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Arbuscular mycorrhizal fungi and *Eucalyptus* species with different levels of water stress tolerance

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Dissertation presented to obtain the degree of Master in Science. Area: Soil and Plant Nutrition

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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RESUMO

Fungos micorrízicos arbusculares e espécies de *Eucalyptus* com diferentes níveis de tolerância ao estresse hídrico

As espécies de eucalipto respondem por quase 80% das florestas plantadas no Brasil, o que faz da silvicultura uma atividade de grande importância no cenário nacional e internacional. Apesar de sua adaptabilidade, o eucalipto está sujeito a danos que as alterações climáticas podem causar, especialmente o déficit hídrico. A maioria das plantas possui estratégias de sobrevivência em situações de déficit hídrico. A associação com fungos micorrízicos arbusculares (FMA) é uma delas, pois aumenta a absorção de água e nutrientes pelas raízes. Assim, o objetivo principal deste trabalho foi avaliar a colonização de fungos micorrízicos em seis espécies de *Eucalyptus* com diferentes níveis de tolerância à seca: *E. brassiana, E. camaldulensis, E. citriodora, E. cloeziana, E. grandis* e *E. urophylla*. Dois ensaios foram realizados: um no campo e outro em casa de vegetação. Em casa de vegetação, os vasos foram submetidos a diferentes níveis de água: 50, 75 e 100% da capacidade de campo, por três meses. No campo, as amostras foram coletadas em dois períodos diferentes: após o período seco e após a estação chuvosa. As amostras de solo e raízes finas coletadas nos dois experimentos foram submetidas à análise microbiológica e molecular. A colonização da raiz por FMA foi avaliada. Os genes ITS foram analisados por PCR quantitativo (*q*PCR) e a estrutura da comunidade fúngica por polimorfismo de fragmentos de restrição (T-RFLP). Dentre as espécies estudadas, *E. urophylla* apresentou maior colonização em ambos os ensaios. A atividade microbiana do solo foi fortemente afetada pela umidade do solo. A estrutura da comunidade fúngica não se diferenciou claramente entre os tratamentos. A idade das plantas e a umidade do solo parecem ter maior efeito no desempenho das espécies de eucalipto do que seu nível de tolerância ao estresse hídrico.

Palavras-chave: Microbiologia do solo; Eucalipto; Micorrizas; Tolerância ao estresse hídrico

ABSTRACT

Arbuscular mycorrhizal fungi and *Eucalyptus* species with different levels of water stress tolerance

Eucalyptus species account for almost 80% of the forests planted in Brazil, which makes silviculture an activity of great importance in the national and international scenario. Despite its adaptability, *Eucalyptus* is subject to damages caused by climate change, especially the water deficit. Most plants have survival strategies in water deficit situations. Association with mycorrhizal fungi (FM) is one of them, since they increase water and nutrients uptake by the roots. Thus, the main objective of this study was to evaluate the colonization of mycorrhizal fungi in six Eucalyptus species with different levels of drought tolerance: *E. brassiana, E. camaldulensis, E. citriodora, E. cloeziana, E. grandis* and *E. urophylla*. Two trials were carried out, one in the field and another in a greenhouse. In the greenhouse, the substrate of the pots were submitted to different levels of water retention, 50, 75 and 100% of the field capacity, for three months. In the field, soil and roots were sampled at two different periods: after the dry period and after the rainy season. The soil samples and fine roots collected from the two experiments were submitted to microbiological and molecular analyses. AMF root colonization was evaluated. The ITS genes were analyzed by quantitative PCR (*q*PCR) and the fungal community structure by restriction fragment polymorphism (T-RFLP). Among the eucalypt species studied, *E. urophylla* presented higher colonization in both trials. Soil microbial activity was strongly affected by soil moisture. The fungal community structure did not differ clearly among the treatments. Age of plants and soil moisture seemed to affect the plant species more than levels of water stress tolerance.

Keywords: Soil microbiology; *Eucalyptus*; Mycorrhiza; Water stress tolerance

INTRODUCTION AND JUSTIFICATIVE

Silviculture has been consolidated across the Brazilian territory and, nowadays, forest products are an important slice of exportations in agribusiness. The activity thrives for many reasons, due to the high adaptability of the genus *Eucalyptus*, which accounts for 75% of the Brazilian planted forests (ABRAF, 2013).

One of the challenges of agriculture is to keep high yields amid climate adversities, especially water deficit, which has been intensifying in the last years according to the global warming (Booth, 2013). Eucalypt plantations in Brazil are concentrated in the Southeast region, where the rainfall is relatively regular, but there is a trend for long dry periods (Gonçalves et al., 2013; Gonçalves et al., 2017). In order to obtain continued crop expansion in more arid regions, it is necessary to explore new means of adaptation to the changing environment (Cha-um et al., 2014).

Root colonization by arbuscular mycorrhizal fungi (AMF) or ectomycorrhiza (ECM) works as an extension of the roots, helping in the uptake of water and nutrients (Cardoso et al., 2016). Therefore, the study of how mycorrhizal fungi behave under different rainfall conditions and in association with different *Eucalyptus* species may enlighten our understanding of the phenomena and probably show interesting results related to the crop establishment in low rainfall regions or survival to long dry periods.

Evaluating the fungal community might result in the identification of species that can be helpful when inoculated into plants during dry periods, and being able to support the healthy development of the trees. Thus, there is a lack in our knowledge and it is urgent to obtain better information in literature correlating the eucalypt tree, mycorrhizal processes and water deficit, as a kickoff to further studies.

CONCLUSION

Our study shows that the AMF colonization was different among *Eucalyptus* species, highlighting *Eucalyptus urophylla*, which is less tolerant to drought and presented more colonized roots, both in the field and in the greenhouse essays. However, the higher rates of colonization were observed in the rainy season and not under water stress as may have been expected.

Following the same trend, attributes such as soil basal respiration and microbial biomass carbon had a significant increase in the rainy season, showing again that soil moisture stimulates microbial activity overall.

The fungal community structure was not enlightening, as we could not identify groupings among the *Eucalyptus* species. To understand these processes we will need to set up new experiments with plants at different ages and with long-time follow-ups. Studies with other groups of microorganisms perhaps may help to get a better understanding of their behavior.

Besides soil moisture, other factors must be taken into account, such as the age of the plants and history of the area. *Eucalyptus* crops have a long cycle and the results we found are related to trees with less than two years of age, and this may change along the time.

It is important to highlight that this study is exploratory and we need further studies to understand better the role played by AMF in drought tolerance in *Eucalpytus* crops.

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