Effects of arbuscular mycorrhizal fungi on the repair capacity of perennial ryegrass (Lolium perenne L.) in the uranium-containing soils

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Abstract. This research inclines to study the ramification on arbuscular mycorrhizal fungi (AMF), including Glomus claroideum (G. claroideum) and G. mossea e strains as well as the repair process of perennial ryegrass (Lolium perenne L.) in the uranium-containing soils. Three times at 120 °C for an hour was required to disinfect the soil mixture. Spraying the uranium (U₃O₈) into the soil mixture and mixing until the ingredients were thoroughly blended, serving the purpose of obtaining the uranium-containing soil mixture with 5 mg/kg U₃O₈. A week later, AMF was inoculated into every flowerpot and chamber facility. Seeds of perennial ryegrass were cultivated with thirty seeds in each flowerpot or each indoor equipment in a fortnight’s time. Perennial ryegrass plants tended to be harvested after sowing and meeting the conditions of 60 days. Three substances are detected by three different detection means (Photosynthetic pigments, soluble proteins, and malondialdehyde correspond to ethanol extraction method, Coomassie brilliant blue method, and thioliferic acid method, respectively). Additionally, testing included activities of alkaline phosphatase and succinate dehydrogenase as well. Moreover, Hitachi H-7650 transmission electron microscope performed the function of observing cellular and subcellular distributions of uranium in plant epidermal cells. Photosynthetic pigment standards of perennial ryegrass are subject to AMF, which not only has a tendency to enhance the soluble protein levels in perennial ryegrass, but also gains the possibility of holding up the uranium-induced improvement of malondialdehyde levels in perennial ryegrass. G. mossea e is properly more efficacious than G. claroideum in the attenuation of uranium damages in cell structures of perennial ryegrass. Our results demonstrate that AMF is capable of developing the capacity of perennial ryegrass on repairing the uranium-related soil contamination.

1. Introduction
Uranium waste rocks, uranium tailings, and uranium-containing waste water[1-3] are likely to be derived from Uranium mining and hydrometallurgy. Therefore, ecosystem is prone to being devastated and individuals are vulnerable to be afflicted with disease. On account of special advantages such as low-cost, simple operation and no secondary pollution, biological remediation is regarded as a sophisticated method of repairing uranium-contaminated soils[4].

Uranium is not an element essential for plant growth, but it has a strong effect on chemical toxicity and radiation damage to plant cells[5]. Arbuscular mycorrhizal fungi (AMF), the most commonly distributed microorganisms in soils, are able to stimulate absorption of nutrients and enhance uptake and resistance of plants to heavy metals and radioactive elements[6]. After inoculation with AMF,
increase of soluble plant protein contents is the characterization of AMF to improve the ability of plants to resist heavy metal toxicity[7]. In contrast, it is by no means certain that whether AMF affects perennial ryegrass (Lolium perenne L.) to uptake uranium from contaminated soils.

According to this study, we have made use of perennial ryegrasses the host plant to investigate the uptake of plant to uranium, with inoculation of the two representative species of AMF which is christened Glomus claroideum (G. claroideum) and G. mosseae strains. Furthermore, infection rate of AMF and the plants adsorption along with transport of AMF have been researched as well.

2. Materials and Methods

2.1. Fungus strains and perennial ryegrass seeds

AMF G. claroideum and G. mosseae strains were acquired from Microbiology Institute of Chinese Academy of Sciences, Beijing, China. The fungi were propagated in our laboratory for this investigation. The seeds of perennial ryegrass (Lolium perenne L.) were vigorously supported by the specimen laboratory of the Northwest Agriculture and Forestry University (Yangling, China). In this study, the seeds without damages by moths, full and uniform sizes, were likely to be chosen for experiment.

2.2. Experimental design

River sand, vermiculites, and soils were blended for the sake of obtaining the soil mixture with a volume ratio of 1:1:1. The soil mixture was sterilized three times at 120°C for an hour. Spraying the uranium (U₃O₈) into the soil mixture and mixing until the ingredients were thoroughly blended, serving the purpose of obtaining the uranium-containing soil mixture with 5 mg/kg U₃O₈. A week later, seeds of perennial ryegrass were cultivated with thirty seeds in each flowerpot or each indoor equipment, which contained 1 kg of soil mixture with or without uranium (U₃O₈). AMF was inoculated into each flowerpot and each chamber facility in a fortnight’s time. Compared with the natural conditions of the flowerpots, the indoor equipment provides more professional growth conditions, including light, nutrition, temperature and humidity.

2.3. Observation of cell ultrastructures by transmission electron microscopy

Cellular and subcellular distributions of uranium in plant epidermal cells were observed by the 5-Br-PADAP method[8-10] using Hitachi H-7650 transmission electron microscope. That is to say, the plant tissues were fixed by using 4% glutaraldehyde, which were then treated by means of acetone dehydration (30%, 50%, 70%, 80%, 90%, and 100%, respectively). The samples were embedded through Epon812 (Hongjin Chemical Co., Ltd., Hengyang, China) and then cut into slices with a thickness of 70 nm by a Leica microtome purchased from Leica Microsystems (Shanghai, China) Co., Ltd., Germany. Those slices were then observed by Hitachi H-7650 transmission electron microscope.

3. Results

3.1. AMF influents photosynthetic pigment levels of perennial ryegrass.

Photosynthetic pigment content illustrates production of photosynthesis in plants. Environmental pressure has an inclination to contribute to reduction and degradation of photosynthetic pigment content. As a consequence, photosynthetic pigment content is an important physiological index of leaf senescence[8]. In this study, to investigate if AMF affects the pigment contents of perennial ryegrass that was planted in the uranium-contained soils (5 mg/kg U₃O₈), photosynthetic pigment contents in plants in two types of environmental conditions were determined.

The perennial ryegrass plants were divided into 8 groups. Plants in soils without uranium but inoculated with G. claroideum, G. mosseae, or water were termed as the Con+Gc, Con+Gm, and Con groups, respectively. The Con group served as the negative control group for the condition without uranium and without AMF inoculation. Plants in soils containing uranium (5 mg/kg U₃O₈) but
inoculated with water, G. claroideum, or G. mosseae were termed as the ConU, ConU+Gc, and ConU+Gm groups, respectively. The ConU group served as the negative control group for the condition with uranium but without AMF inoculation. Furthermore, plants in the compartment cultivation device containing soils with the presence of uranium (5 mg/kg U$_3$O$_8$) but inoculated with G. claroideum or G. mosseae were termed as the ConUcomp+Gc, and ConUcomp+Gm groups, respectively. Perennial ryegrass plants were harvested 60 days after sowing in soils. All plants in their whole growth period have no obvious yellow or withered symptoms with or without the presence of uranium. All experiments in this study were repeated for at least 3 times.

It is clear that the pigment contents of perennial ryegrass were determined according to Fig. 1. Compared with the Con group, the levels of chlorophyll-a (chl-a), chlorophyll-b (chl-b) and carotenoids in the ConU group were descended (p < 0.05), indicating that uranium inhibits the growth of plant. In contrast to the ConU group, the levels of chl-a and carotenoids in the ConU+Gc were significantly ascended (p < 0.05), whereas the chl-b levels were not altered significantly (p > 0.05). In comparison with the ConU group, the levels of chl-a, chl-b, and carotenoids in the ConU+Gm were not changed significantly (p > 0.05). These results demonstrated that only G. claroideum can develop the levels of chl-a and carotenoids of plants in natural conditions.

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Measurements of photosynthetic pigment contents of perennial ryegrass.

To further investigate the effect of AMF on plants in the uranium condition, levels of chl-a, chl-b, and carotenoids in the ConUcomp+Gc and ConUcomp+Gm groups were determined. As shown in Fig.1, levels of chl-a, chl-b, and carotenoids in the ConUcomp+Gc and ConUcomp+Gm groups were significantly elevated than those of ConU+Gc and ConU+Gm groups (p < 0.05). These results suggest in the compartment cultivation devices, AMF can increase resistance of plants against uranium more effectively than in the natural conditions.

### 3.2. AMF increases the soluble protein content levels in perennial ryegrass

When plants are contaminated by heavy metals, heavy metal ions enter into the plants. The heavy metal ions can interact with other compounds to form a metal complex, which inhibits synthesis of plant proteins. Therefore, soluble protein content is an important indicator to measure whether the plant has heavy metal stress[9]. In order to investigate whether AMF have impact on the soluble protein content of perennial ryegrass, experiments which are similar to those written above were performed. It can be seen in Fig. 2 that the soluble protein content levels in plant shoots and roots in the ConU group were both fell down (p < 0.05) in contrast to the Con group, illustrating that uranium represses growth of the plant. Compared with the ConU group, the soluble protein content levels in plant shoots and roots in the ConU+Gc were both ascended (p < 0.05). In comparison with the ConU group, the soluble protein content levels in plant shoots in the ConU+Gm were increased (p < 0.05), whereas those in plant roots were not changed significantly (p > 0.05). These results indicated that G. claroideum and G. mosseae can increase the soluble protein content levels in plant shoots in natural conditions. Similarly, G. claroideum can improve the soluble protein content levels in plant roots of plants.
To further investigate the effect of AMF on plants in the uranium condition, the soluble protein content levels in the ConUcomp+Gc and ConUcomp+Gm groups were determined. As shown in Fig. 2, in comparison with the ConU+Gc group, the soluble protein content levels in plant shoots and roots in the ConUcomp+Gc were both increased (p < 0.05). In comparison with the ConU+Gm group, the soluble protein content levels in plant shoots and roots in the ConUcomp+Gm group were both increased (p < 0.05). These results suggest in the compartment cultivation devices, AMF can increase resistance of plants against uranium more effectively than in the natural conditions.

3.3. AMF represses the uranium-induced increase of malondialdehyde (MDA) levels in perennial ryegrass

In the process of plant senescence or in uranium-contaminated soils, free radicals are produced in the lipid peroxidation, in which malondialdehyde (MDA) is one of the most important products of oxidation of cell membrane lipids. Thus the MDA level reflects the plant membrane lipid over oxidation degree and plant resistance[10].

As a consequence, levels of MDA in the shoots and roots of perennial ryegrass were determined. Fig. 3 shows that the MDA levels in plant shoots and roots in the ConU group were both went up (p < 0.05) in contrast to the Con group, which verified that uranium improves the MDA levels.

After inoculation with AMF (G. claroideum and G. mosseae), it is clear that the number of MDA levels in the shoots and root of plants was slightly fell down in comparison with those of CK5 group. On the contrary, figure of MDA levels in the ConUcomp+Gc and ConUcomp+Gm groups plunged greater in contrast to those of ConU+Gc and ConU+Gm groups (p < 0.05). The levels were even similar to those in the Con+Gc and Con+Gm groups. These results suggest that AMF inhibits the uranium-induced increase of MDA levels in perennial ryegrass. In addition, compared with the limitation in the natural conditions, the inhibition of AMF on MDA levels was more significant in the compartment cultivation devices.
3.4. **Plant growth conditions influence the negative effects on perennial ryegrass roots due to AMF infection.**

In the experiments as described in the above sections, AMF infection rates (%) in plant roots of 6 groups, including the Con+Gc, Con+Gm, ConU+Gc, ConU+Gm, ConUcomp+Gc, and ConUcomp+Gm groups, were investigated. It can be seen in the Fig. 4 that the percentage of infection rates of ConU+Gc group witnessed a fall by 36.18% (p < 0.05) compared with the Con+Gc group. However, in comparison with ConU+Gc, the infection rates of ConUcomp+Gc group were rescued back (p < 0.05), to a level close to that of the Con+Gc group. Moreover, the infection rates of groups infected by G. mosseae had similar trend. The activities of SDH and ALP were also determined. As shown in Fig. 4, the activities of SDH and ALP showed situations similar to those of infection rates of the 6 groups. These results suggest that better growth conditions than the natural condition, such as the compartment cultivation device, can more efficiently attenuate the negative effects of AMF infection to perennial ryegrass roots.

![Figure 4](image)

**Figure 4.** Infection rates of AMF into perennial ryegrass roots.

3.5. **G. mosseae has a more effective function than G. claroideum on AMF-mediated attenuation of uranium damages on cell structures of perennial ryegrass.**

To further investigate if uranium affects perennial ryegrass and if AMF can attenuate those effects, cell ultrastructures of perennial ryegrass shoots and roots were observed. As shown in Fig. 5A, for plants in the Con group without the presence of uranium, shoot cells had normal mitochondria and obviously observed nucleus. Many chloroplasts were observed in cells. For plants in the ConU group (Fig. 5B) with the presence of uranium (5mg/kg), shoot cells had a reduced number of mitochondria. Damaged nucleus and nuclear membranes were found. Chloroplast membrane and cell wall were damaged (Fig. 5B).

For plants in the ConU+Gc group (Fig. 5C) with the presence of uranium (5mg/kg) and inoculation of G. claroideum, shoot cells had a few number of mitochondria. Cell wall was very thin and chloroplasts had expansive deformation. For plants in the ConU+Gm group (Fig. 5D) with the presence of uranium (5mg/kg) and inoculation of G. mosseae, shoot cells had an increased number of mitochondria in comparison with plants in the ConU+Gc group. Furthermore, the cell wall thickness was increased and the number of deformed chloroplasts was decreased in comparison with plants in the ConU+Gc group. In addition, the transmission electron microscopy results showed that the cell ultrastructures of perennial ryegrass roots (Fig. 5E-H) had similar trends to those of shoots (Fig. 5A-D). Altogether, these results suggest that uranium damages the cell structures of perennial ryegrass shoots and roots, which can be attenuated by AMF, with G. mosseae possessing a more efficient effect than G. claroideum.
4. Discussion

The relationship between the heavy metal pollutants and the plant uptake of pollutants is becoming an important research area. This study has manifestly illustrated that AMF may affect photosynthetic pigment levels of perennial ryegrass. AMF improves the soluble protein content levels in perennial ryegrass and represses the uranium-induced increase of MDA levels in perennial ryegrass. G. mosseae is properly more efficacious than G. claroideum in the attenuation of uranium damages in cell structures of perennial ryegrass.

The number of MDA contents in the shoots and roots of perennial ryegrass witnessed a fall after inoculation with AMF. This result manifestly demonstrated that AMF had ability of improving the antioxidant enzymes in the plants effectively while reducing the levels of reactive oxygen species and alleviating the degrees of membrane lipid peroxidation. There are some differences in infection rate and the activity of the roots of perennial ryegrass between two kinds of AMF. In the present study, the infection rate and the activity of the roots of perennial ryegrass inoculated with G. mosseae were all higher than those of G. claroideum. The hyphae of AMF expanded the scope of the plant roots hence the absorption capacity of the plant to the water and mineral nutrients was manifestly increased.

In the uranium-contaminated soils, the infection rate and the activity of the roots of perennial ryegrass roots of G. claroideum and G. mosseae were decreased, which showed that AMF infection in soils was inhibited. And, the inhibitory effect of uranium contamination on the activity of SDH and ALP in the hyphae of AMF was stronger than that of its inhibitory effect on the infection rate of AMF. These results were consistent with the experimental results of Oryowska et al, since they found that in zinc-contaminated soils, AMF mycelium activity of ALP inhibition by zinc pollution is more obvious than the inhibition rate[11].

In this study, the enrichment of G. claroideum and G. mosseae in the shoots and roots of perennial ryegrass showed different characteristics. Uranium enrichment in shoots of perennial ryegrass inoculated with G. claroideum decreased, but uranium enrichment in roots of perennial ryegrass inoculated with G. mosseae increased. This is because after inoculation with G. mosseae, hyphae has certain adsorption capacity to uranium. Moreover, uranium immobilization of mycorrhizal structure reduces transfer of uranium in plants, inhibiting the enrichment of uranium in perennial ryegrass. Inoculation with G. mosseae increased uranium enrichment in roots of perennial ryegrass and therefore decreased damages of uranium to soils. These results are consistent with findings reported previously, which shows that AMF improves the enrichment of Zn in plants[12].

Observation of perennial ryegrass cell ultrastructures by transmission electron microscopy shows that in the presence of uranium (5 mg/kg U₃O₈), the shoot and root cell structures, including mitochondria, nucleus, chloroplast, and double membrane, are altered, different from the plants in the conditions without uranium. G. mosseae can effectively relieve the damage of the ultrastructure of LoliumPerenne L. leaf, and enhance the resistance of LoliumPerenne L. to the radioactive uranium.

Figure 5. Observation of cell ultrastructures of perennial ryegrass using transmission electron microscope.
Our results will be helpful for finding more plants and fungi to resolve uranium contamination problem in the future.

5. Conclusion
This study has manifestly illustrated that AMF may affect photosynthetic pigment levels of perennial ryegrass. AMF can contribute to the increase of the soluble protein content levels in perennial ryegrass and repress the uranium-induced increase of MDA levels in perennial ryegrass. It showed that G. mosseae is properly more efficacious than G. claroideum in the attenuation of uranium damages in cell structures of perennial ryegrass.

Furthermore, the infection rate and the activity of the perennial ryegrass roots with G. mosseae indicated all higher than those of G. claroideum. The hyphae of AMF expanded the scope of the plant roots. As a result, the absorption capacity of the plant to the water and mineral nutrients was increased.

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