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Analysis of bacterial and mycorrhizal diversity in the rhizosphere of maize lines contrasting in phosphorus efficiency, using denaturing gradient gel electrophoresis

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Abstract

The maize improvement program at the Embrapa Maize and Sorghum in Brazil has developed a series of maize lines and hybrids differing in phosphorus acquisition efficiency. Bacterial and arbuscular endomycorrhizal (AM) fungi have an important function in soil nutrient acquisition and mobilization, especially phosphorus (P). Soil and root samples were taken from the root zone of maize cultivars during flowering growing red latosol with low P availability. The root zone soil of P efficient and inefficient maize hybrids and inbred lines were studied. Mycorrhizal and bacterial DNA was extracted from the rhizosphere soil using BIO 101 Kit protocols. The mycorrhizal rDNA fragments were amplified based on nested PCR using fungal universal primers and the ITS/Glomaceae specific primers. Sixteen S rDNA fragments were amplified to study bacterial populations. PCR products were loaded in a 6% polyacrylamide gel with denaturing gradients between 45% and 70% of urea-formamide gels (DGGE) and run for 16h in a BIO-RAD Dcode System, VA, USA. A routine silver staining protocol was used for detection of DNA and the gels were photographed with a digital camera. The mycorrhizal and bacterial-DGGE profiles obtained suggest population differences in the microbial communities in the rhizosphere of P efficient and P inefficient maize genotypes grown under P stress. The profiles showed bands that were present in P efficient lines only, indicating that some mycorrhizal groups are favored with efficient maize genotypes. The results suggest a greater genetic bacterial population diversity in the rhizosphere soil of genotypes under P stress, indicating that certain microbial populations may be favored with P stress.This study demonstrated the potential of using DGGE fingerprints based upon 16S rDNA fragments amplified via nested PCR to characterize the bacterial community in the rhizosphere of maize cultivars. The results demonstrate that the ITS/Glomaceae specific primers used in the present study, and DGGE, can be used to differentiate the diversity and dynamics of complex natural mycorrhizal communities; however more studies will be necessary to discriminate phylogenetically closely related organisms in the rDNA sequences. Additional research is currently being conducted with other primers looking to improve the methodology and identify possible differences between maize genotypes contrasting for P efficiency.

Introduction

Studies of rhizosphere microbial communities have indicated that different plant species or genotypes influence the composition of the mycorrhizal and bacterial community differentially due to the differential response of populations to different root exudation patterns, especially when plants are under environmental stress (Abbott & Robson, 1991). Maize plants have a high growth rate and a high demand for nutrients and are highly colonized by mycorrhizal fungi (Clark & Zeto, 1996). However, little information is available about the development and dynamics of AM and bacterial populations in the rhizosphere in maize genotypes contrasting in P efficiency. AM identification by culturing requires several months of growth in trap cultures in greenhouse conditions which may influence fungal development and survival (Simon *et al.*, 1992). As a result, they may not reflect the population distribution in the field. In this study, DNA based methodology, DGGE generated fingerprints, was used to investigate the diversity and variation of microbial communities in the rhizosphere of maize genotypes contrasting for P efficiency.

Material and Methods

Soil and root samples were taken from maize cultivars at flowering growing in a red latosol under P deficient or sufficient conditions. Two P efficient inbred lines, L3 and L228, and an inefficient line, L22, all developed by the maize breeding program at Embrapa Maize and Sorghum at Sete Lagoas, MG, Brazil were studied. Some samples were taken from the no-tillage (NT) and no-tillage under high P (NTP) and cerrado soil (CS). The mycorrhizal and bacterial DNA was extracted from the rhizosphere soil using BIO 101 Kit protocols. The mycorrhizal rDNA fragments were amplified based on nested PCR using fungal universal primers (NS5 and ITS4) and the ITS/Glomaceae specific primers GLOM1310 and ITS2 with a CG clamp (Redecker, 2000). For bacteria, 16S rDNA fragments were amplified using the 968CG and R1401 primers. PCR products were loaded on a 6% polyacrylamide gel with denaturing gradients between 45 and 70% of urea-formamide gels (DGGE) and run for 16h in a BIO-RAD Dcode System, VA, USA. A routine silver staining protocol was used for detection of DNA and the gels were photographed with a digital camera. The bands of interest were excised from the DGGE gel and amplified with PCR for species identification by sequencing.

Results and Discussion

The mycorrhizal and bacterial DGGE profiles suggest differences in microbial community composition in the rhizosphere between P efficient and P inefficient maize genotypes. The AM profiles showed bands that were present in P efficient lines only (Fig.1-1, see arrows), indicating that some mycorrhizal fungi are favored by P efficient maize genotypes. The results suggested the existence of greater genetic bacterial population diversity in the rhizosphere soil of genotypes under P stress, indicating that certain microbial species may be favored with P stress (Fig.1-2, arrow 1). Some bands occurred (Fig.1-2) in the samples from hybrids at low P, but not at high P.

When comparing the rhizosphere of the hybrid HT cultivated under conventional tillage and under no-tillage, we found some differences in the composition of the mycorrhizal and bacterial communities (Fig.1-1 and 2, arrow 2 and 3; lanes NTP, NT, CS), indicating that the agricultural management may be selecting different bacteria and AM species in the rhizosphere. The productivity of plant communities will depend on the symbiotic effectiveness of the colonizing microbial populations and the P efficiency of genotypes.

This study demonstrated the potential of using DGGE fingerprints to characterize the bacterial population in the rhizosphere of maize cultivars. The results demonstrate that the ITS/Glomaceae specific primers used in the present study, and DGGE, can be used to differentiate the diversity and dynamics of complex natural mycorrhizal communities; however, more studies will be necessary to discriminate phylogenetically closely related organisms in the rDNA sequences. Additional research is currently being conducted with other primers to improve the methodology and identify possible differences between maize genotypes contrasting for P efficiency.

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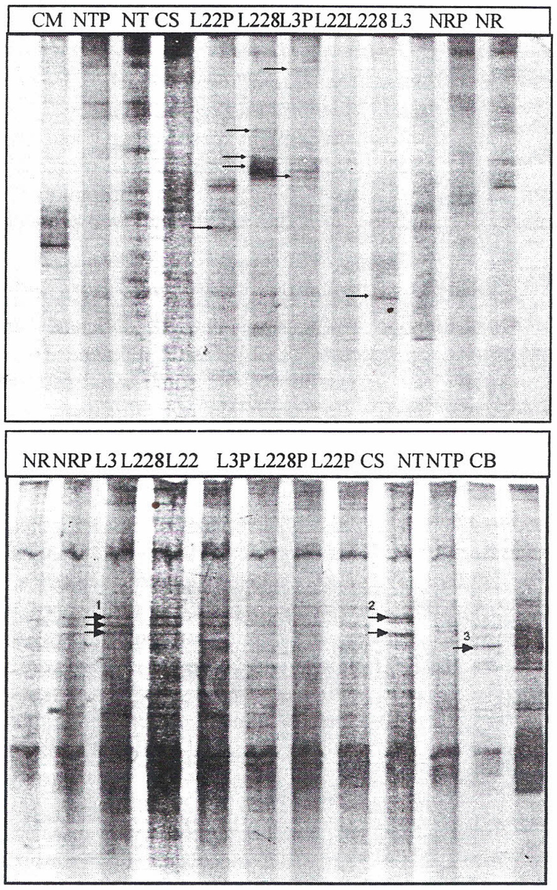

Figure 1. DGGE patterns of rhizosphere soil with P efficient lines (lanes L3 and L228) and P inefficient line (lane L22) obtained from maize genotypes under low P soil, and high P soil (lanes L3P, L228P, L22P); no-tillage (lane NT) and no-tillage under high P (lane NTP) and cerrado soil (CS). Lane CM and CB contain the DGGE markers and the lanes NR and NP contains patterns of no-rhizosphere soil under low P and under high P respectively. 1) Mycorrhizal DGGE; 2) BacterialDGGE. The arrows indicate the soil microorganisms (or bands) for the different treatments.