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Growth Response and Arsenic Uptake of White Clover (*Trifolium repens*) and Evening Primrose (*Oenothera odorata*) Colonized with Arbuscular Mycorrhizal Fungi in Arsenic-Contaminated Soil

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ABSTRACT: A greenhouse experiment was conducted to investigate the role of the arbuscular mycorrhizal (AM) fungus, *Glomus mosseae* (BEG 107) in enhancing growth and arsenic (As) and phosphorus (P) uptake of white clover (*Trifolium repens*) and evening primrose (*Oenothera odorata*) in soil collected from a gold mine having concentrations of 381.6 mg total As kg^{-1} and 20.5 mg available As kg^{-1}. *Trifolium repens* and *O. odorata* are widely distributed on abandoned metalliferous mines in Korea. The percent root colonization by the AM fungus was 55.9 % and 62.3 % in *T. repens* and *O. odorata*, respectively, whereas no root colonization was detected in control plants grown in a sterile medium. The shoot dry weight of *T. repens* and *O. odorata* was increased by 323 and 117 % in the AM plants compared to non-mycorrhizal (NAM) plants, respectively. The root dry weight increased up to 24 % in *T. repens* and 70% in *O. odorata* following AM colonization compared to control plants. Mycorrhizal colonization increased the accumulation of As in the root tissues of *T. repens* and *O. odorata* by 99.7 and 91.7 % compared to the NAM plants, respectively. The total uptake of P following AM colonization increased by 50% in *T. repens* and 70 % in *O. odorata*, whereas the P concentration was higher in NAM plants than in the AM plants. Colonization with AM fungi increased the As resistance of the host plants to As toxicity by augmenting the yield of dry matter and increasing the total P uptake. Hence, the application of an AM fungus can effectively improve the phytoremediation capability of *T. repens* and *O. odorata* in As-contaminated soil.

Key Words: Arbuscular mycorrhizae, Arsenic, Phosphorus, Phytoremediation

INTRODUCTION

Arsenic is a ubiquitous element in soil and nearly all other environmental media. The occurrence of As in the earth's continental crust is generally given as 1.5 to 2 ppm[1)]. Although its concentration in uncontaminated soils is low, arsenic is continuously released into the soil and water from natural deposits through anthropogenic activities such as mining and manufacturing[2,3)]. Arsenic co-exists with such elements as gold (Au) and silver (Ag) in metal deposits, and is a by-product of the extraction of Au and Ag from their ores in gold mines[4)]. In Korea, numerous metalliferous mines were developed in the early 1900s, but economic problem forced many of them to close during the 1970s[5)]. Subsequently, the releases of toxic metalloids such as As from those areas have become a significant environmental problem. Neglected tailing heaps and mine spoils left over from mining activities have resulted in vegetation loss. Efforts have been made to decontaminate the soil and restore the vegetation at these sites. Among these efforts, phytoremediation is an emerging technique that uses green plants to

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eliminate or stabilize contaminants[6,7]. The efficiency of phytoremediation of contaminated soils can be enhanced by inoculating hyper-accumulator plants with mycorrhizal fungi, which play a crucial role in protecting plant roots from heavy metals[8].

Arbuscular mycorrhizal fungi, which form endomycorrhizal associations with their host plants, are the most widespread fungal form on earth, and the majority of plants under natural conditions symbioses with mycorrhizae[9]. One of the principal roles of plant-associated mycorrhizal fungi is to provide P for the host. AM fungi obtain their carbon from the photosynthate of the host plant, and the host plant obtains P via nutrient exchange with the AM fungi[10].

Arsenate behaves as an analogue of the macronutrient phosphate. Thus, plants grown in arsenate-contaminated soils will assimilate high levels of arsenate unless they have altered phosphate transport mechanisms[11]. Sharples et al. (2000)[12] demonstrated enhanced arsenite efflux system as a mechanism operating arsenate resistance. Arbuscular mycorrhizal fungi-infected plants from As-contaminated sites have further enhanced resistance to arsenate by suppressed arsenate uptake[13]; however, the clear mechanism by which AM fungi influence arsenate resistance in host plants and its interrelationship with arsenate/phosphate uptake has yet to be elucidated[14]. Although the use of AM fungi has limited applicable potent due to the depression mechanism of As uptake by the As efflux system in AM-colonized host plants[12], it was recently reported that AM colonization in As-contaminated soil increased the biomass and As accumulation of host plants[15-17].

Here, we report the role of AM fungi in enhancing the growth and arsenate resistance of host plants via AM associations. First, the contributions of AM fungi to the enhanced growth and arsenate resistance of host plants were investigated. Second, the interrelationship of those effects with arsenate/phosphate uptake in AM plants was examined. Finally, the potential benefits of AM fungi in phytoremediation were evaluated.

MATERIALS AND METHODS

Plant materials and AM fungus

The host plants, evening primrose and white clover, used in this experiment, were previously selected based on their As resistance from several mining sites in Korea[18]. The biennial evening primrose (*O. odorata*) belongs to Oenotheraceae family and is widespread in abandoned metalliferous mines in Korea18). A previous study demonstrated that *O. odorata* adapts well to various soil / water regimes and textural classes[19]. White clover (*T. repens*) belongs to the Leguminosae family and is a naturalized weed from Europe, which has been widely distributed across Korea, including in several mining areas[20]. Seeds of *O. odorata* growing indigenously in an abandoned gold mine in Gangwon Province, Korea, were collected in the fall of 2004 and stored at 4 ℃; seeds of *T. repens* were purchased commercially. Because *T. repens* is available commercially, obtaining seeds is easy and direct seeding for revegetation of mine soils is possible[7].

The AM fungus (*Glomus mosseae* BEG 107) was propagated with white clover on sterilized vermiculite and sand (1:1 v/v)[21]. The inoculum comprised 10% (v/v) of the pot volume, where 0.5 g of white clover was used per kg of substrate (i.e., vermiculite and sand). The aerial parts of the host plants were removed after three months and the vermiculite and sand containing the colonized root pieces, spores, and hyphae were used as the fungal inoculum. Approximately 60% of each root was AM-colonized.

Preparation of growth medium

Arsenic-contaminated soil was collected from the surface horizon of the abandoned Geumjeong gold mine in Gyeongbuk Province, Korea. Selected chemical characteristics of this soil were measured (Table 1). The soil had a neutral pH. The levels of total, available, and water-soluble As present in the soil were 381.6, 20.5, and 0.5 mg kg^{-1}, respectively. The levels of cadmium, copper, lead, and zinc were relatively low. The concentration of available P, extracted with sodium bicarbonate, was 150.9 mg kg^{-1}. The soil was air-dried at ambient temperatures and sieved (4 mm) to eliminate coarse gravel. Nutrients were added to the soil to compensate for insufficient macronutrients for plant growth two weeks prior to the start of the experiment. The nutrients were supplied at the following concentrations and in the following forms: 476 mg N (NH_4NO_3), 186 mg K (K_2SO_4), and 19 mg Fe ($FeCl_2$) [kg $soil^{-1}$]. The soils were then allowed to equilibrate for two weeks at 4℃.

Table 1. Chemical properties of the As-contaminated gold mine soil used in the greenhouse experiment

Soil properties	Values
pH	7.53
Total nitrogen (%)	0.032
Organic matter (%)	2.55
CEC (cmol(+)/kg)	9.05
Available P (mg kg^{-1})[a]	0.51
Total P (mg kg^{-1})	680
Water soluble As (mg kg^{-1})	0.52
Available As (mg kg^{-1})[b]	20.55
Total As (mg kg^{-1})[c]	381.6
DTPA extractable heavy metal (mg kg^{-1})	
Cd	0.35
Cu	0.70
Pb	12.37
Zn	148.81
Total heavy metal (mg kg^{-1})[c]	
Cd	1.74
Cu	44.59
Pb	83.49
Zn	486.14

[a, b] Sodium bicarbonate extractable As and P [30]
[c] Microwave assisted acid digestion (USEPA method 3051)

The growth medium was prepared according to the method of Lee and George[22]. Two experimental groups were established: AM and non-AM (NAM). For colonization with the AM fungus group, the prepared fungal inoculum was mixed with autoclaved (121℃, 20 min) mine soil by the amount of 10 % of soil volume. For the NAM group, the same amount of autoclaved (121℃, 20 min) inoculum and a filtrate (Whatman No.2) of non-sterilized mycorrhizal inoculum were added to the sterilized mine soil to compensate for nutrients and microorganisms in the original AM inoculum.

Experimental setup

Plastic pots (1.5 L) were filled with 1.2 kg of the prepared soils with 0.1-mm netting placed at the bottom of the pots to prevent the loss of fine particles. The soil bulk density was adjusted to 1.3 g cm^{-1}. Seeds of *T. repens* and *O. odorata* were sterilized in 10 % H_2O_2 for 10min and then pre-germinated for 6 h in saturated $CaSO_4$. Sixty seeds were sown directly into each pot. The plants were thinned to thirty for *O. odorata* and forty for *T. repens* after germination. The pots were watered with distilled and deionized water to maintain the soil-water content equivalent to 60 % of the field moisture capacity. Four sets of pots were prepared for each treatment. The plants were grown in a greenhouse under 24 ℃ during day and 16 ℃ during night and harvested after 8 weeks. Shoots and roots were harvested and analyzed for fresh and dry weights, as well as element concentrations. Partial fresh roots were analyzed to determine the level of root mycorrhizal colonization after washing with tap water.

Chemical analyses

Plant analysis

The shoots and roots were dried in an oven at 80 ℃ for 48 h and pulverized with grinding machine. The ground samples were digested following the method of Cai et al.[23] to measure for As and P. The As concentration in the digested solutions was analyzed using an ICP-AES (JY 138 Ultrace, Jobin Yvon, Edison, NJ, USA). The P concentration of the same solution was determined using the molybdenum blue assay[24]. The percentage of the root length colonized by AM fungi was determined by trypan blue staining[25] using the gridline-intersect method[26].

Soil analysis

The mine soils were air-dried and ground to pass through a 2-mm sieve before analysis. The soil pH was measured in a soil-water suspension (1:5 ratio) at equilibrium (i.e., after 1 h) using a glass electrode. Total nitrogen was measured by the Kjeldahl method[27], and organic matter was determined by the method of Nelson and Sommers[28]. The cation exchange capacity was measured by neutral ammonium acetate saturated extraction[29]. Available As and P were extracted using sodium bicarbonate[30]. Subsequent measurements of As and P were made using an ICP-AES and the molybdenum blue assay, respectively. The available heavy metals were extracted with DTPA (diethylenetetraminepentaacetic acid)[31], and the total heavy metals were measured by microwave digestion (USEPA method 3051) with subsequent measurement of As using an ICP-AES.

Statistical analysis

To determine significant differences caused by the presence and absence of the mycorrhizal fungus, the

data were subjected to analysis using Student's *t*-tests at $P<0.01$ and 0.05 for comparison of the means using STATISTICA (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Plant growth and root colonization

No root colonization by AM fungi was detected in the control plants grown in sterile medium, whereas the percent root colonization by AM fungi was 55.9 % and 62.3 % in *T. repens* and *O. odorata*, respectively. Our results indicated that G. mosseae BEG 107 was capable of root colonization at the As level (total As 382 mg kg^{-1}) used in this experiment (Table 1). This level is higher than the As levels reported by Liu et al.[16] and Chen et al.[32]. This may imply that the As level in the soil had little or no effect on the colonization of BEG 107 in *T. repens* and *O. odorata*, although this fungus was not isolated from the As-contaminated soil.

After 8 weeks, the shoot dry weights of *O. odorata* and *T. repens* increased significantly after AM colonization by 323% ($P<0.01$) and 117% ($P<0.01$), respectively, compared to NAM plants (Fig. 1a). AM colonization resulted in a 24% and 70% ($P<0.01$) increase in root dry weight for *O. odorata* and *T. repens*, respectively, compared to control plants grown in sterile medium (Fig. 1b). Mycorrhrizal infection slightly increased root biomass production, but no significant differences were detected between the mycorrhizal and non-mycorrhizal roots of *O. odorata* (Fig. 1b). Within the first two weeks after germination, growth depression was observed in both groups of mycorrhizal plants (data not shown). Three weeks later, however, the growth of mycorrhizal plants had recovered and their growth exceeded that of the control plants grown in sterile medium. Koide[33] reported that mycorrhizal colonization caused a transient depression in leaf area relative to non-mycorrhizal plants at the early stages of growth, possibly due to competition for carbon between the host plant and the AM fungi[34]. It has also been reported that as internal seed reserves are depleted, transient growth inhibition occurs as carbon is allocated to the developing AM fungi in early stage[35]. Ultimately, however, the mycorrhizal association established for both plant species grown in As-contaminated soil promoted their growth, and high biomass was obtained after 8 weeks. The increased

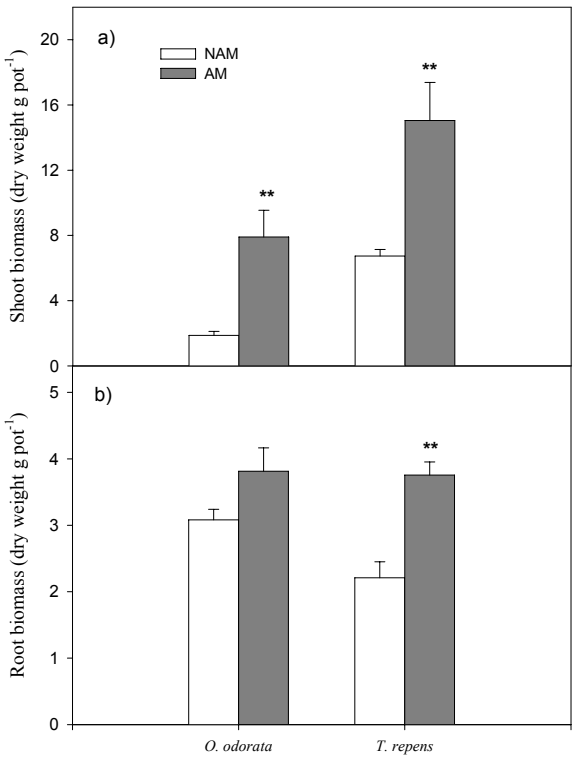

Fig. 1. (a) shoot and (b) root biomass of harvested non-mycorrhizal and mycorrhizal plants inoculated with *Glomus mosseae* after 8 weeks of growth in arsenic-contaminated gold mine soil. The vertical bars above the columns represent the standard error (n=4); * and ** indicate a significant difference between the mycorrhizal and non-mycorrhizal groups for the respective species at $P<0.05$ and $P<0.01$, respectively, as determined by Student's *t*-test.

biomass caused by mycorrhizal colonization may be resulted from an enhanced growth rate and absorptive surface area in the plant root, which in turn increases the plant's nutrient uptake capability. The increase in biomass of *T. repens* and *O. odorata* due to mycorrhizal colonization indicates that they are suitably adapted for As exposure. Although Gonzalez-Chavez et al.[13] reported that AM fungi isolated from mine spoils had evolved arsenate resistance and could confer this enhanced resistance on Holcus lanatus, the AM fungus that we used was isolated from an agricultural field, not from a contaminated area. Still, our results indicate the potential of this system for As resistance.

As and P concentrations in the host plant tissues

The As and P concentrations in the stems, leaves, and root tissues of *O. odorata* were affected by AM

fungi colonization. Whereas there were no significant differences in the As concentrations in the aerial parts of *O. odorata* between the AM and NAM plants, the root As concentrations were higher in the AM plants than in the NAM plants (Fig. 2). In fact, highest As concentration observed (240.14 mg kg^{-1}) was measured in the mycorrhrizal root tissue of *O. odorata* (Fig. 2b). In addition, the level of As tended to be higher in the colonized roots than in the stems and leaves of *O. odorata* (Fig. 2b).

The stem P concentration was higher in NAM *O. odorata* than in the AM plants, whereas the root and leaf P concentrations were unaffected by AM colonization (Fig. 2a). The P concentration of NAM *O. odorata* was higher in the stem and leaves than that in AM *O. odorata*, and was highest in leaf tissue (0.45 %).

The P and As concentrations in stem, leaf, and root tissues of *T. repens* were affected by the AM fungus (Fig. 3). For both elements, *T. repens* showed a similar pattern to *O. odorata*. It was marked that P concentrations in AM and NAM *T. repens* were not significantly different (Fig. 3a); however, the presence of a mycorrhizal association increased the concentrations of As concentration in the root tissues. As concentration of AM *T. repens* showed maximum concentration (250.10 mg kg^{-1}) in root tissue compared to that of NAM groups (Fig. 3b). In accordance with our results, Liu et al.[16] reported that AM-colonized tomato plants had an increased plant biomass and As concentration compared to host plants grown in sterile medium.

The stem/root and leaf/stem As ratios of *T. repens* and *O. odorata* indicate that As accumulated mainly in the root tissues and that its translocation to the aerial parts of the host plants was inhibited by AM colonization (Fig. 4). On the whole, As accumulated

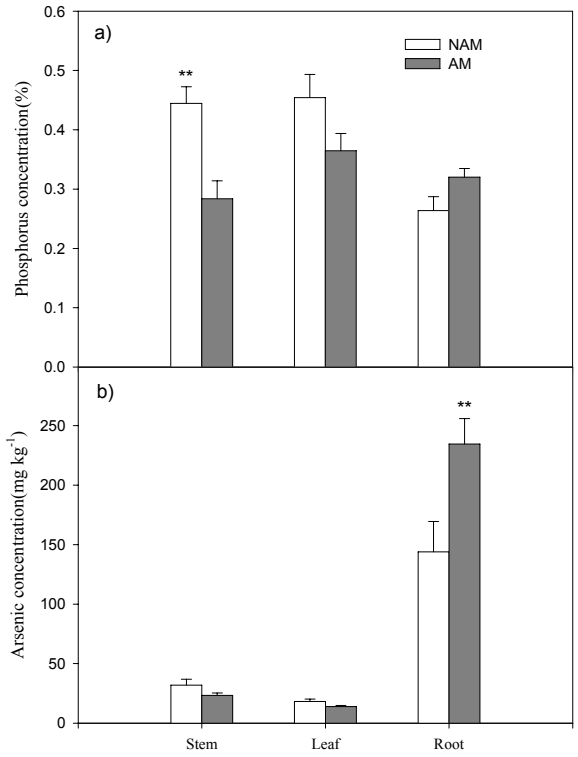

Fig. 2. (a) P and (b) As concentrations in stem, leaf, and root tissues of non-mycorrhizal and mycorrhizal *O. odorata* inoculated with *Glomus mosseae* after 8 weeks of growth in As-contaminated gold mine soil. The vertical bars above the columns represent the standard error (n=4); * and ** indicate a significant difference between the mycorrhizal and non-mycorrhizal groups for the respective species at $P<0.05$ and $P<0.01$, respectively, as determined by Student's *t*-test.

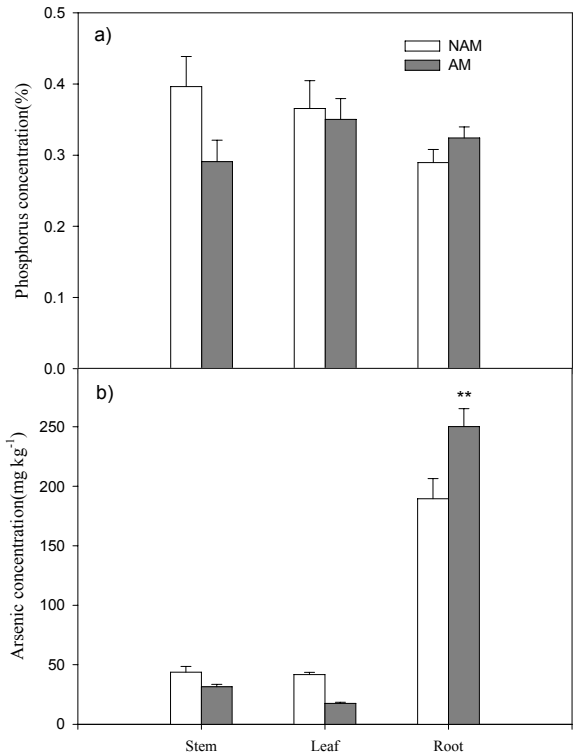

Fig. 3. (a) P and (b) As concentrations in stem, leaf, and root tissues of non-mycorrhizal and mycorrhizal *T. repens* inoculated with *Glomus mosseae* after 8 weeks of growth in As-contaminated gold mine soil. The vertical bars above the columns represent the standard error (n=4); * and ** indicate a significant difference between the mycorrhizal and non-mycorrhizal groups for the respective species at $P<0.05$ and $P<0.01$, respectively, as determined by Student's *t*-test.

more in the stems than in leaves of *T. repens* and *O. odorata* (Fig. 4). The bioconcentration factor is defined as the ratio of metals concentrated in plant shoot versus those in the root[36], and species with values greater than one are considered to be suitable for phytoextraction. In addition, a shoot-to-root metal concentration ratio greater than one indicates efficient root-to-shoot transport[36]. Our analysis indicates that neither *T. repens* nor *O. odorata* is a hyperaccumulator of As because the As bioconcentration factors and S/R ratios of both plants were less than one. Our findings also suggest that As translocation from the root to the shoot tissues was inhibited in both plant species as a result of AM inoculation (Fig. 4). These results are consistent with those of Leung et al.[15], who reported that *C. dactylon* accumulates As primarily in the root tissue and translocation to the shoots was inhibited by the presence of AM fungi. We evaluated the translocation of As in our plants in three distinct parts. The P uptake results showed that the presence of the AM fungi alleviated the As toxicity and maintained the As homeostasis of the host plants by keeping the level of P high. Therefore, As was not actively translocated from the root through the stem to the leaves.

Total uptake of As and P into the host plant tissues

Mycorrhizal colonization increased the leaf and root As content (P<0.05 and 0.01, respectively) in *O. odorata* (Fig. 5), which contradicts the results of Gonzalez-Chavez et al.[13] and Sharples et al.[12], who reported that *Holcus lanatus* colonized with AMF and *Calluna vulgaris* that formed an ericoid-mycorrhizal association achieved arsenate resistance via the suppression of As uptake by mycorrhizal colonization. We found no reduction of As uptake following inoculation of AM fungi. Arsenate is an analogue of

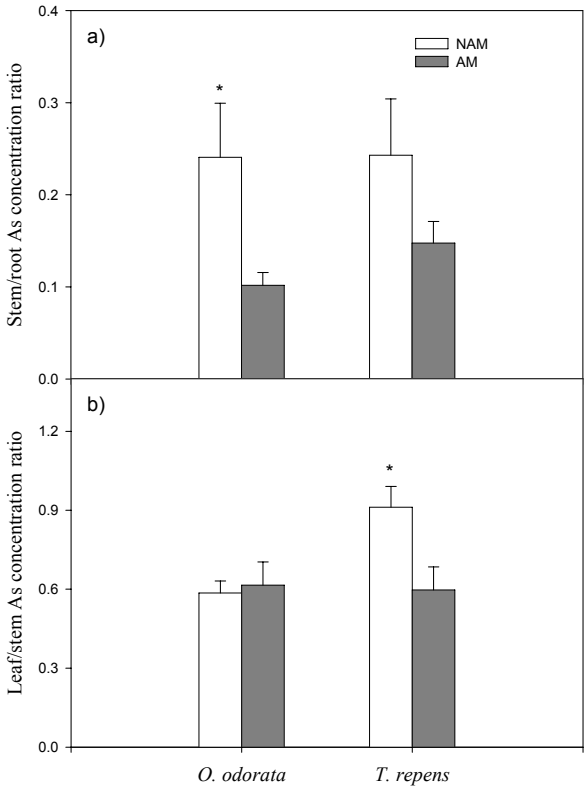

Fig. 4. (a) Stem/ root and (b) leaf/ stem As concentration ratios of *O. odorata* and *T. repens* inoculated with *Glomus mosseae* after 8 weeks of growth in As-contaminated gold mine soil. The vertical bars above the columns represent the standard error (n=4); * and ** indicate a significant difference between the mycorrhizal and non-mycorrhizal groups for the respective species at P<0.05 and P<0.01, respectively, as determined by Student's t-test.

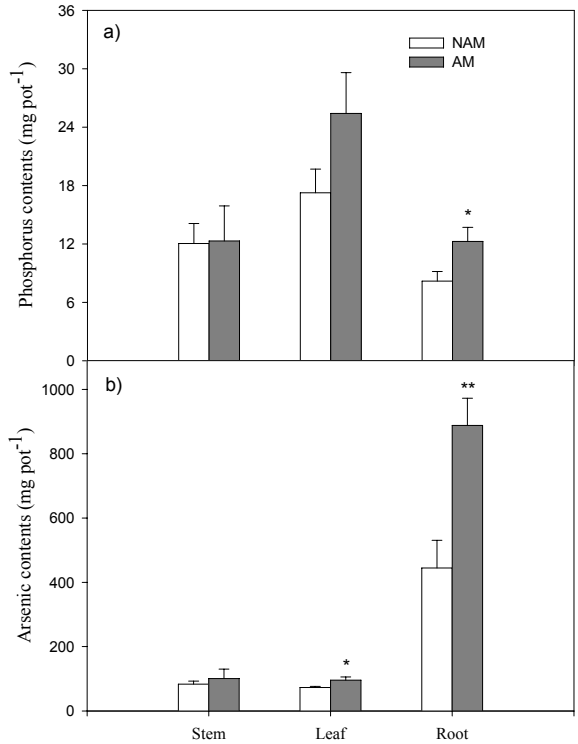

Fig. 5. a) Phosphorus and b) arsenic contents in stem, leaf, and root tissues of non-mycorrhizal and mycorrhizal *O. odorata* inoculated with *Glomus mosseae* after 8 weeks growth in As-contaminated gold mine soil. The vertical bars above the columns represent the standard error (n=4); * and ** indicate a significant difference between the mycorrhizal and non-mycorrhizal groups for the respective species at P<0.05 and P<0.01, respectively, as determined by Student's t-test.

phosphate that competes for the same uptake carriers in the root plasmalemma[11]. Arsenate-resistant plants have high levels of P in their shoots, despite suppressed phosphate uptake[37]. It is remarkable that As and P root contents of *O. odorata* were higher than those of their NAM counterparts. This suggests that *O. odorata* could resist As toxicity by maintaining a high level of P in its root tissues, which increases its biomass. AM fungi are known to enhance the collection of phosphate from soil by increasing the surface area of their fungal hyphae[38], and it has been speculated that AM fungal hyphae may also enhance the capture of As from soil in a similar fashion. Although our experiment did not reveal any suppression of As or P uptake, significantly more P was observed in the leaves than in roots of *O. odorata* (Fig. 5a). The presence of mycorrhizal association increased the total uptake of P in the roots (P<0.05), but this difference was not observed in stems and leaves. However, AM colonization did not result in a difference in P concentration (Fig. 2). This may be explained by a dilution effect caused by the enhanced biomass and P levels resulting from the mycorrhrizal association39). Similar phenomena have been reported for mycorrhrizal-associated tomato plants grown in As-spiked soils[16].

In both *T. repens* and *O. odorata*. mycorrhizal colonization significantly enhanced the accumulation of P in leaf and root tissues (P<0.01). Whereas the level of P highly accumulated in leaf tissues of AM *O. odorata* was high, the highest levels of P were observed in the roots of *T. repens* colonized with AM fungi (Fig. 6a). Arsenic was mainly found in the roots, and the level of accumulation was significantly increased by mycorrhizal colonization (P<0.05). Cao et al.[40] demonstrated that As uptake induced enhancement of P uptake as well as promotion of greater levels of P in roots in the Chinese brake fern suggesting that P plays a role in As detoxification.

Although the P to As ratio of the soil used in our experiment was as low as 1 (Table 1), the ratio in the plants was as high as ranging 15.0 to 266.1 in both host plants (Table 2). Arsenate is taken up via the phosphate uptake system which has a preference for phosphate over arsenate[41], and AM fungi play a role in proving P to the hosts. The enhanced ratio by AM fungi inoculation was observed in leaves of AM *T. repens* significantly (P<0.05), which was attributed

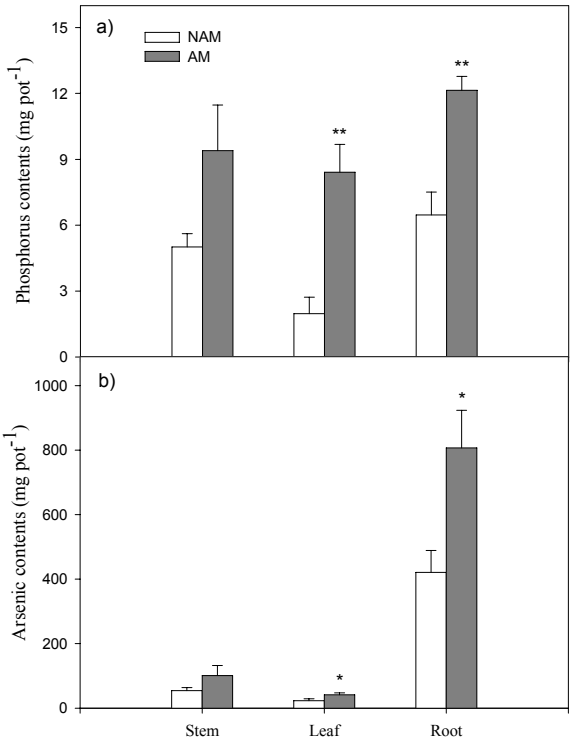

Fig. 6. a) P and b) As contents in stem, leaf, and root tissues of non-mycorrhizal and mycorrhizal *T. repens* inoculated with *Glomus mosseae* after 8 weeks of growth in As-contaminated gold mine soil. The vertical bars above the columns represent the standard error (n=4); * and ** indicate a significant difference between the mycorrhizal and non-mycorrhizal groups for the respective species at P<0.05 and P<0.01, respectively, as determined by Student's *t*-test.

Table 2. Uptake ratio of P / As in non-mycorrhizal and mycorrhizal *O. odorata* and *T. repens* inoculated with *Glomus mosseae* after 8 weeks of growth in As-contaminated gold mine soil

	O. odorata			*T. repens*		
	Leaf	Stem	Root	Leaf	Stem	Root
Non-mycorrhizal	237.1	145.0	18.4	84.7	92.6	15.4
Mycorrhizal	266.1	122.2	13.8	202.8*	93.0	15.0

*** represents a significant difference between inoculation treatments by Student's *t*-test at P<0.05.**

that P uptake in leaves of AM *T. repens* was greater than in NAM. The enhanced ratio was also detected in leaves of AM *O. odorata*, but there was no significant difference ($P<0.05$). However As was still kept high in the roots of both plant species. We can also assume that the host plants possess the cellular detoxification system such as the formation of phytochelatins coping with As toxicity.

The potential application of mycorrhizal fungi for phytoremediation has been reviewed by many researchers[8,42]. Mycorrhizal fungi can act as a barrier to metal toxicity in host plants and improve plant nutrition and biomass production[43]. In particular, for metalloid element such as As, AM fungi act as a filter to maintain a low As concentration in host plants[44]. Recently the effects of inoculation on the suitability of ferns as As hyperaccumulators for phytoremediation were reported[15,17]. The authors concluded that AM fungi-associated ferns had higher yields and improved P nutrition compared to control plants. The plants that we used (i.e., *T. repens* and *O. odorata*) accumulated more As in their root tissues than did the control plants, rather than exhibiting suppressed As uptake. Translocation to the aerial tissues was inhibited, resulting in more P and higher biomass production compared to the control plants.

CONCLUSION

Our results revealed that *Glomus mosseae* was capable of colonization in the *T. repens* and *O. odorata* roots in As-contaminated soils. The inoculation with AM fungi increased the shoot biomass, root As, and P content of the host plants. Higher As level was concentrated in the root than in the leaf and stem tissues of *T. repens* and *O. odorata*. Although both the species we examined were not hyperaccumulators for As, it was found that they became As-tolerant due to dilution effects caused by the augmented biomass production and P levels as a result of the association with AM fungi. The use of As-tolerant plants and those associated with AM fungi seemed to enhance the efficiency of phytoremediation through alleviating As-toxicity and overcoming P-deficiency in As-contaminated soils. Further field studies should be conducted to test the feasibility of the direct AM fungal inoculation of endemic plants around mining areas.

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REFERENCES

1. Adrino, D.C. (2001) Trace elements in terrestrial environments, biogeochemistry, bioavailability, and risks of metals, 2nd ed. Springer p. 222.
2. Azcue, J.M., Mudroch, A., Rosa, F., and Hall, G.E.M. (1994) Effects of abandoned gold mine tailings on the arsenic concentrations in water and sediments of Jack of Clubs Lake, BC. *Environ. Technol*. 15, 669-678.
3. Davis, A., Ruby, M.V., Bloom, M., Schoof, R., Freeman, G., and Bergstorm, P.D. (1996) Mineralogic constraints on the bioavailability of arsenic in smelter-impacted soils. *Environ. Sci. Technol*. 30, 392-399.
4. Alloway, B.J. (1994) Heavy metals in soils, In: O'Neill P. Arsenic. 2nd ed. Blackie Academic & Professional p. 106.
5. Park, Y.H. (1994) Management practices of inactive and abandoned metalliferous mine area in Korea. Korean Environment Technology & Research KETRI/1994/RE-14, 1-14.
6. Cunningham, S.D. and Ow, D.W. (1996) Promises and prospects of phytoremediation. *Plant Physiol*. 110, 715-719.
7. Tordoff, G.M., Baker, A.J.M., and Willis, A.J. (2000) Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* 41, 219-228.
8. Khan, A.G., Kuek, C., Chaudhry, T.M., Khoo, C.S., and Hayes, W.J. (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere* 41, 197-207.
9. Read, D.J. (1993) Mycorrhiza in plant communities. In: Advances in Plant Pathology 9: Mycorrhiza Synthesis. Tommerup IC(eds), Academic Press, San Diego.
10. Pearson, J.N. and Jakobsen, I. (1993) Symbiotic exchange of carbon and phosphorus between

cucumber and three arbuscular mycorrhizal fungi. *New Phytol*. 124, 481-488.
11. Merharg, A.A. and Macnair, M.R. (1992) Suppression of the high-affinity phosphate-uptake system - a mechanism of arsenate tolerance in *Holcus lanatus* L. *J. Exp. Bot*. 43, 519-524.
12. Sharple, J.M., Meharg, A.A., Chambers, S.M., and Cairney, J.W.G. (2000) Mechanism of arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae. Plant Physiol*. 124, 1327-1334.
13. Gonzalez-Chavez, C., Harris, P.J., Dodd, J., and Meharg, A.A. (2002) Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lantus. New Phytol*. 155, 163-171.
14. Meharg, A.A. and Hartley-Whitaker, J. (2002) Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytol*. 154, 29-43.
15. Leung, H.M., Ye, Z.H., and Wong, M.H. (2006) Interactions of mycorrhizal fungi with *Pteris vittata* (As hyperaccumulator) in As-contaminated soils. *Environ. Pollut*. 139, 1-8.
16. Liu, Y., Zhu, Y.G., Chen, B.D., Christie, P., and Li, X.L. (2005) Yield and arsenate uptake of arbuscular mycorrhizal tomato colonized by *Glomus mosseae* BEG 167 in As spiked soil under glasshouse conditions. *Environ. Int*. 31, 867-873.
17. Trotta, A., Falaschi, P., Cornara, L., Minganti, V., Fusconi, A., Drava, G., and Berta, G. (2006) Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern *Pteris vittata* L. *Chemosphere* 65, 74-81.
18. Kim, J.G., Lim, S.K., Lee, S.H., Lee, C.H., and Jeong, C.Y. (1999) Evaluation of heavy metal pollution and plant survey around inactive and abandoned mining areas for phytoremediation of heavy metal contaminated soils. *Kor. J. Environ. Agri*. 18(1), 28-34.
19. Kim, D.Y., Kim, W.H., Lee, J.G., and Kim, J.G. (2005) Effects of soil texture and soil water contents on growth and physiological responses of *Oenthera odorata* Jacq. *Korean Soc. Soil Sci. Fert*. Proceeding, 12-13 May 2005, 38(1), p. 322.
20. Lee, Y.N. (1996) Flora of Korea. Kyo-Hak Publishing Co., Ltd. p. 412.
21. Lee, Y.J. (2002) Contribution of arbuscular mycorrhizal fungi to metal cations uptake in vegetables. Ph.D Dissertation. Hohenheim University, Stuttgart, Germany.
22. Lee, Y.J. and George, E. (2005) Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply. *Plant Soil* 278, 361-370.
23. Cai, Y., Georgiadis, M., and Fourqurean, J.W. (2000) Determination of arsenic in seagrass using inductively coupled plasma mass spectrometry. *Spectrochim. Acta* Part B 55, 1411-1422.
24. Kitson, R.E. and Mellon, M,G. (1944) Colorimetric determination of phosphorus as molybdivanadophosphoric acid. *Ind. Eng. Chem.* 16, 379-383.
25. Koske, R.E. and Gemma, J.N. (1989) A modified procedure for staining roots to detect VA mycorrhizas. *Mycol. Res*. 92, 486-505.
26. Giovanneti, M. and Mosse, B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycohhrhizal infection in roots. *New Phytol*. 84, 489-500.
27. Mremner, J.M. (1996) Nitrogen-Total. In: Sparks, DL. (Eds.), Method of Soil Analysis: Part III-Chemical Methods. American Society of Agronomy, Madison, WI 1085-1121.
28. Nelson, D.W. and Sommers, L.E. (1996) Total carbon, organic carbon, and organic matter. In: Sparks, D.L. (Eds.), Method of soil analysis: Part III-Chemical methods. American Society of Agronomy, Madison, WI 961-1010.
29. Sumner, M.E. and Miller, W.P. (1996) Cation exchange capacity and exchange coefficients. In: Sparks, DL. (Eds.), Method of soil analysis: Part III-Chemical methods. American Society of Agronomy, Madison, WI 1201-1229.
30. Woolson, E.A., Axley, J.H., and Kearney, P.C. (1971) Correlation between available soil arsenic, estimated by six methods, and response of corn (*Zea mays* L.), Soil Sci. *Soc. Amer*. Pro. 35, 101-105.
31. Lindsay, W.L. and Norvell, W.L. (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am*. J. 42, 421-428.
32. Chen, B.D., Zhu, Y.G., and Smith, F.A. (2006) Effects of arbuscular mycorrhizal inoculation on uranium and arsenic accumulation by Chinese brake fern (*Pteris vittata* L.) from a uranium mining-impacted soil. *Chemosphere* 62, 1464-1473.
33. Koide, R. (1985) The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal

infection. *New Phytol*. 99, 449-462.
34. Buwalda, J.G. and Goh, K.M. (1982) Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. *Soil Biol. Biochem*. 14, 103-106.
35. Johnson, N.C., Graham, J.H., and Smith, F.A. (1997) Functioning of mycorrhizal associations along mutualism-parasitism continuum. *New Phytol*. 135(4), 575-586.
36. McGrath, S.P. and Zhao, F.J. (2003) Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotech*. 14, 277-282.
37. Wright, W., Fitter, A., and Meharg, A.A. (2000) Reproductive biomass in *Holcus* clones that differ in their phosphate uptake kinetics and mycorrhizal colonization. *New Phytol*. 146, 493-501.
38. Smith, S.E. and Read, D.J. (1997) Mycorrhizal symbiosis, 2nd ed. San Diego, CA, USA, Academic Press.
39. George, E., Marschner, H., and Jakobsen, I. (1992) Water and nutrients translocation by hyphae of *Glomus mosseae*. *Can. J. Bot*. 70, 2130-2137.
40. Cao, X., Ma, L.Q., and Tu, C. (2004) Antioxidative responses to arsenic in the arsenic hyperaccumulator Chinese brake fern (*Pteris vittata* L.). *Environ. Pollut*. 128, 317-325.
41. Meharg, A.A., Naylor, J., and Macnair, M.R. (1994) Phosphorus nutrition of arsenate-tolerant and nontolerant phenotypes of velvetgrass. *J. Environ. Qual*. 23, 234-238.
42. Fitz, W.W. and Wenzel, W.W. (2002) Arsenic transformations in the soil-rhizosphere-plant system: fundamentals and potential application to phytoremediation. *J. Biotechnol*. 99, 259-278.
43. Leyval, C., Turnau, K., and Haselwandter, K. (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7, 139-153.
44. Sharple, J.M., Meharg, A.A., Chambers, S.M., and Cairney, J.W.G. (2000) Symbiotic solution to arsenic contamination. *Nature* 404: 951-952.