Effects of Different Arbuscular Mycorrhizal Fungi on the Growth and Yield of Soybean in Coal Mine Spoil

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Abstract: A pot culture experiment was carried out to determine the effects of inoculation with different arbuscular mycorrhizal (AM) fungi on the growth and yield of soybean (Glycine max (L.) Merrill cv. Ludou-4) grown in coal mine spoil. Three species of AM fungi, Acaulospora mellea ZZ, Glomus mosseae LY and Glomus caledonium CD, were included. The coal mine spoil was collected from the Zaozhuang coal mine in Shandong Province, China. Among the three fungi, A. mellea ZZ was found to be the best fungus in terms of root colonization ability and effectiveness to promote growth and yield of plants. The plants inoculated with A. mellea ZZ produced greater biomass, grew higher, flowered earlier and produced more flowers and pods and higher yields than non-mycorrhizal plants. In addition, there was a significant increase in P concentrations in mycorrhizal plants compared with non-mycorrhizal plants. The results indicate that AM fungi as an effective and economical biofertilizer can increase growth of host plants and may play a potential role in revegetation and reclamation of coal mine spoils.

Key words: Arbuscular mycorrhiza • Glycine max • coal mine spoil • reclamation

INTRODUCTION

The process of the coal mining not only spoiled great quantities of surface soils and plants and left so many eco-fragile soil belts, but also directly polluted the soils. Restoration of disturbed soils is a difficult challenge and reclamation after mining can take many years. Plantations can be used as a tool for mine spoil restoration as they have ability to restore soil fertility and ameliorate microclimatic conditions [1], however, mine spoils are generally known to be with low pH, poor plant nutrient (P, N and K) and high levels of metals (Al and Mn), which prevents revegetation of plants on such sites. Other assistant tools such as beneficial microorganisms may be incorporated.

Arbuscular mycorrhiza is one of the most common symbioses on earth, which colonize the majority of higher plant species including most crops. AM fungi impart to their hosts a variety of benefits including increased growth and yields due to enhanced nutrient acquisition, water relations, etc. [2]. The most common beneficial effect of AM is increased uptake of immobile nutrients (notably P) by host plants from soil [3]. Actually, AM fungi will not only benefit plant growth and development, but offer the possibility of increasing resistance to stress as well, such as drought, disease, pests, salinity and heavy metals [3]. The application of AM fungi in bioremediation of contaminated soils has attracted more attention [4].

Many studies have shown that AM fungi occur in mined soils including coal mine spoils and can colonize many species of plants in mining sites [5-12]. Mehrotra reported that 13 species of pioneering plants grown to reclaim mine spoil at an openeast coal mine site can be colonized by AM fungi [12]. The roles of AM fungi in biological reclamation of mining spoils have been reported [13, 14]. AM fungi may play an important role in plantation of coal mine spoils and species or isolate-specific screening of AM fungi and of AM-plant combinations needs to be carried out.

As a common crop worldwide, soybean forms symbioses with rhizobia and AM fungi and is well adapted to soils poor in N and P, then it may be used as a pioneering plant in reclamation of mine spoils. An effective AM fungus may help soybean to colonize coal mine spoils, however, mycorrhizal effects may depend on soil status, plant and fungal species/ecotypes. It may be of importance to isolate and select effective AM fungal

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species/ecotypes in reclamation of coal mine spoils. The objectives of the present work were to compare the effects of three AM fungal species on soybean in coal mine spoils and to try to find a more effective one that can be used in revegetation and reclamation of coal mine spoils.

**MATERIALS AND METHODS**

**AM fungal inocula:** (1) *Acacinospora mellea* ZZ: This species of fungus was isolated from the rhizosphere of crabgrass (*Digitaria sanguinalis* (L.) Scop.) grown in coal-mine spoils in the southeastern part of Shandong Province, China. It colonizes host plants well (root colonization rates >60%) in waste mine spoils and the spore number ranges from 20 to 200 per mL soils. (2) *Glomus mosseae* LY: The species was isolated from field soil and can colonize a wide variety of crops well under field conditions. (3) *Glomus caledonium* CD: The fungus, occurred widely, was isolated from the rhizospheres of several wild plants, such as *Vitis amurensis*, *Ziziphus jujuba*, *Amorpha fruticosa*, *Vitex negundo*, grown in forest land of Chang Island, Shandong Province, China.

After single-spore culture [15], the three AM fungal species were trap-cultured on sudangrass (*Sorghum sudanense* (Piper) Stapf.) respectively. After four months of culture, AM spores were wet-sieved and surface-sterilized using 0.4% streptomycin sulphate and 200 mg L⁻¹ chloramin T solution and then washed repeatedly with sterile water.

**Soils:** The soil was collected at 0-20 cm depth from Zaozhuang coal mine in southeastern part of Shandong Province, China. The Zaozhuang coal mine, with more than 100 years' history, was abandoned in 1999 and a large amount of spoils were left. The soil type is brown soil and soil texture is light loam, with the following properties: soil pH (soil:water ratio, 1:2.5) 4.0, 1.07% organic matter, 0.057% total N, 0.032% total P, 4.55 mg kg⁻¹ Olsen P, 82 mg kg⁻¹ Cu, 25 mg kg⁻¹ Pb, 156 mg kg⁻¹ Zn, 58 mg kg⁻¹ Cr, 1.1 mg kg⁻¹ Cd. After sifting through 2 mm sieve, the soil was sterilized (121°C, 2 h) and air-dried and then filled into clay pots (1.2 L).

**Soybean:** Seeds of soybean (*Glycine max* (L.) Merrill cv. Ludou-4) were surface-sterilized with 0.5% NaClO solution and germinated at 28°C for 48 h before sowing.

**Experimental design:** The experiment included four treatments: inoculation with *A. mellea* ZZ (Am), inoculation with *G. mosseae* LY (Gm), inoculation with *G. caledonium* CD (Gc) and the control (without inoculation, C). Air-dried soil (1000 mL) was filled into each pot. One hundred fresh AM fungal spores were put onto a filter paper and then embedded the middle layer of soil (except for the control treatment). Ten germinated seeds of soybean were transplanted into each pot and three seedlings were left after emergence. Pots were arranged in a Randomized Complete Block Design with 10 replicates per treatment. Plants were grown in a sunlit greenhouse with natural light, a day/night temperature 32/22°C and a relative humidity 40%-60%. Plants were watered to maintain soil moisture at about 50% of water holding capacity by adding tap water during the experimental period. Seeds were sown on July 3, 2001. The plants were harvested at mature stage.

**Methods of measurement:** Plant height and leaf number per plant were determined at 20, 40, 60 days after sowing. Seedling stage and flower number per pot were determined at the flowering stage. Shoots (including pods), roots and seeds were harvested separately at mature stage. Root systems were thoroughly washed in running tap water. Fresh weight of shoots and roots were determined. Root mycorrhizal colonization was estimated after clearing and staining [16], using the acid fuchsin staining-grid intersects method [17]. Pod number per pot was determined and seeds were weighed after air-drying for 2 weeks. Leaf and root P contents were measured spectrophotometrically using the molybdate blue method [18].

**Data analysis:** The data were subjected to one-way ANOVA using the SPSS 10.0 software. Means and standard errors were calculated for eight replicate values. Means were compared by the Duncan’s multiple range test and statistical significance was determined at p<0.05 level.

**RESULTS**

**Mycorrhizal colonization rate:** The plants in Am treatment had the highest root colonization rates (28.3%), while there was no significant difference between Gm (15.0%) and Gc (12.5%). No mycorrhizal colonization was detected in non-inoculated treatment.

**Growth and development of soybean plants:** In general, mycorrhizal plants had higher plant height and more leaves than non-mycorrhizal plants at any of the three sample times, especially at 20 days after sowing (Figs. 1 and 2). At 40 days and 60 days after sowing, Gm and Gc did not show significant effects. For mycorrhizal
Fig. 1: Plant height of soybeans in coal mine spoil

Fig. 2: Leaf number per plant of soybeans in coal mine spoil
C: non-mycorrhizal inoculation; Am: inoculation with A. mellea ZZ; Gm: inoculation with G. mosseae LY; Ge: inoculation with G. caledonium CD. Bars indicate standard errors

soybeans, seedling stage was all shorter than non-mycorrhizal plants (Fig. 3a), but only Am increased flower number (Fig. 3b). Compared with the control treatment, although the three fungi all showed positive trends on plant growth, only Am increased shoot and root fresh weights significantly (Fig. 4).
Fig. 3: Seedling stage (a) and flower number (b) of soybeans in coal mine spoil

Fig. 4: Fresh weight of shoots (a) and roots (b) of soybeans in coal mine spoil

Fig. 5: Number of pods (a) and yield (b) of soybeans in coal mine spoil

C: non-mycorrhizal inoculation; Am: inoculation with *A. mellea* ZZ; Gm: inoculation with *G. mosseae* LY; Gc: inoculation with *G. caledonium* CD. Bars indicate standard errors
Pod number and yield of soybeans: Mycorrhizal plants had more pods and higher yields than non-mycorrhizal plants (Fig. 5). Among them, Am showed the most effective effects and there was no significant difference between Gm and Ge.

P concentration of soybean plants: Plants inoculated with AM fungi had higher P concentrations in both leaves and roots than the control (Fig. 6). The Am-treated plants had the highest leaf and root P concentrations, increased by 34.6 and 55.1%, respectively compared to the control plants. No significant difference was observed between Gm and Ge.

DISCUSSION

AM fungi are likely to contribute to the reclamation of mine spoils through the following mechanisms: (1) Increasing the tolerance to stressed environments and survival rate of host plants; (2) Enhancing establishment and growth of plants by increasing nutrient uptake; (3) Maintaining plant biodiversity and ecosystem productivity; (4) Contributing to efficient recycling of nutrients and thus to long term stability; (5) Stabilizing soil structure and improving soil quality. More and more application of AM fungi in reclamation of mine spoils was studied and the results were positive [19, 20]. Lindsey et al. [20] reported the effects of endomycorrhizae on growth of rabbit brush, four-wing salt brush and corn in coal mine spoil material. AM fungi enhanced nutrient absorption and water transport to plant roots in coal mine spoil reclamation [21]. Our results showed that AM fungi increased growth and yield of soybean in coal mine spoil, indicating AM fungi may play a role not only in ecological reclamation of mine spoils but also in sustainable agricultural production.

The beneficial effects of AM fungi are primarily due to increased uptake of P nutrient, especially when available P content in soils was low [2]. Under stressed conditions such as heavy metal or organic pollution, AM fungi still contribute greatly to P uptake of mycorrhizal plants [22, 23]. Our experiment showed similar results. The spoil used in our experiment was low in P content, especially available P, so improvement of P nutrition by AM fungi may be one of main reasons resulted in increased plant growth and yield. However, the effects of AM fungi are diverse and they can influence physiological and biochemical functions of host plants by various ways, which all need further study.

In our results, besides the growth effect, AM fungi also shortened the seedling stage and accelerated flowering (Fig. 3). By modulating phytohormones and improving mineral nutrients, AM fungi may help promote flowering and the maturing of pods. It has been proved that AM fungi induced phytohormone changes in host plants [24], but direct evidence is lacking that AM fungi modulate the flowering time.

There were differences in infectivity and effectiveness among the three fungi used in the experiment, which maybe is closely related to different
ecological adaptability. Generally, AM fungal ecotypes from contaminated sites seemed to be more effective in promoting growth of the host plants in contaminated soils. *A. mellea ZZ* was isolated from coal mine spoils, so it was already adapted to the stress of coal-contaminated soils after the natural selection in its evolution process. However, *G. mosseae* LY and *G. caledonium* CD were originated from nonpolluted habitats, without acclimation and selection in coal-contaminated soils, so they were not as effective as *A. mellea ZZ*.

In our results, *A. mellea ZZ* can easily infect the plants, such as crabgrass and soybean, growing in coal mine waste soils. In addition, *A. mellea ZZ* is easily monocultured and propagated, which is very important for its application and propagation in field, since AM fungi cannot be grown in pure culture. Therefore, *A. mellea ZZ* may be adopted for the rehabilitation of coal mine spoils. However, although the effects of *A. mellea ZZ* on pot-cultured soybeans are satisfactory, whether or not it can show and exert such excellent role when inoculated onto plants grown in the field still needs further study.

The characteristics of coal mine spoils are: much more residue and waste, few species of native-born AM fungi, low nutrient content but high heavy metal content, poor soil structure, etc. Many studies showed the lack in active AM fungi is one of main reasons why it is difficult for plants to grow in waste mine soils [25]. Reintroduction of AM fungi in mine spoils may be critical to plant restoration. Harley and Smith reported that leguminous plants grown in mine soils can accelerate the recovery of N, but P content become a restrictive factor to the growth of leguminous plants [26]. This means mycorrhizal technique can play a role in soil amelioration and ecological restoration and reconstruction. The combination of leguminous plants, AM fungi and nodule bacteria may be an effective tool in reclamation of coal mine spoils. In the future reclamation project, development and application of AM fungi and suitable plants, together with other biological techniques, should be strengthened.

**CONCLUSIONS**

Among the three AM fungi, *A. mellea ZZ* had the greatest mycorrhizal infectivity and significantly increased growth and yields of the soybean plants in the coal mine spoil. Both leaf and root P concentrations of soybean plants were improved by the three AM fungi, especially by *A. mellea ZZ*, which may explain increased growth and yields of mycorrhizal plants. Our results indicate that AM fungi may play a potential role in revegetation and reclamation of coal mine spoils.

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