Comparison of root colonization by arbuscular mycorrhizal fungi in energy crop species cultivated on arable land contaminated with heavy metals

To cite this article: K Nowak et al 2019 IOP Conf. Ser.: Earth Environ. Sci. 214 012030

View the article online for updates and enhancements.
Comparison of root colonization by arbuscular mycorrhizal fungi in energy crop species cultivated on arable land contaminated with heavy metals

K Nowak1,*, A Szada-Borzyszkowska1, J Krzyżak1, S Rusinowski1, M Soja1, M Pogrzeba1

1 Institute for Ecology of Industrial Areas, Poland

E-mail: k.nowak@ietu.pl

Abstract. Arbuscular mycorrhiza is a widespread terrestrial symbiosis, involving 80 – 85% of vascular plants in almost all ecosystems. AM fungi increase plant tolerance to heavy metals by developing mechanisms allowing immobilization metals within or near the root and preventing their translocation to the shoot. Therefore, AMF could be involved in phytostabilization of heavy metals contaminated soils. The aim of the presented study was to explore the effect of heavy metals on arbuscular mycorrhizal fungi (AMF) root colonization of selected energy crop species: Miscanthus x giganteus, Panicum virgatum and Spartina pectinata cultivated on heavy metals contaminated arable land under different fertilisation (NPK vs. microbial inoculation). All examined plant species formed arbuscular mycorrhizal associations and differences in degree of mycorrhizal colonization among experimental variants were found. Influence of soil fertilization on AMF root colonization was species-dependent. Both NPK fertilization and microbial inoculation resulted in reduction of mycorrhizal colonization in Miscanthus x giganteus, whereas in Panicum virgatum degree of AMF root colonisation was increased. Soil fertilisation had no effect on degree of Spartina pectinata root mycorrhization, since they were almost fully colonized independently on fertilization. In conclusions, contamination with heavy metals did not result in restriction of mycorrhizal colonization, which could be a consequence of AMF adaptation to such conditions. Nevertheless, among tested species, higher level of mycorrhizal colonization was found for Spartina pectinata roots. It indicates the highest susceptibility of this species to development of mycorrhizal structures regardless of heavy metal pollution and fertilization.
1. Introduction

Intense industrialization is one of the factors that decreases quality of agricultural production, consistently negatively affecting human health [1,2]. Therefore degraded and heavy metals contaminated lands seem to be appropriate to energy crop production [3].

To improve yields and quality of crops, different fertilizers are used, including chemical fertilization and microbial inoculation. The most common chemical fertilization is based on using nitrogen, phosphorus and potassium to soil treatment, in order to increase availability of nutrients for plant development [4]. In turn, using microbial inoculation can promote organic matter decomposition and increase nutrient concentrations in the soil. This kind of inoculants can consist of Plant-Growth Promoting Bacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) [2, 5, 6].

Associations between plants and AMF, consisting of the infection of plant roots by fungal species belonging to phylum Glomeromycota are called arbuscular mycorrhiza [7-11]. The essence of this symbiosis is cooperation roots of host with fungal hyphae, which enables enhancing plants ability for extracting nutrients from the soil [12, 13]. Recently more and more often emphasizes the role of mycorrhiza in reducing stress reactions associated with environmental pollution with heavy metals [14-19]. Arbuscular mycorrhizal fungi (AM) is presumably the most common mycorrhizal type. It is estimated, that 70 – 90 % of all plant species can undergo a symbiosis with arbuscular mycorrhizal fungi [8, 9, 11]. The presence of arbuscular mycorrhizal fungi was observed in the soils of most ecosystems, also in the soils contaminated with heavy metals. Mycorrhizal effects are diversified and may depend on elements, plant and fungal species/ecotypes [13]. AMF can abolish plant stress caused by toxic concentration of heavy metals through several mechanisms, such as: immobilization of heavy metals within or near the root, chelating of metals inside the fungus, adsorption in fungal cell walls or precipitation in polyphosphate granules in the soil [20].

The aim of the present study was to explore the effect of heavy metals on arbuscular mycorrhizal fungi (AMF) root colonization of selected energy crop species: Miscanthus x giganteus, Panicum virgatum and Spartina pectinata cultivated on heavy metals contaminated arable land under different fertilisation (NPK vs. microbial inoculation).

2. Material and methods

2.1. Site description

The experiment was conducted on contaminated arable land in Bytom (Upper Silesia), Poland (50°20'43.0"N 18°57'19.6"E) on the experimental site of the Institute for Ecology of Industrial Areas. Soil was characterized by heavy metals contamination, resulting from short distance from closed-down Pb/Zn smelter. Total soil Pb, Cd and Zn concentration exceed the maximum threshold values proscribed by Polish government (Dz.U. 2016 poz. 1395), excluding this area from food production. Average values of temperature and total precipitation measured during 2015 and 2016 growing season were 22/18°C and 120/350 mm respectively (Institute of Meterology and Water Management, Poland).

2.2. Experiment design

Soil samples were collected from each experimental plot established in May 2014 before planting investigated species (May 2014). Subsequently, the plots were used to cultivation of Miscanthus x giganteus, Panicum virgatum and Spartina pectinata (Figure 1). On each plot 49 plants were planted over an area of 16 m² with a buffer zone of 4 m between each plot. Each plot was treated in a different manner:
a) P1 – Control (without treatment);
b) P2 – NPK standard fertilization, applied directly to the soil before planting (M. x giganteus – nitrogen 70 kg ha\(^{-1}\), phosphorus 30 kg ha\(^{-1}\) as \(\text{P}_2\text{O}_5\) and potassium 45 kg ha\(^{-1}\) as \(\text{K}_2\text{O}\); P. virgatum – nitrogen 70 kg ha\(^{-1}\), phosphorus 30 kg ha\(^{-1}\) as \(\text{P}_2\text{O}_5\) and potassium 45 kg ha\(^{-1}\) as \(\text{K}_2\text{O}\); S. pectinata – nitrogen 80 kg ha\(^{-1}\), phosphorus 50 kg ha\(^{-1}\) as \(\text{P}_2\text{O}_5\) and potassium 75 kg ha\(^{-1}\) as \(\text{K}_2\text{O}\));
c) P3 – Commercial microbial inoculum Emfarma Plus\(^{\circledR}\) ProBiotics Poland (Lactic Acid Bacteria \(> 3.0 \times 10^5\text{cfu ml}^{-1}\), Yeast \(< 1.0 \times 10^6\text{cfu ml}^{-1}\), and Purple Non-Sulfur Bacteria \(> 1.0 \times 10^4\text{cfu ml}^{-1}\) in molasses suspension). Eight liters of 10% water solution of Emfarma Plus\(^{\circledR}\) was sprayed on the soil surface; additionally the roots of the seedlings were soaked in this solution at the beginning of the experiment. Furthermore, 10% water solution of Emfarma Plus\(^{\circledR}\) as aerosol was spread on the leaves in the middle of every month during the growing season.

In October 2016, root and soil samples were collected for further analysis. Soil samples were air dried and sieved through 2 mm (for texture, organic matter content, pH, EC) and then ground \(< 0.25\text{ mm}\) for further analysis (total and bioavailable metal concentration). The root material after collecting, was washed with tap water and then with deionized water.

**Figure 1.** Three species of energy crops: Miscanthus x giganteus (a), Panicum virgatum (b), Spartina pectinata (c).

### 2.3. Soil analysis

Soil physico-chemical parameters (soil pH and soil organic matter content) were measured on soil sieved through a 2 mm sieve. Soil pH was measured in \(\text{H}_2\text{O}\) and 1 M KCl (ratio 1:2.5 m/v) with a combination glass/calomel electrode (OSH 10-10, METRON, Poland) and a pH-meter (CPC-551, Elmetron, Poland) at 20°C. Soil organic matter content (OM) estimation was carried out as follows: air dry soil was dried at 105°C for 24 h and then (5 g) treated with 550°C for 4 hours. The bioavailable forms of metals in the soil were extracted using 0.01 M \(\text{CaCl}_2\) [21]. During extraction, 5 g of air-dried soil (\(< 0.25\text{ mm}\)) was inserted in 50 mL 0.01 M \(\text{CaCl}_2\) solution and shaken for 2 hours. Bioavailable metal concentrations (Cd, Pb, Zn) were measured in filtrate by a flame atomic absorption spectrometry (iCE 3500 FAAS, Thermo Scientific). The total concentrations of metals (Cd, Pb, Zn) were obtained using hot plate digestion (concentrated \(\text{HNO}_3\) and \(\text{HClO}_4\), ratio 4:1 v/v) and flame atomic absorption spectrometry (iCE 3500 FAAS, Thermo Scientific).

### 2.4. Arbuscular mycorrhiza colonization measurement

For the estimation of root colonization level by AMF, the roots were prepared according to a modified method of Philips and Hayman [22]. After washing in tap and deionized water, the roots were dipped in 7% KOH at 80 °C according to selected time dependent on tested species, i.e. 25 min., 35 min. and 45 min. for S. pectinata, M. x giganteus and P. virgatum, respectively. Subsequently, roots were acidified in 5% lactic acid for 24 h and also stained with 0.05% aniline blue in lactic acid.
for 24 h. Subsequently, the evaluation of AMF colonization was conducted according to Trouvelot method [23].

3. Results

3.1. Soil characteristics

3.1.1. Soil physico-chemical parameters

Soil pH was slightly acidic and values did not differ between all variants. Analysis of soil organic matter content (OM) did not show any significant differences between plots (Table 1).

| Parameters                  | Variants | P1                                         | P2                                         | P3                                         |
|-----------------------------|----------|--------------------------------------------|--------------------------------------------|--------------------------------------------|
| Concentration of elements in soils |          | Cd total 17.43 ± 2.95 a                    | 17.53 ± 3.85 a                            | 17.90 ± 3.84 a                            |
|                             |          | Pb total 480.27 ± 93.42 a                  | 459.70 ± 93.04 a                          | 465.47 ± 90.29 a                          |
|                             |          | Zn total 1617.00 ± 234.44 a                | 1684.67 ± 399.15 a                        | 1705.00 ± 361.98 a                        |
|                             |          | Cd bioavailable 1.02 ± 0.49 a              | 1.14 ± 0.26 a                             | 0.92 ± 0.25 a                             |
|                             |          | Pb bioavailable 480.27 ± 93.42 a          | 459.70 ± 93.04 a                          | 465.47 ± 90.29 a                          |
|                             |          | Zn bioavailable 1617.00 ± 234.44 a        | 1684.67 ± 399.15 a                        | 1705.00 ± 361.98 a                        |
|                             |          | pH (H₂O) 6.65 ± 0.25 a                    | 6.61 ± 0.12 a                             | 6.67 ± 0.13 a                             |
|                             |          | pH (KCl) 6.14 ± 0.31 a                    | 6.06 ± 0.11 a                             | 6.17 ± 0.13 a                             |
|                             |          | OM (%) 4.91 ± 0.79 a                      | 5.01 ± 0.88 a                             | 5.21 ± 0.87 a                             |

Values are means ± SE (n = 3). Lower case letters (a, b, c) denote significant differences among parameters at P ≤ 0.05 according to Fisher LSD test. P1 - Control; P2 – NPK fertilization; P3 – Microbial inoculum fertilization; OM – Organic matter.

3.1.2. Elements concentration in the soil

Elements concentration in the soils are presented in Table 1. Total soil Cd, Pb and Zn concentrations did not differ between plots. Bioavailability of Cd and Zn was comparable within each plot. Bioavailable lead concentrations were below the quantification limit.

3.2. Mycorrhizal studies

Studies show the presence of arbuscular mycorrhiza structures, i.e. intracellular hyphae, arbuscules and vesicles in all examined plants (Figure 3-5).

In the assessment of mycorrhizal colonization three mycorrhizal parameters were taken into consideration: the frequency of mycorrhiza in the root system (F%), intensity of the mycorrhizal colonization in the root system (M%), and arbuscule abundance in the root system (A%).

The relative arbuscular richness in roots of analyzed plants grown on different plots is shown in Figure 2a. Both NPK fertilization and microbial inoculation caused decrease of arbuscule abundance in the root system (A%) in Miscanthus x giganteus. Different tendency in Panicum virgatum was observed, where arbuscule abundance was increased under fertilization. No significant differences were observed between Spartina pectinata grown on differently treated plots. Among plants grown on P1 plot, the highest values were found in M. x giganteus and S. pectinata and also they did not differ between themselves. In contrast, lack of soil fertilization caused reduction of arbuscule abundance in P. virgatum roots. Both in P2 and P3, in M. x giganteus roots fertilizations caused decrease of A%, in contrast to the others, which did not differ between themselves.
Frequency of mycorrhiza in the root system (F%) of analyzed plants grown on different plots is shown in Figure 2b. No significant differences were observed between *M. x giganteus* grown on differently treated plots. In *P. virgatum*, mycorrhizal frequency, similar to arbuscular richness in roots, was fertilization-dependent, due to lower values obtained in plants grown on P1 plot (control). No significant differences were observed between *S. pectinata* grown on differently treated plots. Among plants grown on P1 plot, the highest values were found in *S. pectinata* and there were no differences between *M. x giganteus* and *P. virgatum*, which were characterized by lower values of this parameter. Both in P2 and P3, the highest F% was reported in *S. pectinata* roots, in contrast to *M. x giganteus*, which characterized by the lowest values.
Figure 2. Mycorrhizal colonization in *Miscanthus x giganteus*, *Panicum virgatum*, *Spartina pectinata* roots. The relative arbuscular richness (A%) (a), mycorrhizal frequency (F%) (b), relative mycorrhizal roots (M%) (c). Values are means ± SE (n=3). Lower case letters (a, b) denote significant differences between plants in each experimental plot at P ≤ 0.05 according to Fisher LSD test.

The intensity of root colonization (M%) of analyzed plants grown on different plots is shown in Figure 2c. Both NPK fertilization and microbial inoculation caused decrease of this parameter in *M. x giganteus*. Different tendency in *P. virgatum* was observed, where intensity of root colonization was increased under fertilization. No significant differences were observed between *S. pectinata* grown on differently treated plots. Among plants grown on P1 plot, the highest values were found in *M. x giganteus* and *S. pectinata* and also they did not differ between themselves. In contrast to them, lack of soil fertilization caused reduction of intensity of root colonization in *P. virgatum* roots. Both in P2 and P3, in *M. x giganteus* roots, fertilizations caused decrease of M%, in contrast to the others, which did not differ between themselves.

Figure 3. Mycorrhizal structures in *Miscanthus x giganteus* roots. Apr (appressorium); c (coil); Ih (intracellular hyphae); S (spore); Eh (extracellular hyphae); Arb (arbuscules).
Figure 4. Mycorrhizal structures in *Panicum virgatum* roots. v (vesicle); c (coil); Ih (intracellular hyphae); Arb (arbuscules).

Figure 5. Mycorrhizal structures in *Spartina pectinata* roots. Eh (extracellular hyphae); c (coil); Ih (intracellular hyphae); s (spore); Arb (arbuscules); v (vesicle); DSE (dematiaceous septate hyphae and microsclerotia of dark septate fungal endophytes).

Additionally, DSE were found in *S. pectinata*, while they were absent in other root samples. The mycelium was brownish and occurred mainly in root fragments where arbuscules were not present. Both dematiaceous septate hyphae and microsclerotia were observed (Figure 5j).

4. Discussion

The arbuscular mycorrhizal associations in such energy crops as *Miscanthus x giganteus*, *Panicum virgatum*, and *Spartina pectinata* were already the subject of earlier studies. Nevertheless, most of them, were based on experiments carried out in controlled conditions with the use of mycorrhizal inoculum [19, 24, 25]. This study is an attempt to assess degree of energy crops roots colonization by native arbuscular mycorrhizal fungi, which is rarely found in literature. Additionally, during research
the effect of different fertilizations on the naturally developing arbuscular mycorrhizal associations was investigated.

Commonly used agricultural practice is fertilization aimed at improving crop yields. It is known, that the nitrogen, phosphorus and potassium compounds present in fertilizers can cause different effects dependent of plant species and experiments. Fertilizers with P and K can lead to improved microbiological properties of the soil by increasing the availability of minerals and improving the metabolic capacity of soil microorganisms, which consequently causes increase the diversity of microflora [26, 27]. However, there are the reports, that mineral fertilization can have a profound negative effect on mycorrhizal fungi, which presumably results in reduction of their root colonization [28]. Results of this study confirmed this dependence in M. x giganteus, in which degree of colonization was the highest for soil without any treatments and under NPK fertilization, there is dramatic decrease of this parameter. The negative effect of soil N content on AMF root colonization in plants has been documented by several authors [29, 30]. Also Corkidi et al. [31] reported, that fertilization with nitrogen reduces effectiveness of AM fungi in root colonization, which could be resulted from receptivity to N eutrophication. Nitrogen presumably reduces species diversity of AM fungal communities and changes in the species composition. Also, lower root AMF colonization as a result of phosphorus fertilization has been described [32]. Lower degree of AMF root colonization in M. x giganteus for P3 was also observed. It was presumably connected with competition between microorganisms derived from inoculum and native AMF (2, 33].

Different results were obtained for P., in which both NPK fertilization and microbial inoculation resulted in increase of AMF root colonization. It confirms observations of Wilson et al [34], wherein N enrichment resulted in an increase in mycorrhization and simultaneously increase plant allocation to the mycobionts. Therefore, AMF response to N fertilization is highly variable, which could be dependent on fungal and plant diversity and also differences in specific fungal-plant interactions. It could be related also to morphological changes in plant roots caused by high nitrogen availability [35].

Arbuscular mycorrhizal fungi show a degree of host specificity in plants, and are not irregularly distributed. Additionally, host plant species affects the structure of their associated AM fungal communities [36]. Root colonization by AMF in S.pectinata was fertilization-independent and in all experimental variants (P1, P2, P3) similar values were found. It could have been the result of the highest susceptibility of this species to AMF root colonization regardless of soil conditions. S. pectinata as the host plant, evidently had direct effects on AMF root colonization. Variation in diversity and degree of colonization of host’s root may be dependent to several mechanisms, including its phenology, mycorrhizal dependency and host plant-mediated modifications of the soil microenvironment [37]. Due to the presence of dark septate endophytic fungi only in S. pectinata, we hypothesized, that they may contribute in enhancing intensity of root colonization by arbuscular mycorrhizal fungi. These observations are confirmed by results obtained by Scervino et al. [38], who reported that, exudates of DSE stimulate the hyphal branching of the AMF, which presumably could be the reason of increasing in root colonization. Nevertheless, in literature there is no numerous studies referring to interactions between DSE and AMF.

Studies have shown, that influence of soil fertilization on AMF root colonization is species-dependent. It is characteristic, that these soil microbes occur in almost all habitats, including metal contaminated soils. However, it should be noted, that polluted areas contain reduced AMF community, including autochtonous strains, which are heavy metals tolerant (39). Despite high level of soil contamination, no effect of heavy metals on the degree of AMF root colonization was observed. It may indicate, that native microorganisms, including AMF, occurring in metal-contaminated sites have developed tolerance against metal toxicity and are well adapted to extreme conditions [40, 41]. Therefore, using native AMF inoculum could be biotechnological tool for renovation of degraded areas.
5. Conclusions
In conclusions, contamination with heavy metals did not result in restriction of mycorrhizal colonization, which could be a consequence of AMF adaptation to such conditions. Nevertheless, application of both fertilizers reduced root colonization by arbuscular mycorrhizal fungi (AMF) in Miscanthus x giganteus, which could be resulted from depletion of AMF diversity in soil and also increased competition between microorganisms derived from inoculum and native AMF. In turn, positive influence of fertilization on degree of AMF root colonization in Panicum virgatum could eventuate from differences in specific fungal-plant interactions. Among tested species, the highest level of mycorrhizal colonization was found for Spartina pectinata roots in each experimental variant. It indicates the highest susceptibility of this species to development of mycorrhizal structures regardless of heavy metal pollution and fertilization.

Acknowledgements
Research financed by the PHYTO2ENERGY project – 7FP for Research, Technological Development and Demonstration of the European Union, under the Grant Agreement No. 610797 and Polish Ministry of Science and Higher Education (Institute for Ecology of Industrial Areas statutory founds).

References
[1] Ladwani K D, Ladwani K D, Manik V S, and Ramteke D S 2012 International Journal of Life Sciences Biotechnology and Pharma Research 1, 73-80
[2] Pogrzeba M, Rusinowski S, Sitko K, Krzyżak J, Skalska A, Małkowski E and Kalaji H M 2017 Environmental Pollution 225 163-174
[3] Zurek G, Pogrzeba M, Rybka K, Krzyżak J and Prokopiuik K 2013 E3S Web of Conferences vol 1 (Rome: EDP Sciences) 13006
[4] Dong W, Zhang X, Wang H, Dai X, Sun X, Qiu W and Yang F 2012 PloS One 7 e44504
[5] Van Der Heijden M G, Bardgett R D and Van Straalen N M 2008 Ecology Letters 11 296-310
[6] Rashid M I, Mujawar L H, Shahzad T, Almeelbi T, Ismail I M, and Oves M 2016 Microbiological Research 183 26-41
[7] Huang H, Zhang S, Wu N, Luo L, Christie P 2009 Soil Biology and Biochemistry 41 726-734
[8] Bonfante P, Genre A 2010 Nature Communications 1 48
[9] Bothe H, Regvar M, Turnau K 2010 Soil Heavy Metals (Springer Berlin Heidelberg) pp. 87-111
[10] Gianinazzi S, Gollotte A, Binet M N, van Tuinen D, Redecker D, Wipf D 2010 Mycorrhiza 20 519-530
[11] Zhu X C, Song F B, Liu S Q, Liu T D, Zhou X 2012 Plant Soil Environ 58 186-191
[12] Acosta – Avalos D, Alvarado – Gil J J, Vargas H, Frias-Hernández J, Olalde-Portugal V, Miranda L C M 1996 Plant Science 119 183-190
[13] Banni A S, Faituri M Y 2013 Middle-East Journal of Scientific Research 17 77
[14] Andrade S, Silveira A P D 2008 Brazilian Journal of Plant Physiology 20 39-50
[15] Cicitelli A, Lingua G, Todeschini V, Biondi S, Torrigiani P and Castiglione S 2010 Annals of Botany 106 791-802
[16] Zhu X, Song F, Xu H 2010 Mycorrhiza 20 325-332
[17] Carrasco L, Azcón R, Kohler J., Roldán A and Caravaca F 2011 Science of the Total Environment, 409 1205 – 09
[18] Guca – Przepióra E 2012 Wiadomości Botaniczne 56 5-19
[19] Firmin S, Labidi S, Fontaine J, Laruelle F, Tisserant B, Nsanganwimana F and Shirali P 2015 Science of the Total Environment 527 91-99
[20] Rahmaty R, Khara J 2011 Turkish Journal of Biology 35 51-58
[21] Peijnenburg W J, Zablotskaja M and Vijver M G 2007 *Ecotoxicology and Environmental Safety* **67** 163-179

[22] Phillips J M and Hayman D S 1970 *Transactions of the British Mycological Society* **55** 158IN16-161IN18

[23] Trouvelot A 1986 *Mycorrhizae: Physiology and Genetics* 217-221

[24] Ghimire S R, Charlton N D and Craven K D 2009 *BioEnergy Research* **2** 51-58

[25] Moore C V, Dzantor E K and Hui D 2015 *Journal of Applied Biosciences* **89** 8263-71

[26] Ge Y, Zhang J B, Zhang L M, Yang M and He J Z 2008 *Journal of Soils and Sediments* **8** 43-50

[27] He J Z, Zheng Y, Chen C R, He Y Q and Zhang L M 2008 *J. Soils Sed.* **8** 349–358

[28] Gryndler M, Larsen J, Hršelová H, Řezáčová V, Gryndlerová H and Kubát J 2006 *Mycorrhiza* **16** 159-166

[29] Egerton-Warburton L M, Johnson N C and Allen E B 2007 *Ecological Monographs* **77** 527-544

[30] van Diepen LTA, Lilleskov EA, Pregitzer KS, Miller RM 2007 *New Phytologist* **176** 175-183

[31] Corkidi L, Rowland D L, Johnson N C and Allen E B 2002 *Plant and Soil* **240** 299-310

[32] Baar J 2008 *Mycorrhiza* (Springer Berlin Heidelberg) pp. 361-373

[33] Biró B, Köves-Péchy K, Vörös I, Takács T, Eggenberger P and Strasser R J 2000 *Applied Soil Ecology* **15** 159-168

[34] Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC 2009 *Ecology* **12** 452-461

[35] Porras-Alfaro A, Herrera J, Natvig D O and Sinsabaugh R L 2007 *Plant and Soil* **296** 65-75

[36] Eom A H, Hartnett D C and Wilson G W 2000 *Oecologia* **122** 435-444

[37] Wang F Y, Liu R J, Lin X G and Zhou J M 2004 *Mycorrhiza* **14** 133-137

[38] Scervino J M, Gottlieb A, Silvani V A, Pérzola M, Fernández L and Godeas A M 2009 *Soil Biology and Biochemistry* **41** 1753-56

[39] Zhan F, He Y, Zu Y, Zhang N, Yue X, Xia Y and Luo Y 2013 *African Journal of Microbiology Research* **7** 3943-52

[40] Khan A G 2005 *Journal of Trace Elements in Medicine and Biology* **18** 355-364

[41] Karimi A, Khodaverdiloo H, Sepehri M and Sadaghiani M R 2011 *African Journal of Microbiology Research* **5** 1571-1576