Rhizosphere and Root Mycorrhizal Diversity of Maize Genotypes Contrasting for Phosphorus Efficiency

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INTRODUCTION

Tropical soils are generally characterized by low pH and low fertility, in which crops are continuously exposed to several stresses. Among them, one of the major limiting factors for crop production in tropical acids soils is the low phosphorus (P) availability (Wright et al., 2005). Great effort has been applied to improve crop yields on these areas through soil fertility management and cultivars more adapted to acid soils conditions. However, the use of microbial resources to explore soil nutrients by the root is an important strategy to increase the use efficiency and the bioavailability of restricted nutrients, which are key point for sustainable agriculture (Barea et al. 2005). Plants have evolved several strategies to improve P acquisition, including alterations in root morphology, reducing pH or exudation of organic anions in the rhizosphere (Kpomblekou-A et al., 2003) and by symbiotic associations with arbuscular mycorrhizal (AM) fungi (Smith and Read 1997). The ecology, genetics and evolution of AM fungi are poorly understood mainly due to the inability of obtaining axenic cultures and the difficulties with morphology identification. Molecular studies with 18S ribosomal DNA (rDNA) have provided a powerful mean of characterizing AM fungi diversity in a wide range of environmental samples (Gomes et al., 2003; Van Elsas et al., 2000). The aim of this work was to compare the molecular diversity of AM fungi species recovered from the rhizosphere and roots of two maize inbred lines contrasting for P use efficiency, in a soil with low P. AM fungi were characterized by 18S rDNA fragments cloning e sequencing. In addition, denaturing gradient gel electrophoresis (DGGE) of 18S rDNA was used to analyze the genetic profiles, providing information on the ribotypes presented in maize genotypes under P stress.

METHODOLOGY

Maize inbred lines characterized as P efficient (L3) and P inefficient (L22), developed by the Embrapa Maize and Sorghum, Sete Lagoas, Brazil, were planted in a red latosol, clay texture, in a regular growing season under P stress condition. Soil from the rhizosphere and root samples were collected at flowering stage. Roots were cut into pieces of 1 cm long, fixed in ethanol 50% (v/v), clarified with KOH 10% (m/v) and stained with 0.05% trypan blue (w/v) for 20 min in lactoglycerol acid. Root colonization was quantified using grid line intersect method (Giovannetti & Mosse, 1980) and the spores were counted using a stereomicroscope. Mycorrhizal DNA was extracted from the rhizosphere soil and the roots using the FastDNA® Spin Kit protocols (BIO 101). Mycorrhizal rDNA fragments were amplified by nested PCR using fungal universal primers - NS1, NS4 and NS21 – (White et al., 1990) and the specific primer – VANS1 (Simon et al., 1992). PCR products were cloned into the pGEM-T Easy

Vector (Promega) and transformed *E. coli* DH 5-α. Recombinant clones were sequenced in an ABI Prism 3100 using Big Dye terminator kit (Applied Biossystems). Sequences were compared to those available in the GenBank/EMBL database using the BlastN program (Altschul et al., 1997). PCR amplified products were also analyzed by DGGE in a 6% polyacrylamide gel composed by denaturing gradients between 45 and 70% of urea-formamide, electrophoresed for 16h in a BIO-RAD Dcode System (USA).

RESULTS AND DISCUSSION

In the rhizopheric soil, there was no difference in the number of AM fungi spores between the two contrasting maize lines. However, the P efficient line (L3) showed 95% of root colonization by AM fungi, while in the P inefficient line (L22) no mycorrhizal infection was observed, which is a remarkable difference between these lines (Fig. 1). AM fungi were specifically amplified by the nested PCR, which were mainly represented by the genus *Scutelospora* (family Gigasporaceae) after rDNA sequence analysis. AM fungi diversity in the rhizosphere was similar in L3 and L22 lines, as well as the rDNA profiles in DGGE, in agreement with the amount of spores in these two lines. Genetic diversity in the microbial community of the root was reduced when compared to the rhizosphere, and there were some differences in the AM fungi species between the two lines. Significant differences were also observed in the DGGE profiles of AM fungi extracted from roots of L3 and L22, where at least two bands were present only in the P efficient line, suggesting that some specific root-mycorrhizal interaction might exist (Fig. 1). Besides, these results are based on only two contrasting genotypes, there are strongly evidences that the microbial association roots plays an important role in the improvement of P bioavailability mechanisms in maize.

CONCLUSIONS

- Molecular characterization using rDNA sequences and DGGE profiles was able to detect differences in the genetic diversity of AM fungi present on the maize roots contrasting for P efficient.
- Mycorrhizal association with maize roots is an important mechanism involved in P use efficiency, presenting a great potential to enhance P bioavailability in acid soils

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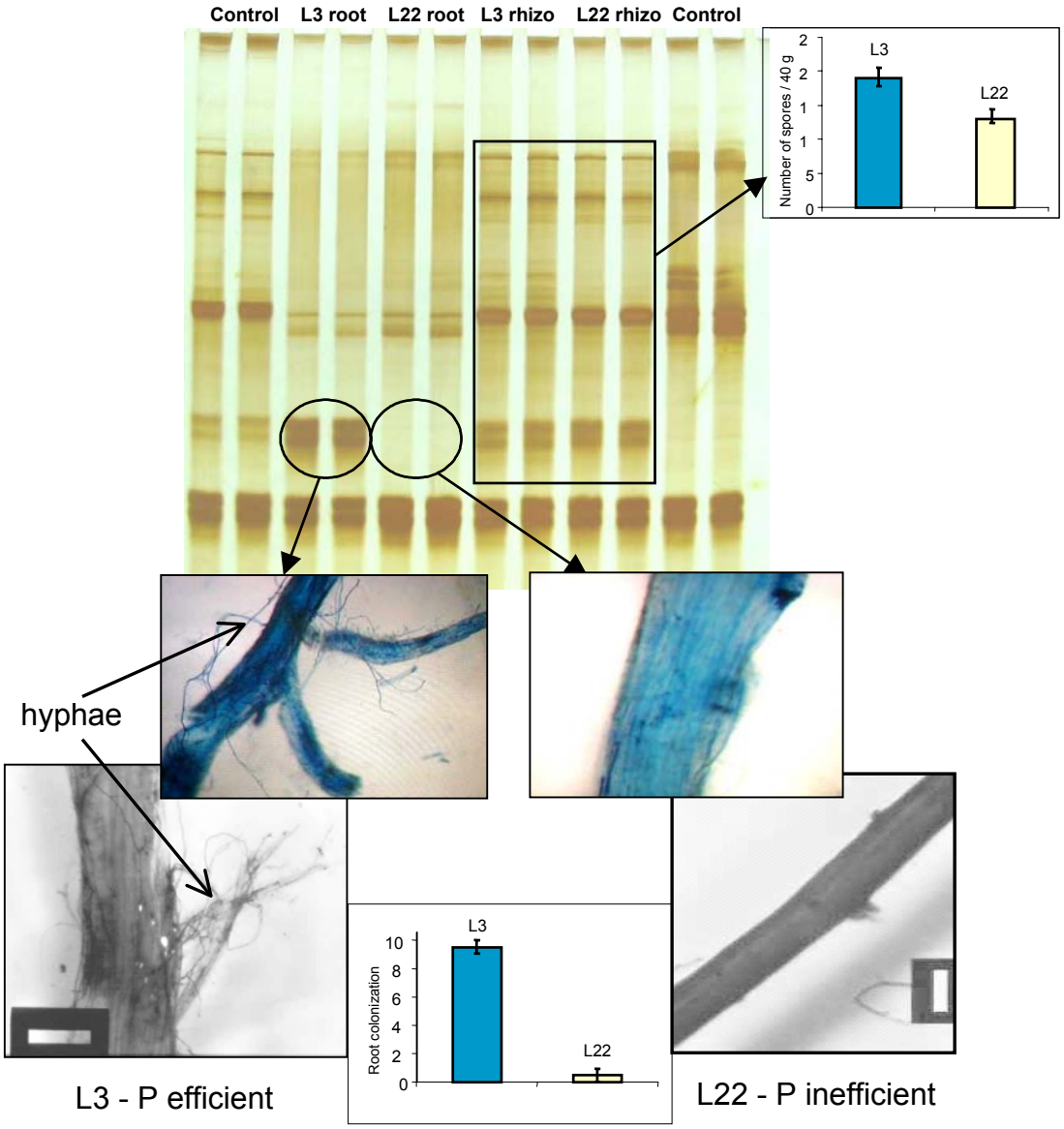

Figure 1. DGGE patterns, number of spores and mycorrhizal colonization of maize root and rhizosphere contrasting for P efficiency.