

ADAPTABILITY STUDY OF DIFFERENT *GLOMUS MOSSEAE* STRAINS TO SOIL HEAVY METAL CONTENT

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Introduction

The emission of heavy metals increased worldwide in the last few decades as a consequence of anthropogenic activities such as mining, metal-working industries or phosphate fertilizers. For the rehabilitation and revegetation of degraded industrial sites stress-tolerant plants are required. Using plants in remediation of soils is regarded as an environmental friendly clean-up method (Gáspár et al., 2004) and used widely due to its various types (phytostabilization, phytoextraction, rhizofiltration etc.) according to the aim of remediation (Simon et al., 2005). As the availability of heavy metals to plants and their toxicity depend on the reaction of the microbial activities in the rhizosphere (Schützendübel et al., 2002), the efficiency of phytoremediation can be enhanced using by adequate selected, metal-tolerant inoculums of root symbionts such as arbuscular mycorrhizal fungi (AMF). AMF form obligatory mutualistic associations with a wide range of plant species. Many studies indicated the ability of AMF in improving of the nutrient supply of plants and protecting their hosts against heavy metal toxicity (Marschner et al., 1994; Leyval et al., 1997). However, to date little is known about what kind of factors may play the important roles in developing of tolerance and adaptation to heavy metals.

The aim of this study was to compare the adaptability to the high soil Cd content of the five *Glomus mosseae* strains isolated from different soil types. The changing of infectivity and effectiveness of the strains were investigated in a pot experiment in soils treated with Cd at three levels (0, 50, 100 mg kg⁻¹) harvested after 11 and 20 weeks.

Methods

The soil originating from Nagyhörcsök (Hungary) is classified as a calcareous loamy chernozem with the following characteristics: pH_(H2O): 8.06, pH_(KCl): 7.79, humus content: 1.99%; the soil KCl-exchangeable NH₄⁺-N: 5.99 mg kg⁻¹, KCl-exchangeable-NO₃⁻-N: 29.83 mg kg⁻¹, AL (Ammonium-Lactate)-soluble K₂O: 112.5 mg kg⁻¹, AL-soluble P₂O₅: 106 mg kg⁻¹. The solution of sulphates of the trace element Cd (3 CdSO₄ 8 H₂O) was added to the soils at 3 levels (0, 50, 100 mg total Cd kg⁻¹ dry soil). The total and available Cd concentration of the soils were 0.76; 52.75; 108 mg Cd kg⁻¹ soil and 0.22; 41.15; 87.55 mg Cd kg⁻¹ soil at the three rates of Cd application. White clover (*Trifolium repens* L.) plants were inoculated with the five cultures of *Glomus mosseae* (5 w/w % AMF inoculum pot⁻¹). We used non-inoculated plants as control in every treatment. The isolates of *G. mosseae* originated from different soil types and geographical areas: *G. mosseae*-M (isolated from a calcareous loamy chernozem soil polluted with Cd (90 mg total Cd kg⁻¹ soil) and probable adapted to Cd, Nagyhörcsök); *G. mosseae*-S (salt-affected soil, Nyírápalos); *G. mosseae*-HS 1-2 (humus sandy soil, Örbottyán); *G. mosseae*-BEG12 (European Bank of Glomeromycota). The test plants were grown for 11 and 20 weeks in a growth chamber under controlled climatic conditions. After staining of the root samples as described by Phillips et al. (1970) root colonization was estimated by the method of Trouvelot et al. (1986) – data of the arbusculum richness (A%)

are only shown. The biomass production and Cd concentrations of the shoots were determined. Data are presented as means of four replications with least significant differences (LSD) and were statistically evaluated by analysis of variance ($P < 0.05$). F tests were used to test treatment effects on the different measured parameters.

Results and discussion

Changes of three parameters of AMF infectivity (arbuscular richness) and efficiency (plant growth response, shoot Cd concentration) were investigated as parameters of adaptability to soil Cd content (Table 2.). The beneficial effects of all the AMF treatments on their hosts could be observed well either at the 11-week-old or at 20-week-old mycorrhizal plants. However, significant differences were shown among the various *G. mosseae* strains in case of all the parameters at each Cd application of soil (Munkvold et al., 2004). Three cultures (*G. mosseae*-HS2, -S, -M) could be emphasized from the point of view of application in phytoremediation. The arbuscular richness (A%) of strain isolated from Cd-polluted soil (*G. mosseae*-M) was not affected neither by the rate of Cd application nor harvesting time. The A% values of strains isolated from non-polluted soils (*G. mosseae*-HS2 and -S) decreased in time at each Cd application and showed significantly lower values than those of *G. mosseae*-M strain in the roots of 20-week-old plants. While the biomass production of 11-week-old plants inoculated with *G. mosseae*-M decreased significantly with increasing soil Cd content (Chen et al., 2004), the high Cd concentration of soils had no effect on biomass production of 20-week-old plants inoculated with the probable Cd-adapted *G. mosseae*-M strain.

Table 2. The arbuscular richness (A%) in the root of mycorrhizal plants colonized with different *G. mosseae* isolates, the shoot dry matter (g) and the shoot Cd concentration (mg kg^{-1}) of mycorrhizal and non-mycorrhizal plants in soils treated with Cd (0, 50, 100 mg Cd kg^{-1} soil) harvested after 11 and 20 weeks

AMF treatments	0 mg Cd kg^{-1} soil		50 mg Cd kg^{-1} soil		100 mg Cd kg^{-1} soil	
	After 11 weeks	after 20 weeks	after 11 weeks	after 20 weeks	after 11 weeks	after 20 weeks
	Arbuscular richness (A%)					
<i>Glomus mosseae</i> -BEG12	36.86	6.4	43.93	21.41	48.03	18.37
<i>Glomus mosseae</i> -HS1	66.24	13.36	44.43	12.67	70.06	32.52
<i>Glomus mosseae</i> -HS2	57.74	51.02	60.94	37.13	75.49	54.64
<i>Glomus mosseae</i> -S	74.2	63.26	79.9	64.78	79.43	55.86
<i>Glomus mosseae</i> -M	65.06	59.29	69.34	68.3	70.14	71.55
LSD _{5%}	7.65	10.76				
	Shoot dry matter (g)					
Control	3.05	5.9	1.73	2.33	1.06	1.55
<i>Glomus mosseae</i> -BEG12	3.62	8.24	1.36	5.77	1.49	4.05
<i>Glomus mosseae</i> -HS1	2.14	6.79	1.35	6.83	2.17	4.33
<i>Glomus mosseae</i> -HS2	4.16	9.35	3.52	9.03	2.65	7.75
<i>Glomus mosseae</i> -S	4.45	9.69	3.55	8.12	2.97	7.41
<i>Glomus mosseae</i> -M	3.55	6.72	2.65	5.97	1.37	6.16
LSD _{5%}	0.65	1.81				
	Shoot Cd concentration (mg kg^{-1})					
Control	0.02	*	1.21	1.09	2.86	3.99
<i>Glomus mosseae</i> -BEG12	0.02	*	1.91	1.12	2.87	2.29
<i>Glomus mosseae</i> -HS1	0.047	*	2.98	1.24	3.19	2.82
<i>Glomus mosseae</i> -HS2	*	*	1.4	0.87	2.1	1.33
<i>Glomus mosseae</i> -S	*	*	1.59	1.04	2.13	1.58
<i>Glomus mosseae</i> -M	*	*	1.86	1.11	2.92	1.49
LSD _{5%}	0.24	0.17				

* Concentration are below detection limit. Detection limit for Cd: 0.02 mg kg^{-1}

However, the shoot weight of plants inoculated with *G. mosseae*-HS2 and -S were significantly higher than plants inoculated with *G. mosseae*-M in most cases of Cd application and in time, too. As the mycorrhizal infection increased the „root” absorption area, the shoot Cd concentration of 11-week-old mycorrhizal plants also enhanced at 50 mg Cd kg^{-1} soil. On the other hand, at 100 mg Cd kg^{-1} soil pollution approximately equal or lower shoot Cd concentration could be observed in mycorrhizal plants than in shoots of control plants harvested after 11 weeks. The shoot Cd concentrations of the plants inoculated with *G. mosseae*-HS2, -S and -M decreased strongly in time. The observed alleviation of phytotoxicity is caused by a dilution effect as a consequence of higher biomass production which can be explained with higher infectivity of the *G. mosseae*-HS, -S, -M strains. In summary, the *G. mosseae*-M strain isolated from Cd polluted soil can be regarded as a Cd-adapted strain. However, the beneficial effect of this strain on their host had appeared only after 20 weeks. While *G. mosseae*-HS2 and -S strains isolated from non-polluted soil showed tolerance to Cd, these cultures may become adapted to Cd and will be suited to use in phytoremediation.

Conclusions

Present report demonstrated that the alleviation of Cd phytotoxicity can be enhanced using by metal tolerant mycorrhizal strains. The probable high intraspecific variability of AMF could be utilized in phytoremediation from the point of view of selection and adaptation. Moreover, greater attention should be paid to choose sampling time because the beneficial effect of some AMF strains couldn't be observed within a short time. Further investigations are obviously required to reveal that which kinds of defense system are used by AMF and cause tolerance and adaptability to heavy metals.

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