

# Mycorrhizal Diversity of Acaulosporaceae and Glomaceae Families in the Sorghum Rhizosphere in Brazilian Acid Soils

IE Marriel<sup>1\*</sup>, R Raposeiras, EA Gomes<sup>1</sup>, AC Oliveira<sup>1</sup>, CA Oliveira<sup>2</sup>, VMC Alves<sup>1</sup>, AA Neves<sup>1</sup>, AM Coelho<sup>1</sup> and RE Schaffert<sup>1</sup>

1. Embrapa Maize and Sorghum, CP 151, 35701-970 Sete Lagoas, MG, Brazil.

2. University Center Sete Lagoas, Av Marechal Castelo Branco, 2765 Sete Lagoas, MG, Brazil.

\* Corresponding author; imarriel@cpnms.embrapa.br

## ABSTRACT

The genetic diversity of arbuscular mycorrhizal fungi (AMF) was characterized in the rhizosphere of two sorghum genotypes contrasting for tolerance to aluminum toxicity, using molecular techniques. The plants were cultivated in an oxisol of the Brazilian cerrado with three levels of aluminum saturation (0, 20% and 40%). DNA samples were amplified based on nested PCR using fungal universal primers and specific primers for Glomaceae and Acaulosporaceae families. DGGE profiles clearly revealed that the diversity (number of amplicons) found was higher for Acaulosporaceae than Glomaceae. Both the sorghum cultivar and the level of aluminum saturation affected the AM fungi community structure.

## INTRODUCTION

The expansion of Brazilian agriculture has been increasing in the cerrado biome where there are extensive areas of potential new agricultural land. Oxisol, the most common soil type in this ecosystem, presents a set of chemical constraint limiting plant growth and development, particularly, low pH, low levels of nutrients and high phosphorus adsorption capacity (Novais & Smith, 1999). Fertilizer applied to these soils, particularly phosphates, presents low utilization efficiency by plants and is responsible for a significant portion of production cost.

The use of acid soil tolerant sorghum cultivars provides an environmentally and economical friendly component contributing to sustainable crop production. Plants have developed several physiological and biochemical systems of adaptation to Pi-deficiency stress. Marschner (1991) has concluded that the strategy of avoidance, such as: 1) root-induced changes in the rhizosphere such as pH increase, 2) release of chelators for Al and increased activity of ectoenzymes (acid phosphatases), and 3) increase in root surface area via mycorrhizae. Mycorrhizal symbiosis, a mutualistic plant-fungus, is an essential feature of the biology and ecology of most terrestrial plants, since it influences their growth in a multifunctional nature (Rosendahl, 2008; Nielson et al., 2004; Smith and Read, 1997; Newsham et al., 1995). Molecular studies have been used with success to investigate the phylogenetic relationship and genetic variation among the glomalean fungi (Simon, 1996; Sanders et al., 1996; Lanfranco et al., 1998) and for identifying the presence of three families of

the Glomales (Clapp et al., 1995; Rodecker, 2000), using ribosomal RNA (rRNA) genes and polymerase chain reaction-PCR/denaturing gradient gel electrophoresis-DGGE (Muyzer and Smalla, 1998).

Studies of the soil root interface are essential for a better understanding of the mechanisms by which plants adapt to acid and mineral soils. The characterization and identification of dominant groups of AMF and their role in the rhizosphere of adapted maize and sorghum genotypes to Brazilian acid soil are currently poorly understood. The objective of this study was: 1) to analyze the AMF communities associated with sorghum plants in acid soils, 2) to compare the inter- and intra-family genetic diversity of AMF in sorghum rhizosphere, and 3) to determine the influence of aluminum saturation in the soil on AMF community structure in the sorghum rhizosphere of acid soils.

## MATERIALS AND METHODS

### Sampling

Oxisol samples were taken from the rhizosphere of two sorghum lines contrasting in aluminum tolerance (G1-tolerant and G2-sensible) developed by the Embrapa Maize and Sorghum, Minas Gerais, Brazil, located at latitude 19° 28'S and longitude 44° 15'W. The genotypes were grown in a typical oxisol, with three levels of aluminum saturation (0, low; 20%, intermediate; 40%, high). The soil adhering to the roots of five plants/plot was pooled and considered as the rhizosphere soil. For control, soil without roots (bulk soil) was used, also collected 90 days after sorghum emergence. The samples were kept at -20°C until DNA

extraction.

## Molecular and data analysis

Soil samples of 500 mg were used for total DNA extraction using BIO 101 Kit protocols, following the manufacturer's recommendations. AMF community structures were examined by PCR-DGGE. The small subunit (SSU) ribosomal RNA gene was amplified from a total soil DNA extract based on nested PCR using fungal universal primers (NS5 and ITS4) and ITS/Glomaceae and ITS/Acaulosporaceae specific primers attached with the sequence clamp, ITS2/GLOM1310CG and ITS2/ACAU1660 CG, respectively (Redecker, 2000). PCR products were loaded by electrophoresis in a 6% polyacrilamide gel composed of denaturing gradients between 45% and 70% of urea-formamide gels (DGGE) and run for 16 h in a BIO-RAD Dcode System, VA, USA. Cluster analysis was performed using the Jaccard's coefficient of similarity and the unweighted pair group method (UPGMA) using the NTSYS software package (version 2.02).

## RESULTS AND DISCUSSION

The plant exudation pattern affects the structural and functional diversity of rhizosphere microbial communities (12). Based on DGGE patterns, the data allow the comparison of variation in the AM fungal community composition among plants genotypes, between the presence and absence of plants and between levels of aluminum saturation. There was a strong shift in the mycorrhizal communities due to Al saturation, principally in the bulk soil samples and in the rhizosphere soil for the Acaulosporaceae family, with reduced diversity in the high Al saturation environment. An apparent paradoxical increase in AMF richness was noted in the tolerant sorghum genotype, with a high diversity AMF at the high Al saturation level for Glomaceae. The results also revealed a higher diversity for the Acaulosporaceae family than the Glomaceae family. The cluster analysis identified two principal groups for Glomaceae; group I included samples of soil without roots, and group II included the rhizosphere soil samples. Three principal groups encountered for Acaulosporaceae; group I, rhizosphere soil samples without aluminum; group II, bulk soil without plants, and group III, samples from rhizosphere soil in the presence of  $Al^{3+}$ . The data show the influence aluminum saturation and sorghum genotype on the

composition of the AMF studied. The high diversity and variation of the AMF observed in this study indicate their importance ecologically and thus may have the potential to influence adaptation of sorghum cultivars to acid soil. There is evidence that AMF may be functionally distinct in phosphorus acquisition and overall benefit to the host plant (Sanders et al., 1996). This study showed that the fungal community composition in the rhizosphere is affected by a complex interaction between sorghum plant genotypes, AM fungi genotypes and aluminum saturation; and that there is a higher diversity of Acaulosporaceae than Glomaceae in the sorghum rhizosphere of an acid soil.

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