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Water availability and formation of propagules of arbuscular mycorrhizal fungi associated with sorghum

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) promote greater tolerance to the negative effects of water stress in their host plants, yet may also be influenced by the availability of water in the production of their infective propagules. The objective of this study was to assess the effect of water availability in the soil on the sporulation of *Claroideoglomus etunicatum*,*Gigaspora albida* and *Scutellospora heterogama*, and the influence of this condition on the infective potential and number of nuclei in glomerospores of these species. The reduction of water availability from 75 to 25% did not decrease significantly the sporulation of *C. etunicatum*, but resulted in decrease of sporulation of *G. albida* (600 to 7 glomerospores per 30 g^{-1} soil) and *S. heterogama* (274 to 2 glomerospores per 30 g^{-1} soil). The water availability at 75 and 71% promoted maximum sporulation of *G. albida* and *S. heterogama*, respectively. While *G. albida* and *S. heterogama* had greater sporulation than *C. etunicatum*, the infective potential of these species was lower, which may be related to the life-strategy and type of infective propagules of each species. The number of nuclei per glomerospore varied only among the species ($p < 0.05$), with *C. etunicatum* and *G. albida* presenting the higher number of nuclei when compared to *S. heterogama*, but no differences were found among the treatments of water availability ($p > 0.05$). These results suggest that AMF have distinct sporulation strategies and the amount of glomerospores is not directly related to the infectivity of the inoculum. Possibly, the differences in the life-strategies among the species were greater than the effects of water availability.

1. Introduction

The climatic projections show that semi-arid areas will be more affected due to climate change, promoting more severe drought (Bates et al., 2008). In semi-arid areas, the phenomenon of drought is frequent, requiring adaptations for coexistence and sustainable management of water for agriculture (Rockström et al., 2010). The use of biotechnological tools to maintain quality production, even under a limited water condition, is of utmost importance for these regions. Therefore, arbuscular mycorrhizal fungi (AMF) play an important role in this context. AMF occur in the soil and promote benefits to plants (Smith and Read, 2008), increasing the transport of water and nutrients through the mycelium (Egerton-Warburton et al., 2007; Ruth et al., 2011; Tobar et al., 1994) and consequently influencing the plant's water balance (Augé, 2001).

Improved water relations can promote higher growth potential and yield in mycorrhized plants, particularly under water-limited or nutrient-limited conditions (Jayne and Quigley, 2014). In addition, physiological and biochemical mechanisms of defense against injuries caused by the water stress are enhanced with mycorrhization, as increase in expression of genes that encode for aquaporins, which are proteins that channel the passage of water through the cell membrane (Uehlein et al., 2007), increase the stomatal conductance (Cho et al., 2006) and the concentration of solutes that promote osmotic adjustment in the cell (Bohnert and Jensen, 1996), among others.

On the other hand, the AMF effects depend on the level of water deficiency, because the water deficit should not affect AMF survival and growth. Few studies emphasize the effects of drought stress on the AMF propagule production, and the glomerospore constitute the most important infective propagules for the majority of AMF

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species (Smith and Read, 2008). Besides, the water availability in the soil can affect the production of glomerospores differently depending on the isolate of AMF. In the field contrasting results are observed, low water availability in the soil can reduce the AMF propagules production (Cui and Nobel, 1992) or increase of the glomerospores number and negatively affect the mycorrhizal colonization (Panwar et al., 2011). These differences in the number of glomerospores in the field can be related to the AMF species community composition, since the infectivity, persistence and formation of propagules vary with the AMF group (Klironomos and Hart, 2002).

Under experimental conditions, a gradual reduction of soil moisture over four weeks resulted in increased production of external mycelium and sporulation of *Glomus intraradices* Schenck & Smith (Neumann et al., 2009). However, in extreme drought conditions a reduction of AMF sporulation occurs, but an increase in the formation of propagules can additionally occur depending on the form and duration of water stress, which is influenced also by the host and fungal species (Augé, 2001).

The different responses of the AMF species in promoting plant growth under water stress may indicate that some isolates of AMF can be more efficient than others in this condition (Ruiz-Lozano et al., 1995), due to differences in their ability to absorb water from the soil (Marulanda et al., 2003). Furthermore, glomerospores with different nucleotypes can present distinct mycorrhizal efficiency (Angelard et al., 2010). Viera and Glenn (1990) suggested that adaptation of the AMF to adverse environmental conditions can also be related to the large amount of nuclei, and it was demonstrated that this number can vary between species of AMF (Hosny et al., 1998). Marleau et al. (2011) showed that the number of nuclei has a linear relationship with spore diameter, indicating also that the variation in number of nuclei can be related to spore maturity.

The nuclei show variation in the number and also can be genetically distinct within the same glomerospore (Kuhn et al., 2001). This genetic variability within populations of AMF should be considered for the development and selection of mycorrhizal inoculants because this would ensure the possibility of gene expression under different conditions (Sanders, 2004).

In the symbiosis between plants and AMF, water restriction can reduce mycorrhizal colonization (Manoharan et al., 2010), which can influence the production of spores and number of nuclei in a spore. Studies related to sporulation of AMF under conditions of water stress and the number of nuclei in the glomerospores are scarce, and it is important to understand some aspects of the fungal genetics to expand our understanding of this symbiosis (Sanders and Croll, 2010), in order to make more efficient use of AMF.

The knowledge about the effects of water restriction on the sporulation of species of AMF is of great importance to the selection of species better adapted to this condition, allowing for the AMF isolate to survive and promote the development of plant. In this study we addressed the influence of water availability on different aspects of the biology of AMF, testing the hypothesis that these fungi have distinct strategies regarding to production of glomerospores and other propagules under different levels of water availability. For this, isolates of *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) Walker & Schüßler, *Gigaspora albida* N.C. Schenck & G.S. Sm. and *Scutellospora heterogama* (T.H. Nicolson & Gerd.) Walker & Sanders were tested in association with plants of *Sorghum bicolor* (L.) Moench var. Ponta Negra. The objective of this work was to test the hypothesis that water availability in the soil affects the sporulation of *C. etunicatum*, *G. albida* and *S. heterogama*, which can thus lead to modifications in the infective potential and number of nuclei in glomerospores of these species.

2. Material and methods

2.1. Preparation of soil and inoculum

2.1.1. Soil characteristics

The soil used for the experiment was a sandy loam soil type upper layer of a Typic Haplustults according to the classification Soil Survey Staff (2010), with 769 g sand kg^{-1}, 215 g silt kg^{-1} and 16 g clay kg^{-1}, with the following chemical characteristics: pH 5.2; electrical conductivity 0.22 dS m^{-1}; P = 3.4 mg kg^{-1}; K^+ = 87.5 mg kg^{-1}; Ca^{2+} = 316.1 mg kg^{-1}; Mg^{2+} = 60.8 mg kg^{-1}; Na^+ = 3.0 mg kg^{-1}; and Al^{3+} = 8.9 mg kg^{-1}; cation exchange capacity (CEC) = 5.24 cmolc dm^{-3}. The soil was previously sterilized in an autoclave for two periods of one hour each at 120 °C.

2.1.2. Experimental design and data analysis

The experimental design was randomized blocks in factorial arrangement of three AMF (*G. albida*, *S. heterogama*, and *C. etunicatum*) × four levels of water availability (up to 25%; 50% – between 30 and 50%; 75% – between 55 and 75%; and 100% – between 80 and 100%), in 5 replicates. For the number of glomerospores, Tukey test was performed, the data were transformed into $\sqrt{(x+0.5)}$ and when data were significant ($p < 0.05$) for ranges of field capacity, a regression analysis was performed. For the number of nuclei, the data were subject to analysis of variance (ANOVA) and when significant the means were compared by the Tukey test (5% of probability). The statistical analyses were performed with the aid of the Statistica.

2.1.3. Determination of levels of water availability

To assist in the management of water availability in the treatments, tests were done to determine the water-retention capacity of the soil. Ten pots (11.3 cm height × 9.4 cm diameter) with 557 g of soil were used which were closed at the top after the application of water to saturation, to prevent evaporation. After 24 h, the volumes of drained water were collected and measured. Immediately after the removal of drained water, the pots were weighed. From the average of the weights, the standard weight was determined for maximum water retained by the soil; it was considered to be field capacity. This information was utilized to adjust the water availability during the experiment. To more accurately determine the quantity of water needed to maintain field capacity during the experiment, extra pots with plants inoculated with the mix of *G. albida*, *G. etunicatum* and *S. heterogama*, were produced under the conditions of the experiment. Plants were quantified weekly and these values were subtracted from pot weight to yield the remaining wet weight of soil for the calculation of water addition. This procedure was made to avoid interference of the plant biomass weight in the standard weight of the pots, in each irrigation treatment (Bernardo et al., 2006; Klar, 1991). Daily the moisture content of the substrate was kept to the maximum values of the treatments up to 25% (Treatment 1); from 30 to 50% (Treatment 2); from 55 to 75% (Treatment 3) and 80 to 100% (Treatment 4) of field capacity, until the end of the experiment.

The water volume to be applied per pot in each treatment (FC (field capacity), 0.75FC, 0.50FC and 0.25FC) was calculated using the following equations:

$$Ww_{FC} = \{Wcp_{FC} - [Wa5pl_{FC} - (Wa4pl_{FC})]\} \quad \text{(Eq.?1)}$$

$$Ww_{0.75FC} = \{Wcp_{0.75FC} - [Wa5pl_{0.75FC} - (Wa4pl_{0.75FC})]\} \quad \text{(Eq.?2)}$$