is much lower on average (Coyne *et al.*, 2006), but appears to be increasing over time during the stored period (Kolombia *et al.*, 2014). Previously, synthetic chemical nematicides have been a primary means of controlling root-knot nematodes on yam (Ayodele and Agbaje, 2007). However, their highly hazardous nature has led to many of these products being removed from the market and their use discontinued] (FAO, 2013). Other nematode management practices on yam tubers, such as hot water treatment (Speijer, 1996; Coyne *et al.*, 2009), organic fertilizers (Agu, 2008; McSorley, 2011; Osei *et al.*, 2013) or cultural control (Claudius-Cole *et al.*, 2014) have been explored for yam with some success. Screening for resistant cultivars has also been conducted with varying levels of resistance identified (Onyeke and Akueshi, 2012; Ettien *et al.*, 2013; Osei *et al.*, 2015). Recent progress in biotechnology has also shown that *in vitro* meristem tissue culture of yam can provide disease- and pest-free planting material (IITA, 2006). However, as sterile plantlets free of pathogens, they are also free of their natural protective endophytic microorganisms, which normally reside *in planta*, often with beneficial impact (Cassells, 2012) such microorganisms may be arbuscular mycorrhizal fungi (AMF). AMF are important elements of the soil microflora in agroecosystems, which form a mutualistic symbiosis with most plant species, including almost all plants currently micropropagated (Smith and Read, 2008). Root colonization by AMF favors plant development by increasing nutrient uptake, hormonal activity, growth rate and consequently yield (Naher *et al.*, 2013; Lone *et al.*, 2016). Previous studies were conducted on the protective effect of AMF root colonization against soil borne pathogens such as nematodes and the

mechanism involved (e.g. Azcon-Aguilar and Barea, 1997; Borowicz, 2001; Hol and Cook, 2005; Schouteden *et al.*, 2015). Indigenous or introduced AMF have been shown to benefit production of various crops, such as vegetables (Affopkon *et al.*, 2011), cassava (Straker *et al.*, 2010), Sweet potatoes (Halder *et al.*, 2015) and potatoes (Carpio *et al.*, 2005; Farmer *et al.*, 2006; Wu *et al.*, 2013) to name a few. On yam a number of studies have demonstrated the high mycorrhization of the crop with a range of AMF species and the apparent high level of association under natural conditions (Tchabi *et al.* 2009), including the efficiency of AMF colonization on yam growth and yield (Oyetunji and Afolayan, 2007; Tchabi *et al.*, 2010; Lu *et al.* 2015).

Furthermore, few studies regarding the effect of AMF on yam nematodes have shown the suppressions of *Scutellonema bardys* (Tchabi, 2008) in the greenhouse conditions. Moreover there is no report regarding a tripartite interaction including AMF, *Meloidogyne* spp. and yam plants. The present study evaluates the effect of two commercially available AMF products, based on *Funneliformis mosseae* and *Glomus dussii*, on infection of yam by *Meloidogyne* spp.

Materials and Methods

Experimental site

The experiment was conducted in 2 litre pots in the greenhouse at the IITA-station, Cotonou, Benin, situated between 6°25.256N and 2°19.719E, at an altitude of 23 m asl. The site is characterized by sub-equatorial climate with two rainy seasons from March to July and from September to November and two dry seasons in between. The ambient temperature varies between 29-34°C during the day, 24 – 27°C during the

night and the relative humidity between 70% and 85%.

Substrate used for the experiment

The soil used for the experiment was collected from up to a depth of 0–15 cm at IITA-Benin. The soil is characterized as laterite with a pH of 5.20, 30 g organic C kg⁻¹, 2 g K Kg⁻¹ of soil. Total nitrogen and phosphorus were 5 g N kg⁻¹ and 2.1 mg P kg⁻¹, respectively. The soil was sieved through a 1 mm diameter mesh to remove stones and roots, autoclaved at 80 ± 1°C for 3 days, and then air-dried. No fertilizer was applied during the experiment.

Experiment design

The experiment involved three factors: AMF (*Funneliformis mosseae*, *Glomus dussii* and non-inoculated control); yam cultivar (TDa98-165, TDa98-01183, TDr745, TDr97-00551); *Meloidogyne* spp. (500 infective juveniles (J₂) non-inoculated control). The experiment involved 24 treatments with 10 replications, giving in total 240 pots.

A single acclimatized tissue culture yam plants were planted in pots containing 1000 cm³ sterilized. For AMF treatments pots, 80 g of inoculum representing 500 spores, colonized roots and hyphal fragments from each inocula, were placed in a planting hole (1.5 cm diameter and 8 cm depth) made with a cylindrical stick, which was disinfected with 70% alcohol in between each pot.

Non-mycorrhizal controls received 80 g of autoclaved AMF inoculum. *Funneliformis mosseae* and *G. dussii* were commercial inocula obtained from BIORIZE[®] (Dijon, France). The pots were maintained in the greenhouse over 7 months.

Meloidogyne spp. inoculum preparation and inoculation procedure

Meloidogyne spp. were originally extracted from an infested yam tuber (*D. rotundata*, cv. laboko) collected from markets in Cotonou, Benin. The collected tubers were peeled, finely chopped and nematodes were extracted using a modified Baermann method (Coyne *et al.*, 2007). The nematodes were maintained for 4 months in the greenhouse at IITA-Benin on tomato (cv. Pello) plants before removing, rinsing the roots which were finely chopped and macerated in 1.0 % NaOCl for 4 min to release eggs and J₂ of *Meloidogyne* spp. Eggs and J₂ were collected on a 20 µm aperture sieve after passing through nested sieves, rinsing in five changes of tap water and then maintained in tap water for 10 days at room temperature. Hatched J₂ were inoculated (two months after planting) into soil aside seedlings in a shallow trench of ~5 cm radius and 5-10 cm deep. The rate of *Meloidogyne* spp. inoculation was 500 J₂/plant in 10 ml of water. Control plants received 10 ml of tap water. All plants were subsequently watered with 300 ml tap water per plant the day of planting and furthermore each two days. Prior to inoculation, the suspension was adjusted to 500 J₂ per 10 ml with tap water, by estimating from 3 replicates of 10 ml aliquots using a Leica Wild M3C stereomicroscope and adjusting accordingly.

Estimation of AMF root colonization

Soil core samples were removed 24 h prior to harvest the yam tubers, according to Oehl *et al.* (2003) using two separate soil cores of a combined total of 30 cm³ (sampling depth 10 cm) from each pot. Roots were extracted by wet sieving and decantation. Roots colonized by AMF were determined according to Brundrett *et al.* (1996), using trypan blue to stain the mycorrhizal

structures. The gridline-intersect technique (Giovannetti and Mosse, 1980) was used to analyse AMF root colonization under a Leica Wild M3C at 90x magnification.

Assessment of yam growth parameters

At harvest (seven months after planting) the shoots were cut to soil level, tubers and roots were gently removed by hand. Shoots, roots and tubers from each pot were gently rinsed with tap water, air dried, labelled and stored in paper bags. Dry weight of shoots, roots and tubers were recorded following oven-drying in a well-ventilated Gallenkamp oven at 80°C for 72 h at IITA-Cotonou, Benin. Total dry root weights were calculated after taking into account material removed to determine mycorrhizal colonization and nematode density.

Assessment of nematode density and tuber galling symptoms

All tubers harvested were scored for galling severity on a scale of 1-5 (Claudius-Cole *et al.*, 2005) where 1 = clean tuber, 2 = 1-25% of tuber symptoms (low level of damage), 3 = 25-50% symptoms (low to moderate level of damage); 4 = 51-75% symptoms (moderate to severe level of damage); 5 = 76-100% symptoms (high level of damage). All tubers were scored and the mean scores were calculated when more than one tuber per pot was present. Nematodes were extracted from the soil of each pot by mixing all the contents of each pot, removing a 100 ml sub-sample and extracting using a modified Baermann plate method (Coyne *et al.*, 2007). *Meloidogyne* spp. were also extracted from roots and tubers peels using the same technique after chopping into small pieces of 0.1 to 0.2 cm and removing 2 x 5 g sub samples and macerating in 1.0% NaOCl for 4 min. Nematodes were counted with a Leica Wild M3C microscope

Data analysis

All data were analysed using STATGRAPHICS, version 9.1 in Windows 2007. Three-Way ANOVA was used to compare yam growth parameters between treatments. Data on nematodes and on mycorrhization were analysed by one-way ANOVA. Prior to analysis, AMF spore density and nematode population were $\log_{10}(x+1)$ transformed, while data on mycorrhizal colonization were $\arcsin(x/100)$ transformed in order to normalise data. Fischer's Least Significant Difference (LSD) Test was used to separate the means across treatments. Pearson's correlation was used to assess the association between root colonization and various growth parameters.

Results and Discussion

Root colonization by AMF

The AMF root colonization was significantly influenced by *Meloidogyne* spp. inoculation ($p = 0.028$) and also appears as a function of AMF species and yam cultivar compatibility (Table 1). Moreover, the AMF root colonization was very low (0.3% to 6%) (figure 1.a). *Funneliformis mosseae* inoculation resulted in higher root colonization for TDa98-01183 than *G. dussii* ($p = 0.03$), while the root colonization was higher for Tdr cultivars with *G. dussii* than with *F. mosseae* ($P=0.03$) (Figure 1.a).

Effect of AMF and *Meloidogyne* spp. inoculation on yam plant growth

Across cultivars, without *Meloidogyne* spp. inoculation, there was a significant interaction ($p = 0.054$) with AMF species for tubers dry weight. Inoculation with AMF led to higher tuber yields ($p = 0.001$, table 1; figure 1b), an observation consistent for *Meloidogyne* spp. inoculated plants ($p = 0.041$; table 1). Root dry weights were also

significantly higher ($P = 0.018$,) with plantlets inoculated with AMF + *Meloidogyne* spp. compared to plantlets inoculated with *Meloidogyne* alone (figure 1d). Moreover, there were interactions between yam cultivars and *Meloidogyne* spp. inoculation ($p = 0.0097$) regarding shoot dry weight (Table 1). Shoot dry weight was significantly higher ($p = 0.03$) for TDa98-01183 when inoculated with *G. dussii* and *Meloidogyne* spp. compared to control (Figure 1 c).

Across yam cultivars, AMF root colonization was positively correlated with tuber weight ($p = 0.0006$) and *Meloidogyne* spp. densities in soil ($p = 0.017$) (Table 2). Positive correlations were observed between root colonization and tuber dry weight for TDa98-01183 ($p = 0.04$) and TDa98-165 ($P < 0.001$) and between root colonization and root dry weight for TDr745 ($p = 0.028$) while no correlation was observed between root colonization and tuber dry weight for TDa98-165 and TDr97-00551 (Table 2). Interesting, negative correlation was observed between root colonization and nematodes galls damages on root for all cultivars (Table 2)

Effect of AMF inoculation on *Meloidogyne* spp. density and yam tuber quality

At harvest, yam tuber galling was significantly lower ($p = 0.04$) on plants inoculated with combined AMF species and *Meloidogyne* spp. than on plants having only *Meloidogyne* spp. inoculation (Table 1). Plantlets inoculated with *Meloidogyne* spp. had no roots at harvest and high galling damage of tubers, compared to plants inoculated with both AMF species and *Meloidogyne* spp. (Figure 2). For individual yam cultivars, only *F. mosseae* significantly suppressed *Meloidogyne* spp. tuber density on TDa98-165 ($p \leq 0.03$) and *Meloidogyne*

spp. root density on TDa98-01183 ($p \leq 0.01$) (Table 3). Galling symptoms on tubers were statistically similar across treatments. Visually the application of AMF improved tuber quality of plants inoculated with *Meloidogyne* spp. (Figure 2).

The present study is the first known to assess the interaction and protective potential of AMF against *Meloidogyne* spp. on yam using *in vitro* plantlets. Usually, most plantlets at this stage would not be challenged or come in contact with nematodes, but for this study, it serves as an initial indicator on host reaction to AMF, the potential of AMF to protect against nematodes and the potential for using AMF as bio-enhancement of such planting material. The study also serves to help identify or assess suitable plant growth stages for inoculation, which will require further evaluation. It was suggested that the particular growth stage at which tissue-cultured plantlets are inoculated with AMF is important but varies according to plant genotype (Smith and Read, 2008). The results showed clearly that there was relatively low root colonization by the AMF in the current study; while previously, the same combinations yielded up to 90% of root colonization (Tchabi, 2008). A possible explanation could relate to the difference of soil chemical properties. The substrate in the present study is more acid, with less extractable phosphorus than in Tchabi *et al.* (2016). Such differences in root colonization due to soil chemical composition were observed in *Acer rubrum* (Wiseman and Wells, 2005). Furthermore, the difference can be attributed to others abiotic factors, such as low availability of oxygen, which affected plant root colonization in salinized soil (Levy *et al.*, 1983; Heikham *et al.*, 2009).

Despite the low level of root colonization, the results of our study clearly show that

there is a potential of AMF application on yam plantlets, in terms of improved production under non-pest challenged conditions. Without nematode challenge, our results demonstrated that application of commercially available AMF products can improve micro-tuber growth and development under glasshouse conditions, but that the degree of effectiveness is dependent on genotype or cultivar and on AMF strain/species. Previous studies have also noted this interaction between AMF and yam genotype under both greenhouse (Tchabi *et al.*, 2010) and field conditions in Taiwan (Lu *et al.*, 2015) and Nigeria (Oyetunji and Afolayan, 2007). In the current study AMF products commercially produced in Europe were used due to their availability, which may not be the most suitable or compatible for tropical conditions. Although the same products led to some high levels of yam root colonization in previous studies compared to populations locally isolated (*Glomus etunicatum*, *G. hoi*, *G. clarium* and *Acaulospora scorbiculata*) in Benin (Tchabi *et al.*, 2010). Indeed survey studies in Benin and in Côte d'Ivoire showed that yam is highly mycorrhizal and associated with a wide diversity of AMF species (Tchabi *et al.*, 2009; Nandjui *et al.*, 2013).

Furthermore, studies have demonstrated that AMF positively influences plant growth in crops that are harvested for underground parts or tuber crops, such as cassava, potato, and sweet potato (Ekin *et al.*, 2013; Hijri, 2016). It would appear therefore that yam is a suitable candidate for bio-enhancing with AMF and that by more precisely determining appropriate genotype x AMF strain combinations, suitable recommendations can be established, both for tissue culture micro-tuber conditions and for field conditions across climatic zones (Khosro *et al.*, 2011; Berruti *et al.*, 2016).

Table.1 Analysis of variance table for yam cultivar, inoculation of Arbuscular mycorrhizal fungi and *Meloidogyne* spp. factor effects on micropropagated yam plantlet growth, AMF development and nematode parameters

Variable	Factors						
	Cultivar (A)	AMF ¹ (B)	<i>Meloidogyne</i> spp. ² (C)	AxB	AxC	BxC	AxBxC
Colonization							
Df	3	2	1	6	3	2	6
F	0.25	4.02	100.21	5.88	34.8	42.36	18.41
p	0.86	0.02	0.028	0.94	0.69	0.130	0.496
TDW							
Df	3	2	1	6	3	2	6
F	2.28	6.87	4.29	2.18	1.06	0.08	0.68
p	0.086	0.001	0.041	0.054	0.36	0.92	0.66
SDW							
Df	3	2	1	6	3	2	6
F	3.21	0.71	2.03	1.93	4.06	1.21	0.19
p	0.027	0.49	0.15	0.086	0.0097	0.30	0.97
RDW							
Df	3	2	1	6	3	2	6
F	1.63	1.09	5.87	0.57	0.11	0.01	0.42
p	0.18	0.34	0.018	0.75	0.95	0.99	0.86
Galling score							
Df	3	2	1	6	3	2	6
F	3.28	3.20	259.6	0.97	1.21	4.15	0.96
p	0.025	0.046	0.0001	0.45	0.309	0.019	0.460
Density J₂ root							
Df	3	2	1	6	3	2	6
F	1.38	1.00	12.62	1.10	1.38	1.00	1.10
p	0.25	0.37	0.001	0.36	0.25	0.37	0.36
Density J₂ soil							
Df	3	2	1	6	3	2	6
F	0.83	1.36	18.15	0.95	0.83	1.36	0.95
p	0.21	0.25	0.001	0.46	0.48	0.25	0.46
Density J₂ tuber							
Df	3	2	1	6	3	2	6
F	0.83	1.36	18.15	0.95	0.83	1.36	0.95
p	0.21	0.25	0.001	0.46	0.48	0.25	0.46

Tuber galling severity were assessed on a scale from 1 to 5 (Claudius-Cole *et al.*, 2005) where 1 = clean tuber; 2 = 1-25% tuber galling symptoms (low level of damage); 3 = 25-50%; 4 = 51-75%; 5 = 76-100%.

¹AMF inoculated at rate of 300 spores per pot at yam plantlet transplanting into individual pot.

²*Meloidogyne* spp. inoculated at a rate of 500 J₂ (juveniles) two months after AMF inoculation. TDW = tuber dry weight; SDW = shoot dry weight; RDW = root dry weight.

Table.2 Pearson correlation analysis between Arbuscular mycorrhizal fungal root colonization and yam plant growth parameters (tuber number, tuber fresh weight) or nematode damage scores (galling) and J2 density.

Cultivars		TDW	RDW	Galling	J2 density
All cultivars	<i>r</i> value	0.32024	0.00019	-0.0144	0.03015
	<i>P</i> value	0.006	0.99	0.04	0.6421
TDa98-165	<i>r</i> value	0.36800	0.12556	-0.2746	-0.0746
	<i>P</i> value	< 0.001	0.33	0.03	0.5709
TDa98-01183	<i>r</i> value	0.39071	0.21152	-0.10099	0.16321
	<i>P</i> value	0.0484	0.10	0.044	0.2128
TDr 745	<i>r</i> value	0.31367	0.42968	-0.0878	-0.0154
	<i>P</i> value	0.19	0.0285	0.050	0.90
TDr87-00551	<i>r</i> value	0.17042	0.11005	0.07170	0.06486
	<i>P</i> value	0.019	0.40	0.05862	0.62

Fig 1 Percentage root colonization (a) and effect of Arbuscular mycorrhizal fungal *Funneliformis mosseae* and *G. dussii*) and *Meloidogyne* spp. inoculation on micropropagated yam plantlet growth(b, c and d) of four yam cultivars (TDr745, TDr97-00551,TDa98-165, TDa98-01183) in pots under greenhouse conditions. Values are means (□ SE) of ten replicates at harvest, seven and five months after AMF and *Meloidogyne* spp. inoculation, respectively.

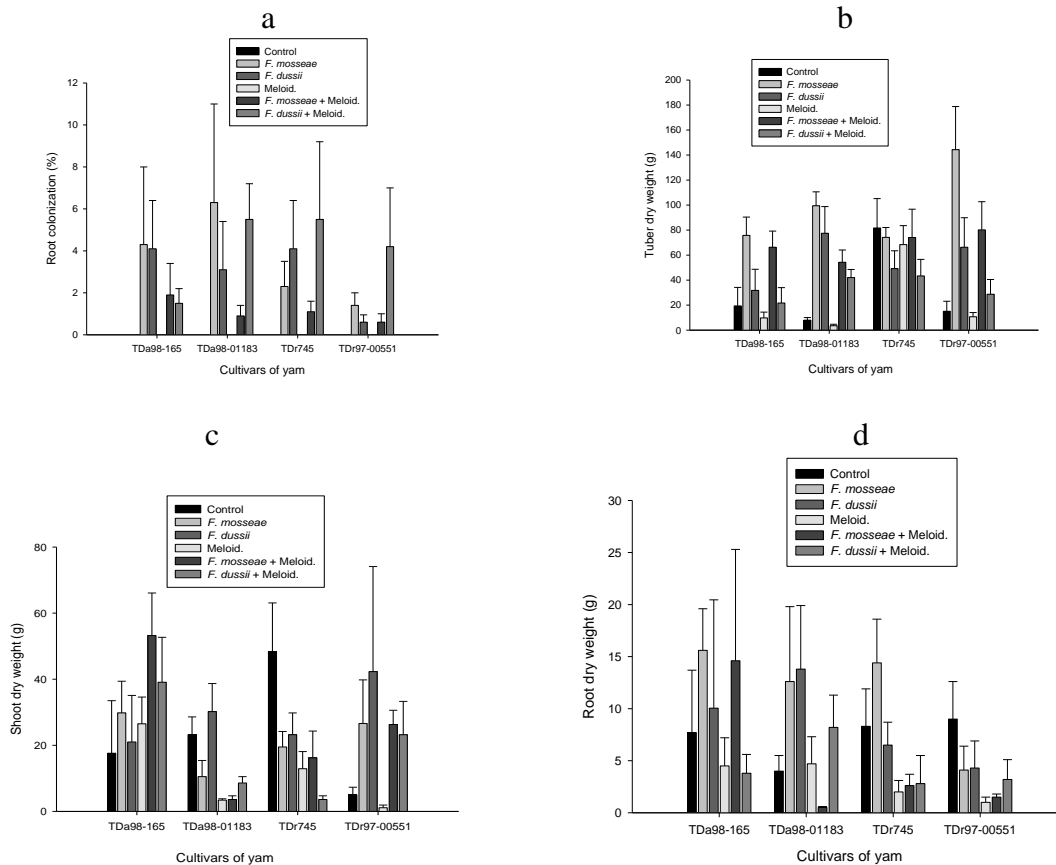


Table.3 Tuber galling and the mean population density of *Meloidogyne* spp. at harvest seven and five months after Arbuscular mycorrhizal fungi (*F. mosseae* and *G. dussii*) and *Meloidogyne* spp. inoculation, respectively, to yam micropropagated plantlets of two cultivars of *Dioscorea rotundata* (TDr745, TDr97-00551) and two cultivars of *D. alata* (TDa98-165; TDa98-01183) under screen house conditions

Treatments	Tubers galling		<i>Meloidogyne</i> spp. density ¹ (root)		<i>Meloidogyne</i> spp. density ² (soil)		<i>Meloidogyne</i> spp. Density ³ (tuber)	
	non-inoc	inoc	non-inoc	-inoc	non-inoc	inoc	non-inoc	inoc
TDa98-165								
Control	0.8±0.2	5±0.3a	-	2977.6±1615.3	-	734.2±356.9	-	77±0.2b
<i>G. mosseae</i>	1±0.0	3±0.6b	-	1687.3±716.7	-	148.3±55.9	-	56±0.6a
<i>G. dussii</i>	0.8±0.4	3.2±0.4b	-	236.4±167.5	-	202.5±138.1	-	63±0.3b
p-value	0.67	0.02		0.20		0.15		0.03
TDa98-01183								
Control	1.0±0.0	4.8±0.9	-	560.8±324.9a	-	727.3±655.8	-	85±0.3
<i>G. mosseae</i>	1.0±0.0	3.0±1.3	-	457.5±354.1a	-	1055.6±724.5	-	69±0.4
<i>G. dussii</i>	1.0±0.1	3.3±0.5	-	135.2±85.5b	-	354.3±117.9	-	71±0.6
p-value	0.9	0.03		0.01		0.12		0.48
TDr745								
Control	1.0±0.1	4.7±0.6	-	1308.3±530.5	-	1048.3±581.8	-	102±0.2
<i>G. mosseae</i>	1.0±0.0	3.6±0.2	-	178.3±110.3	-	85.8±44.6	-	59±0.0
<i>G. dussii</i>	1.0±0.2	3.7±0.1	-	110.2±39.6	-	155.5±82.3	-	58±0.2
p-value	0.81	0.05		0.3		0.26		0.71
TDr97-00551								
Control	1.0±0.0	4.0±0.3	-	53.3±13.2	-	225.6±142.4	-	156±0.1
<i>G. mosseae</i>	1.0±0.1	2.3±0.6	-	2720.3±1516.3	-	125.3±72.7	-	73±0.1
<i>G. dussii</i>	0.7±0.3	3.5±0.3	-	2053.3±1309.5	-	460.8±170.8	-	63±0.2
p-value	0.6	0.04		0.51		0.23		0.39

Roots and soil from non-inoculated treatments were nematode free. - = no data collected. Analysis and means separation of nematode densities were undertaken on log₁₀(x+1) transformed data. ¹Nematode density per 5 g of root; ²Nematode density per 50 g soil. ³Values were mean (± SE) of six replicates non transformed data. For each yam cultivar means were compared by columns. Means followed by the same letter were not significant difference (*P* > 0.05) according to the Protected Least Significant Different Test (LSD). Inoc = inoculated and non-inoc = non-inoculated with *Meloidogine* spp. Tuber galling severity were assessed on a scale from 1 to 5 (Claudius-Cole *et al.*, 2005) where 1 = clean tuber; 2 = 1-25 % tuber galling (low level of damage); 3 = 25-50%; 4 = 51-75%; 5 = 76-100%.

Fig.2 Yam tubers and roots at harvest seven months after planting following Arbuscular mycorrhizal fungi inoculation at planting and *Meloidogyne* spp. inoculation two months after planting.



Legend: A = Control, B = *F. mosseae*+*Meloidogyne* spp., C= *F. mosseae*, D= *Meloidogyne* spp.

Regarding suppression of nematodes and nematode damage by AMF and the current study provided further confirmation of the positive impact of AMF on such biotic challenges. Again, the interaction between yam genotype and AMF affected the response, with the cv. TDa98-01183 responding more efficiently in terms of tuber yield to inoculation with *F. mosseae* and *Meloidogyne* spp., compared to *Meloidogyne* spp. alone. This result also compares favourably with earlier studies assessing the impact of AMF on the yam nematode *Scutellonema bradys* (Tchabi *et al.*, 2016). The current study, however, indicates that AMF inoculation enables yam plantlets to recover from *Meloidogyne* spp. injuries, possibly through the p-compensation phenomena (Harrier and Watson, 2004; Schouteden *et al.*, 2015). Although the AMF had no influence on nematode densities, galling damage symptoms were lower in the presence of AMF and tubers visibly healthier (cleaner) and of better quality (Fig 2). It has been

shown that the intensity of colonization by AMF can be a determining factor on the response of mycorrhized plants to nematode parasitism (Smith *et al.*, 1986). *Meloidogyne hapla* for example, was absent in cortical tissues of alfalfa roots when over 10% of roots were colonized (Grandison and Cooper, 1986), while in cotton, *M. incognita* was inhibited only when roots were 50% colonized by *Glomus intraradices* (Smith *et al.*, 1986).

In our study, the 6% yam root colonization by *F. mosseae* and *G. dussii* was likely insufficient to suppress the development of *Meloidogyne* spp. on the host root. These results reflect those of Ryan *et al.* (2003), who reported that with less than 50% of root colonization, the population of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) was not suppressed on potato plants inoculated with the commercial product Vaminoc[®] (combination of three *Glomus* spp.). However, our results were contradictory to former reports, which

established the suppression (roots and soil) of *M. incognita* in tomato (Grandison and Cooper, 1986; Talavera *et al.*, 2001), *M. hapla* on onion (MacGuidwin *et al.*, 1985) and on banana (Jaizme-Vega *et al.*, 1997) with low root colonization. The difference between our results and those former studies on AMF suppressing *Meloidogyne* spp. population densities may be explained by the fact that a single species of *Meloidogyne* spp. is more often used in these inoculation studies, while in the present study, the inoculum of the *Meloidogyne* spp. included a mixture of two species (*M. incognita* and *Meloidogyne* spp).

The reduction in number of galls, observed on roots of yam plantlets could be due to the competition between the pathogen and the symbiont for infection sites, but other factors such as increase of lignin and phenols (Umesh *et al.*, 1988) or nematicide substances, such as phenylalanine and serine (Suresh *et al.*, 1985) can be involved. Our present results reflect those of Kellam and Schenck (1980) who registered lower quantity of galls in mycorrhizal soybean plants than in non-mycorrhizal.

In conclusion, the current study provides further supporting evidence on the use of AMF for the protection of crops against nematode pests. It also supports the use of bio-enhancing tissue culture plantlets for improving their growth and production, in this case yam micro-tubers. However, AMF species influence on plant growth appears highly dependent on the plant genotype with which they are associated. Taking into consideration tuber weight, AMF attributes and nematode management, the product based on *F. mosseae* was more effective than the *G. dussii*-based product at improving growth and reducing nematode damage. Although, prior to further application of the present result in the field,

a broader assessment should be recommended for selecting the best combination AMF strain/species x yam genotype. Assessment of available commercial products enables a relatively rapid ability to determine the suitability of products that are currently accessible, while assessment of local indigenous population provides a longer term strategy for development of products based around populations that may be more acclimatized to local conditions.

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