Mycorrhizal diversity analyzed by denaturing gradient gel electrophoresis in the rhizosphere of maize (*Zea mays*) genotypes contrasting for phosphorus efficiency

Christiane Abreu de Oliveira[1], Nadja Maria Horta de Sá[3], Eliane Aparecida Gomes[2], Maria Rita Scotti Muzzi[3], Robert E. Schaffert[2], Sidney Neto Parentoni[2], Cláudia Teixeira Guimarães[2], Ivanildo Evódio Marriel[2], Vera Maria Carvalho Alves[2]

[1]McKnight CCRP/Embrapa Maize and Sorghum Fellow; CP 151, CEP 35701-970, Sete Lagoas, MG, Brazil. E-mail:ampaiva@netzero.net, [2]Embrapa Maize and Sorghum, CP 151, CEP 35701-970, Sete Lagoas, MG, Brazil. [3]Universidade Federal de Minas Gerais, Departamento de Botânica, CP 486, *CEP 31270901, Belo Horizonte, MG, Brazil.*

Vesicular-arbuscular endomycorrhizal (VAM) fungi have an important function in soil nutrient acquisition and mobilization, mainly phosphorus (P). Studies of rhizosphere microbial communities have indicated that the presence of different plant species or genotypes influence the composition of the mycorrhizal community due to the differential response of fungal population to different root exudation patterns, especially when plants are under environmental stress (Abbot & Robson, 1991). Maize plants have a high growth rate and a high demand for nutrients, frequently having micotrophic interactions (Clark & Zeto, 1996). However, little information is available on interactions among maize genotypes contrasting for P efficiency and the development and dynamics of VAM in the rhizosphere. Recognizing, identifying, and quantifying of VAMs involved are difficult since these fungi are unable to grow in pure culture. Culturing required several months to grow in trap cultures in greenhouse conditions effecting fungal development and survival (Simon et al., 1992). As a result, population distribution in the field may be misinterpreted. DNA based methodology, developing DGGE generated fingerprints, was used to investigate the diversity and variation of VAM communities in the rhizosphere of maize genotypes contrasting for P efficiency. Soil and root samples were taken from the root zone of maize cultivars growing in a red latosol, clay texture soil during flowering. Three P efficient maize hybrids (HT, HS1, HS2), two efficient inbred lines (L3 and L228), two inefficient hybrids (HS3, HS4) and an inefficient line (L22), all developed by the maize breeding program at Embrapa Maize and Sorghum at Sete Lagoas, MG, Brazil were studied. The mycorrhizal DNA was extracted from the rhizosphere soil using BIO 101 Kit protocols and the VAM amplified rDNA fragments of 18S fungal ribosomal genes were separated based on nested PCR using fungal universal primers (NS1, NS4) and the specific primers to VAM populations attached with the CG sequence clamp, VANS1 and NS21 (Simon et al., 1992). PCR products were loaded by electrophoresis and run for 16h in a BIO-RAD Dcode System, VA, USA. A routine silver staining protocol was used for detection of DNA in DGGE gels. Root samples were used to determine the percentage of roots colonized by VAM. The roots were washed with water, cut into 1.0 cm pieces, fixed in ethanol, clarified and stained with trypan blue. The stained roots were examined stereomicroscopically and percent colonization was assessed using the grid line intersect method of Giovanneti & Mosse, 1980.

The mycorrhizal-DGGE profiles reflected population differences in the mycorrhizal community in the rhizosphere of P efficient and P inefficient maize genotypes. The profiles showed bands that were presents in P efficient lines only (Fig.1-3, see arrows), indicating that some mycorrhizal groups are present in efficient maize genotypes. The inefficient hybrid HS3 had a lower number of bands (Fig.1-2). Comparison of the rhizosphere of HT cultivated under conventional tillage and under no-tillage, indicated some differences in the composition of the mycorrhizal community (Fig.1-1, see arrows), indicating that different production systems may influence the VAM species present in the rhizosphere. This study demonstrated the potential of using DGGE fingerprints based upon 18S rDNA fragments amplified via nested PCR to characterize the VAM population in the rhizosphere of maize cultivars. Stereomicroscope observations of mycorrhizal colonization in roots

obtained from genotypes contrasting for P efficiency indicated significant differences for root colonization confirming mycorrhiza genetic diversity (Figs. 2 and 3). Mycorrhizal root colonization was highest in roots of the P efficient maize cultivars, especially HS2 with low soil P.

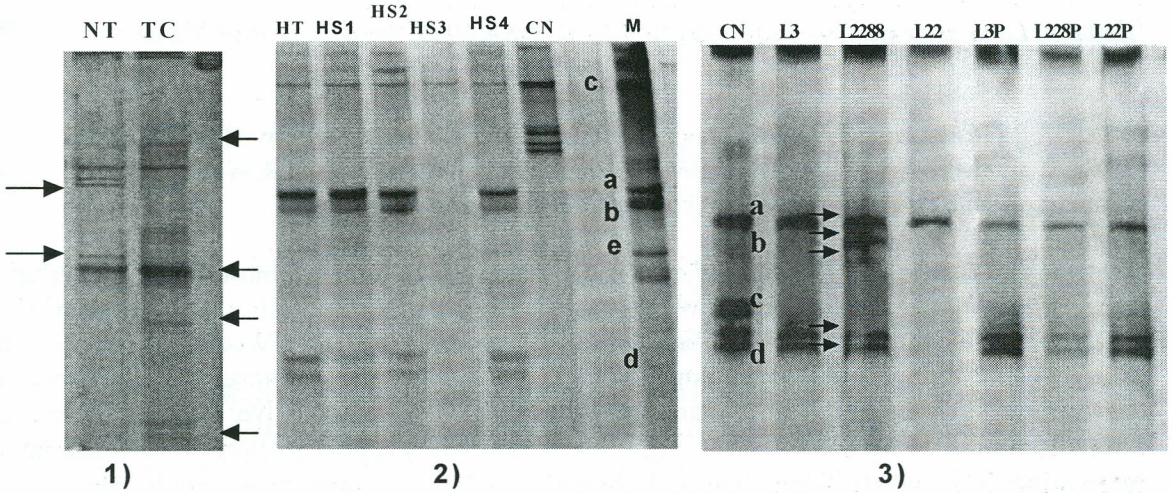

Figure 1. DGGE patterns of rhizosphere soil obtained from maize genotypes, grown in two different P levels. **1)** rhizosphere soil of hybrid HT under no-tillage (lane NT) and under conventional tillage (lane CT); **2)** rhizosphere soil with P efficient hybrids HT, HS1, HS2 and inefficient hybrids HS3 and HS4 , lane CN and M contains the DGGE markers; (a) *Acaulospora morronoe*, (b) *Acaulospora serobienta*, (c) *Glomus clarum*, (d) *Gigaspora margarita*, (e) *Glomus etunicatum*.; **3)** rhizosfere soil with P efficient lines L3 and L228 P inefficient line L22 under low P soil (lanes L3, L228, L22) and high P soil (lanes L3P, L228P, L 22P). The arrows indicate the soil microorganisms (or bands) for the different treatments.

Colonization was lowest in the P inefficient cultivars (Fig. 2) and highest in the efficient hybrids, but without significant differences between the P levels. Colonization (Figure 3), was highest in the efficient genotype, L3 under P stress (100% root colonization) and about 50% more than under high P soil. The results demonstrate that the VAM 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.

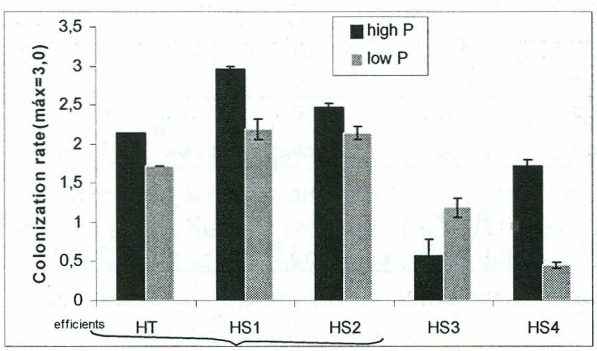

Figure 2. Mycorrhizal colonization of maize roots of contrasting hybrids for P efficiency under P stress and no stress.

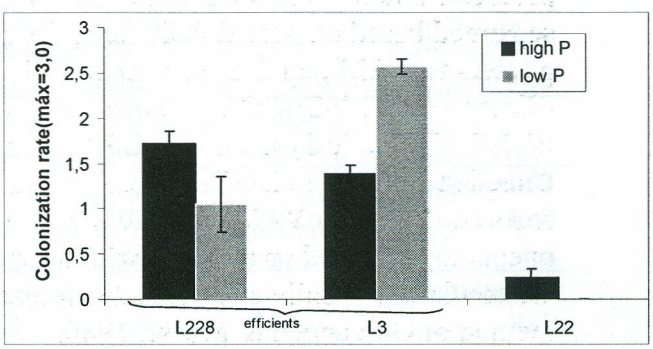

Figure 3. Mycorrhizal colonization of maize lines contrasting for P efficiency under P stress and no stress.

L.K. Abbott, A.D. Robson (1991) *Ecosystems and Environment*, 35, 121-150.
R.B. Clark, R.B.S.K. Zeto (1996) *Soil Biol. Biochem.*, 28, 1505-1511.
M. Giovanetti, B. Mosse (1980) *New Phytologist*, 84, 482-500.
L.Simon, M. Lalonde, T.D.Bruns (1992). *Appl. Environ. Microbiol.* 54, 2908-2915.