Repair capacity of perennial ryegrass (*Lolium perenne* L.) based on arbuscular mycorrhizal fungi on the in uranium contaminated soil

Zhang Shi-qi¹, Lv Yi-jin¹, Zhang Yu¹, Peng Xiaoyong¹, Liu Yingjiu, Rong Lishan¹,*

¹Hunan Provincial Key Laboratory of Pollution Control and Resources Technology, University of South China, Hengyang, 421001, P. R. China

*Corresponding author e-mail: 2jieronglisha@163.com

**Abstract:** This study is to research effects of arbuscular mycorrhizal fungi (AMF) on the repair capacity of perennial ryegrass (*Lolium perenne* L.) in the uranium-containing soils. Perennial ryegrass plants were harvested 60 days after sowing in soils. Photosynthetic pigments, soluble proteins, and malondialdehyde were determined by ethanol extraction method, Coomassie brilliant blue method, and thiobarbituric acid method, respectively. Activities of alkaline phosphatase and succinate dehydrogenase were also determined. And, cellular and subcellular distributions of uranium in plant epidermal cells were observed with Hitachi H-7650 transmission electron microscope. AMF influents photosynthetic pigment levels of perennial ryegrass. AMF increases the soluble protein levels in perennial ryegrass and represses the uranium-induced increase of malondialdehyde levels in perennial ryegrass. It suggested that AMF can effectively increase the ability of perennial ryegrass on repairing the uranium-related soil contamination.

1. Introduction

Some effluents generated by mining industry include large quantities of toxic substances like heavy metals which do harm to living creatures and ecosystems [1]. Living organisms are affected by these toxic heavy metals and it may lead to the biodiversity decreasing in some way [2]. Uranium mining and hydrometallurgy often produce uranium waste rocks, uranium tailings, and uranium-containing waste water [3-5], which threat the ecological environment and human health [6-7]. Biological remediation is a new method of repairing uranium-contaminated soils, because of the low costs, relatively simple operations, and no secondary pollutions [8-10]. A large number of studies [11-12] show that, under the condition of heavy metal pollution, the plant’s resistance to external stress may be enhanced and the toxicity of heavy metals to plants may be reduced. At present, it is not clear that the mechanism of how to improve the plant’s resistance to uranium contamination [14-15].

Arbuscular mycorrhizal fungi (AMF) are the most widely distributed microorganisms in soils, and they can promote absorption of nutrients and increase uptake and resistance of plants to heavy metals and radioactive elements [16-17]. 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 [18]. Moreover, the increase of soluble protein levels can decrease the osmotic potential of plant cells [19]. However, it is not known if AMF affects perennial ryegrass (*Lolium perenne* L.) to uptake uranium from contaminated soils.
In this study, it is used perennial ryegrasses the host plant to study the uptake of plant to uranium, with inoculation of the two representative species of AMF, *Glomus claroideum* (*G. claroideum*) and *G. mosseae* strains. Infection rate of AMF and the plant’s adsorption and transport of AMF have also been investigated.

2. Material and Methods

2.1 Fungus strains and perennial ryegrass seeds
AMF, *G. mosseae* strains were provided by Microbiology Institute of Chinese Academy of Sciences, Beijing, China. The fungi were propagated in our lab for this study. The seeds of perennial ryegrass (*Lolium perenne L.*) were kindly provided by the specimen laboratory of the Northwest Agriculture and Forestry University (Yangling, China). The seeds of full and uniform sizes, without damages by moths, were chosen for use in this study.

2.2 Experimental design
River sand, vermiculites, and soils were mixed to obtain the soil mixture with a volume ratio of 1:1:1. The soil mixture was sterilized three times at 120°C for 1 hour. The U₃O₈ solution was evenly sprayed into the soil mixture, which was mixed well to obtain the uranium-soil mixture with 5 mg/kg U₃O₈. After a week, seeds of perennial ryegrass were planted with thirty seeds in each flowerpot or each chamber device, which contained 1 kg of soil mixture with or without uranium (U₃O₈). After one more week, AMF was inoculated into each flowerpot and each chamber device. The chamber device provided better growth conditions including light, nutrition, temperature, and humidity, than the flowerpot under the natural conditions.

2.3 Determination methods
Photosynthetic pigments, soluble proteins, and malondialdehyde were determined by ethanol extraction method, Coomassie brilliant blue method, and Thiobarbituric acid method, respectively. All experiments were repeated for at least three times. Briefly, the plant tissues were fixed by using 4% glutaraldehyde, and then treated with acetone dehydration (30%, 50%, 70%, 80%, 90%, and 100%, respectively). The samples were embedded using Epon812 (Hongjin Chemical Co., Ltd., Hengyang, China) and then made into slices with a thickness of 70 nm by a Leica microtome purchased from Leica Microsystems (Shanghai, China) Co., Ltd., Germany. Hitachi H-7650 transmission electron microscope was used to observe the slices.

3. Results and Discussions

3.1 AMF influences photosynthetic pigment levels of perennial ryegrass
Photosynthetic pigment content reflects production of photosynthesis in plants. Environmental stress may lead to reduction and degradation of photosynthetic pigment content. Therefore, photosynthetic pigment content is an important physiological index of leaf senescence [20]. As shown in Fig. 1, the pigment contents of perennial ryegrass were determined. When compared with the Con group, the levels of chlorophyll-a (chl-a), chlorophyll-b (chl-b), and carotenoids in the ConU group were decreased (p < 0.05), suggesting that uranium represses the plant growth. When compared with the ConU group, the levels of chl-a and carotenoids in the ConU+Gc were significantly increased (p < 0.05), but 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 indicated that in natural conditions, only *G. claroideum* can increase the levels of chl-a and carotenoids of plants.
3.2 AMF increases the soluble protein content levels in perennial ryegrass

To investigate if AMF can affect the soluble protein content of perennial ryegrass, experiments similar to those as describe above were performed. As shown in Fig. 2, when compared with the Con group, the soluble protein content levels in plant shoots and roots in the ConU group were both decreased (p < 0.05), suggesting that uranium represses the plant growth. When compared with the ConU group, the soluble protein content levels in plant shoots and roots in the ConU+Gc were both increased (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), but those in plant roots were not changed significantly (p > 0.05). These results indicated that in natural conditions, *G. claroideum* and *G. mosseae* can increase the soluble protein content levels in plant shoots. And, *G. claroideum* can increase the soluble protein content levels in plant roots of plants.

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

Therefore, levels of MDA in the shoots and roots of perennial ryegrass were determined. As shown in Fig. 3, when compared with the Con group, the MDA levels in plant shoots and roots in the ConU group were both increased (p < 0.05), suggesting that uranium increases the MDA levels. After inoculation with AMF (*G. claroideum* and *G. mosseae*), the levels of MDA in the shoots and root of plants were slightly decreased when compared with those of CK5 group. However, 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. Moreover, in the compartment cultivation devices, the inhibition of AMF on MDA levels was more significant than in the natural conditions.
In this study, it was found that AMF influences photosynthetic pigment levels in perennial ryegrass. AMF increases the soluble protein content levels in perennial ryegrass and represses the uranium-induced increase of MDA levels in perennial ryegrass. *G.* mosseaes has a more effective function than *G.* claroideum on the attenuation of uranium damages on cell structures of perennial ryegrass.

After inoculation with AMF, the contents of MDA in the shoots and roots of perennial ryegrass were decreased, indicating that AMF could improve the antioxidant enzymes in the plants effectively, reduced the levels of reactive oxygen species, and alleviated the degrees of membrane lipid peroxidation. The two kinds of AMF had somehow differences in the infection rate and the activity of the roots of perennial ryegrass. In the present study, the infection rate and the activity of the roots of perennial ryegrass inoculated with *G.* mosseaes were all higher than those of *G.* claroideum. The hyphae of AMF expanded the scope of the plant roots, and therefore the absorption capacity of the plant to the water and mineral nutrients was increased.

**Acknowledgement**

This work was financially supported by Innovation Platform Open Fundation of Hunan Province (16K078).

**References**

[1] Azapagic A.. Developing a framework for sustainable development indicators for the mining and minerals industry. Journal of Cleaner Production. (2004). 12(6), 639–662.

[2] Peters, K., Bundschuh, M., & Schafer, R.. Review on the effects of toxicants on freshwater ecosystem functions. Enviromental Pollution. (2013). 180, 324–329.

[3] Ruedig, E, Johnson TE. An evaluation of health risk to the public as a consequence of in situ uranium mining in Wyoming, USA. J Environ Radioact. (2015).150:170-178.

[4] Lancia, C., et al. "Marginal structural models with dose-delay joint-exposure for assessing variations to chemotherapy intensity. " Statistical Methods in Medical Research (2018).

[5] Wang Wei-Hong, Luo Xue-Gang, Liu Lai,Zhang Yan,Zhao Hao-Zhou. Ramie (Boehmeria nivea)'s uranium bioconcentration and tolerance attributes [J].  Journal of environmental radioactivity,2018,184-185.

[6] Yelena P. Katsenovich, Claudia Cardona,Jim Szecsody,Leonel E. Lagos,Walter Tang. Assessment of calcium addition on the removal of U(VI) in the alkaline conditions created by NH 3 gas[J]. Applied Geochemistry,2018,92.

[7] Krawczyk-Barsch E, Lütke L, Moll H, Bok F, Steudtner R, Rossberg A. A spectroscopic study on UVI biominerализation in cultivated Pseudomonas fluorescens biofilms isolated from granitic aquifers. Environ SciPollut Res Int. 22 (2015). 6:4555-4565.
[8] O. Neves, EM Vicente. Vicente. Vicente. Uptake of uranium by Lettuce (Lactuca sativa L.) in natural uranium contaminated soils in order to assess chemical risk for consumers. Water Air Soil Pollut. 195 (2008): 73-84.

[9] Burken JG, Schnoor JL. Phytoremediation: plant uptake of atrazine and role of root exudates. Journal of Environmental Engineering. 122 (2015) 11:958-963.

[10] Testa, A., Ballarini, F., Giesen, U., Gil, O. M., Carante, M. P., & Tello, J., et al. Analysis of radiation-induced chromosomal aberrations on a cell-by-cell basis after alpha-particle microbeam irradiation: experimental data and simulations. Radiation Research. (2018).

[11] Clemens S, Ma JF. Toxic Heavy Metal and Metalloid Accumulation in Crop Plants and Foods. Annual review of plant biology. 67 (2016): 489-512.

[12] Luo Z B, He J, Polle A, Rennenberg H. Heavy metal accumulation and signal transduction in herbaceous and woody plants: Paving the way for enhancing phytoremediation efficiency. Biotechnology Advances. 34 (2016) 6: 1131-1148.

[13] Liu, Yaping, et al. "Bacterial diversity among the fruit bodies of ectomycorrhizal and saprophytic fungi and their corresponding hyphosphere soils." Scientific Reports 8(2018).

[14] Hodge A, Storer K. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant and soil. 386 (2015) 1-2: 1-19.

[15] Wagg C, Bender SF, Widmer F, van der Heijden MG. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences. 111 (2014) 14: 5266-5270.

[16] Nascimento F J A, Svendsen C, Bradshaw C. Combined effects from γ radiation and fluoranthene exposure on carbon transfer from phytoplankton to zooplankton. Environmental science & technology. 49 (2015) 17: 10624-10631.

[17] Herman D J, Firestone MK, Nuccio E, Hodge A. Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiology Ecology. 80 (2012) 1: 236-247.

[18] Cozzolino V, De Martino A, Nebbioso A, Di Meo V, Salluzzo A, Piccolo A. Plant tolerance to mercury in a contaminated soil is enhanced by the combined effects of humic matter addition and inoculation with arbuscular mycorrhizal fungi. Environmental Science and Pollution Research. 1-11 (2016).

[19] Thalmann M R, Pazmino D, Seung D, Horrer D, Nigro A, Meier T, Kölling K, Pfeifhofer HW, Zeeman SC, Santelia D. Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants. Plant Cell. 28 (2016) 8:1860-1878.

[20] Li H S, et.al. The experiment principle and technique on plant physiology and biochemistry. Beijing: Higher Education Press. (2000):164-261.