ZIBELINE INTERNATIONAL PUBLISHING

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Asian Pacific Journal of Microbiology Research (AJMR)

DOI : http://doi.org/10.26480/ajmr.01.2018.01.04

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SIMULATION STUDY ON RELIEFING NEGATIVE INFLUENCE OF DAMAGE ROOTS IN COAL MINING SUBSIDENCE AREA

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ARTICLE DETAILS

Article History:

Received 12 November 2017
Accepted 12 December 2017
Available online 1 January 2018

ABSTRACT

In the process of coal mining, surface subsidence leads to plant root injury. In this study, the mitigation effects of an arbuscular mycorrhizal fungus (AMF) inoculation on the growth of root injured alfalfa were investigated by five-chamber system matrix internal injury root method, which artificially simulated root damage caused by coal mining. The results indicated that AMF inoculation alleviated the adverse effects caused by root harm and contributed to the growth of alfalfa. The average dry weight per plant of the inoculation group was higher than that of the control group. In addition, AMF inoculation significantly promoted the alfalfa to take up mineral elements from the soil and increased the content of hyphae length and organic matter which existed in the rhizosphere soil of the injured alfalfa. The contents of total hyphae length and organic matter in the different test design showed the same rules that the inoculation on both sides were higher than the inoculation on one side. AMF inoculation improved the micro-environment of rhizosphere and made a contribution to the amelioration and fertilization of degraded soil in the mining area. It will provide technical support for land reclamation and ecological reconstruction by studying the effects of AMF on the growth of damaged plants.

KEYWORDS

Arbuscular mycorrhizal fungus, root damage, mycorrhizoremediation

1. INTRODUCTION

Coal mining causes vegetation damage in two ways. Firstly, surface subsidence, ground fissure and other factors caused by coal mining may directly damage surface soil structure and lead to vegetation degradation and withering. Secondly, surface subsidence and ground fissure may destroy the underground aquifer structure and mine drainage may lead to the falling water level in mine lots, which may further result in vegetation degradation and death. The root system damage problem has always been the bottleneck of reclamation and ecological reconstruction in mine lots. At present, some scholars think in the manual-control environment, through guaranteeing reasonable supplies of soil moisture and nutrient a certain amount of the root system pruning can promote the root system occurrence and become an efficient means of the root system regulation.

According to a study, there are few reports on the studies of the plant root system damage caused by coal mining at home and abroad [1]. This mainly lies in that the plant root system in soil forms black box, which is difficult to observe it. Together with irregular applied force of the plant root system on the soil distribution and surface subsidence, it is more difficult to have overall studies on the plant root system in mining area [2-4]. Study showed there is no perfect technology of the root system repair of damaged plants in mining area. Physic-chemical method can ease the adverse effects of coal mining to certain degree, but it cannot fundamentally solve the environmental degradation problems in mine lots caused by the root system damage. At the same time, the governance costs are high. Thus, it is difficult to be widely applied in mining areas [5-8]. According to a researcher, biological means is one of the advocated methods at home and abroad [9]. Among them, arbuscular mycorrhizal fungi are taken as one good biological bacterial manure and owns great application value and potential in the treatment of the ecological environment [10]. This paper uses matrix trauma root treatment method to study the damage function and effect of mycorrhiza fungi on the root system and verify whether extraradical mycelium of mycorrhizal fungi can maintain the balance between plant root cap.

2. MATERIALS AND METHODOLOGY

The experiment adopts five-chamber culture method. The experiment device is made of organic glass. Chamber 1 and chamber 5 are taken as root chamber and are filled with sandy soil to plant Alfalfa. Chamber 2 and chamber 4 are taken as buffer chamber and filled with loamy soil and nothing is planted. Chamber 3 is mycelia chamber and is filled with quartz sand of 1 mm grain size. Chambers 1 to 5 are separated by nylon nets of 30 μm bore diameter, with serial number from A to D.

The size of the culture system is length*width*height=(14*10*10) cm3, respectively with length of root chamber, buffer chamber and mycelia chamber 3 cm, 2 cm and 4 cm. Mycelia can pass through chamber 2 and chamber 4 and enter into middle mycelia chamber. While the root system cannot pass through Nylon net A and Nylon net D to enter into chamber 2 and chamber 4.

The experiment is treated as inoculation group and contrast group and there are four experiment designs, with inoculation in chamber 1 and

chamber 5, with inoculation in chamber 1 and no inoculation in chamber 5, without inoculation in chamber 1 and with inoculation in chamber 5, without inoculation in chamber 1 and chamber 5 (Table 1). Root damage belongs to matrix internal root damage and chamber 5 is designed into embedded U-tube mode to conveniently cut the plant root system with matrix (Figure 1 and Figure 2). Root damage only occurs in the left side of chamber 5 while the other side of chamber 1 maintains undisturbed. After 45-day plant growth, root damages are treated according to 3 volumes, 0, 1/3 and 1/2. Each treatment is repeated 4 times. Then plants are harvested after 45-day root damage. The experiment is five-factor design, 1 (inoculation treatment)*1 (plant)*3 (root damage treatment)*4 (root damage modes)*4 (repetition) =48 pots. 50 alfalfa seeds are sowed in each chamber. Before being put into pot, alfalfa seeds are implemented surface disinfection with 10% H2O2. After deionized water is used to wash several times, they are put in 25℃ constant temperature oven to accelerate germination for reserve. After seedlings emerge, each chamber owns final singling of 10 strains.

In the whole experiment process, the soil moisture content maintains around 60%~80% of the soil maximum saturation moisture capacity. Black shade is used to cover chamber 2 and growth of mycelia in chamber 3. Nitrogen fertilizer, phosphate fertilizer and potassium fertilizer are respectively applied in chamber 1 and chamber 5 when seeding and root damage, with concentration of N (NH4NO3) 100 mg/kg, P (KH2PO4) 15 mg/kg and K (KNO3) 150.

Table 1: The design of experiment.

Experiment unit					Experiment design
chamber 1	chamber 2	chamber 3	chamber 4	chamber 5	
Inoculated alfalfa	surge chamber	Hyphae chamber	surge chamber	Inoculated alfalfa	+M丨+M
Inoculated alfalfa				Contrast alfalfa	+M丨CK
Contrast alfalfa				Inoculated alfalfa	CK丨+M
Contrast alfalfa				Contrast alfalfa	CK丨CK

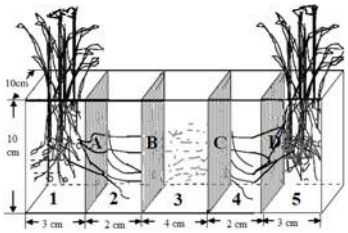

Figure 1: Five compartment cultivation system.

Notes: Number 1-5 represent compartment which separated by nylon mesh screens; letter A-D represent the number of nylon mesh screen; hyphae is represented by broken lines; roots are represented by solid lines.

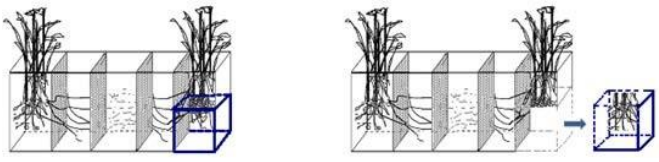

Figure 2: Root damage process.

Notes: The left type U tube was not root damage state, the right U tube pull out root damage process; the root volume remove were 1/3 and 1/2.

When harvest, spoon is used to transfer the fungi with quartz sand in mycelia chamber to 380 μm screen and distilled water is used to wash carefully. Finally, 30 μm screen is adopted to recycle the mycelia.

After root damage, deionized water is used to wash the cut the root system in chamber 5 and then its wet weight is measured. A small amount of root segment is selected to measure the mycorrhiza infection rate and dried under 70℃ to weight the dry weight. When harvest, the overground part and the root system in chamber 5 are separated. Deionized water is used to wash the root system to measure the wet weight. Then cut it into root segments of 1 cm, few root segments are used to measure mycorrhiza infection rate. The left root segments and overground part are carried out deactivation of enzymes under 105℃ for 30 min. Then they are dried under 70℃ to weight the dry weight

3. RESULTS

After 45-day growth, alfalfa is treated with root damage. U-tube in Chamber 5 is taken to cut the root system according to the proportion of root damage. The cut the root system is collected after passing through 1 mm screen and then its dry weight is measured. For 1/2 root damage, the dry weights of the root system in four experimental designs all present the same rules and reach significant difference: + M

丨+ M > CK 丨+ M > + M 丨 CK > CK 丨 CK, which

indicate that inoculation mycorrhiza can effectively increase the underground the root system biomass of alfalfa (Figure 3)

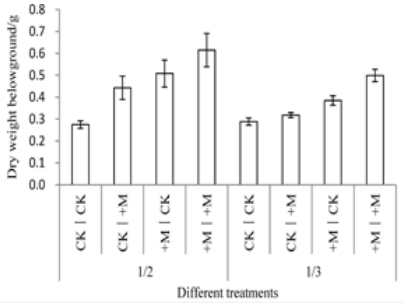

Figure 3: The influence of different alfalfa root damage treatment on dry weight belowground.

Alfalfa is harvested after 45-day root damage. The dry weights of the overground part and underground part after harvest are measured. For 0 root damage, dry weights of the overground part and underground part in four experimental designs both reach significant difference, which indicate that inoculation mycorrhiza can promote the growth and development of alfalfa and effectively increase the underground biomass of alfalfa.

For the dry weights of the overground part and underground part in 1/2 root damage and 1/3 root damage treatment, the dry weight of the overground part of + M 丨+ M is significantly higher than those of the other three experiment designs, but there is slight

difference between M and CK in the dry weight of the underground part (Figure 4). It can be seen that the root system damage seriously influences the development the underground part of alfalfa. Compared with the severe influence of 1/2 root damage treatment on the root system, the dry weights of the overground part and underground part of + M

丨+ M in 1/3 root damage treatment both reach significant difference with the other three treatments (Figures 5-7)

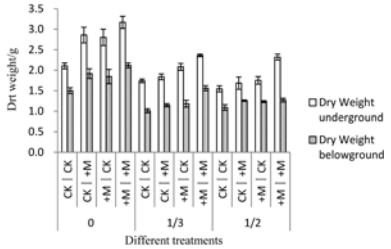

Figure 4: The influence of different alfalfa root damage treatment on dry weight underground and belowground

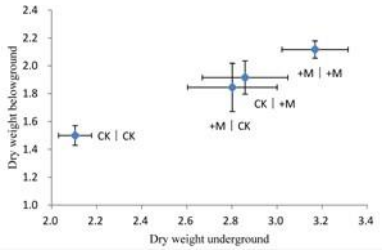

Figure 5: The influence of 0 alfalfa root damage treatment on wet and dry weight underground and belowground

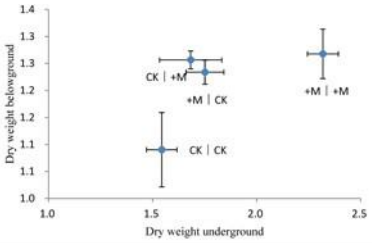

Figure 6: The influence of 1/2 alfalfa root damage treatment on wet and dry weight underground and belowground

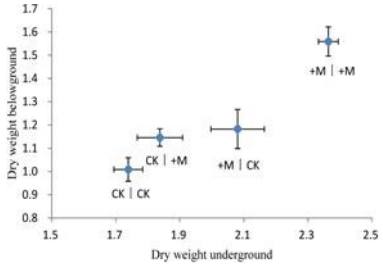

Figure 7: The influence of 1/3 alfalfa root damage treatment on wet and dry weight underground and belowground.

The influences of different treatments on alfalfa rhizosphere mycelia density and mycorrhiza infection rate are shown in figure. The alfalfa rhizosphere mycelia density and mycorrhiza infection rate in Chamber 5 after root damage differ slightly in four experimental designs but they all are significantly higher than M and CK, indicating in the early growth stage, mycorrhiza fungi is closely connected with the root system and forms stable plant-mycorrhiza symbiont. Alfalfa regrows for 45 days. The measured mycelia density and mycorrhiza infection rate after harvest both decrease compared with 0 root damage treatment (Figures 8 and 9)

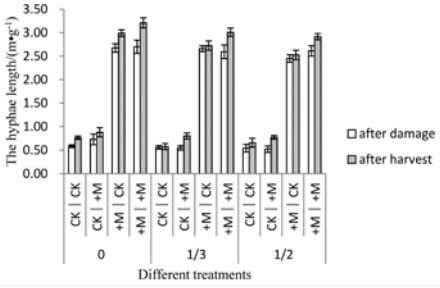

Figure 8: The hyphae length in different root damage treatments

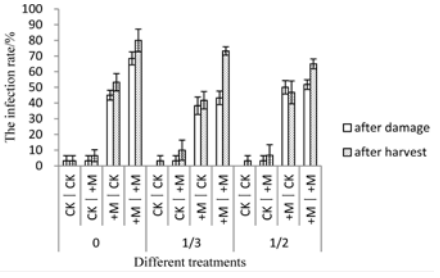

Figure 9: The infection rate in different root damage treatments

The influences of different treatments on alfalfa rhizosphere acid phosphatase activity and available phosphorus content are shown in Table 3. After the root damage, the acid phosphatase activity in four different experiment designs owns the following rules that different root damage treatments differ slightly, and they can decrease the acid phosphatase activity while they are not significant (Table 2).

Available phosphorus contents in the rhizosphere soil after root damage and after harvest vary slightly. This indicates that root damage has obvious influences on the underground mycelia network, whose damage makes the available phosphorus content difficult to maintain at certain level and causes the deficit area of available phosphorus content around the alfalfa rhizosphere.

Table 2: The impact of different treatment on rhizosphere microenvironment.

Experiment design	Root damage	Acid phosphatase activityphenol (mg·g soil^{-1})		Olsen-P (mg/kg)	
		After damage	After harvest	After damage	After harvest
+ M | + M	0	2.21 a ± 0.24	2.46 a ± 0.26	11.15 a ± 0.77	11.23 a ± 0.42
	1/2	2.06 a ± 0.28	1.76 b ± 0.20	11.23 a ± 0.42	10.20 ab ± 0.16
	1/3	2.46 a ± 0.21	2.17 a ± 0.18	11.43 a ± 0.47	10.93 a ± 0.13
CK | + M	0	1.75 b ± 0.13	2.07 a ± 0.29	10.73 b ± 0.94	10.33 ab ± 0.63
	1/2	1.69 b ± 0.13	1.67 b ± 0.15	10.58 b ± 0.84	10.23 ab ± 0.81
	1/3	1.67 b ± 0.14	1.79 b ± 0.16	10.33 bc ± 0.71	10.33 ab ± 0.71
+ M | CK	0	1.40 c ± 0.05	1.65 b ± 0.10	11.18 a ± 0.63	9.40 c ± 0.26
	1/2	1.25 d ± 0.10	1.35 c ± 0.06	10.15 c ± 0.58	9.83 b ± 0.38
	1/3	1.40 c ± 0.05	1.57 b ± 0.07	11.35 a ± 0.65	9.60 b ± 0.27
CK | CK	0	1.29 c ± 0.08	1.39 c ± 0.04	10.58 bc ± 0.31	9.08 c ± 0.34
	1/2	1.13 d ± 0.06	1.33 c ± 0.07	11.23 a ± 0.42	9.58 b ± 0.39
	1/3	1.30 c ± 0.07	1.49 c ± 0.06	11.23 a ± 0.69	9.55 c ± 0.26

Notes: The values in this table were the means of four replicates. Means followed by different letters indicated significant difference at 5% level.

4. DISCUSSION

This paper adopts five-chamber culture method. The experiment device is improved on the glass bead chamber culture system by Chen Baodong, aiming at extracting mycelium from mycelia chamber 3 to measure the concentration of mineral elements. However, many influence factors in the experiment are difficult to control, for example, watering and pollution prevention in the mycelia chamber. Though the experiment has acquired certain data, the experiment device still needs further improvement in terms of the damage mechanism of the root system and strives to minimize the external influences on experimental results [11-15]. On the other hand, this experiment adopts the simulation treatment of matrix root system, namely, it only needs to extract the cut root system from the matrix while the left root system is undisturbed. However, how to simulate the plant root system damage in coal mining subsidence areas more real also needs further study. The experiment results in this paper has proved that the root system damage can influence the plant biomass of the overground part and underground part and decreases the enzyme activity of plant rhizosphere soil, but how to quantitatively analyze the function of mycorrhiza fungi still needs further exploration.

5. CONCLUSIONS

Root damage treatment can decrease the overground and underground biomass of alfalfa and reduce the alfalfa rhizosphere acid phosphatase activity, available phosphorus content and root activity. The influences of different experiment designs after the root damage and harvest on plant biomass and riphzosphere environment show that both-side inoculation is higher than those of single-side inoculation and the control group;

The mycelia density and mycorrhiza infection rate after root damage in different root damage treatments present that both-side inoculation is significantly higher than those of single-side inoculation and higher than those of the control group. The mycelia density and mycorrhiza infection rate after harvest in different root damage treatments both decrease compared with those before root damage. This indicates that the root system damage has negative influences on the plant-mycorrhiza symbiont.

After the root damage treatment, mineral element contents in mycelia in 1/3 and 1/2 root damage treatments decrease compared with 0 root damage treatment. This may be relevant to the flow of mineral element contents in mycelia.

ACKNOWLEDGEMENTS

This paper was funded by Xi'an University of science and Technology Basic Fund (201306) and Xi'an University of science and Technology Research Fund for the Doctoral Program (2014 QDJ061).

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