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Effects of *arbuscular mycorrhizal fungi* on the drought resistance of the mining area repair plant Sainfoin

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ABSTRACT

To solve the problem of lack of water in arid areas and barren soil in the process of ecological restoration, the growth and drought resistance of Sainfoin (*Onobrychis viciifolia* Scop.) was studied by inoculation of AMF (*arbuscular mycorrhizal fungi*), under drought stress. The results show that the inoculation of AMF can significantly improve the growth and mycorrhizal infection rate of Sainfoin and accelerate the absorption of water and nutrients by the roots compared with uninoculated plants. On the 40th day under drought stress, the relative water content, total nitrogen and total phosphorus content of the leaves of inoculated plants increased by 7.27%, 4.21% and 2.40%, respectively, the osmotic adjustment property in plant cells was improved, the protective enzyme system was improved and membrane lipid peroxidation was reduced. The inoculation of AMF can slow down the damage from drought, improve the drought resistance of Sainfoin, and will be of great significance to the ecologic restoration in arid areas.

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1. Introduction

The continued exploitation of mineral resources has caused serious environmental problems, such as land desertification and soil erosion [1]. In recent years, much research on land reclamation and ecological rehabilitation at home and abroad has been carried out. Great progress has been made in comprehensive management of the ecology and environment of mining wasteland, selection and cultivation of pioneer plants with strong resistance, soil improvement and afforestation, and biological reclamation technology [2]. However, the lack of water resources in arid areas, barren soil, dysplasia, and low biological diversity slow down ecological restoration. The artificial vegetation coverage rate of current mining areas is still relatively low and the vegetation type is unitary with poor resistance. This requires regular watering and full-time care, which wastes a lot of materials and human resources and shows poor repair effects. Therefore, the choice of drought resistant plants, enhancement of soil fertility, and soil ecological community reconstruction are the main problems to be solved in arid area ecological restoration.

AMF (*arbuscular mycorrhizal fungi*) is a beneficial soil bacterium which can join commensally with most plants [3]. It has been found that AMF is able to improve the drought resistance of plants because its mycelium can enlarge the absorption range of roots, accelerate the absorption of water and nutrients, particularly P element which moves 10 times faster in mycelium than in roots [4]. Mycorrhiza can also improve soil structure, increase the biomass and stabilize the soil ecosystem [5]. Therefore, inoculation of AMF is a new method for re-vegetation in arid areas. Sainfoin (*Onobrychis viciifolia* Scop.) is a bird's-foot plant with strong drought resistance due to its well developed roots with many root nodules and with good fertilization ability [6]. Such a plant used to afforest a coal mine reclamation area, could provide a large amount of organic material and improve the physical and chemical properties of the soil [7].

Our experiments were designed for the features of lack of water, barren soil, less biodiversity, and difficulty in ecological restoration of arid areas. Relying on the properties of AMF and Sainfoin, with *Glomus mosseae* as the inoculant, we studied the influence of the inoculate on the growth and drought resistance of Sainfoin in order to provide a theoretical basis for the application of mycorrhizal technology and Sainfoin in the ecologic restoration of arid areas.

2. Experimental

2.1. Materials and methods

The test plant used was Sainfoin, and the inoculant was *G. mosseae* (BGC NM03D). Soil for the test was selected from the

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China University of Mining and Technology. It was sterilized at high temperature, high pressure and steam (121 °C, 2 h), dried in air and mixed with boiled sand in a proportion of 1:1 after screening by a 2 mm mesh. Physicochemical properties of the mixed substrate: pH: 7.3, organic content: 0.62%, alkali-hydrolyzable nitrogen: 9.96 mg/kg, and rapidly available phosphorus: 15.29 mg/kg. Foam boxes (48 cm × 34 cm × 20 cm) were used with each box being filed with 10 kg of mixed sandy soil.

The tests were based on two substrate water contents: drought stress (relative water content in the substrate was 50% of the maximum water-holding capacity of the substrate, L) and normal water supply (relative water content in substrate was 80% of the maximum water-holding capacity of the substrate, H). For each substrate water contents, two groups were made: inoculated (G+) and uninoculated (G−), 3 boxes each and the 12 boxes were randomly arranged.

The boxes were seeded on May 25, 2013 and inoculated on June 12, with each box receiving a dosage of 40 g. Normal water supply for each group started on August 25. Plants with similar growth trends were selected every 10 days to test the contents of water, soluble proteins and malonaldehyde, the antioxidase activity in leaves, and the total nitrogen and phosphorus in the plants and substrate. All plants were harvested on October 4. Weighing was used to measure the relative water contents of the soil in the control boxes.

2.2. Indicator and assay method

The ink and vinegar staining method was used to measure the mycorrhizal infection rate [8], the oven drying method for relative water content, the ultraviolet spectrophotometer method for the content of soluble proteins, the spectro-photometer method for MDA content, the nitroblue tetrazolium photochemical reduction method for SOD activity, the guaiacol method for POD activity [9,10], the ultraviolet absorption method for POD activity, molybdenum antimony anti absorption spectrophotometry for total phosphorus, and the H_2SO_4–H_2O_2 heating digestion method for total nitrogen.

2.3. Data processing

ORIGIN software was used for drawing, DPS7.05 software and the Duncan multiple comparison methods were used for analysis of the significant difference of data.

3. Results and discussion

3.1. Influences of different treatments on the growth and mycorrhizal infection of Sainfoin

Table 1 shows that under normal water supply conditions, the inoculation of AMF improved the plant height, root length, dry weights of above ground parts and below ground parts by 10.38%, 6.07%, 6.58%, and 6.25%, respectively, which shows its promotion effect on growth, while under drought stress, the four figures increased by 18.98%, 11.78%, 14.29% and 12.20%, respectively. From this it can be seen that water is a key factor affecting the growth of Sainfoin and inoculations promoted Sainfoin's growth and decreased the infection rate from 78.96% to 67. 29% under drought stress, which is consistent with the results of Guo et al. on *Astragalus adsurgens Pall*, under the same drought stress [11]. Mycorrhizal dependency refers to, in comparison with unvaccinated plants. AMF inoculation has influence on the growth of plants [12]. Under drought stress, mycorrhizal dependency increased from 6.56% to 14.16%, which indicates that drought stress has inhibited the infection of AMF, so AMF inoculation's promotion effect is larger compared to uninoculated plants.

3.2. Influences of different treatments on the relative water content and content of soluble proteins of Sainfoin

Fig. 1 shows that, under normal water conditions, the relative water content in the leaves of Sainfoin was at first slowly reduced, but in the later stage, compared to uninoculated plants, inoculated plants had higher water content. Under drought stress, the relative water content in the leaves of Sainfoin was quickly reduced, significantly lower than in the normal water supply group. After AMF inoculation, the water content in the leaves of these plants increased by 3.87% and 7.27%, respectively on the 20th day and 40th day when compared to uninoculated plants. From this it can be seen that inoculation of AMF improves the water content in plants under drought stress.

Osmotic adjustment substances in plants mainly include polybasic alcohol, nitrogenous compounds (such as proline and soluble proteins) and organic acid [13]. Research shows that inoculation of AMF could promote the accumulation of osmotic adjustment substances in plants under drought stress, maintain the water content, and normal metastasis. In the case of normal water supply, the content of soluble proteins in Sainfoin slightly changed during the experiments and inoculated plants had a higher content compared to uninoculated plants. Under drought stress, the content of soluble proteins in Sainfoin significantly changed, reaching the peak value on the 30th day, followed by reduction. After inoculation of AMF, the content of soluble proteins in the plants increased by 8.03%, 14.31%, 14.72% and 24.56% on the 10th, 20th, 30th and 40th day respectively, compared to uninoculated plants. This indicates that drought stress accelerates the accumulation of soluble proteins, and the inoculation of AMF can further improve the content, which leads to an increase of osmotic pressure, reduces water loss, and slows down lack of water caused by drought.

3.3. Influences of different treatments on the total nitrogen and phosphorus of Sainfoin

Under drought, movement of NO^{3-} in soil is constrained and the potential lack of nitrogen also restricts growth. AMF can improve

Table 1
Effect of different treatments on growth and mycorrhizal infection status of Sainfoin.

Treatments		Plant height (cm)	Root length (cm)	Shoot dry mass (g)	Root dry mass (g)	Infection rate (%)	Mycorrhizal dependency (%)
LG−		4.90 ± 0.66c	9.34 ± 0.31c	0.63 ± 0.02d	0.041 ± 0.003c	2.05 ± 0.23c	
LG+		5.83 ± 0.83b	10.44 ± 0.67bc	0.72 ± 0.03c	0.046 ± 0.004d	67.29 ± 5.03b	14.16
HG−		6.65 ± 0.34ab	11.40 ± 0.24ab	0.76 ± 0.02b	0.048 ± 0.003a	2.57 ± 0.27c	
HG+		7.34 ± 0.35a	12.11 ± 0.85a	0.81 ± 0.04a	0.051 ± 0.004b	78.96 ± 4.30a	6.56
Significance	W	**	**	**	**	*	
	G	*	*	**	**	**	
	W × G	NS	NS	NS	NS	*	

Note: L, relative water content of 50%; H, relative water content of 80%; G−, uninoculated; G+, inoculated with BGC NM03D; NS, no significant difference. Letters with the figures in the same line represent a distinct difference at a level of 5%.
* $P < 0.05$.
** $P < 0.01$.

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