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Initial development and chemical components of sugarcane under water stress associated with arbuscular mycorrhizal fungi

Carmem C. M. de Sousa[1], Elvira M. R. Pedrosa[2], Mario M. Rolim[2], Uided M. T. Cavalcante[3], Inácio P. Monte Júnior[4] & João V. Pereira Filho[5]

[1] Engenheira Agrícola. Fortaleza, CE. E-mail: crismareco@hotmail.com (Corresponding author)
[2] Departamento de Engenharia Agrícola/Universidade Federal Rural de Pernambuco. Recife, PE. E-mail: elvira.pedrosa@deagri.ufrpe.br; rolim@deagri.ufrpe.br
[3] Departamento de Biologia/Universidade Federal de Pernambuco. Recife, PE. E-mail: umaaze@yahoo.com.br
[4] Biológo. Recife, PE. E-mail: inaciopascoal@hotmail.com
[5] Departamento de Engenharia Agrícola/Universidade Federal do Ceará. Fortaleza, CE. E-mail: joao_valdenor@hotmail.com

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ABSTRACT
The objective of this study was to evaluate the effects of water stress levels in the soil and a mix (or: a mixed inoculum of four species: *Claroideoglomus etunicatum*, *Gigasporas rosea*, *Acaulospora longula*, *Fuscutata heterogama*) of arbuscular mycorrhizal fungi (AMF) on initial vegetative growth, fresh and dry biomass production, root colonization, phosphorus, proteins, enzymes and amino acid of the sugarcane variety RB 857515 under greenhouse conditions. The experiment was set in a randomized block design in a 2 x 2 factorial scheme with four treatments (T1 - 50% PC - pot capacity, with AMF; T2 - 100% PC with AMF; T3 - 50% PC without AMF; T4 - 100% PC without AMF) with 16 replicates. The water stress level of 50% PC decreased stem diameter and shoot and root fresh weight of sugarcane plants, as well as AMF in the soil and in plant roots. However, AMF and the water stress level of 50% PC, separately or combined, did not affect plant height, number of leaves, dry matter and contents of phosphorus, total soluble proteins, catalase, ascorbate peroxidase, polyphenoloxidase, peroxidase and proline of the sugarcane variety RB857515.

Palavras-chave:
colonização micorrízica
proteínas
prolina

Desenvolvimento inicial e componentes químicos da cana-de-açúcar sob estresse hídrico associado a fungos micorrízicos arbusculares

RESUMO
Objetivou-se, neste estudo, avaliar os efeitos de níveis de estresse hídrico no solo e um mix (ou: um inóculo misto de quatro espécies: *Claroideoglomus etunicatum*, *Gigasporas rosea*, *Acaulospora longula*, *Fuscutata heterogama*) dos fungos micorrízicos arbusculares (FMA) no desenvolvimento vegetativo inicial, produção de biomassa fresca e seca, colonização micorrízica, teores de fósforo, proteínas, enzimas e aminoácido da cana-de-açúcar variedade RB 857515, em condições de casa de vegetação. O delineamento estatístico adotado foi blocos ao acaso em esquema fatorial 2 x 2 com 4 tratamentos (T1- 50% CP – capacidade do pote com FMA; T2 -100% CP – com FMA; T3 - 50% CP sem FMA; T4 - 100% CP sem FMA) com 16 repetições. O nível de estresse hídrico de 50% CP diminuiu o diâmetro do colmo, a biomassa fresca da parte aérea e da raiz da cana-de-açúcar, além dos FMA no solo e raiz da planta; entretanto, os FMA e o nível de estresse hídrico de 50% CP, isoladamente ou em conjunto, não afetaram a altura da planta, número de folhas, matéria seca e teores de fósforo, proteínas solúveis totais, catalase, ascarbato peroxidase, polifenoloxidase, peroxidase nem a prolina da cana-de-açúcar variedade RB857515.

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Introduction

The use of arbuscular mycorrhizal fungi (AMF) has major importance for the growth of various plant species, due to the great potential for a more efficient crop development and to the reduction in the use of fertilizers, especially in the beginning of plant development (Russomanno et al., 2008; Subramanian et al., 2011). AMF increase the absorption of soil nutrients, especially phosphorus, and plant resistance to biotic and abiotic stresses (Russomanno et al., 2008; Smith & Read, 2008; Kiriachek et al., 2009; Silva & Amorim, 2009; Ngwene et al., 2010; Subramanian et al., 2011). According to Abdel-Fattah & Asrar (2012), in addition to the nutritional effects, the association provides plants with tolerance to water stress, contributing to aggregate stability, increase in soil aeration and subsequent water availability (Borie et al., 2008), since plants with mycorrhizae maintain higher stomatal conductance during water deficit periods and, in return, higher diffusive dependence (Zhang et al., 2011).

Studies on the interaction between AMF and sugarcane, however, are scarce and, although the sugarcane response to AMF in field conditions is not expressive, there is a great potential to use this technology in the production of pathogen-free seedlings, with increases in plant survival indices and higher initial vigor, making them more adapted to the transplanting in the field (Silva et al., 2009).

On the other hand, it is important to develop studies on water use efficiency with the arbuscular mycorrhizal symbiosis aiming to increase the production with minimum use of the available water resources (Asrar et al., 2012). Given the above, this study aimed to evaluate the effect of water stress levels in the soil and inoculation with AMF on the initial vegetative development, fresh and dry biomass production, mycorrhizal colonization and contents of phosphorus, proteins, enzymes and amino acid of sugarcane.

Material and Methods

The experiment was carried out in a greenhouse (8° 01' 05" S; 34° 56' 48" W; 4 m) of the Federal Rural University of Pernambuco (UFRPE) in Recife-PE, Brazil, with minimum and maximum temperatures of 23.8 and 36.7 ºC, respectively, and average relative air humidity of 77.38%.

Pots with 30 cm of height, 17 cm of diameter and a volume of 3.8 m^3 were filled with soil material of a distrophic Yellow Argisol, with sandy-loam texture, collected in the municipality of Carpina, Pernambuco, from the layer of 0.5-0.9 m, with the following characteristics: pH (H_2O) = 4.6; Ca^{2+} = 0.37 $cmol_c\ kg^{-1}$; Mg^{2+} = 0.51 $cmol_c\ kg^{-1}$; Na^+ = 0.03 $cmol_c\ kg^{-1}$; K^+ = 0.04 $cmol_c\ kg^{-1}$; $H^+ + Al^{3+}$ = 2.84 $cmol_c\ kg^{-1}$; Al^{3+} = 0.91 $cmol_c\ kg^{-1}$; C = 7.0 g kg^{-1}; N = 0.3 g kg^{-1}; O.M. = 23 g kg^{-1}; assimilable P = 8 mg kg^{-1}; and K = 0.04 mg dm^{-3}. The soil was initially sieved (5-mm grid), autoclaved twice (120 °C, 101 kPa), for 1 h and 30 min, with an interval of 24 h, and used 30 days after disinfection.

The sugarcane variety used in the experiment was RB 857515. The micropropagated seedlings were provided by the Biofactory of the Northeast Center for Strategic Technologies (CETENE). The seedlings remained 20 days in acclimation in pots with humus, in a greenhouse, and then were transplanted to pots with autoclaved soil.

The pot capacity (PC) was considered as being the content of water retained by the soil after saturation and the action of gravity, until drainage stopped (Souza et al., 2000). The irrigation of the pots was controlled by daily weighing, using a precision scale with sensitivity of 1 g, between 7 and 9 a.m., for later replenishment of the water evapotranspired in the period, maintaining the soil in the pots close to the field capacity, which began 50 days after transplanting the seedlings into the pots.

The statistical design adopted was randomized blocks, in a 2^2 factorial scheme, with 4 treatments (T1 – 50% PC with AMF; T2 – 100% PC with AMF; T3 – 50% PC without AMF; T4 – 100% PC without AMF) and 16 replicates.

The AMF used in the mix were: *Claroideoglomus etunicatum* URM-FMA 03W. N. (Becker & Gerd) C. Walker & A. Schüβler, *Gigaspora rosea* URM-FMA 01T. H. Nicolson & N. C. Schenck, *Acaulospora longula* URM-FMA 07 Spain & N. C. Schenck and *Fuscutata heterogama* URM-FMA 04 Oehl, F. A. Souza, L. C. Maia & Sieverd., from the Mycorrhiza Laboratory of the Federal University of Pernambuco (UFPE). The AMF were separately multiplied, for three months, in pots planted with millet (*Panicum miliaceum* L.), maize (*Zea mays* L.), sunflower (*Helianthus annus* L.) and sorghum (*Sorghum bicolor* L. Moench). The pots contained 3 kg of soil sterilized in an autoclave at 120 ºC, in two periods of 1 h and 30 min, on consecutive days. The AMF (200 spores $plant^{-1}$) were deposited around the roots of the sugarcane seedlings, 20 days after transplantation into a greenhouse. The harvest was performed 90 days after AMF inoculation.

The evaluated variables were based on the morphological growth (stem length, stem diameter and number of leaves), on shoot and root fresh matter, shoot dry matter, number of glomerospores in the soil, mycorrhizal colonization rate, shoot phosphorus content, total soluble proteins, the enzymes catalase, ascorbate peroxidase, polyphenoloxidase and peroxidase, and on proline.

The morphological growth was evaluated by measurements using a tape measure and a caliper rule, on the 90th day after mycorrhizal inoculation. For dry matter determinations, the collected samples were stored in previously identified paper bags, and then weighed and dried in a forced-air oven at 65 ºC, until constant mass. After determining shoot dry mass, the samples were ground and placed in polyethylene bags, while aliquots were used for phosphorus (P) analysis using the method of Miyazawa et al. (1984), at the Plant Chemistry Laboratory of the UFRPE.

AMF were extracted from 100 g of soil, using the technique of wet sieving (Gerdemann & Nicolson, 1963) followed by water centrifugation and sucrose solution (Jenkins, 1964) at 50% (w/v). Mycorrhizal colonization was estimated in a hundred 1 cm root fragments (Giovannetti & Mosse, 1980), after clearing with KOH (10%) and H_2O_2 (10%), stained with Trypan blue at 0.05% (Phillips & Hayman, 1970), mounted in glass slides and observed in the microscope.

The biochemical analysis was performed using the third leaf of each plant, which, after harvested, was submersed in liquid nitrogen and stored in a freezer. Sample extract was

prepared for the analysis through the homogenization of 0.1 g of fresh matter in 4 mL of the buffer sodium phosphate 0.1 M (pH 6.5), mixed with 0.05 g of polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10.000 × *g* at 4 ºC for 10 min (Zeraik et al., 2008), and then analysed for total soluble proteins (Bradford, 1976) and the enzymes catalase (Berrs & Sizer 1952), ascorbate peroxidase (Nakano & Asada, 1981), polyphenoloxidase (Kar & Mishra, 1976) and peroxidase (Fatibelho Filho & Vieira, 2002). For the analysis of the amino acid proline, an extract obtained from 1 g of dry matter in 5 mL of sulfosalicylic acid at 3% was used. The homogenate was centrifuged at 2.000 x g for 10 min, filtered using No. 2 filter paper and determined according to Bates et al. (1973).

The data were subjected to analysis of variance and, when significant, subjected to Tukey test ($p \leq 0.05$) using the statistical software SAS.

Results and Discussion

There was no interaction between AMF and water stress, and no effect of AMF on stem length, stem diameter, number of leaves, shoot and root fresh matter, shoot dry matter and P content in the plant (Tables 1 and 2). Similar results were obtained by Russomanno et al. (2008), in rosemary (*Rosmarinus officinalis* L.) and basil plants (*Ocimum basilicum* L.), and by Silva et al. (2009) in sweet passion fruit plants (*Passiflora alata*).

Water stress significantly affected stem diameter and shoot and root fresh matter. On the other hand, there was interaction between water stress and AMF; water stress affected the number of glomerospores in the soil and the AMF root colonization rate of sugarcane seedlings. However, the concentration of assimilable P in the studied soil was equal to 8 mg kg^{-1}, which is considered as intermediate for sugarcane, since high P availabilities would inhibit AMF root colonization (Zhang et al., 2011), considering that plants would not need to spend photoassimilates with AMF. In this case, the fungus would behave as a parasite and could interfere with plant development. For this reason, most studies on AMF are performed in soils either poor or dependent on phosphorus (Smith & Read, 2008; Kiriacher et al., 2009).

Table 1. Analysis of variance for stem length (SL), stem diameter (SD), number of leaves (NL), shoot fresh matter (SFM), root fresh matter (RFM) of RB85751 sugarcane seedlings subjected to water stress levels and inoculated with AMF, in greenhouse.

Source of variation	DF	Mean squares				
		SL	SD	NL	SFM	RFM
Stress (S)	1	3066.70^{ns}	0.34*	25.25^{ns}	1.10*	0.61*
AMF	1	0.24^{ns}	0.04^{ns}	0.02^{ns}	0.000002^{ns}	0.004^{ns}
S × AMF	1	113.70^{ns}	0.27^{ns}	0.17^{ns}	0.057^{ns}	0.07^{ns}
Residue	58	108.80	0.06	1.67	0.032	0.029
C.V. (%)	-	16.79	23.29	17.71	9.19	11.64

*Significant at 0.05 probability level; ns - not-significant by F test

Table 2. Summary of analysis of variance of shoot dry matter (SDM), number of glomerospores (NGS), AMF root colonization rate (RCR) and plant phosphorus content (PP) of RB 857515 sugarcane seedlings subjected to water stress levels and inoculated with AMF, in greenhouse.

Source of variation	DF	Mean squares			
		SDM	NGS	RCR	PP
Stress (S)	1	0.81^{ns}	30938.20*	0.81*	0.055^{ns}
AMF	1	0.001^{ns}	27.04*	0.001*	0.01^{ns}
S × AMF	1	0.03^{ns}	208.43*	0.03*	0.04^{ns}
Residue	58	0.04	2050.20	0.04	0.02
C.V. (%)	-	16.01	17.71	16.01	20.28

* Significant at 0.05 probability level; ns - not-significant by F test

Beltrano & Ronco (2008) reported significant increases in maize (*Zea mays* L.) and wheat (*Triticum* spp.), respectively under moderate and extreme water stress, inoculated with *Glomus claroideum*. According to Sánchez-Blanco et al. (2004) and Goicoechea et al. (2005), this symbiosis (AMF) has proved to increase the tolerance to water stress, although the mechanisms involved are controversial, because under drought conditions the leaf water potential may or may not be lower in plants with AMF. This decrease, however, is lower in plants with AMF and under stress, possibly due to the fact that in low soil water potentials, plants with AMF can absorb more water compared with AMF-free plants, as mentioned by Porcel & Ruiz-Lozano (2004).

The stress level of 50% PC was enough to change the development and the vegetative growth of sugarcane seedlings, even in the presence of AMF, with decrease of 75, 83.55 and 80.54% in stem diameter, shoot fresh matter and root fresh matter, respectively (Table 3). Russomanno et al. (2008) obtained the opposite result for root fresh biomass, in rosemary plants, with no differences between the AMF-inoculated species (*C. etunicatum* and *G. clarum*), while in basil plants, root fresh biomass increased in 93.10% when compared with the control, non-inoculated, which also occurred in other crops associated with AMF (Silva et al., 2009).

The obtained results are contrary to the ones found by Silva et al. (2009), who observed that seedlings of yellow passion fruit did not show differences in stem diameter when inoculated with *G. albida* and *S. heterogama*.

The highest results for AMF population occurred when sugarcane was not subjected to water stress, indicating that AMF develop better (both in roots and in soil) when sugarcane is supplied with water, i.e., when the soil is at field capacity (Table 4).

With the water stress, both colonization and sporulation were reduced in 25 and 35%, respectively (Table 4). Inoculation was performed with 200 glomerospores per pot, which corresponds to approximately 6.66 glomerospores per 100 g soil. The percentage of root colonization was low for the plants in the two water systems, which suggests that a few

Table 3. Mean values of stem diameter (SD), shoot fresh biomass (SFB) and root fresh biomass (RFB) of sugarcane variety RB857515 under water stress (50% PC) 90 days after inoculation in greenhouse

Stress	SD	SFB	RFB
	(mm)	(g)	
With (50% PC)	0.63 B	64.69 A	19.83 A
Without (100% PC)	0.84 A	77.43 A	24.62 A
LSD	0.24	23.75	7.93

Different letters in the columns have significantly different means by Tukey test at 0.05 probability level; With – 50% of the pot capacity; Without – 100% of the pot capacity; LSD – Least significant difference

Table 4. Mean values of the number of glomerospores of arbuscular mycorrhizal fungi (AMF) in the soil (NGS) and AMF root colonization rate (RCR) in the sugarcane variety RB 857515 under water stress (50% PC) 90 days after inoculation in greenhouse

Stress	NGS (100g^{-1} soil)		RCR (%)	
	AMF		AMF	
	With	Without	With	Without
With	853.10 aB	0.00 bA	4.89 aB	0.00 bA
Without	2.406.30 aA	0.00 bA	19.37 aA	0.00 bA
LSD	54.41		4.48	

Different letters have significantly different means for each variable by Tukey test at 0.05 probability level, and lowercase letters refer to rows and uppercase letters refer to columns; LSD - least significant difference.
PC = pot capacity, according to Souza et al. (2000)

glomerospores managed to germinate, penetrate and colonize the roots. Usually, mycorrhizal efficiency is related to the amount of external mycelium formed in soil, varying with AMF and plant species (Rossumanno et al., 2008).

Other factors that reflect on mycorrhizal efficiency are related to edaphoclimatic conditions and aspects of the fungus-plant relationship, i.e., they depend on fungus species, plant species and environmental conditions (Zhang et al., 2011; Asrar et al., 2012). Ngwene et al. (2010), studying cowpea (*Vigna unguiculata*) inoculated with AMF, found that the colonization was severely reduced when plants received phosphate and nitrogen fertilization. Bonfim et al. (2010) observed higher number of AMF spores in the system with arboreal component (larger root area) and in coffee crop (*Coffea arabica* L.) in dry period, indicating that the restriction in water availability in this season has induced microorganism association with plants and the manifestation of adaptation mechanisms, like increase in sporulation. However, for both evaluated seasons (dry and rainy), there was no correlation between number of spores and leaf water potential, indicating that, regardless of water regime, AMF act normally in the crop and in the soil. Similar results were observed by Amorim et al. (2004), in Brazilian pepper (*Schinus terebinthifolius* Raddi) and 'umbu' trees (*Spondias tuberosa* L.) under water stress conditions.

The summary of analysis of variance of the enzymes present in tissues of sugarcane plants subjected to different levels of water stress and inoculated with AMF is shown in Table 5.

There was no interaction between AMF and water stress, and no isolated effect of AMF and water stress for the enzymes catalase, peroxidase, ascorbate peroxidase, polyphenoloxidase, soluble protein and the amino acid proline. Similar results for total protein activity in wheat leaves, with and without water stress, were observed by Beltrano & Ronco (2008), although plants with AMF had higher protein concentration than plants without AMF under severe stress.

Table 5. Analysis of variance for the enzymes catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), polyphenoloxidase (PPO), soluble protein (SP) and the amino acid proline (PRO) in RB 857515 sugarcane plants subjected to water stress levels and inoculated with AMF in greenhouse

Source of variation	DF	Mean squares					
		CAT	POD	APX	PPO	SP	PRO
Stress (S)	1	0.013^{ns}	3.67^{ns}	0.002^{ns}	2298.31^{ns}	1.02^{ns}	0.0002^{ns}
AMF	1	2.89^{ns}	2519.07^{ns}	0.014^{ns}	633.71^{ns}	1.67^{ns}	0.0002^{ns}
S × AMF	1	0.51^{ns}	1779.86^{ns}	0.003^{ns}	811.17^{ns}	2.95^{ns}	0.009^{ns}
Residue	58	0.42	777.33	0.018	375.69	5.69	0.005
CV (%)	-	34.59	12.32	8.99	24.01	19.89	8.61

*Significant at 0.05 probability level; ns – not-significant by F test

Conclusions

1. The water stress levels of 50 and 100% pot capacity were not enough to interfere with the arbuscular mycorrhizal colonization, and with growth and initial development of the sugarcane variety RB857515.

2. The water stress level of 50% pot capacity (PC) affected the initial development of sugarcane seedlings and the AMF colonization in the soil and in plant roots. The AMF species *Claroideoglomus etunicatum*, *Gigaspora rosea*, *Acaulospora longula* and *Fuscutata heterogama*, and the water stress level of 50% PC, separately or combined, did not affect the initial development, dry matter production and the contents of P, total soluble proteins, catalase, ascorbate peroxidase, polyphenoloxidase, peroxidase and proline of the sugarcane variety RB857515.

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