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Growth media and mycorrhizal species effect on acclimatization and nutrient uptake of banana plantlets

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ABSTRACT

The objective of this study was to investigate the acclimatization and performance of banana (Dwarf Cavendish) in two substrates inoculated with different AM fungi (*Glomus. caledonium* and *G. macrocarpun)* and assess the plantlets dependency on inoculation for phosphorus (P) and zinc (Zn) uptake along with biomass development. In addition, to investigate the role of mycorrhizal fungi in supporting acclimatization phase, a plant growth promotion study was set-up in greenhouse using micropropagated plantlets. Two growth media, as GM-I and GM-II were used accompanied by *G. caledonium* and *G. macrocarpum*. In first phase, 9 weeks acclimatization study was conducted and in second phase, acclimatized plants were propagated for 16 weeks in both inoculated and non-inoculated conditions. Plantlets acclimatization and nutrient uptake were recorded along with other parameters. Mycorrhizal inoculation significantly increased banana plantlets growth, root infection and P uptake. Plantlets inoculated with *G. caledonium* exhibited increase in shoot and root dry mass, P and Zn concentration, and root infection in the GM-I. The shoot and root dry mass, P and Zn concentration, and root infection were higher in GM-I than Konaktas soil series. Banana plantlets are mycorrhizal dependent (MD) and soil-grown banana plantlets are more MD than plants grown in the GM-I. Mycorrhizal inoculation seems to be a significant factor in decreasing mortality and increasing production of high-quality banana plantlets under micropropagation conditions.
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1. Introduction

Bananas are the second premier fruit crop, widely grown in developing countries and makes with production of 106 million tons followed by citrus (FAO, 2013; Madhulatha et al., 2004). Banana is a sensitive crop which faces a number of diseases on growing via vegetative propagation. To eliminate such risks, and develop disease-free planting material, bananas are micropropagated on a large scale, which has additional benefits of high multiplication rate, optimized use of resources (plant material) and the production of high-quality plant.

As the micropropogated plants are more sensitive to environmental changes (Vestberg et al., 2002), early inoculation with *G. intraradices* enhances the growth of bananas by improving nutrient uptake (Pinochet et al., 1997). Phosphorus makes 0.2% dry mass of banana plantlets and it's availability become more crucial for tissue-cultured plantlets in changing environment (Schachtman et al., 1998).

Arbuscular mycorrhizal (AM) fungi are widely recognized for plant growth improvement by nutrient uptake and assistance stress tolerance (Barea et al., 2005; Declerck et al., 2002; Johansson et al., 2004). Wide extension of mycorrhizal hyphae increase ability of roots to absorb nutrients by enhancing surface area mainly for depleted soils (Smith and Read, 2008). AM fungi facilitate in plant growth under nursery conditions (Douds et al., 1993; Jefwa et al., 2009; Kavoo-Mwangi et al., 2013) by colonizing 80% of the terrestrial plants (Brundrett, 2002). Banana roots are also colonized and increase plant vigour which enhances water absorption, mineral nutrient uptake, and mainly the phosphorus. Micropropagated plantlets are expected to be microbe free including pathogenic and non-pathogenic bacteria and AM fungi (Yano-Melo et al., 1999). Besides that, different species of *Glomus* are effective in promoting banana plantlets growth (Mwashasha et al., 2011; Yano-Melo et al., 1999).

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Fig. 1. Banana plantlets acclimatized up to 9 weeks and propagated further for 16 weeks in GM-I and GM-II.

Survival and development of mycorrhizal propagules depend on environmental factors such as soil types, fertility status, plant species and fungal strain which assist in establishment of symbiosis (Plenchette, 2000). In addition, survival of micropropagated banana plantlets is low on transplanting into sterile soil, therefore use of mycorrhizal fungi and other rhizospheric organisms is worthwhile to support plantlets survival and growth. At the beginning of weaning stage, growth response of micropropagated banana plantlets depends on plantlet variety and type of AM fungal inoculation (Declerck et al., 1995). The climate of Turkey is supportive for banana cultivation, however the banana production rely on nutrient management where, supply of P and Zn through AM fungi resides key role; therefore, the AM fungi application enabling sufficient nutrient supply could be a novel option along with chemical-P fertilizer for banana cultivation in Turkey. Deficiency of Zn is a worldwide problem among humans, where one-third of the human population suffers from Zn deficiency (Hotz and Brown, 2004).

The objective of this study was to investigate the performance of banana in two substrates inoculated with different AM fungi and assess the plantlets dependency on inoculation for nutrient uptake and biomass development. To the best of our knowledge, mycorrhizal species (*G. caledonium* and *G. macrocarpum*) and substrates have not been previously investigated to determine their role in nutrient uptake and biomass development of banana plantlets (*Dwarf Cavendish*). The present study could be baseline for use of specific mycorrhizal fungi in promoting banana plantlets growth under greenhouse conditions.

Table 1
Chemical properties of soil, compost and andesitic tuff.

Properties	Unit	Soil	Compost	Andesitic tuff
pH	(1:1 H_2O)	7.4	7.91	–
Clay	g kg^{-1}	330	–	–
Silt	g kg^{-1}	200	–	–
Sand	g kg^{-1}	470	–	–
CEC	cmol$^+$ kg^{-1}	16.57	–	–
C_{org}	%	2.47	54.2	–
N	%	–	1.13	–
P	kg ha^{-1}	32.6	0.18	0.03
K	%	0.006	0.98	4.3
Ca	%	0.21	2.5	–
Mg	%	0.22	0.27	–
Zn	mg kg^{-1}	0.64	40	0.1
Fe	mg kg^{-1}	3.76	1005	2
Cu	mg kg^{-1}	3.21	12	0.2
Mn	mg kg^{-1}	0.95	141	3.6

2. Materials and methods

2.1. Plant material and culture collection

Banana plantlets were produced via micropropagation technique by preparing the plant material and culture. The banana cultivar Dwarf Cavendish was obtained from a commercial banana grower and cultured according to the standard protocol (Kaçar et al., 2010).The MS basal salt mixture was supplemented with Morel vitamins 1 mg L^{-1} each (Ca pantothenate, Thiamine HCl, Pyridoxine HCL and Nicotinic acid), 0.01 mg L^{-1} Biotin and 100 mg L^{-1}

myo-inositol, 4.44 μM Benzyladenine (BA) was used as the initiation and multiplication media, and 4.92 μM Indole-3-butyric acid (IBA) for the rooting medium. Sucrose (4%) was added as a carbon source and agar–agar (Sigma A-1296) was added at 7 g L^{-1} as a gelling agent. All media were adjusted to pH 5.7, sterilized at 1.05 kg/cm^2 and 121 °C for 15 min. Shoot tip cultures of banana were incubated at 25 ± 2 °C in darkness for the 4 weeks of initiation stage in 10 cm^2 culture vessels. Explants were transferred to fresh medium after 30 days and cultured in a 16/8-h light/dark photoperiod using cool-white fluorescent light (50 μmoL m^{-2} s^{-1}). Rooted shoots of 6–10 cm were washed free off agar medium and taken to the micropots with rooting medium to induce root formation. Healthy rooted plants were transferred in soil to the greenhouse after 6 weeks (Kaçar and Faber, 2012).

2.2. Growth media setup

The experiments were conducted under greenhouse conditions, using the following two substrates as a growth medium (GM):

1- Andesitic tuff + soil + compost (6:3:1 v:v:v) where tuff is a local substrate with 0.5–1 mm granulometery as GM-I

2- Soil from the Konaktaş soil series (Lithic Xerorthent Ortent Entisol by USDA classification) as GM-II

Soil material was collected from surface horizons (0–20 cm) in the Çukurova Basin. The physical and chemical characteristics of the growth media were analysed according to Page et al. (1982) in the Rhizosphere Laboratory at Çukurova University (37.0573°N, 35.3552°E) in Adana Province of Turkey and are presented in Table 1. Each GM was sterilized in the autoclave for 2 h at 121 °C and re-inoculated with the crude soil filtrate to balance microbial activities except AM fungi.

2.3. Mycorrhization

Two mycorrhizal species were used: *Glomus caledonium* (Gerdemann and Nicolson, 1963) Rothamsted isolate, UK, and *G. macrocarpum* (Tulasne and Tulasne, 1844), USA with sorghum plants as the trap culture plant for mycorrhizal spore propagation. The growth medium consisted of a mixture of soil, sand, and peat at a ratio of 3:1:1 by volume. The inoculum potential was estimated based on the number of spores in 100 g of inoculum, and approximately 1000 spores were placed about 30–40 mm below the plantlets. In non-mycorrhizal treatments, each plantlet received the same amount of mycorrhizael fungi-free substrate.

2.4. Acclimatization and pot cultivation

The experiment was conducted in two phases, the first was to observe the acclimatization of transplanted plants in both growth media in the presence of mycorrhizal fungi. For this purpose, 15 plants were transplanted and the percentage of acclimatized plantlets was recorded after 9 weeks. In the second phase of the experiment for pot cultivation, survived plantlets were transferred into 3 kg growth medium in plastic pots with 4 replications. After transplanting, the plantlets were grown for 16 weeks in a controlled greenhouse with an average day-night temperature of 25 ± 3 °C and 80 ± 5% humidity. Distilled water was used to maintain the moisture at 75% of field capacity.

2.5. Measurements

2.5.1. Plant sampling

After growing plantlets for 16 weeks, they were harvested, and root and shoot dry mass (g $plant^{-1}$) were recorded. A Tema mill, RM100 (Retsch Solutions in Miling and Sieving, Haan, Germany) was used for grinding dried plant material and 0.2 g of the ground plant material was ashed at 550 °C, followed by dissolution in 3.3% HCl.

Table 2
Acclimatization and root colonization of micropropagated plantlets in two different growth media.

Media	Treatment	Acclimatization of plantlets	Acclimatized plantlets (%)
GM-I	Control	05	33.3 ± 0.09
	G. caledonium	06	40.0 ± 0.10
	G. macrocarpum	10	66.7 ± 0.17
GM-II	Control	08	53.3 ± 0.14
	G. caledonium	10	66.7 ± 0.17
	G. macrocarpum	10	66.7 ± 0.17

2.5.2. Root colonization

During harvesting, shoots were separated from the roots at 0.5 cm above the soil surface and soil was separated from the roots by washing with running tap water, followed by distilled water. Root sub-samples were taken and preserved in a mixture of ethanol, glacial acetic acid and formalin to determine mycorrhizal infection. Preserved roots were stained (Koske and Gemma, 1989) and root colonization by mycorrhizal fungi was determined using the gridline-intersect method (Gioannetti and Mosse, 1980).

2.5.3. Mycorrhizal dependency

At harvest, the mycorrhizal dependency (MD) of shoot dry mass (SDM) was calculated for inoculated (M) plants (Plenchette et al., 1983):

$$Mycorrhizal < MML : MSPACEWIDTH = "0.4EM" HEIGHT = "0.0EX" DEPTH =$$

2.6. Experimental analysis and statistical analysis

In plant shoot material, P concentration was determined using a colorimetric method (Murphy and Riley, 1952). Atomic absorption spectrophotometry was employed to determine Zn concentration. The variation in shoot and root dry mass, root colonization and nutrient concentrations, attributed to the effects of growth medium and mycorrhizal species, were assessed according to an entirely randomized design with a 2 × 3 factorial arrangement of analysis of variance procedure (SAS, 2009). Means were compared using the Tukey Multiple Range Test at $P < 0.05$.

3. Results

3.1. Acclimatization and root colonization

In first phase of the experiment, acclimatization of micropropagated plantlets was observed significant for GM-II than GM-I, as growth medium determines the plant acclimatization depending on its composition and environment. Plantlets inoculated with *G. caledonium* and *G. macrocarpum* were more acclimatized than non-inoculated plantlets. In both GM, *G. macrocarpum*-inoculated plantlets exhibited 66.7% acclimatized plantlets, *G. caledonium*-inoculated plantlets had an acclimatization of 40% and 66.7% for GM-I and GM-II, respectively (Table 2). Non-inoculated plantlets had a 33.3% acclimatized plantlets in GM-I and 53.3% in GM-II.

The mycorrhizal colonization was 68.8% and 71.2% for *G. macrocarpum*-inoculated plantlets in GM-I and GM-II respectively while for *G. caledonium* it was 50.8% and 55.3%. Root colonization was higher in GM-I, while acclimatization was supported to the greatest degree by the inoculum of *G. macrocarpum,* i.e., 66.7%, and little by the control, at 33.3%. In GM-II, root infection was also higher in the *G. caledonium*-inoculated treatment, in comparison to the

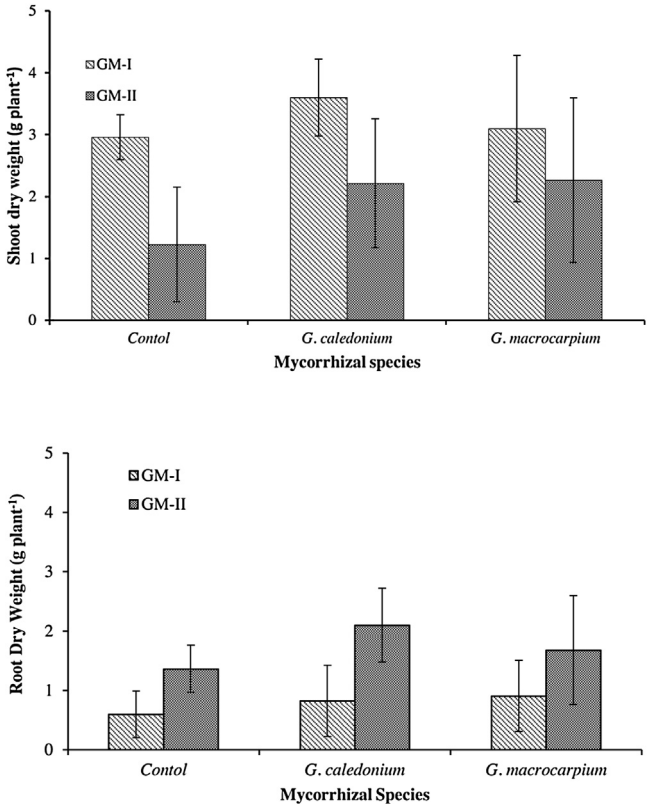


Fig. 2. (a) Shoot dry mass (SDM) and (b) root dry mass (RDM) of the inoculated and non-inoculated banana plants under two different growth media (GM).

control, while acclimatization was equally supported by inoculum of *G. macrocarpum* and *G. caledonium,* i.e., 66.7%, and least by the control, 53.3%.

3.2. Mycorrhizal dependency (MD)

The results shown in Fig. 3, indicate that banana plantlets are highly mycorrhizal – dependent; however, significant difference between growth media is there. When plantlets were grown in GM-I, *G. caledonium*-inoculated plantlets exhibited 17.8% dependency and *G. macrocarpum*-inoculated plants showed 4.6%. However in GM-II, it was 44.7% for *G. caledonium* and 45.9% for *G. macrocarpum*.

3.3. Dry mass and nutrient concentration

Producing micropropagated bananas under inoculated and non-inoculated conditions resulted in different shoot and root growth in different growth media (Fig. 1). The effect of growth media was significant for SDM and RDM (Fig. 2a). *G. caledonium* in GM-I showed highest mass of 3.59 g plant^{-1}. In contrast, *G. macrocarpum* and the control in GM-I were similar in terms of SDM. Similarly, the SDM trend in GM-II was slightly different for banana plantlets inoculated with *G. caledonium* and *G. macrocarpum* and they produced a biomass of 2.21 and 2.26 g plant^{-1} respectively. The effect of GM was significant ($P < 0.019$) for micropropagated banana SDM and mycorrhizal inoculation ($P < 0.004$).

The RDM trend in two GM was contrary to SDM. GM-II-grown plantlets had a higher RDM than plantlets grown in GM-I. *G. caledonium* in GM-I produced 2.09 g plant^{-1} mass, while it was 1.67 g plant^{-1} for *G. macrocarpum* (Fig. 2b). Similarly, in GM-II, *G. macrocarpum* and *G. caledonium* produced high RDM than control at 0.90 and 0.82 g plant^{-1}, respectively where GM-II significantly ($P < 0.001$) enhanced RDM followed by mycorrhizal treatments ($P < 0.0004$).

Furthermore, mycorrhizal inoculation was significant ($P < 0.001$) for P uptake and root colonization. In GM-I, P concentration was 1.8 g kg^{-1} in *G. caledonium*-inoculated plantlet higher than other (Table 3). For both GM, mycorrhizal inoculation had significant effect on plant P concentration. When plantlets were grown in GM-II, P concentration was 1.3 g kg^{-1} in *G. macrocarpum*-inoculated plantlet. In banana plantlets tissues, Zn concentration for GM-I, non-inoculated plantlets contained 26.9 mg kg^{-1} of Zn, however *G. caledonium*- and *G. macrocarpum*-inoculated plantlets contained 26.0 and 29.7 mg kg^{-1} of Zn respectively (Table 3).

Correlation of evaluated parameters in the study is shown in Table 4 where colonization and P are highly significant. All variables of this study were positively correlated, RDM and SDM are significantly correlated ($P < 0001$). Phosphorus percentage in the shoot is directly related to SDM and is significantly correlated ($P < 0.0238$). Root colonization significantly ($P < 0.020$) increased the SDM and P concentration ($P < 0.0001$). In the same way, Zn concentration was found to be significantly ($P < 0.0064$) correlated to root conolization.

4. Discussion

During acclimatization, *G. macrocarpum*-inoculated plantlets had the highest survivorship by 67% for both growth media. In soil as growth medium, plantlets acclimatization was high which is dependent on plant species and the soil type (Kavoo-Mwangi et al., 2013). Furthermore, banana plantlets exhibited high acclimatization percentage in GM-I than GM-II, which may be related to physical conditions of growth medium (Ansar and Yusuf, 2016). The bulk density and porosity of GM may have had an effect on early stages of plantlet root development. Soil porosity in GM-II is low which might stunt the roots. The results show that growth media is a crucial factor in plant response to mycorrhizal inoculation, which may be related to nutrient concentration in growth medium.

The SDM and RDM of inoculated banana plantlets were significantly higher than control. *Glomus*-inoculated plantlets were superior in most of the evaluated growth parameters (Yano-Melo et al., 1999). Mycorrhizal species have significant effect on plant attributes. Under both GM, *G. caledonium* was effective than *G. macrocarpum.* Our findings are in harmony with Declerck et al. (1995)'s results in which they indicated that plants inoculated with *G. macrocarpum* grew better than *G. mosseae*-inoculated plants. For growth media, GM-II exhibited high dry mass than GM-I plantlets. The magnitude of AM fungal species effect on plantlet growth varied with growth media. Soil type had a significant effect on banana plantlet growth (Plenchette, 2000) whereas effect of commercial inoculant is dependent on soil type and plant development stage (Kavoo-Mwangi et al., 2013).

The root colonization was significantly affected by GM type and mycorrhizal species used. For both growth media, *G. caledonium*-inoculated plantlets exhibited a higher root colonization. Significant differences in colonization of banana cultivars by AM fungal isolates were observed (Declerck et al., 1995). In addition, under greenhouse conditions 50–70% root colonization was observed; however under field conditions, it was 37–57% (Jefwa et al., 2012). Plant mycorrhizal dependency and root colonization depend upon several soil and plant factors, as well as AM fungal species (Mwashasha et al., 2011; Ortas and Ustuner, 2014).

Banana plantlets have a high mycorrhizal dependency for optimum growth (Elsen et al., 2003; Jaizmevega and Azcon, 1995). It is possible that addition of compost to GM influenced MD as a result of nutrient richness. Mycorrhizal dependency of banana plantlets has been calculated and magnitude of response vary between and within species (Ortas and Ustuner, 2014). In particular, soil fertil-

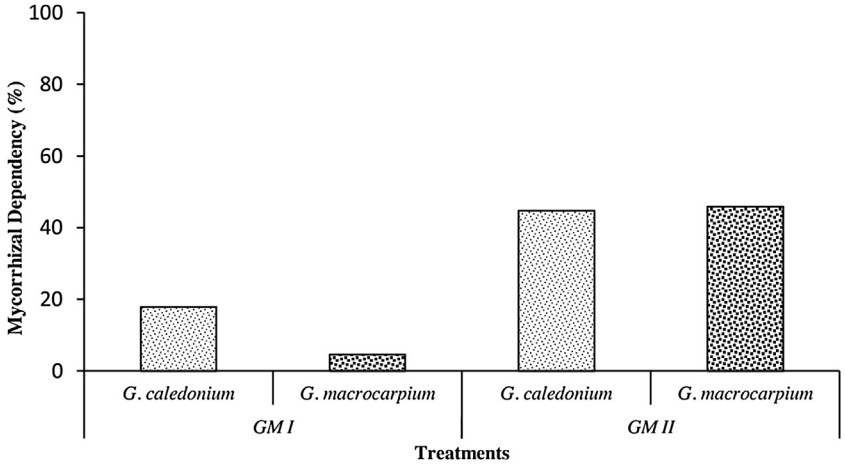


Fig. 3. Mycorrhizal dependency of the inoculated and non-inoculated banana plants under two different growth media (GM).

Table 3
Root colonization and nutrient uptake concenteration in plantlets leaf for different treatments in GM.

		Root colonization	P (g kg^{-1})	Zn (mg kg^{-1})
GM – I	Control	0.0 ± 0.0 c	0.8 ± 0.0 c	26.9 ± 2.8 b
	G. *caledonium*	50.8 ± 6.9 b	1.8 ± 0.0 a	36.0 ± 3.6 a
	G. *macrocarpum*	68.8 ± 5.0 a	1.4 ± 0.0 b	29.7 ± 3.5 b
GM – II	Control	0.0 ± 0.0 b	0.6 ± 0.0 c	25.9 ± 5.3 b
	G. *caledonium*	55.3 ± 11.0 a	2.1 ± 0.0 a	31.7 ± 8.9 ab
	G. *macrocarpum*	71.2 ± 11.4 a	1.3 ± 0.0 b	36.3 ± 9.4 a
Significancy				
Growth Media (GM)		0.4595	0.4221	0.4975
Mycorrhizae (M)		<0.0001	<0.0001	0.1452
GM x M		0.5746	0.0174	0.7858

Table 4
Relationships among plant growth variables, nutrient uptake and colonization expressed as Pearson's correlation coefficient.

	SDM	RDM	P%	Zn mg kg^{-1}	Colonization%
SDM	1				
RDM	0.83310 <0.0001*	1			
P%	0.45977 0.0238*	0.31689 0.1314	1		
Zn	0.18130 0.3965	0.07362 0.7325	0.31517 0.1336	1	
Colonization	0.47016 0.0204*	0.38423 0.0638	0.88155 <0.0001*	0.54052 0.0064*	1

* P < 0.05 significant.

ity and mainly P concentration is an important parameter (Ortas, 2012). Mwashasha et al. (2011) indicated that mycorrhizal dependent banana plants may improve adaptation in harsh environments by absorbing more nutrients. Konaktas soil contains 0.64 mg kg^{-1} of Zn soil, as the Zn concentration in this soil is above the critical level (>0.5), all plants either inoculated with mycorrhizal fungi or not inoculated, also contained Zn tissue concentrations which were over the critical level (>20 mg kg^{-1}) (Jones, 1998). Positive effects of P supply on root development help to increase Zn uptake by roots (Zhang et al., 2016). The results (de Oliveira and de Oliveira, 2005) reveled that mycorrhizae association was significatly correalated with banana leaf P and Zn concentation.

The selection of an efficient combination of AM fungus/growth medium cultivars should be a consideration in the improvement of banana plantlets production under greenhouse conditions and subsequent transplantation to the field.

5. Conclusion

Our acclimatization and propagation experiment illustrated that, banana plantlets (Dwarf Cavendish) are strongly dependent on growth media and soil is found to be best substrate for root colonization and mycorrhizal dependency. Both mycorrhizae are growth media specific resulted in variability of banana plantlets acclimatization and nutrient uptake. Mycorrhizal inoculation specificity for the substrate propagated plant dry mass by assisting in nutrient uptake. Considering the widespread and complex nature of the plant-AM fungi symbiosis, our results indicate the need for future research on interaction among micropropagated plant, AM fungi and substrates including the dry mass production, root colonization, nutrient uptake, yield and substrate quality.

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