J. Microbiol. Biotechnol. (2015), 25(7), 978–987
http://dx.doi.org/10.4014/jmb.1408.08002

jmb

Arbuscular Mycorrhizal Fungal Communities in the Roots of Maize Lines Contrasting for Al Tolerance Grown in Limed and Non-Limed Brazilian Oxisoil

Eliane A. Gomes*, Christiane A. Oliveira, Ubiraci G. P. Lana, Roberto W. Noda, Ivanildo E. Marriel, and Francisco A. de Souza

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

Received: August 4, 2014
Revised: February 3, 2015
Accepted: February 5, 2015

First published online
February 12, 2015

*Corresponding author
Phone: +55-31-3027-1290;
Fax: +55-31-3027-1279;
E-mail:
eliane.a.gomes@embrapa.br

pISSN 1017-7825, eISSN 1738-8872

Aluminum (Al) toxicity is one of the greatest limitations to agriculture in acid soils, particularly in tropical regions. Arbuscular mycorrhizal fungi (AMF) can supply plants with nutrients and give protection against Al toxicity. The aim of this work was to evaluate the effects of soil liming (*i.e.*, reducing Al saturation) on the AMF community composition and structure in the roots of maize lines contrasting for Al tolerance. To this end, we constructed four 18S rDNA cloning libraries from L3 (Al tolerant) and L22 (Al sensitive) maize lines grown in limed and non-limed soils. A total of 790 clones were sequenced, 69% belonging to the Glomeromycota phylum. The remaining sequences were from Ascomycota, which were more prominent in the limed soil, mainly in the L3 line. The most abundant AM fungal clones were related to the family Glomeraceae represented by the genera uncultured *Glomus* followed by *Rhizophagus* and *Funneliformis*. However, the most abundant operational taxonomic units with 27% of the Glomeromycota clones was affiliated to genus *Racocetra*. This genus was present in all the four libraries, but it was predominant in the non-limed soils, suggesting that *Racocetra* is tolerant to Al toxicity. Similarly, *Acaulospora* and *Rhizophagus* were also present mostly in both lines in non-limed soils. The community richness of AMF in the non-limed soils was higher than the limed soil for both lines. The results suggest that the soil Al saturation was the parameter that mostly influences the AMF species composition in the soils in this study.

Keywords: Aluminum saturation, arbuscular mycorrhizal fungi (AMF), *Zea mays* L., molecular diversity, 18S rDNA

Introduction

Tropical soils are in general highly weathered and dystrophic, exposing plants to various forms of stress during their growth cycle [13, 56]. One of the limiting factors of agricultural expansion in regions with such soils is the aluminum (Al) toxicity and the high binding capacity of phosphorus (P) to the soil matrix, resulting in low availability of this nutrient to the plants [27]. Ideally, sustainable agriculture should be based on cultivars and/or plant-microbial associations adapted to stress conditions to improve the efficiency of soil nutrient acquisition in these areas and therefore increase production yield [38].

One alternative to prevent the occurrence of Al toxicity in acid soils is to apply lime to raise the soil pH. However, liming may also affect the activity and composition of microbial populations, and although being a common agricultural practice, relatively little is known about its impact on the microbial communities in tropical soils.

Symbiosis establishment among arbuscular mycorrhizal fungi (AMF) and the majority of the land plant roots has a great potential to increase plant growth in tropical soils by mediating Al toxicity in the mycorrhizosphere. Mycorrhizal fungi are known for their ability to enhance plant nutrient uptake, particularly P, improving nutrient acquisition, which may be important under Al exposure [22, 39, 46, 57].

They have been shown to increase the productivity of most crop species, including maize [52], one of the most important crops in the world with high growth rate and high demand for nutrients. Additionally, AMF can also alleviate stresses such as drought and root pathogen attack, improve soil structure, and confer tolerance to metal in plants [3, 7, 49, 51].

Previous studies have shown that soil acidity and liming appear to be an edaphic factor with great influence on the distribution of AMF species, although the effects vary in different fungal species and isolates [10, 15, 18, 33, 48, 55]. van Aarle *et al*. [55] evaluated the response of the AMF *Scutellospora calospora* and *Rhizophagus intraradices* in association with *Plantago lanceolata* to two different pH treatments. They observed that in high soil pH, the total AMF root colonization decreased for both fungi and the arbuscule and vesicle formation was reduced in *R. intraradices*. On the other hand, Göransson *et al*. [15] sampled tussocks of four grass species together with samples of the surrounding soil, in oak forests of varying soil pH, and they observed low AMF colonization of roots in the most acidic soils, which was ascribed to Al toxicity.

According to previous studies by our group, there are differences in AMF root colonization and denaturing gradient gel electrophoresis (DGGE) profiles of maize genotypes, contrasting in their P efficiency and Al tolerance cultivated in soils with different P concentrations, suggesting that these genotypes may influence the community of AMF in the rhizosphere [30]. However, in this study, the authors did not access the phylogeny or the composition and structure of the AMF communities present inside the roots of these maize genotypes.

In the present study, we examined the same maize lines evaluated by Oliveira *et al*. [30] to assess whether these genotypes harbor differences in the root AMF communities and also if the soil liming affects AMF community composition and structure. To this end, we constructed four 18S rDNA cloning libraries from roots of two maize lines contrasting in Al tolerance, grown on soil with high (non-limed soil) and low Al saturation (limed soil). We found that both genotypes cultivated in soil with high Al saturation exhibited high AMF community richness and that uncultured *Glomus* was the dominant genus in the majority of libraries. *Rhizophagus* and *Funneliformis* were other important genera predominately under non-limed and limed conditions, respectively. In addition, we report a high frequency of non-AM fungi on limed soils, mainly in the Al-tolerant maize line. These results shed light into the factors controlling AMF phylotypes colonizing roots of contrasting maize genotypes and its interaction with limed and non-limed soils. Understanding the effects of the interaction between plant genotypes and soil liming upon AMF communities might help to develop strategies to improve the benefits of this symbiosis that are applicable to sustainable agriculture in acidic tropical regions.

Materials and Methods

Field Experimental Design

The field experiment was performed in a red Oxisol soil of the Brazilian Savanna Biome (Cerrado) during the summer (December through March) at Embrapa Maize and Sorghum located in Sete Lagoas, Minas Gerais, Brazil, at latitude 19º28′S and longitude 44º15′W, at an altitude of 732 m. The local climate is the Aw, according to the Köppen classification, with a mean temperature of 22°C, rainfall of 1,300 mm, and mean relative humidity of 70%. The experiment had a 2 × 2 factorial design, with the maize genotypes and Al saturation as the factors, using a randomized complete block design with three replicates. The maize genotypes consisted of two lines: L3 (Al-tolerant) and L22 (Al-sensitive), according to Parentoni *et al*. [31]. The soil pH was raised from 4.9 to 6.1 by liming, with the consequence that the Al saturation dropped from 32% to 2% (Table 1). Each experimental plot consisted of two rows, 5 m long, with 0.8 m between rows and 0.2 m between plants.

Sampling

Samples were collected 60 days after sowing during the flowering period of the crop. The root samples of five random

Table 1. Chemical soil properties at the study sites.

Treatments	pH[a] (H_2O)	H + Al	Al	Ca	Mg	K	P	SBC	CEC	BS	Al sat	Organic matter
		$cmol_c$/kg				mg/kg		$cmol_c$/kg		(%)		dag/kg
High Al saturation	4.9	7.10	1.13	1.77	0.36	82	9	2.34	9.44	25	32	3.48
Low Al saturation	6.1	3.31	0.13	3.75	0.98	82	13	4.94	8.25	60	2	3.16

[a]soil/water ratio 1:2.5 (w/v).
SBC = sum of bases cations.
CEC = cation exchange capacity.
BS = base saturation.
Al sat. = Aluminum saturation.

individual plants were pooled for each of the three replicate field plots per experimental treatment. In total, 12 root samples were collected (two plant genotypes, two types of soils, and three replicates). The fine roots were stored in a cool box during transport to the laboratory, washed to remove soil debris, frozen in liquid nitrogen, and stored at −80ºC until DNA extraction.

Root DNA Extraction and PCR Amplification

DNA was extracted from 100 mg of fine roots of the three replicates of each treatment as described by Saghai-Maroof *et al*. [40]. The rDNA of the AMF was amplified separately for each replicate, using a semi-nested PCR approach. The first step was performed with the small subunit ribosomal DNA primers using the universal primer NS31 [47] and mycorrhizal fungi-specific primer AM1 [19]. One microliter of the resulting PCR product was used as template for the semi-nested PCR, performed with the forward primer NS31 and the reverse primer FM7 (5′-GCT TTCGCAGWAGTTAGTCTTCA-3′). This primer set generates products approximately 397 bp in length with enhanced specificity toward AMF as compared with the NS31:AM1 set (data not shown). Nevertheless, it also amplified Ascomycota when tested using primer BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). Amplification reactions were performed in a total volume of 50 µl, containing 20 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM of each dNTP, 1 unit of *Taq* DNA polymerase, and 50 ng of DNA. The reactions were run in a Veriti thermocycler (Life Technologies, Foster City, CA, USA), programmed for 94°C for 1 min, followed by 40 cycles for the first step and 25 cycles for the semi-nested step at 95°C, 1 min; 94°C, 20 sec; 55°C, 35 sec; 72°C, 1 min; and a final extension step of 5 min at 72°C. Negative controls with no DNA template were included in each experiment.

DNA Cloning, Sequencing, and Phylogenetic Analysis

Amplified fragments from three replicates were purified from the agarose gel with the QIAquick system (Qiagen, Hilden, Germany) and cloned using the pJET2.1 vector (Fermentas International, Inc., ON, Canada) following both manufacturers' instructions. Based on Renker *et al*. [37], DNAs were amplified separately and pooled, and the amplicons were used for constructing four clone libraries. Recombinant clones of *Escherichia coli* were sequenced on an ABI Prism 3100 automatic sequencer using the M13 primers and Big Dye terminator ver. 3.1 kit (Life Technologies). Four libraries were constructed from the root samples collected from L3 and L22 genotypes cultivated in non-limed soil of high Al saturation (L3HS, L22HS) and limed soil of low Al saturation (L3LS, L22LS), respectively.

Processing parameters for sequences were analyzed by Phred quality ≥13 (error probability less than 5%) and sequence size ≥100 nucleotides [12].

The partial sequences of the clones obtained in this study were compared with the SILVA database (http://www.arb-silva.de) [34] using the algorithm BLAST-N [2] to identify the most similar sequences. The sequences were also aligned by the program Clustal W and manually edited by SeaView [16].

The Glomeromycota taxonomy and classification used in this study were that proposed by Oehl *et al*. [29]; however, we considered *Rhizophagus* and *Sclerocystis* members of the Glomeraceae, as proposed by Schüßler and Walker [45] and Redecker *et al*. [36].

Forty representatives of those operational taxonomic units (OTUs) containing two or more sequences generated by Mothur ver. 1.30.0 [44] and 37 reference sequences from public databases were used to construct a phylogenetic tree. The sequences were aligned using MUSCLE [11], improved by manual and automatic corrections, and the poorly aligned regions were removed from the final alignment using G-blocks [53]. The phylogenetic trees were obtained by maximum likelihood with the program PhyML 3.0 [17] implemented in SeaView 4.4.0 [16] using the GTR model, with six rate categories for across site rate variation, empirical nucleotide equilibrium frequencies, optimized percentage of invariable sites, and option "best" of NNI and SPR for the tree search. The initial tree was obtained by BioNJ. Bootstrap values were estimated from 1,000 replicates. The OTUs were grouped into several clades within the phylogenetic tree, and assigned to classes, orders, families, and genera of AMF, according to the current literature.

The partial rDNA gene sequences of the 547 AMF clones reported in this study have been deposited in GenBank under accession numbers KF488843−KF489389.

Statistical Analysis

All the statistical analyses were performed after normalization, by the Mothur program, based on the smallest AMF clone number of 93, corresponding to the L3LS library (Table 2). The pairwise distances were used as input for the program Mothur in order to group the sequences into OTUs at a defined sequence identity. A threshold of 97% nucleotide identity, corresponding to 0.03 dissimilarity ($OTU_{0.03}$), was used to define OTUs, rarefaction curves, Chao diversity estimators, Shannon-Wiener diversity index, coverage, and evenness, and to draw a Venn diagram using the Mothur program.

Results

We wanted to know about the diversity of AMF communities harbored by different maize genotypes and

Table 2. Number of total clones sequenced and clones of arbuscular mycorrhizal fungi (AMF).

Libraries[a]	Total clones	Clones of AMF
L22HS	202	170
L22LS	190	143
L3HS	179	141
L3LS	219	93

[a]See Fig. 1 for clone library identity.

the effects of soil conditions on these communities. To this end, we constructed four rDNA libraries from the roots of maize genotypes contrasting for Al tolerance cultivated in limed and non-limed soils. OTUs were identified and used to construct a phylogenetic tree to understand the diversity and structure of the AMF communities in these soils. This approach provided an overview of the profiles of the AMF communities that emerged in response to the change of soil condition due to liming, which is known to decrease Al toxicity.

Identities of the Sequences in the Clone Libraries

We combined BLAST searches and phylogenetic analysis to determine the phylogenetic affiliation of the clones obtained from the four libraries, L3HS (L3 in non-limed soil), L3LS (L3 in limed soil), L22HS (L22 in non-limed soil), and L22LS (L22 in limed soil). From a total of 790 clones sequenced from the four libraries, 547 clones (69.2% of the total) showed high similarity to sequences of taxa belonging to the phylum Glomeromycota, distributed in 70 OTUs as L3HS (78.8%), L3LS (42.5%), L22HS (84.1%), and L22LS (72.3%) (Table 2). The 243 remaining sequences were from the phylum Ascomycota, mainly members of the genus *Fusarium*. They were detected in all libraries, but they were more prominent in the L3 and L22 libraries obtained from limed soil (57.5% and 24.7%, respectively) (Fig. 1).

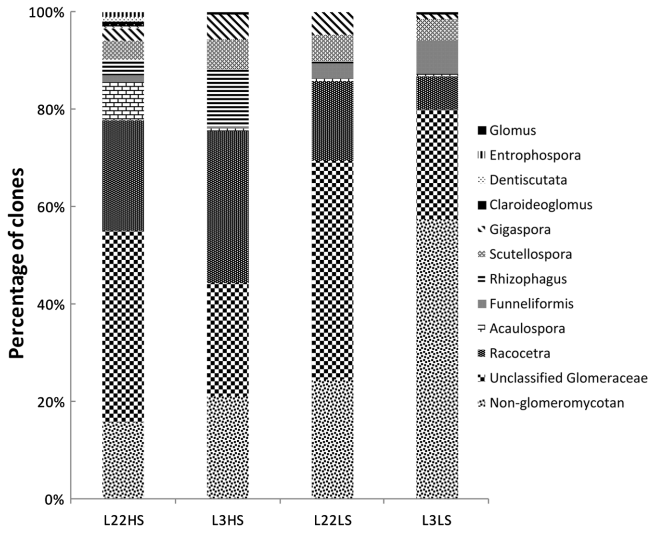


Fig. 1. Percent fraction of arbuscular mycorrhizal fungi (AMF) clones per AMF genus.

The sequences were recovered from four clone libraries of PCR amplicons of root DNA extracts and assigned to AMF taxa, using BLAST similarity searches against the public sequence databases. L22HS (L22 genotype cultivated in high Al saturation soil) L22LS (L22, low Al saturation soil), L3HS (L3, high Al saturation soil), and L3LS (L3, low Al saturation soil).

OTU and Phylogenetic Analysis of AMF Sequences

In order to analyze the OTUs of the AMF communities in the four libraries, only sequences belonging to Glomeromycota were selected for the subsequent analysis. The distribution of 547 Glomeromycota sequences among the libraries and the number of AMF OTUs at 97% similarity, calculated after normalization based on the smallest AMF clone number (93 clones corresponding to the L3LS library) are shown in Tables 2 and 3.

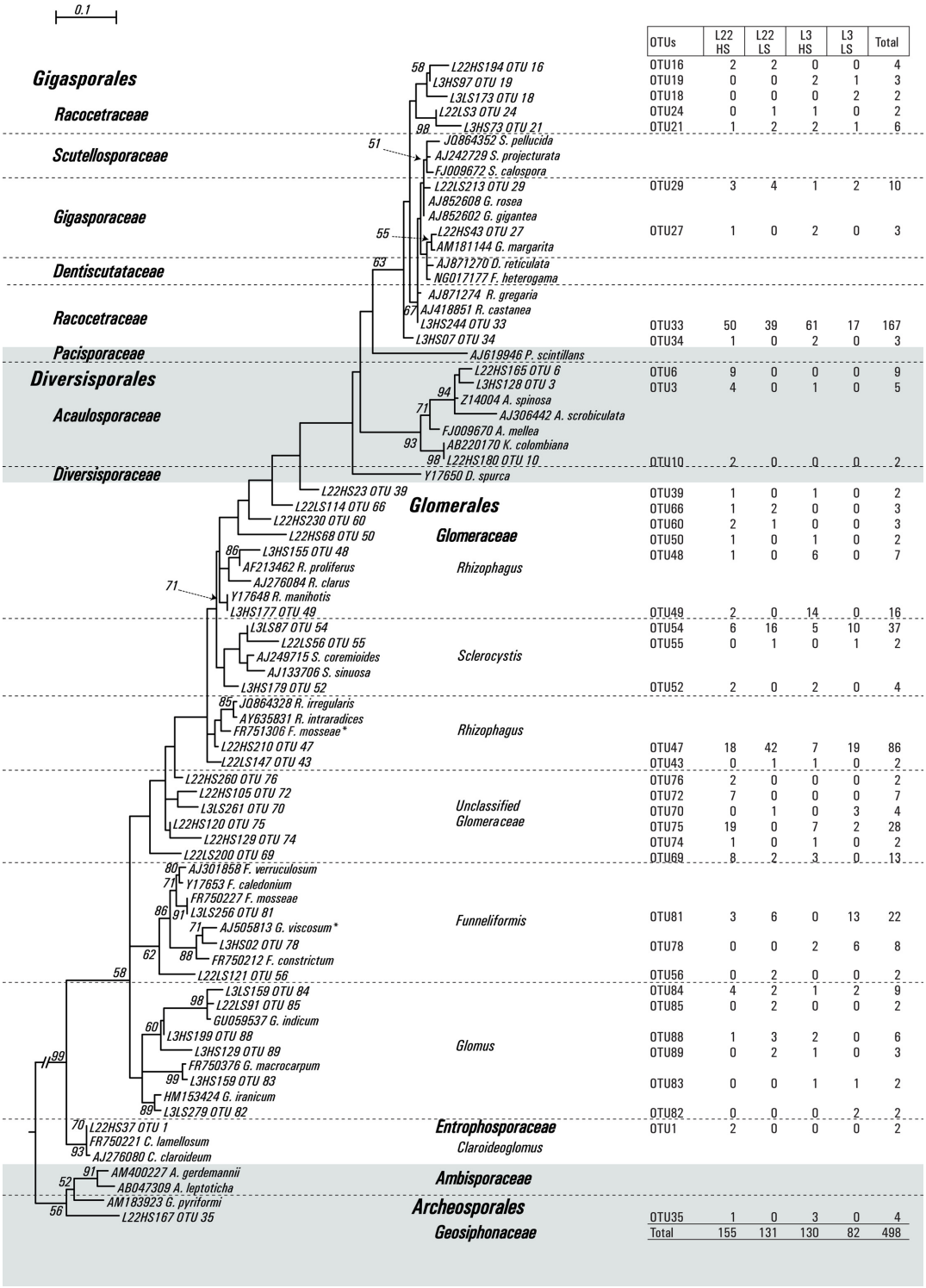
To minimize the possibility of incorporating artifacts and sequencing error, only OTUs containing two or more sequences were considered for phylogenetic analysis. All the sequences after alignment were trimmed to 372 bp. In total, representative sequences of 40 OTUs generated by program Mothur and 37 reference sequences from public databases were used to construct a maximum likelihood phylogenetic tree (Fig. 2). The results of the phylogenetic tree were in good concordance with the distribution of the clones by the BLAST analysis. By far, the most abundant OTU, with 167 sequences (27% of the Glomeromycota clones) represented in the four libraries, was affiliated to the genus *Racocetra* (Gigasporales order). However, the next six most frequent OTUs belonged to the order

Table 3. Observed versus estimated (Chao non-parametric richness estimators), Shannon diversity index, coverage, and eveness of arbuscular mycorrhizal fungi OTUs, at 97% sequence identity.

Libraries	Number of observed OTUs	Chao estimator	Shannon diversity	Coverage	Evenness
L22HS	28	52 (35;109)[a]	2.66 (2.41;2.91)	0.83	0.80
L22LS	20	29 (22;57)	2.09 (1.83;2.34)	0.88	0.70
L3HS	31	66 (43;136)	2.44 (2.12;2.77)	0.77	0.71
L3LS	25	46 (31;98)	2.51 (2.27;2.75)	0.84	0.78

All the calculations were done after normalization by the Mothur program, based on the smallest AMF clone number of 93, corresponding to the L3LS library. See Fig. 1 for clone library identity.

[a]95% confidence interval.



OTUs	L22 HS	L22 LS	L3 HS	L3 LS	Total
OTU16	2	2	0	0	4
OTU19	0	0	2	1	3
OTU18	0	0	0	2	2
OTU24	0	1	1	0	2
OTU21	1	2	2	1	6
OTU29	3	4	1	2	10
OTU27	1	0	2	0	3
OTU33	50	39	61	17	167
OTU34	1	0	2	0	3
OTU6	9	0	0	0	9
OTU3	4	0	1	0	5
OTU10	2	0	0	0	2
OTU39	1	0	1	0	2
OTU66	1	2	0	0	3
OTU60	2	1	0	0	3
OTU50	1	0	1	0	2
OTU48	1	0	6	0	7
OTU49	2	0	14	0	16
OTU54	6	16	5	10	37
OTU55	0	1	0	1	2
OTU52	2	0	2	0	4
OTU47	18	42	7	19	86
OTU43	0	1	1	0	2
OTU76	2	0	0	0	2
OTU72	7	0	0	0	7
OTU70	0	1	0	3	4
OTU75	19	0	7	2	28
OTU74	1	0	1	0	2
OTU69	8	2	3	0	13
OTU81	3	6	0	13	22
OTU78	0	0	2	6	8
OTU56	0	2	0	0	2
OTU84	4	2	1	2	9
OTU85	0	2	0	0	2
OTU88	1	3	2	0	6
OTU89	0	2	1	0	3
OTU83	0	0	1	1	2
OTU82	0	0	0	2	2
OTU1	2	0	0	0	2
OTU35	1	0	3	0	4
Total	155	131	130	82	498

Fig. 2. Maximum likelihood phylogeny of partial 18S rRNA gene sequences of 40 representatives of operational taxonomic units (OTUs) of arbuscular mycorrhizal fungi recovered from maize roots (labeled with the library name) and 37 reference sequences from public databases (labeled with the accession number).

Bootstrap values estimated from 1,000 replicates larger than 50% are indicated above the lines. The scale bar indicates the number of nucleotide substitutions per site. The table beside the phylogenetic tree shows the number of sequences belonging to a specific library. See Fig. 1 for clone library identity.

Glomerales, with 202 sequences (37% of the clones) among all the treatments, and uncultured *Glomus* was the predominant genus (Fig. 2).

Representatives of the genera *Acaulospora* and *Rhizophagus* were generally present in the non-limed soils. Sixteen clones of the genus *Acaulospora* occurred only in the L22HS library, whereas the other three clones of this genus were distributed equally in the other libraries (Figs. 1 and 2). The genus *Rhizophagus* was also almost exclusively present in soil of high Al saturation, with 21 clones in L3HS and six in the L22HS library and only one clone in L22LS. The genera *Geosiphon* (four sequences in L3HS and L22HS), and *Entrophospora* and *Dentiscutata* (two sequences each in L22HS) were also found in high Al saturation soil (Figs. 1 and 2). Sequences from Archaeosporaceae and Paraglomeraceae were not detected as expected after using the AM1 primer, which misses these taxa [35].

According to the Venn diagram (Fig. 3), 212 Glomeromycota sequences were shared by all the four libraries distributed in four OTUs, including the most abundant OTU affiliated to genus *Racocetra*, at 97% similarity. The Venn diagram also shows that the two libraries obtained from soils with high Al saturation (L3HS and L22HS) shared the most OTUs (12) and sequences number (136) in relation to the other libraries.

Diversity and Structure of the AMF Communities

To analyze and to compare the richness and diversity indices, the coverage, and the evenness, the number of Glomeromycota clones of all libraries was normalized to 93 sequences (based on the smallest library L3LS) such that each sequence contributes equally to the distance calculated. The community richness estimation using 97% sequence identity revealed the highest values for the Chao estimator for the libraries L22HS and L3HS, with coverage of 83%, 88%, 77%, and 84% to libraries L22HS, L22LS, L3HS, and L3LS, respectively (Table 3). The library L22LS also showed the smallest Shannon diversity index, which measures the diversity of the community. In addition, the evenness values, which shows the distribution of individuals among OTUs, were higher in the libraries L3LS and L22HS than in the others libraries.

To determine whether samplings of clones obtained from each library were representative of the AMF diversity found in the samples, a rarefaction curve was constructed for each library, based on OTUs defined by a similarity level of 97% (Fig. 4). The rising tendency of the curve suggests that the sequencing of additional clones would lead to a large increase in the number of phylotypes found and that the sequence diversity of the community sample was not totally covered.

Discussion

In this work, we constructed 18S rDNA clone libraries of maize roots to investigate whether contrasting maize lines to Al tolerance differ in the composition and structure of the AMF communities when grown in soils with low and high Al saturation (soil limed and non-limed, respectively). The findings of this study may indicate that the level of Al

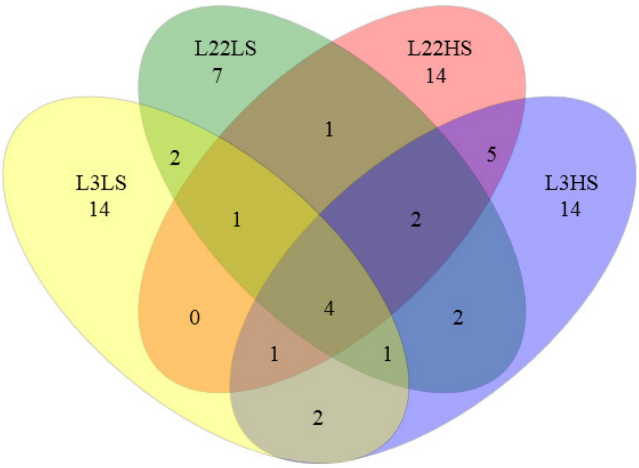


Fig. 3. Venn diagram at 97% nucleotide sequence identity similarity, showing the number of operational taxonomic units shared among the clone libraries.
See Fig. 1 for clone library identity.

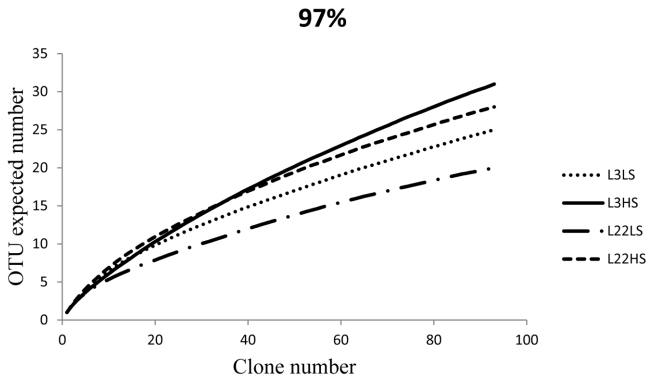


Fig. 4. Sampling effort curves showing the relationships between operational taxonomic unit richness of arbuscular mycorrhizal fungi and the number of clones at the sequence divergences of 97% recovered from four clone libraries of maize root samples.
See Fig. 1 for clone library identity.

saturation of soils can differentially affect the structure of AMF communities in roots. This study also revealed a higher frequency of endophytic Ascomycota in roots of maize cultivated in soil of low Al saturation than such cultivated in soil of high Al toxicity.

AMF and Non-AMF Representation in the Clone Libraries

Sequencing of the clones revealed an increased frequency of Ascomycota-related sequences in the L3 genotype cultivated in limed soil (57.5% of 219 clones), when compared with the others libraries (Fig. 1). Interestingly, the library with the second most clones of Ascomycota was L22 (24.7% of 190 clones) cultivated in limed soil of low Al saturation (Fig. 1). A possible explanation for this difference is that in soils of high Al saturation, plants exude organic acid such as citrate, malate, or oxalate in response to exposure to Al [24], stimulating the roots colonization by a diverse group of AMF in stressed plants, while in the non-stressed plants only the more competitive fungi could colonize the roots. Moreover, the community richness estimated for the libraries from the stressed plants were higher than the non-stressed plants (discussed in section below). As this is the first study to show an increase in the frequency of endophytic Ascomycota in relation to the limed soil, additional studies are necessary to understand if such associations are beneficial or detrimental to the plants.

The primer pair AM1/NS31 used in this study, which covers a central region of about 550 bp of the small subunit rDNA, has been widely used in studies of molecular diversity of AMF in previous studies [4, 5, 10, 19]. The lack of specificity of this primer pair for the phylum Glomeromycota has been already reported in the literature [5, 41, 54]. Santos-González *et al*. [41] detected 16.6% of the sequences similar to Ascomycota in roots of two perennial forbs in semi-natural grasslands in a study using the same primer combinations. These authors suggested that studies on the distribution of mycorrhizal fungi should pay more attention to the distribution of other groups of fungi colonizing roots. Borrielo *et al*. [5] recovered 41% sequences affiliated to Ascomycota in maize fields. Similarly, Toljander *et al*. [54] ascribed 59% of their sequences to Ascomycota in a study of maize roots in a long-term fertilization trial. According to these authors, it may indicate that plant roots in agricultural systems are more heavily colonized by non-mycorrhizal fungi. For the purpose of this study, we designed an additional primer (FM7) to reduce the problem of lack of AMF-specificity, but this primer also amplified sequences of Ascomycota.

Identities of AMF Sequences Based on BLAST Similarity Searches and Phylogenetic Analysis

The results of BLAST analysis as well the topology of the phylogenetic tree indicated that, among the AMFungi, the most abundant clones were related to the family Glomeraceae, represented mostly by the uncultured genus *Glomus* (45.3% of all AMF sequences) followed by *Rhizophagus* and *Funneliformis* (Figs. 1 and 2). Other authors also demonstrated that species of *Glomus* were the main colonizers of maize [5, 42], particularly in high-input managed agrosystems [20, 26]. Sasvári *et al*. [42] showed that the mycorrhizal fungi *Glomus*, *Funneiliformis,* and *Rhizophagus* (former *Glomus* group A) dominate the AMF community of maize, and they found a relatively high richness of phylotypes within this group, even under conditions where maize has been grown in exclusive monoculture for half a century. The dominance of *Glomus* species in such a system may be related to their ability to disseminate *via* fragments of mycelium or mycorrhizal root fragments. These structures can be easily found in fields upon soil physical disturbance imposed either through agricultural use or by heavy machinery, such as plowing between crop cycles [26, 43].

According to some authors, many *Glomus* species are acidic and/or Al sensitive [6, 32], whereas *Scutellospora*, *Racocetra*, *Gigaspora,* and *Acaulospora* were thought to be less sensitive. In this work, the genus *Racocetra* was found in all libraries, but predominantly in the genotypes cultivated in the non-limed soils (Figs. 1 and 2). Siqueira *et al*. [48] observed that acidic soil inhibited mycorrhizal formation by *F. mosseae* in maize through its strong fungistatic effect on spore germination. Here, we found the genus *Funneliformis* mostly in the Al-tolerant genotype cultivated in limed soil. In contrast, the genus *Rhizophagus* was present in soil of high Al saturation, where it colonized mainly the Al-tolerant maize genotype (Figs. 1 and 2). Klugh-Stewart and Cumming [23] observed that plants colonized by *R. clarus* and *Dentiscutata heterogama* (formerly *G. clarum* and *S. heterogama*) did not exhibit reduction in growth when exposed to Al, produced high concentrations of Al-chelating organic acids, and showed low concentrations of free Al in their root zones. *R. clarus* was also the most aggressive colonizer and provided the most benefits to yellow poplar plants exposed to Al [22].

To answer the question if these genera are important to Al tolerance of these maize genotypes, it will be necessary

to analyze more samples to a greater coverage as possible *via* next-generation sequencing.

Arbuscular Mycorrhizal Fungal Community Richness

The L22 and L3 lines of maize cultivated in non-limed soil (L22HS and L3HS, respectively) hosted AMF communities of higher estimated OTU richness compared with the same lines grown in limed soil (Table 3). Besides this, the two libraries from maize cultivated in limed soils presented the highest number of non-mycorrhizal fungi (Fig. 1).

Soil acidity may have a direct effect on AMF distribution as well as an indirect effect by influencing nutrient availability [18]. The Al tolerance in maize genotypes is mainly associated with an Al-exclusion mechanism mediated by citrate exudation in root tips [24]. Previous studies have demonstrated that the L3 line grown in a nutrient solution increased citrate exudation with exposure time and with increasing Al activities. Other less-tolerant maize lines in the presence of Al also increased the organic acids exudation compared with non-stress condition (data not published). Based on this, the highest AMF community richness observed in the lines grown in the non-limed soil may be associated with their citrate exudation, which can support more colonization and growth of AMF. Guo *et al*. [18] also observed that the AMF phylotypes diversity decreased in soils and plant roots when long-term lime was applied compared with the non-limed treatments. These authors suggest that the low AMF diversity in the non-stressed plants may be due to the response of AMF species to reduced soluble carbohydrate in root exudates in limed plants, which could consequently support less mutualistic AMF than nutrient-stressed plants in non-limed soils. This may be attributed to the differential exudation once plants grown in soils with nutritional stresses can exudate functional substances, such as organic acids, jasmonic acid, phosphatases, and phenolic compounds, which can stimulate colonization and growth of the AMF and influence host-AMF specificity [14, 21, 25, 50].

However, the explanation of the observed differences based on competition between AMF species and differential root exudation is still a working hypothesis that requires the analysis of more data, obtained with a higher number of plant genotypes and different soil conditions, in order to be fully tested. Future studies are also required to select the most efficient AMF groups for the condition of limed soil in Brazil.

In general, the soil chemistry, rather than genotypes, may determine the distribution of AMF in acid soils [1, 8, 9, 15, 18, 28]. In our study, both genotypes cultivated in non-limed soil showed high AMF OTU richness and diversity, suggesting that the Al saturation was more important than genetic identity of the host plant as determinants of the AMF communities in roots (Table 3). Similarly, the Venn diagram showed that L3 and L22 genotypes grown in a non-limed soil shared 12 OTUs and 136 sequences, while the same genotype in different Al saturation shared smaller number of OTUs (8 for each pair of lines: L22HS/L22LS and L3HS/L3LS). This result may indicate that the soil Al saturation was the most important factor affecting the distribution of AMF. Oehl *et al*. [28] also showed that land use intensity and soil type are major determinants of the composition and species richness of AMF communities in Central European soils. Furthermore, they concluded that soil pH is the parameter that mostly influences the AMF species composition in soils, especially under conditions of lower land use intensity.

The findings of this study may be important in efforts to more effectively use and manipulate native AMF communities in different types of tropical acidic soils, using practices of management of soil microbial communities to enhance plant growth. In extensive annual crops such as maize, inoculation with AMF is not widely used owing to the large volume of inoculum required [49]. An alternative would be the management of AMF adapted to the local soil conditions. AMF are present naturally in most agricultural soils in not sufficient numbers to trigger positive nutritional responses in fast growing and short-lived crop plants. Moreover, as AMF may play a role in conferring Al tolerance to the plant hosts [22, 39, 46, 57], their use as biofertilizers should be considered in environmentally friendly low input agriculture on acidic soils with phytotoxic Al levels.

Acknowledgments

This work was funded by the Brazilian Agricultural Research Corporation (Embrapa), the Brazilian Council of Research and Scientific Development (CNPq), and the Foundation of Research Support of Minas Gerais State (FAPEMIG).

References

1. Alguacil MM, Torrecillas E, Roldán A, Díaz G, Torres MP. 2012. Perennial plant species from semiarid gypsum soils support higher AMF diversity in roots than the annual *Bromus rubens*. *Soil Biol. Biochem*. **49:** 132-138
2. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST:

a new generation of protein database search programs. *Nucleic Acids Res*. **25:** 3389-3402.
3. Augé RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11:** 3-42.
4. Bedini S, Turrini A, Rigo C, Argese E, Giovannetti M. 2010. Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice. *Soil Biol. Biochem*. **42:** 758-765.
5. Borriello R, Lumini E, Girlanda M, Bonfante P, Bianciotto V. 2012. Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. *Biol. Fertil. Soils* **48:** 911-922.
6. Clark RB. 1997. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant Soil* **192:** 15-22.
7. Clark RB, Zeto SK. 1996. Growth and root colonization of mycorrhizal maize grown on acid and alkaline soil. *Soil Biol. Biochem*. **28:** 1505-1511.
8. da Mota FF, Gomes EA, Marriel IE, Paiva E, Seldin L. 2008. Bacterial and fungal communities in bulk soil and rhizospheres of aluminum-tolerant and aluminum-sensitive maize (*Zea mays* L.) lines cultivated in unlimed and limed Cerrado soil. *J. Microbiol. Biotechnol*. **18:** 805-814.
9. Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, *et al*. 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytol*. **190:** 794-804.
10. Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME J*. **4:** 337-345.
11. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res*. **32:** 1792-1797.
12. Ewing B, Hillier L, Wendl MC, Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Res*. **8:** 175-185.
13. Fageria NK, Baligar VC, Zobel RW. 2007. Yield, nutrient uptake and soil chemical properties as influenced by liming and boron application in common bean in a no-tillage system. *Commun. Soil Sci. Plant Anal*. **38:** 1637-1653.
14. Gianinazzi-Pearson V, Branzanti B, Gianinazzi S. 1989. *In vitro* enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. *Symbiosis* **7:** 243-255.
15. Göransson P, Olsson PA, Postma J, Falkengren-Grerup U. 2008. Colonisation by arbuscular mycorrhizal and fine endophytic fungi in four woodland grasses – variation in relation to pH and aluminium. *Soil Biol. Biochem*. **40:** 2260-2265.
16. Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol*. **27:** 221-224.
17. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk M, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 30. *Syst. Biol.* **59:** 307-321.
18. Guo YJ, Ni Y, Raman H, Wilson BAL, Ash GJ, Wang AS, Li GD. 2012. Arbuscular mycorrhizal fungal diversity in perennial pastures; responses to long-term lime application. *Plant Soil* **351:** 389-403.
19. Helgason T, Daniell TJ, Husband R, Fitter AH, Young JP. 1998. Ploughing up the wood-wide web? *Nature* **394:** 431.
20. Hijri I, Sýkorová Z, Oehl F, Ineichen K, Mäder P, Wiemken A, Redecker D. 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Mol. Ecol*. **15:** 2277-2289.
21. Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* **237:** 173-195.
22. Klugh KR, Cumming JR. 2007. Variations in organic acid exudation and aluminum resistance among arbuscular mycorrhizal species colonizing *Liriodendron tulipifera*. *Tree Physiol.* **27:** 1103-1112.
23. Klugh-Stewart K, Cumming J. 2009. Organic acid exudation by mycorrhizal *Andropogon virginicus* L (broomsedge) roots in response to aluminum. *Soil Biol. Biochem*. **41:** 367-373.
24. Kochian L, Hoekenga O, Piñeros M. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol.* **55:** 459-493.
25. Ladygina N, Hedlund K. 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. *Soil Biol. Biochem*. **42:** 162-168.
26. Lin X, Feng Y, Zhang H, Chen R, Wang J, Zhang J, Chu H. 2012. Long-term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in North China revealed by 454 pyrosequencing. *Environ. Sci. Technol*. **46:** 5764-5771.
27. Marschner P, Solaiman Z, Rengel Z. 2006. Rhizosphere properties of Poaceae genotypes under P-limiting conditions. *Plant Soil* **283:** 11-24.
28. Oehl F, Laczko E, Bogenrieder A, Stahr K, Bösch R, van der Heijden M, Sieverding E. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biol. Biochem.* **42:** 724-738.
29. Oehl F, Sieverding E, Palenzuela J, Ineichen K, Alves da Silva G. 2011. Advances in Glomeromycota taxonomy and classification. *IMA Fungus* **2:** 191-199.
30. Oliveira CA, Sá NMH, Gomes EA, Marriel IE, Scotti MR, Guimarães CT, *et al*. 2009. Assessment of the mycorrhizal community in the rhizosphere of maize (*Zea mays* L) genotypes contrasting for phosphorus efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE). *Appl. Soil Ecol*. **41:** 249-258.
31. Parentoni SN, de Souza Jr CL, Alves VMC, Gama EEG, Coelho AM, Oliveira AC, *et al*. 2010. Inheritance and

breeding strategies for phosphorus efficiency in tropical maize (*Zea mays* L). *Maydica* **55:** 1-15.
32. Porter WM, Robson AD, Abbott LK. 1987. Factors controlling the distribution of vesicular-arbuscular mycorrhizal fungi in relation to soil pH. *J. Appl. Ecol*. **24:** 663-672.
33. Postma JWM, Olsson PA, Falkengren-Grerup U. 2007. Root colonisation by arbuscular mycorrhizal fine endophytic and dark septate fungi across a pH gradient in acid beech forests. *Soil Biol. Biochem*. **39:** 400-408.
34. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, *et al*. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41:** 590-596.
35. Redecker D. 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza* **10:** 73-80.
36. Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* **23:** 515-531.
37. Renker C, Weißhuhn K, Kellner H, Buscot F. 2006. Rationalizing molecular analysis of field-collected roots for assessing diversity of arbuscular mycorrhizal fungi: to pool, or not to pool, that is the question. *Mycorrhiza* **16:** 525-531.
38. Richardson AE, Lynch JP, Ryan PR, Delhaize E, Smith FA, Smith SE, *et al*. 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil* **349:** 121-156.
39. Rufyikiri G, Dufey JE, Nootens D, Delvaux B. 2000. Effect of aluminium on bananas (*Musa* spp.) cultivated in acid solutions. I. Plant growth and chemical composition. *Fruits* **55:** 367-379.
40. Saghai-Maroof MA, Soliman KA, Jorgensen RA, Allard RW. 1984. Ribossomal DNA spacer length polymorphism in barley: mendelian inheritance, chromosomal location, and populations dynamics. *Proc. Natl. Acad. Sci. USA* **81:** 8014-8018.
41. Santos-González JC, Finlay RD, Tehler A. 2007. Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. *Appl. Environ. Microbiol.* **73:** 5613-5623.
42. Sasvári Z, Hornok L, Posta K. 2011. The community structure of arbuscular mycorrhizal fungi in roots of maize grown in a 50-year monoculture. *Biol. Fertil. Soils* **47:** 167-176.
43. Schalamuk S, Cabello M. 2010. Arbuscular mycorrhizal fungal propagules from tillage and no-tillage systems: possible effects on Glomeromycota diversity. *Mycologia* **102:** 261-268.
44. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, *et al*. 2009. Introducing Mothur: open-source platform-independent community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75:** 7537-7541.
45. Schüßler A, Walker C. 2010. The Glomeromycota. A species list with new families and new genera. Available at http://wwwlrzde/wschuessler/amphylo/amphylospecieshtml.
46. Seguel A, Cumming JR, Klugh-Stewart K, Cornejo P, Borie F. 2013. The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. *Mycorrhiza* **23:** 167-183.
47. Simon L, Lalonde M, Bruns TD. 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Appl. Environ. Microbiol.* **58:** 291-295.
48. Siqueira JO, Hubbell DH, Mahmud AW. 1984. Effect of liming on spore germination, germ tube growth and root colonization by vesicular-arbuscular mycorrhizal fungi. *Plant Soil* **76:** 115-124.
49. Siqueira JO, Lambais MR, Sturmer SL. 2002. Fungos micorrízicos arbusculares: características associação simbiótica e aplicação na agricultura. *Biotecnolog. Ciênc. Desenvolv*. **25:** 12-21.
50. Siqueira JO, Safir GR, Nair MG. 1991. VA-mycorrhizae and mycorrhiza stimulating isoflavonoid compounds reduce plant herbicide injury. *Plant Soil* **134:** 233-242.
51. Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*, 3rd Ed. Academic Press, Cambridge.
52. Sylvia DE, Hammond LC, Bennet JM, Hass JH, Linda SB. 1993. Field response of maize to a VAM fungus and water management. *Agron. J*. **85:** 193-198.
53. Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* **56:** 564-577.
54. Toljander JF, Santos-González JC, Tehler A, Finlay RD. 2008. Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial. *FEMS Microbiol. Ecol.* **65:** 323-338.
55. van Aarle IM, Söderström B, Olsson PA. 2003. Growth and interactions of arbuscular mycorrhizal fungi in soils from limestone and acid rock habitats. *Soil Biol. Biochem*. **35:** 1557-1564.
56. von Uexkull H, Mutert E. 1995. Global extent development and economic impact of acid soils. *Plant Soil* **171:** 1-15.
57. Yano K, Takaki M. 2005. Mycorrhizal alleviation of acid soil stress in the sweet potato (*Ipomoea batatas*). *Soil Biol. Biochem*. **37:** 1569-1572.