Selection of arbuscular mycorrhizal fungi for sugarcane in four soils with the presence of dark septate endophytes

Rosalba Ortega Fors[1]* [iD]**, Orivaldo José Saggin Júnior[2], Marco Aurélio Carbone Carneiro[3] and Ricardo Luis Louro Berbara[1]**

[1]Departamento de Solos, Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, BR-465, km 07, 23897-000, Seropédica, Rio de Janeiro, Brazil. [2]Empresa Brasileira de Pesquisa Agropecuária, Seropédica, Rio de Janeiro, Brazil. [3]Departamento de Ciência do Solo, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. *Author for correspondence. E-mail: rosalbaof@gmail.com

ABSTRACT. The present study aimed to select efficient arbuscular mycorrhizal fungi (AMF) for sugarcane growth and P nutrition in four soils that spontaneously contained dark septate endophytes (DSE). The effect of nine AMF isolates was evaluated individually in sugarcane presprouted seedlings (SP81-3250) grown under greenhouse conditions for a 120-day period. The isolates that stimulated plant growth in the soils with low P availability were *Acaulospora colombiana* (ACOL)*, Claroideoglomus etunicatum* (CETU)*, Gigaspora margarita* (GMAR)*, Rhizophagus clarus* (RCLA) and *Scutellospora calospora* (SCAL). Compared to the Yellow Argisol, which had the highest P level, the Red-Yellow Argisol, with an intermediate P content, increased plant height. Compared to the other treatments, inoculation with ACOL, RCLA, and SCAL resulted in higher foliar P content in plants grown in soils with high to intermediate P levels. Root colonization by AMF and DSE was verified in the plants, with the coexistence of both fungal groups in the same plant and/or root fragment. However, AMF colonization was low compared to DSE colonization. The cooccurrence of DSE and AMF was higher in the plants inoculated with ACOL, RCLA, SCAL, and *Dentiscutata heterogama*. ACOL, CETU, GMAR, RCLA, and SCAL are AMF isolates that have the potential to establish a mycorrhizal inoculant for sugarcane that would be effective in several soils.

Keywords: *Saccharum* sp.; glomeromycota; inoculants; plant nutrition; presprouted seedlings.

Introduction

The demand for alternatives that reduce dependence on agricultural external inputs, such as fertilizers, has increased in recent years (Owen, Williams, Griffith, & Withers, 2015; Sruthilaxmi & Babu, 2017). Phosphorus (P) stands out as a macronutrient that is essential for plant development and highly necessary for Brazilian soils. The national reserves are igneous and low consisting of approximately 337 million tons of P_2O_5, which represents approximately 0.72% of the global reserves (Souza & Fonseca, 2009). Since Brazil is the fourth largest consumer of fertilizers in the world, dependence on foreign reserves of this nutrient can become a risk factor for Brazilian agriculture (Cordell & White, 2011). Additionally, the indiscriminate use of this nutrient in the soils of the country has been responsible for the eutrophication of water bodies, resulting in a serious environmental problem (Klein & Agne, 2012). Therefore, reducing the dependence on phosphate fertilization is a matter of sustainability and sovereignty for Brazilian agriculture.

The development of biotechnologies that contribute to the reduction of P use in the primary crops represents a promising advance for the agricultural economy (Owen et al., 2015). Arbuscular mycorrhizal fungi (AMF) (Phylum Glomeromycota) can be considered a viable alternative to improve the efficiency of P use and absorption by plants. AMF establishes a mutualistic symbiosis with plants, including most of the agricultural crops (Varma, 2008). In addition to other benefits, this symbiosis increases the vegetal absorption of water and nutrients, especially P (Cardoso, Cardoso, Nogueira, Baretta, & Paula, 2010).

The benefit of AMF for P absorption can result in great savings in Brazil's extensive crops, such as sugarcane, which is planted on approximately nine million hectares. Brazil is the largest sugarcane producer worldwide, with 674 million tons harvested in 2018 (IBGE, 2018). Due to this production, sugarcane consumes approximately 15% of the fertilizers used in Brazilian agriculture (Rosetto, Dias, & Vitti, 2010).

Any reduction of P use on this crop could save large amounts of money for the country. Presprouted seedlings, a relatively new sugarcane planting system (Landell et al., 2013), offer a favorable opportunity for easy inoculation with AMF.

Few studies have been conducted on the efficiency of AMF inoculation in sugarcane, and the results obtained remain controversial. Some authors report a low dependence of this crop on mycorrhizal symbiosis (Kelly, Edwards, Thompson, & Magarey, 2005). However, under other conditions, Surendran and Vani (2013) observed mycorrhizal colonization of up to 97% in sugarcane plants inoculated with AMF. These authors reported increments of the Pure Obtainable Cane Sugar (POCS) and brix%, and reduction of 25% of the P fertilizer for soils with medium P levels, when sugarcane plants were inoculated with mycorrhizae. Due to the diversity of soils and crop managements on which the sugarcane crop grows in the Brazilian territory, the selection of efficient AMF will have to be performed in the presence of associated biota and different fertility conditions. This way, fungi selected to promote the phosphate nutrition of sugarcane can be added as an inoculant comprised of multiple species that can function in different soils.

In this context, this study aimed to select efficient AMF isolates for sugarcane growth and P nutrition in four different soils with the spontaneous presence of dark septate endophytes.

Material and methods

A greenhouse experiment was conducted at the Embrapa Agrobiology in a factorial 11 x 4 (11 inoculation x 4 soil treatments) randomized block design with five replicates. The nine AMF isolates inoculated individually were: ACOL: *Acaulospora colombiana* A15; AMOR: *Acaulospora morrowiae* A79; ASCR: *Acaulospora scrobiculata* A38; CETU: *Claroideoglomus etunicatum* A44; DHET: *Dentiscutata heterogama* A2; ECON: *Entrophospora contigua* A28; GMAR: *Gigaspora margarita* A1; RCLA: *Rhizophagus clarus* A5 and SCAL: *Scutellospora calospora* A80. The AMF isolates were obtained from the Collection of Mycorrhizal Fungi of Embrapa Agrobiology (COFMEA) belonging to the Biological Resource Center Dr. Johanna Döbereiner (CRB-JD) in Seropédica, Rio de Janeiro State, Brazil. Inoculation treatments were completed with two controls that were not inoculated.

The second factor of the experiment consisted of four soils collected in the 0-20 cm depth layer (Table 1). The first two soils were collected in Quatá, São Paulo and included a Red-Yellow Argisol (RYA) from a new sugarcane cultivation area (22.138361° S 50.757750° W, 497 m) and a Yellow Argisol (YA) from an 18-year-old sugarcane monoculture (22.191500° S 50.682389° W, 436 m). The third soil corresponded to a Red Argisol (RA) collected in a road cut ravine at Itumirim, Minas Gerais (21.281416° S 44.860550° W, 944 m). The fourth soil was a Haplic Planosol (HP) collected in the experimental field "Terraço" of Embrapa Agrobiology at Seropédica, Rio de Janeiro (22.749274° S 43.666626° W, 26 m).

Plant and microbial propagules in the soils were reduced by the application of 0.72% formaldehyde (50 mL per kg of soil), followed by solarization for 72 consecutive hours in transparent polyethylene bags. Subsequently, the soils were limed and fertilized based on the chemical analyses (Table 1) and the recommended amount of fertilization for sugarcane in the State of Rio de Janeiro, Brazil (Freire et al., 2013). All the AMF inoculation treatments and the noninoculated control (CNI) received ⅓ P and ½ K of the recommended dose of fertilizer applied in the form of KH_2PO_4. The noninoculated control with total fertilization (CNI + F) received the full P and K fertilization recommended for sugarcane. Hoagland nutrient solution, modified by Jarstfer and Sylvia (1995), was applied weekly (50 mL pot^{-1}).

Table 1. Chemical characterization of the soils under greenhouse conditions used to select AMF isolates efficient in promoting sugarcane growth and P nutrition.

Soil	pH in water 2:1	P	K	Ca	Mg	Al	H + Al	C
		mg dm^{-3}		------------- cmol$_c$ dm^{-3} -------------				g kg^{-1}
RYA	5.35	6.60	39	1.12	0.45	0.03	1.62	2.8
YA	5.98	9.35	44	1.65	0.60	0.01	1.95	3.2
RA	5.30	0.55	30	0.46	0.07	0.00	2.05	3.4
HP	5.07	2.54	21	0.61	0.19	0.21	2.56	3.0

RYA: Red-Yellow Argisol; YA: Yellow Argisol; RA: Red Argisol; HP: Haplic Planosol. Analyses conducted as described by Nogueira and Souza (2005).

Sugarcane seedlings, variety SP81-3250, were obtained from one-cm-long single bud cuttings based on the presprouted seedlings system (Landell et al., 2013). The cuttings were submitted to heat (52°C for 30 minutes) and an antifungal (Comet® BASF S.A., São Paulo, Brazil- Pyraclostrobin 2.5 g L^{-1} for 3 minutes) treatments before being planted in sterile sand: vermiculite (2:1 v/v). One presprouted seedling was planted per pot containing 3 kg of soil, and approximately 100 AMF spores were inoculated per plant in the form of soil inocula.

The phosphate nutrition status of the plants was evaluated periodically by sampling 8 mm discs from the middle third of the newest totally developed leaf (leaf+1). The P content and quantity in the discs were analyzed (Aziz & Habte, 1987) at 60 and 120 days after the transplantation and inoculation of the plants. The plant height up to the insertion of the leaf+1 was measured, and the plant leaf area was estimated according to Hermann and Câmara (1999).

The plants were collected 120 days after transplantation and inoculation. The leaves were dried in a laboratory humidity drying oven (60°C), ground in a Wiley mill and analyzed for P content in the total leaf tissue (Silva, 2009). Approximately 0.5 g of fresh roots was sampled from each plant. The root samples were clarified and stained as described in the Manual of Basic Techniques for Arbuscular Mycorrhizae published by Novais et al. (2017), and colonization by AMF and DSE was evaluated. Colonization was analyzed in root segments of 1 cm placed on microscope slides using glycerol (50%) as the mounting medium. The percentage of root colonization was estimated using an optical microscope in 100 visual fields of 40x. The simultaneous colonization of AMF and DSE in the same visual field and of each of these fungi alone was quantified. The spores of AMF (glomerospores) were extracted from the soil by wet sieving and decanting as described by Novais et al. (2017). The density of glomerospores, expressed as the total number of spores extracted from 50 cm^3 of soil, was quantified under a stereoscopic microscope. Statistical analysis of variance (ANOVA) and the means comparison by Scott-Knott test ($p < 0.05$) were conducted using the SISVAR program (Ferreira, 2011).

Results and discussion

The plants grown in the Argisol from the new sugarcane cultivation area (RYA) had higher height and leaf area at 60 and 120 days after their transplantation and AMF inoculation. In the Planosol (HP) and the Yellow Argisol (YA), the plants had intermediate height and leaf areas. Use of the Red Argisol (RA) resulted in the lowest height and leaf area of the sugarcane plants (Table 2).

P nutrition was analyzed using the P content and quantity in the sampled discs and the P content in the total leaf tissue. The latter variables showed a clear and concordant soil effect. The highest values of the P content in the leaves were verified in the plants grown in the YA (Table 2), followed by those of the RYA and the HP. However, the lowest foliar P content and quantity corresponded with the plants of the RA.

Table 2. Height, leaf area, P content and quantity in foliar discs, and P content in total leaf tissues of sugarcane grown in different soils 60 and 120 days after inoculation with arbuscular mycorrhizal fungi. Primary effects of the soil factor.

Soil	Height (cm)		Leaf area (cm^2)		P content in foliar discs (g kg^{-1})		P quantity in foliar discs (µg P $disc^{-1}$)		P content (g kg^{-1})
	60	120	60	120	60	120	60	120	120
RYA	216 a	431 a	734 a	768 a	1.05 b	1.03 b	4.12 a	4.19 b	0.96 b
YA	187 c	379 c	592 b	689 b	1.22 a	1.17 a	4.00 a	4.78 a	1.11 a
RA	117 d	174 d	258 c	271 c	0.57 d	0.60 d	1.79 c	1.95 d	0.41 d
HP	199 b	409 b	593 b	680 b	0.89 c	0.89 c	3.39 b	3.77 c	0.80 c

RYA: Red-Yellow Argisol; YA: Yellow Argisol; RA: Red Argisol; HP: Haplic Planosol. Letters in columns compare means using the Scott-Knott test ($p < 0.05$).

The effect of the inoculation treatments on the growth of the plants was significant up to 60 days after the start of the experiment and decreased near the end of the experiment at 120 days after inoculation. This finding indicates a quick response of the sugarcane plants to AMF inoculation, probably due to the rapid growth and vigor of this crop. A longer inoculation effect on the growth of the plants could exist. However, the pots used in this experiment rapidly became limited due to the development of the plants (Figure 1). Future studies involving the sugarcane inoculation with AMF should consider the limitations of the rapid growth of the sugarcane in pots.

Figure 1. Root system of sugarcane plants completely filling the soil volume of a pot at 120 days after inoculation with arbuscular mycorrhizal fungi.

The height of the sugarcane plants was influenced by inoculation treatments in the RA and HP only, which were the soils low in available P. However, this effect was not observed in the RYA and YA, which had higher P levels (Table 3). In the RA, the sugarcane plants inoculated with ACOL, CETU, ECON, GMAR, and SCAL were taller than the CNI but not different in height from CNI + F plants. In the HP, inoculation with ACOL, ASCR, CETU, GMAR, RCLA, and SCAL resulted in higher plants compared to those of the control treatments (CNI and CNI + F). The effect of the inoculation effect on the height of the plants was not observed in any of the soils 120 days after the inoculation. Alternatively, significant differences between the leaf areas in the treatments were only detected in the RYA at 60 days after the inoculation, while the plants inoculated with RCLA and SCAL had higher leaf area values than those of the two control treatments (Table 3).

Phosphorus nutrition, evaluated by the P content and quantity in leaf discs, was positively influenced by some of the inoculation treatments in the RYA and YA but not in the RA and HP (Table 3). The foliar discs clearly reflected the P availability in each of the four soils and were very sensitive to the effect of the treatments on the P nutrition of the plants up to 120 days after the inoculation. The AMF species that stimulated P nutrition by the plants included ACOL, CETU, DHET, RCLA, and SCAL. In the RYA, the plants inoculated with these AMF had a greater content and quantity of P in their leaves than did CNI plants, and the levels of P were as high as those in CNI + F plants. For plants grown in the YA, the P nutrition of the plants grown in the YA was better only when they were inoculated with ACOL and CETU.

The total P content of the leaves, evaluated at the end of the experiment, was consistent with the results obtained from measuring the P concentration and quantity in foliar discs (Table 3). The diagnosis of the sugarcane nutritional state has several limitations (Rossetto et al., 2010), but leaf+1 is recommended for field analyses from samples of 100 plants. Therefore, our results strongly recommend utilizing P disc analysis for sugarcane nutritional assessments in experiments, particularly those involving AMF inoculation.

In the RYA, plants inoculated with ACOL, AMOR, DHET, GMAR, RCLA, and SCAL had a higher P content than did the CNI. These AMF species and soil combinations resulted in P contents as high as those of the plants that received total fertilization (CNI + F). In the plants grown in the YA, only those inoculated with ACOL, RCLA, and SCAL had higher values of P content than did the CNI. In the HP, compared with the CNI, only SCAL stimulated higher levels of P absorption. Inoculation with AMF had no effect on the total foliar P content of the plants grown in the RA.

The results involving the P nutrition suggest that improvements in the uptake of P are the primary benefit of the mycorrhizal association for plants (Cardoso et al., 2010; Basu, Rabara, & Negi, 2018). At the same time, they suggest that some of the AMF species inoculated favor P absorption, at least in the soils collected from the sugarcane plantations located in Quatá, São Paulo State. For the RA, which had very low

available P, there was no effect on the P content in the total leaf tissues of the plants from the effects of inoculation or complete fertilization, indicating that this soil demonstrates a high capacity to fix phosphate.

Table 3. Height, leaf area, P content and quantity in foliar discs and the P content in total leaf tissues of sugarcane plants at 60 and 120 days after inoculation with arbuscular mycorrhizal fungi.

Inoculation treatments	Height(cm)		Leaf area(cm²)		P content in foliar discs (g kg⁻¹)		P quantity in foliar discs (µg P disc⁻¹)		P content (g kg⁻¹)
	60	120	60	120	60	120	60	120	120
Inoculation factor unfolding within Red-Yellow Argisol (RYA)									
ACOL	211 a	408 a	742 b	739 a	1.18 a	1.13 a	4.74 a	4.43 a	1.00 a
AMOR	212 a	438 a	658 c	828 a	1.01 b	1.05 a	3.80 b	4.17 a	1.02 a
ASCR	209 a	419 a	550 c	786 a	0.93 b	0.84 a	3.74 b	3.59 b	0.81 b
CETU	213 a	413 a	765 b	836 a	1.19 a	1.09 a	4.88 a	4.38 a	0.89 b
DHET	216 a	413 a	572 c	785 a	1.25 a	1.24 a	5.04 a	4.84 a	1.08 a
ECON	226 a	429 a	767 b	777 a	0.83 b	0.85 a	3.33 b	3.67 b	0.82 b
GMAR	219 a	426 a	723 b	792 a	1.00 b	0.99 a	3.87 b	3.76 b	0.99 a
RCLA	219 a	413 a	897 a	714 a	1.14 a	1.05 a	4.33 a	4.24 a	1.00 a
SCAL	233 a	453 a	950 a	621 a	1.02 b	1.02 a	3.90 b	4.32 a	0.96 a
CNI	205 a	461 a	773 b	751 a	0.88 b	0.94 a	3.22 b	3.77 b	0.87 b
CNI + F	210 a	464 a	681 c	818 a	1.15 a	1.17 a	4.44 a	4.96 a	1.07 a
Inoculation factor unfolding within Yellow Argisol (YA)									
ACOL	207 a	370 a	531 a	689 a	1.65 a	1.57 a	4.72 a	5.76 a	1.23 a
AMOR	190 a	385 a	642 a	623 a	1.16 b	1.17 b	3.88 a	4.99 a	1.09 b
ASCR	179 a	377 a	449 a	710 a	1.06 b	1.05 b	3.58 a	4.22 b	1.02 b
CETU	193 a	381 a	625 a	704 a	1.26 b	1.36 a	4.03 a	5.43 a	1.10 b
DHET	168 a	339 a	724 a	688 a	1.05 b	0.99 b	3.74 a	4.31 b	1.10 b
ECON	184 a	396 a	644 a	650 a	1.38 a	1.01 b	4.44 a	4.12 b	0.98 b
GMAR	202 a	377 a	629 a	676 a	1.07 b	1.03 b	3.73 a	4.29 b	0.96 b
RCLA	181 a	345 a	560 a	682 a	1.26 b	1.19 b	4.21 a	5.03 a	1.23 a
SCAL	186 a	360 a	595 a	657 a	1.01 b	1.14 b	3.33 a	4.85 a	1.18 a
CNI	177 a	409 a	468 a	797 a	1.23 b	1.12 b	4.14 a	4.38 b	1.06 b
CNI + F	192 a	434 a	647 a	703 a	1.24 b	1.20 b	4.19 a	5.24 a	1.27 a
Inoculation factor unfolding within Red Argisol (RA)									
ACOL	128 a	198 a	264 a	326 a	0.69 a	0.59 a	1.92 a	1.86 a	0.39 a
AMOR	107 b	162 a	218 a	247 a	0.61 a	0.62 a	1.99 a	1.94 a	0.42 a
ASCR	92 b	181 a	192 a	300 a	0.56 a	0.64 a	1.75 a	2.07 a	0.41 a
CETU	127 a	167 a	315 a	267 a	0.56 a	0.54 a	1.92 a	1.75 a	0.40 a
DHET	111 b	197 a	258 a	333 a	0.65 a	0.72 a	2.06 a	2.24 a	0.42 a
ECON	124 a	162 a	257 a	208 a	0.46 a	0.55 a	1.42 a	1.89 a	0.44 a
GMAR	120 a	162 a	277 a	217 a	0.54 a	0.57 a	1.49 a	1.85 a	0.42 a
RCLA	113 b	167 a	257 a	206 a	0.53 a	0.57 a	1.72 a	1.66 a	0.40 a
SCAL	141 a	192 a	307 a	358 a	0.54 a	0.61 a	1.68 a	2.30 a	0.39 a
CNI	92 b	140 a	205 a	218 a	0.62 a	0.65 a	1.82 a	1.91 a	0.42 a
CNI + F	130 a	187 a	287 a	301 a	0.56 a	0.57 a	1.89 a	1.98 a	0.40a
Inoculation factor unfolding within Haplic Planosol (HP)									
ACOL	212 a	440 a	551 a	642 a	0.95 a	0.95 a	3.44 a	3.96 a	0.75 b
AMOR	182 b	390 a	599 a	629 a	0.83 a	0.93 a	3.24 a	3.87 a	0.79 b
ASCR	210 a	421 a	692 a	783 a	0.77 a	0.91 a	2.92 a	3.72 a	0.75 b
CETU	217 a	431 a	549 a	709 a	0.88 a	0.91 a	3.14 a	3.89 a	0.79 b
DHET	191 b	376 a	548 a	621 a	0.86 a	0.82 a	3.36 a	3.55 a	0.77 b
ECON	190 b	422 a	512 a	777 a	0.87 a	0.88 a	3.25 a	4.00 a	0.78 b
GMAR	200 a	414 a	608 a	667 a	0.94 a	0.88 a	3.73 a	3.78 a	0.72 b
RCLA	215 a	418 a	679 a	735 a	0.78 a	0.86 a	3.08 a	3.58 a	0.66 b
SCAL	201 a	440 a	594 a	648 a	1.09 a	0.89 a	3.81 a	3.58 a	0.89 a
CNI	186 b	365 a	518 a	563 a	0.81 a	0.85 a	3.05 a	3.65 a	0.83 b
CNI + F	184 b	379 a	669 a	703 a	1.07 a	0.87 a	4.24 a	3.84 a	1.01 a

ACOL: *A. colombiana;* AMOR: *A. morrowiae;* ASCR: *A. scrobiculata;* CETU: *C. etunicatum;* DHET: *D. heterogama;* ECON: *E. contigua;* GMAR: *Gi. margarita;* RCLA: *R. clarus;* SCAL: *S. calospora;* CNI: control noninoculated; CNI + F: control noninoculated with total fertilization. Letters in columns compare means by the Scott-Knott test (p < 0.05) within each soil.

P is an essential nutrient for plants and, at the same time, it is the most limiting factor for Brazilian agriculture, in addition to serving as a regulator of the mycorrhizal response of plants (Simpson et al., 2011; Nouri, Breuillin-Sessoms, Feller, & Reinhardt, 2014). Studies have reported that the arbuscular mycorrhizal symbiosis is negatively influenced by extreme (very low and high) values of available P in

the soil (Kiriachek, Azevedo, Peres, & Lambais, 2009; Nouri et al., 2014). Therefore, the YA and the RA may have contained very high and low concentrations of P, respectively, hindering any mycorrhizal benefit as was described by Kelly et al. (2005). However, a recent study shows that the soil nutrient availability, and P availability in particular, does not necessarily represent the best predictor of AMF inoculation success (Köhl, Lukasiewicz, & Heijden, 2016). The authors suggest that other factors, such as the inoculum potential and the native AMF community composition, may have a greater influence on the response of the plants to the AMF inoculation. In this study, the native AMF community could have influenced the effect of the inoculated species, since the soils used for the experiment were not exempt from native AMF. The application of formaldehyde, followed by solarization, did not guarantee that the soil was sterile; therefore, the native AMF communities could have simply been weakened.

The roots of the sugarcane plants showed typical structures of the arbuscular mycorrhizal symbiosis and of DSE (Figure 2). The rates of root colonization by the treatment within each of the soils are shown in Figure 3. Regardless of the inoculation treatment, the plants grown in the YA were characterized as being totally colonized by the DSE that always exceeded 85%. In the RA, the average colonization of the roots by this fungal group was 100%. In the RYA and HP, some AMF inoculation treatments caused decreases in the DSE root colonization of the plants. Among these two soils, in the RYA, the rate of occurrence of DSE in the plants inoculated with ASCR, GMAR, and RCLA ranged from 60 to 70%. In the HP, the lower values of the total DSE colonization in the plants were related to the inoculation with ACOL, ASCR, CETU, and SCAL in addition to the CNI. The total DSE in these treated plants ranged from 10 to 50%, while for the rest of the treatments, the rate of colonization by DSE always exceeded 75%.

The role of DSE in promoting plant growth has been previously studied (Mandyam & Jumpponen, 2015; Spagnoletti, Tobar, Prado, Chiocchio, & Lavado, 2017; Vergara et al., 2017). However, the research is recent, and a more thorough understanding of the functionality of this fungal group is still needed. Their coexistence with AMF has been reported in several plant groups (Nagaraj, Priyadharsini, & Muthukumar, 2015; Gucwa-Przepióra, Chmura, & Sokołowska, 2016; Thangavelu & Raji, 2016). Among the few studies on DSE in sugarcane, Nasim, Ali, Munawar, and Bajwa (2008) reported that 80% of the root samples were colonized by the DSE, suggesting that DSE have a role in the biocontrol of sugarcane diseases. Xie et al. (2013) evaluated the inoculation effect of six strains of DSE fungi that originated in Japan on sugarcane growth and verified increases in height (69%) and plant mass (57%) compared to the noninoculated control, especially with the strain LtVB3 (*Meliniomyces variabilis*).

In general, colonization by DSE and AMF was higher in the plants inoculated with ACOL, DHET and SCAL. However, in the HP, the cooccurrence of these two fungal groups was not influenced by any of the inoculation treatments. The total AMF colonization was higher in the plants inoculated with DHET, ACOL, AMOR, and RCLA than in the RYA. The inoculation with ACOL, DHET, RCLA, and SCAL in the YA favored higher values of the total AMF colonization in the plants. In the RA, this variable was higher when the ACOL, DHET, and SCAL were inoculated, while in the HP, only the SCAL induced higher colonization levels.

The presence of abundant DSE root colonization in this study might be due to potential infection. The first possible source is the soils themselves, since they were not sterilized and were exposed to diluted formaldehyde and solarization only. Another possibility might have been the introduction of DSE with the soil inoculum used to inoculate AMF. However, DSE were also abundant in the noninoculated controls, which strongly suggests that this was not the case. A third alternative is the introduction of DSE with the irrigation water. Finally, the sugarcane stalks, which were cut to presprout the seedlings, are another potential source of DSE. Posterior cuts made in the sugarcane stalks near the buds showed fungal structures similar to those of DSE. This observation strongly suggests that sugarcane stalks may be the primary source of inoculum for DSE colonization. The occurrence of fungal endophytes in various plant organs is common (Jin et al., 2013; Hardoim et al., 2015). However, DSE have been reported only in plant roots. Thus, their occurrence in sugarcane stalks could be confirmed by future studies.
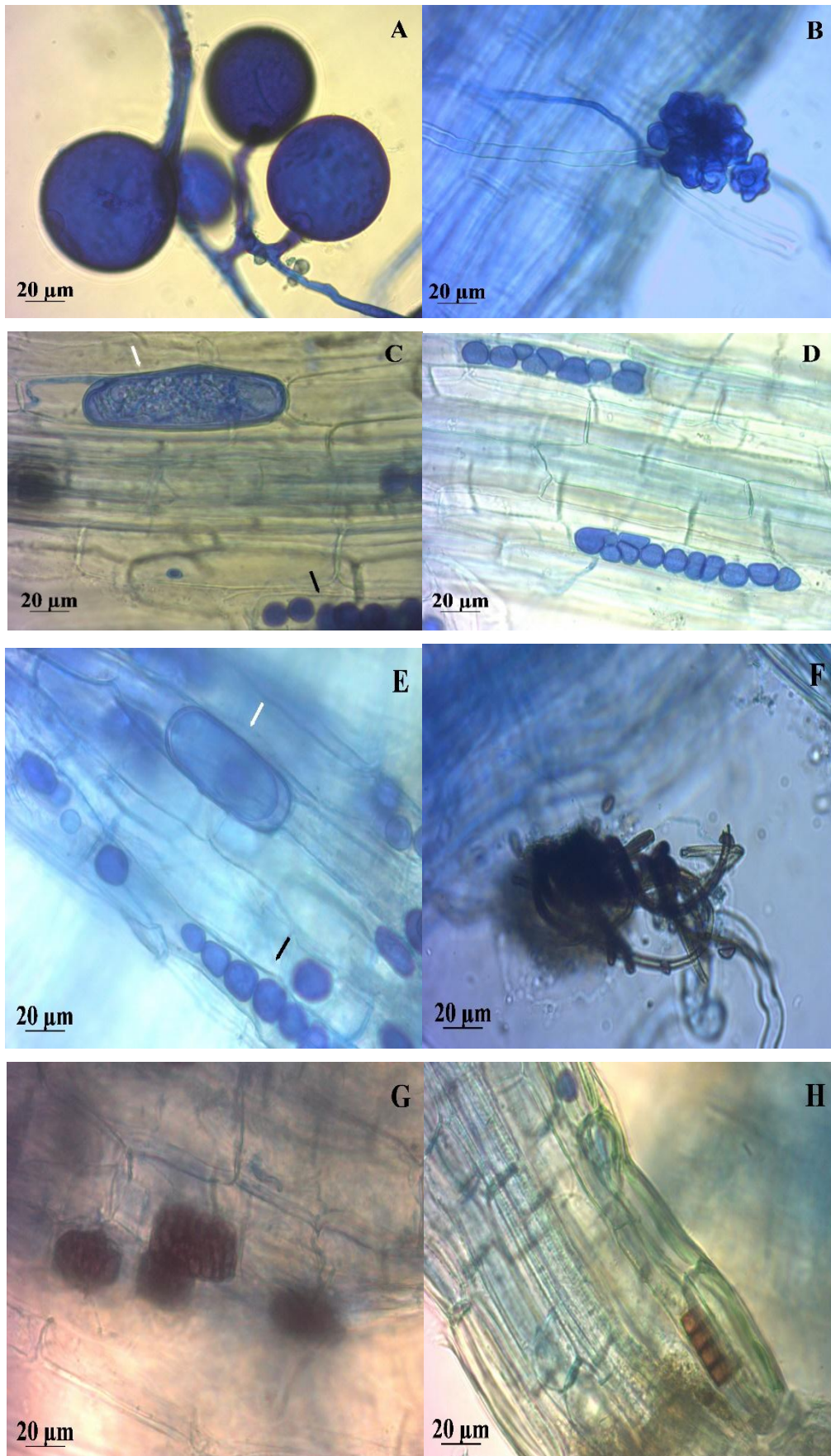
Figure 2. Fungal structures in the sugarcane roots 120 days after inoculation with arbuscular mycorrhizal fungi (AMF) (A-H). A: Glomoid spores of AMF; B: AMF auxiliary cells; C: AMF spore (white arrow) and chlamydospores of the dark septate endophytes (DSE) (black arrow); D: DSE microsclerotia; E: AMF spore (white arrow) and DSE chlamydospores (black arrow); F: coiled hyphae of DSE; G and H: DSE microsclerotia.
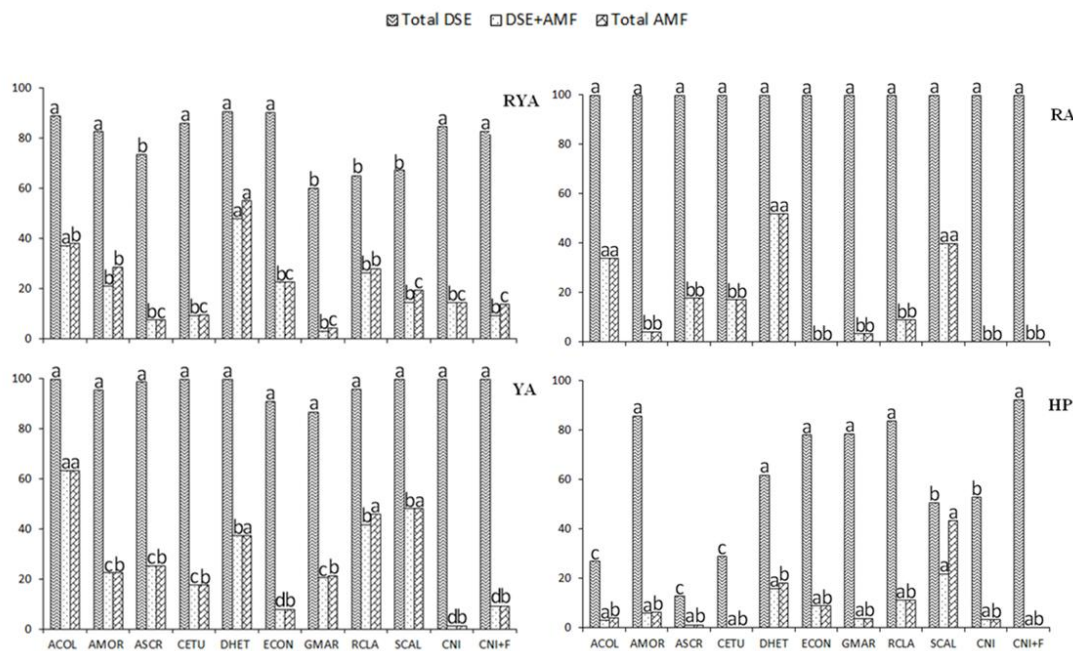
Figure 3. Dark septate endophytes (DSE) and arbuscular mycorrhizal fungi (AMF) root colonization in sugarcane 120 days after inoculation with the AMF. DSE + AMF: DSE and AMF conjunct colonization in the same root segment (CV%: 83.64). Total DSE: Total colonization of DSE, including DSE and DSE + AMF (CV%: 18.49); Total AMF: Total colonization of AMF, including AMF and DSE + AMF (CV%: 81.4%); ACOL: *A. colombiana*; AMOR: *A. morrowiae*; ASCR: *A. scrobiculata*; CETU: *C. etunicatum*; DHET: *D. heterogama*; ECON: *E. contigua*; GMAR: *Gi. margarita*; RCLA: *R. clarus*; SCAL: *S. calospora*; CNI: control noninoculated; CNI + F: control noninoculated with complete fertilization. Letters on same pattern bars compare means using the Scott-Knott test (p < 0.05).

Several researchers have reported the simultaneous colonization of AMF and DSE in different plant species (Gucwa-Przepióra et al., 2016; Thangavelu & Raji, 2016). However, the functioning and importance of the triple interaction "DSE: AMF: plants" is not commonly studied; therefore, it is not fully understood (Debnath et al., 2015; Della Monica, Saparrat, Godeas, & Scervino, 2015). As mentioned above, some studies report niche sharing by both symbionts. However, few studies have evaluated the possible links between AMF and DSE. One pioneering study in this area is that of Scervino et al. (2009) who verified the effect of different concentrations of the exudates of *Dreschlera* sp. (DSE) on the development of *Gigaspora rosea* (AMF). The exudates of *Dreschlera* sp. were modulators of AMF hyphae development, stimulating or inhibiting their growth, depending on their concentration.

The effect of the soil on the sporulation of AMF fungi is shown in Table 4. In general, the highest number of glomerospores (AMF spores) was unaffected by the inoculation status and was recovered from the YA, followed by the RYA, the HP and the RA. However, the number of glomerospores of the inoculated AMF species was higher in the YA and the HP compared to the other two soils. At the species level, *Oehlia diaphana*, a noninoculated and probable native species had abundant sporulation in the RYA. Alternatively, the glomerospores of *Glomus* sp, a noninoculated/native species, AMOR and GMAR were more numerous in the YA compared to the other three soils. In the RA and HP, the species that sporulated the most was ACOL. The density of the glomerospores of the other inoculated species did not differ between the four soils (Table 4).

Table 4. Density of the spores of arbuscular mycorrhizal fungi (AMF) (glomerospores) in 50 cm^3 of four soils cultivated with sugarcane 120 days after inoculation with AMF. Primary effect of the soils.

Soil	Total	Inoculated	ODIA	ACOL	AMOR	GSP	GMAR	SCAL	AMEL	ECON	ASCR	DHET	CETU
RYA	398 b	49 b	272 a	6 b	103 b	1 b	0 b	7 a	3 a	0 a	0 a	4 a	0 a
YA	673 a	271 a	51 b	5 b	435 a	4 a	6 a	14 a	0 a	12 a	2 a	12 a	46 a
RA	119 c	57 b	18 b	28 a	56 b	1 b	0 b	3 a	0 a	0 a	5 a	10 a	0 a
HP	257 c	165 a	0 b	41 a	190 b	1 b	0 b	13 a	0 a	0 a	7 a	6 a	0 a

RYA: Red-Yellow Argisol; YA: Yellow Argisol; RA: Red Argisol; HP: Haplic Planosol. Letters in columns compare means using the Scott-Knott test (p < 0.05). Total: all extracted glomerospores; Inoculated: Glomerospores of inoculated fungi; Glomerospores of ODIA: *Oehlia diaphana*; ACOL: *Acaulospora colombiana*; AMOR: *Acaulospora morrowiae*; GSP: *Glomus* sp; GMAR: *Gigaspora margarita*; SCAL: *Scutellospora calospora;* AMEL: *Acaulospora mellea*; ECON: *Entrophospora contigua*; ASCR: *Acaulospora scrobiculata*; DHET: *Dentiscutata heterogama*; CETU: *Claroideoglomus etunicatum*.

Conclusion

Soil P availability influences the response of sugarcane to AMF inoculation. ACOL, CETU, GMAR, RCLA, and SCAL increase the height of the plants in low P soils (RA and HP). ACOL, RCLA, and SCAL improve P foliar content of the plants in soils with intermediate and high P levels (YA and RYA). Simultaneous root colonization by AMF and DSE is stimulated by ACOL, RCLA, SCA, and DHET, fungi that enhance the growth and P nutrition of the plants. ACOL, CETU, GMAR, RCLA, and SCAL are AMF with the potential to compose a mycorrhizal inoculant for sugarcane, effective in several soils.

Acknowledgements

References

Aziz, T., & Habte, M. (1987). Determining vesicular-arbuscular mycorrhizal effectiveness by monitoring P status of leaf disks. *Canadian Journal of Microbiology*, *33*(12), 1097-1101. DOI: 10.1139/m87-191

Basu, S., Rabara, R. C., & Negi, S. (2018). AMF: The future prospect for sustainable agriculture. *Physiological and Molecular Plant Pathology*, *102(1)*, 36-45. DOI: 10.1016/j.pmpp.2017.11.007

Cardoso, E. J. B. N., Cardoso, I. M., Nogueira, M. A., Baretta, C. R. D. M., & Paula, A. M. (2010). Micorrizas Arbusculares na aquisição de nutrientes pelas plantas. In J.O. Siqueira, F. A. Souza, E. J. B. N. Cardoso, & S. M. Tsai (Eds.), *Micorrizas*: 30 anos de pesquisas no Brasil (p. 153-214). Lavras, MG: UFLA.

Cordell, D., & White, S. (2011). Peak phosphorus: clarifying the key issues of a vigorous debate about long-term phosphorus security. *Sustainability*, *3(10)*, 2027-2049. DOI: 10.3390/su3102027

Debnath, A., Karmakar, P., Debnath, S., Roy Das, A., Saha, A. K., & Das, P. (2015). Arbuscular mycorrhizal and dark septate endophyte fungal association in some plants of Tripura, North East India. *Current Research on Environmental & Applied Mycology*, *5*(4), 398-407. DOI: 10.5943/cream/5/4/12

Della Monica, I. F., Saparrat, M. C. N., Godeas, A. M., & Scervino, J. M. (2015). The co-existence between DSE and AMF symbionts affects plant P pools through P mineralization and solubilization processes. *Fungal Ecology*, *17(1)*, 10-17. DOI: 10.1016/j.funeco.2015.04.004

Ferreira, D. F. (2011). Sisvar: a computer statistic alanalysis system. *Ciência e Agrotecnologia*, *35*(6), 1039-1042. DOI: 10.1590/S1413-705420raji11000600001

Freire, L. R., Balieiro, F. C., Zonta, E., Anjos, L. H. C., Pereira, M. G., Lima, E., ... Polidoro, J. C. (2013). *Manual de calagem e adubação do Estado do Rio de Janeiro*. Brasília, DF: Embrapa; Seropédica, RJ: Editora Universidade Rural.

Gucwa-Przepióra, E., Chmura, D., & Sokołowska K. (2016). AM and DSE colonization of invasive plants in urban habitat: a study of Upper Silesia (southern Poland). *Journal of Plant Research*, *129*(4), 603-614. DOI: 10.1007/s10265-016-0802-7

Instituto Brasileiro de Geografia e Estatística [IBGE]. (2018). *Indicadores IBGE*: *estatística da produção agrícola.* Retrieved on September 10, 2019 from https://sidra.ibge.gov.br/home/lspa/brasil

Hardoim, P. R., Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., ... Sessitsch, A. (2015). The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*, *79*(3), 293-320. DOI: 10.1128/MMBR.00050-14

Hermann, E. R., & Câmara, G. M. S. (1999). Um método simples para estimar a área foliar da cana-de-açúcar. *Revista da Sociedade dos Técnicos Açucareiros e Alcooleiros do Brasil*, *17*(5), 32-34.

Jarstfer, A. G., & Sylvia, D. M. (1995). Aeroponic culture of AMF. In A. Varma, & B. Hock (Ed.), *Mycorrhiza*: *Structure, function, molecular biology and biotechnology* (p. 427-441). Berlin, GE: Springer Verlag.

Jin, H., Yan, Z., Liu, Q., Yang, X., Chen, J., & Qin, B. (2013). Diversity and dynamics of fungal endophytes in leaves, stems and roots of *Stellera chamaejasme* L. in northwestern China. *Antonie van Leeuwenhoek*, *104*(6), 949–963. DOI: 10.1007/s10482-013-0014-2

Kelly, R. M., Edwards, D. G., Thompson, J. P., & Magarey, R. C. (2005). Growth responses of sugarcane to mycorrhizal spore density and phosphorus rate. *Australian Journal of Agricultural Research*, *56*(12), 1405-1413. DOI: 10.1071/AR04185

Kiriachek, S. G., Azevedo, L. C. B. D., Peres, L. E. P., & Lambais, M. R. (2009). Regulation of arbuscular mycorrhizae development. *Revista Brasileira de Ciência do Solo*, *33*(1), 1-16. DOI: 10.1590/S0100-06832009000100001

Klein, C., & Agne, S. A. A. (2012). Fósforo: de nutriente à poluente! *Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental*, *8*(8), 1713-1721. DOI: 10.5902/223611706430

Köhl, L., Lukasiewicz, C. E., & Heijden, M. G. A. (2016). Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. *Plant, Cell and Environment*, *39*(1), 136-146. DOI: 10.1111/pce.12600

Landell, M. G. A., Camapana, M. P., Figuereido, P., Xavier, M. A., Anjos, I. A., Dinardo-Miranda, L. L, ... Miguel, P. E. M. (2013). *Sistema de multiplicação de cana-de-açúcar com uso de mudas pré-brotadas (MPB), oriundas de gemas individualizadas*. Campinas, SP: IAC.

Mandyam, K. G., & Jumpponen, A. (2015). Mutualism-parasitism paradigm synthesized from results of root-endophyte models. *Frontiers in Microbiology*, *5*(1)*,* article 776, 1-13. DOI: 10.3389/fmicb.2014.00776

Nagaraj, K., Priyadharsini, P., & Muthukumar, T. (2015). Mycorrhizal and septate endophytic fungal associations in gymnosperms of southern India. *Anales de Biología*, *37(1)*, 83-94. DOI: 0.6018/analesbio.37.8

Nasim, G., Ali, A., Munawar, A., & Bajwa, R. (2008). Seasonal dynamics of AM fungi in sugarcane (*Saccharum officinarum* L. CV SPF-213) in relation to red rot (*Colletotrichum falcatum*) disease from Punjab, Pakistan. *Pakistan Journal of Botany*, *40*(6), 2587-2600.

Nogueira, A. R. A., & Souza, G. B. (2005). *Manual de laboratórios*: *solo, água, nutrição vegetal, nutrição animal e alimentos*. São Carlos, SP: Embrapa Pecuária Sudeste.

Nouri, E., Breuillin-Sessoms, F., Feller, U., & Reinhardt, D. (2014). Phosphorus and Nitrogen Regulate Arbuscular Mycorrhizal Symbiosis in *Petunia hybrida*. *PLoS ONE*, *9*(3), 1-14. DOI: 10.1371/journal.pone.0090841

Novais, C. B., Borges, W. L., Saggin Júnior, O. J., Sbrana, C., Giovannetti, M., & Siqueira, J. O. (2017). *Técnicas básicas em micorrizas arbusculares*. Lavras, MG: UFLA.

Owen, D., Williams, A. P., Griffith, G. W., & Withers, P. J. A. (2015). Use of commercial bio-inoculants to increase agricultural production through improved phosphorus acquisition. *Applied Soil Ecology*, *86(1)*, 41-54. DOI: 10.1016/j.apsoil.2014.09.012

Rosetto, R., Dias, F. L. F., & Vitti, A. C. (2010). Fertilidade do solo, nutrição e adubação. In L. L. Dinardo-Miranda, A. C. M. P. Vasconcelos, & M. G. A. Landell (Eds.), *Cana-de-açúcar* (p. 221-237). Campinas, SP: IAC.

Sceverino, J. M., Gottlieb, A., Silvani, V. A., Pérgola, M., Fernández, L., & Godeas, A. M. (2009). Exudates of dark septate endophyte (DSE) modulate the development of the arbuscular mycorrhizal fungus (AMF) *Gigaspora rosea*. *Soil Biology & Biochemistry*, *41*(8), 1753-1756. DOI: 10.1016/j.soilbio.2009.04.021

Silva, F. C. (2009). *Manual de análises químicas de solos, plantas e fertilizantes*. Brasílila, DF: Embrapa Informação Tecnológica.

Simpson, R. J., Oberson, A., Culvenor, R. A., Ryan, M. H., Veneklaas, E. J., Lambers, H., ... Richardson, A. E. (2011). Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. *Plant and Soil*, *349*(1), 89-120. DOI: 10.1007/s11104-011-0880-1

Souza, A. E., & Fonseca, D. S. (2009). *Fosfato*. Brasília D.F.: Departamento Nacional de Produção Mineral. Economia Mineral do Brasil (DNPM). Retrieved on Oct. 24, 2017 from http://www.dnpm.gov.br/dnpm/publicacoes/serie-estatisticas-e-economia-mineral/outras-publicacoes-1/7-2-fosfato

Spagnoletti, F. N., Tobar, N. E., Prado, A. F., Chiocchio, V. M., & Lavado, R. S. (2017). Dark septate endophytes present different potential to solubilize calcium, iron and aluminium phosphates. *Applied Soil Ecology*, *111*(1), 25-32. DOI: 10.1016/j.apsoil.2016.11.010

Sruthilaxmi, C. B., & Babu, S. (2017). Micorbial bio-inoculants in Indian agriculture: Ecological perspectives for a more optimized use. *Agriculture, Ecosystems and Environment*, *242*(1), 23-25. DOI: 10.1016/j.agee.2017.03.019

Thangavelu, M., & Raji, M. (2016). Arbuscular mycorrhizal and dark septate endophyte fungal association in *Asparagus*. *Turkish Journal of Botany*, *40*(1), 662-675. DOI: 10.3906/bot-1602-11

Surendran, U., & Vani, D. (2013). Influence of arbuscular mycorrhizal fungi in sugarcane productivity under semiarid tropical agro ecosystem in India. *International Journal of Plant Production, 7*(2), 269-278. DOI: 10.22069/IJPP.2012.986

Varma, A. (2008). *Mycorrhiza state of art, genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics*. Berlin, GE: Springer. DOI: 10.1007/978-3-540-78826-3

Vergara, C., Araujo, K. E. C., Urquiaga, S., Schultz, N., Balieiro, F. C., Medeiros, P. S., … Zilli, J. E. (2017). Dark septate endophytic fungi help tomato to acquire nutrients from ground plant material. *Frontiers in Microbiology*, *8*(2437), 1-12. DOI: 10.3389/fmicb.2017.02437

Xie, L., Zhang, W., Qin, L., Lan, T., Narisawa, K., Li, S., … Liao, S. (2013). Effects of dark septate endophytes (DSE) on sugarcane seedling growth. *Journal of Southern Agriculture*, *44*(11), 1827-1830. DOI: 10.3969/j