

Effects of Arbuscular Mycorrhizal Fungi on *Elaeagnus mollis* Diels Seedlings' Growth and Root

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Abstract: Based on a pot experiment of *Elaeagnus mollis* in greenhouse, which is a two-tier protection of endangered plants in china, this research focused on the effects of inoculating with arbuscular mycorrhizal fungi on *Elaeagnus mollis* seedlings' growth and root. The design was divided into three groups: inoculating with *Glomus mosseae* (GM), inoculating with *Acaulospora delicate* (AD), inoculating with inactivated fungi (CK). After determination of parameters such as mycorrhizal infection rate, biomass, root absorption activity, content of mineral elements and resistance related enzymes, results showed: (1) seedlings' root can form mycorrhiza by inoculating with GM and AD; compared with control seedlings, the morphology and biomass of seedlings with mycorrhiza were greatly different and significantly positively correlated with mycorrhizal infection rate ($P<0.05$). (2) Root activity of seedlings with mycorrhiza increased strikingly and there was significant positive correlation between the ratio of active absorption area in root and the content of N and K ($P<0.05$). (3) In the peak and late growth periods, the root peroxidase activity in seedlings with mycorrhiza was much higher than that in control seedlings, otherwise much lower than that in control seedlings in the later growth period, indicating a negative correlation ($P<0.05$). The root polyphenol oxidase activity in seedlings with mycorrhiza was much higher than that in control seedlings ($P<0.05$), coming to the highest in the peak period, however there was no big difference between seedlings inoculated with different fungi. Results demonstrated that compared with control seedlings, inoculating with fungi increased the root absorption activity and enzyme system activity, but also was good for seedlings resisting to various stresses and of great significance on enhance the distribution region of *Elaeagnus mollis* Diels.

Key words: Arbuscular Mycorrhizal Fungi • *Elaeagnus mollis* Diels • Radicular absorption region • Peroxidase • Polyphenol oxidase

INTRODUCTION

Elaeagnus mollis Diels, of *Elaeagnus*, *Elaeagnaceae*, is a national two-tier protection of rare and endangered plants, Chinese endemic species, mainly distributed in the the Yellow River basin and Shan Weihe basin. This superior tree species can afforest barren mountains, improve soil conditions and prevent soil erosion. While, some factors, such as special structure of fruit, short life of seed, lack of competitiveness of *Elaeagnus mollis* seedlings and serious damage from humans' activities have caused its endangerment [1].

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that establish beneficial relationship with more than 70% of plant species [2]. Lots of studies have shown that inoculating with AM fungi may contribute to

an increased absorption of mineral nutrition and water, further to a promotion of growth of the plant, which can play an important role in the establishment, evolution and development process of forest ecosystem, even a decisive role sometimes, This effect has been widely studied [3-10]. Many Chinese scholars have carried out investigations on AM fungi resources and researches on inoculation effects, initially identified that among Chinese northern AM fungi, *Glomus* is the most main species and *Glomus mosseae* is the dominant [11,12]. Because little information is available concerning the effects of arbuscular mycorrhizal fungi on *Elaeagnus mollis* seedlings in reports, the author focused on the root morphological and physiological changes caused by arbuscular mycorrhizal fungi, through inoculating with *Glomus*, the dominated arbuscular mycorrhizal fungi on *Elaeagnus mollis*

seedlings, in order to provide scientific basis for improvement of the degradation in ecosystem and the endangered situation of *Elaeagnus mollis* Diels.

MATERIALS AND METHODS

Sources and Treatments of Fungi and Seeds: The inocula of AM fungi involved *Glomus etunicatum* (GE), *Glomus mosseae* (GM), *Acaulospora delicate* (AD) and *Glomus aggregatum* (GA), which were provided by Bank of Glomales in China (BGC), founded by Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences.

The host plant was *Elaeagnus mollis* Diels seed from Ganquan forest farm in Yicheng country, Shanxi province, China. Pretreatment measures of seeds included: Placed seeds in the refrigerator with -20° for one day, then moved seeds into 4° surroundings to store for 17 days, washed them with running water for 3 days, peeled epicarp and mesocarp, surface sterilization with 0.3% potassium permanganate solution, sowing.

Seedling substrate was mixed media with 70% wildwood rhizosphere soil from Ganquan forest farm in Yicheng country, Shanxi province, China and 30% sand, with pH 7.33 and disinfected with 0.1% formaldehyde solution. After packing the fully mixed substrates tightly with plastic cloth, it would be ready after 10 days' sun exposure.

Nutritional bowls were filled with soil instantly after they were soaked in 0.1% potassium permanganate solution for one hour. 2.0kg seedling substrate and 10g mycorrhizal inocula were used in each nutritional bowl. Using inactivation mycorrhizal fungus inoculum as the control group, 30 bowls were repeated for each treatment. All seedlings were growing in greenhouse with 15-20 g/m² nitrogenous fertilizer for rush seedling and watered with nutrient solution every two weeks, after establishing 2 seedlings left per nutritional bowl.

Observation of Infection Process and Determination of Physiological Indexes: The Determination of Seedling Biomass and Mycorrhizal Infection Rate: At the end of seedling growing season, 10 standard seedlings were sampled to measure ground diameter of seedling, main root length, number of lateral roots, root biomass (65±36h) and so on. 100-150 fresh root segments were sampled absolutely randomly and fixed by FAA solution; infected segments number were determined by slide microscopy with KOH destaining-acid fuchsin staining [13], then

calculated several indexes likewise mycorrhizal infection rate=(number of mycorrhiza root segments /measured root segments)×100%, mycorrhizal dependence MD= dry weight of mycorrhizal plant/ dry weight of non-mycorrhizal plant×100% and so on [14].

The Determination of Total Absorption Area of Roots and Active Absorption Area of Roots: It would be measured by methylene blue absorption [15].

The Determination of Total Nitrogen, Total Phosphorus and Total Potassium: Total nitrogen was determined by Kjeldahl method; Total phosphorus and total potassium were determined by Mo-Sb colorimetric method and flame photometry, respectively [16].

The Determination of Peroxidase Activity and Polyphenol Oxidase Activity of Roots: Enzymatic Activity was measured according to Maehly and Chance (1954) light-absorption method and calculated with enzyme content per milligram protein per minute [17].

Statistical Analysis: Data was analyzed with SPSS10.0 software and Microsoft Excel software. Experiment data was analyzed by Glm process in SPSS for variance analysis and followed by SSR of Duncan method for multiple comparisons testing error at 5% level.

RESULTS AND DISCUSSION

Mycorrhizal Infection Rate and Root Growth: Based on data in four different growth periods (50 days, 70 days, 90 days, 110 days) of *Elaeagnus mollis* seedlings, the infection number rate, infection section rate and infection strength of seedlings with mycorrhiza all showed some difference compared with control seedlings.

Comprehensively evaluating the mycorrhizal infection rates of 4 strains, infection appeared only in the two treatments inoculating with GM and AD, while the other two treatments not and the infection process was divided into 5 stages (Fig.1). Infection section rates of treatments inoculating with GM and AD differed (Fig.2), but mycorrhizal infection rates of both treatments reached the highest (72.5%, 42.4%) in the peak growth period of seedlings and a great amount of hyphae were found in the rhizosphere at the harvest period. Biological characteristics of GM and AD, media components and environmental conditions should affect the infection mechanism of treatments inoculating with GM and AD. By determination of non-inoculated seedlings, no mycorrhiza was found, indicating that there were no native AM fungi in testing media.

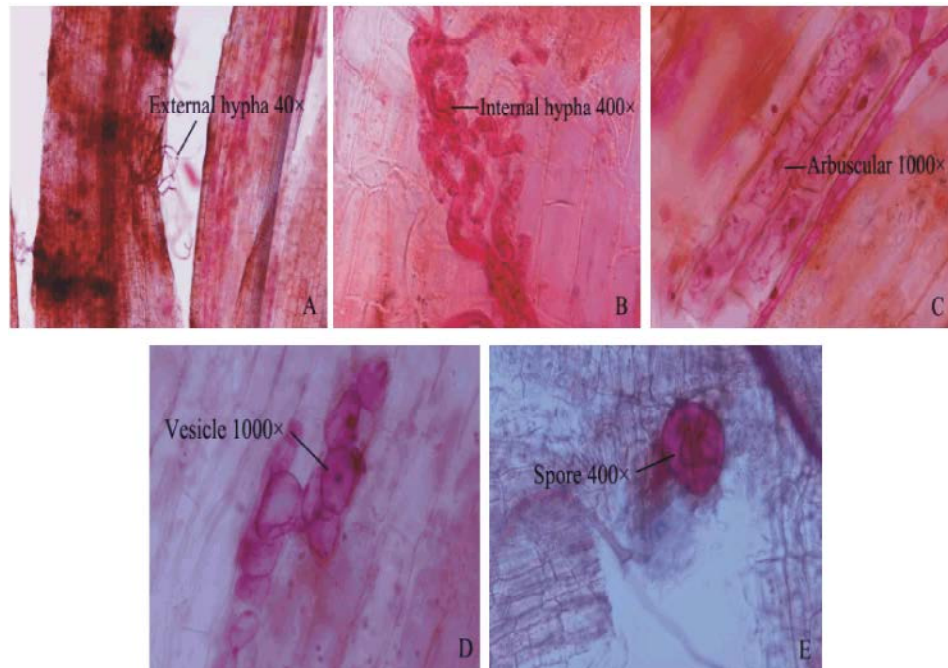


Fig. 1(A-E): The process of AM infection root of *Elaeagnus mollis* Diels (five stages)

- A. Hyphae on the root surface of host intrusion (40×);
- B. Internal hypha (400×);
- C. The formation and development of dendritic (1000×);
- D. Cystic formation and development (1000×); E. Spores formation in roots (400×).

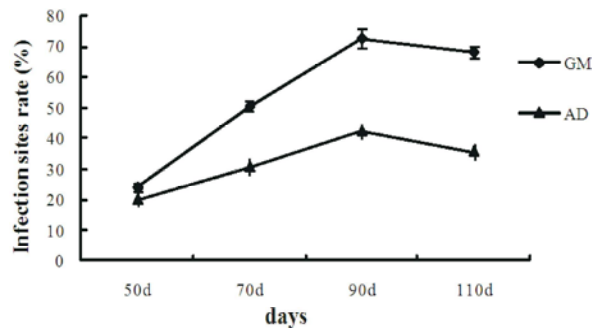


Fig. 2: Mycorrhizal infection

At the end of growth period, but before leaves falling, seedling traits were measured (Table 1). The biggest total dry biomass of seedlings appeared in the treatment inoculating with GM and was 4.98 times higher than CK treatment, showing a significant correlation between differences of different treatments and infection rate ($P < 0.05$). There were remarkable differences between seedlings in two treatments inoculating with GM and AD ($P < 0.05$). In normal water supply conditions, the percentage of moisture of seedlings' underground part was not affected by the AM mycorrhizal fungi.

Gerdemann (1975) [18] firstly put forward the concept, 'Mycorrhizal Dependence' (MD), which of mycorrhizal GM is 500%, showing a much strong mycorrhiza dependence. For example, data in table 1 indicates that mycorrhiza plays a crucial role in biomass increase of *Elaeagnus mollis* seedlings, suggesting that *Elaeagnus mollis* is one mycorrhiza dependent plant.

Effects of AM on Root Absorption Area and Inorganic Nutrient Content: Root volume and absorption area are morphological parameters determining the potentiality of root absorption. In the peak growth period of seedlings (mid-August), root total absorption area and active absorption area were determined. Results showed in Table 2, that root volume and total absorption area in inoculated treatments are bigger than those in control treatment and were greatly differed compared to control treatment.

Nitrogen, phosphorus and potassium are major nutrient elements of plant growth and development. By nutrient elements analysis of seedlings root which had absorption area data, nitrogen content of root in different treatments differed slightly, while the root dry weights in

Table 1: Effectsof arbuscular mycorrhiza on the seedling growth of *Elaeagnus mollis* Diels (mean \pm SE, n=10)

Treat-ment	Height (cm)	Diamet-er (cm)	Root length (cm)	Lateral root length (cm)	Number of lateral roots (root)	Root fresh weight (g)	Root dry weight (g)	Root moistu-re (%)	MD (%)
GM	20.50a	0.24a	28.30a	11.60a	22.6a	2.00a	1.06a	47.00	498
AD	16.20b	0.19b	22.50b	7.40b	17.4b	1.36b	0.72b	47.06	309
CK	10.40c	0.17c	17.00c	6.40c	11.4c	0.34d	0.18d	47.06	100

Values within the same column with different letters are significantly different at $p < 0.05$.

AD, *Acaulospora delicate*; CK, control; GM, *Glomus mosseae*

Table 2: The results of the total absorption and inorganic nutrient contents of the different seedlings

Treatment	Root absorption area, volume		Root mass fraction of inorganic nutrients (%)				
	Root volume (mL)	The total absorption area (m ²)	Active absorption area (m ²)	Area and volume ratio (%)	N	P	K
GM	2.40a	1.994a	1.182a	83.08	1.48a	0.22a	2.05a
AD	1.96b	1.480b	0.779b	75.51	1.43a	0.25a	1.86a
CK	1.85c	1.302c	0.511c	70.4	1.40a	0.16b	0.95b

Table 3: The activity measurement results of peroxidase and polyphenol oxidase in the roots

Treatment	Peroxidase activity / (OD _{470nm} / g • frw • min • cm)			Polyphenol oxidase activity / (OD _{398nm} / g • frw • min • cm)		
	70d	90d	110d	70d	90d	110d
CK	3.118b	4.115a	4.552b	4.580b	4.353b	4.021b
GM	3.469a	4.050a	4.658a	5.452a	5.423a	5.196a
AD	3.389a	4.030a	4.599a	5.176a	5.098a	4.876a

All data are means of 3 replications

inoculated treatments are much higher than those in control treatment, thus a higher nitrogen content of root in inoculated treatments than control treatment (Table 2). Phosphorus and potassium content of root in inoculated treatments differed greatly from those in control treatment ($P < 0.05$), but inside inoculated treatments nitrogen, phosphorus and potassium content of root differed slightly. The ratio of root absorption area has a significant positive correlation with phosphorus and potassium content ($r = 0.942$, $P < 0.05$; $r = 0.978$, $P < 0.05$).

Effects of AM on Peroxidase Activity and Polyphenol Oxidase Activity: Taking samples and determining the peroxidase activity and polyphenol oxidase activity were respected in the peak growth period of seedlings (70D), the later period (90D) and the end period (110D). Based on results in Table 3, because of different sampling times, the activity of the two enzymes changes regularly. As seedlings growing, the peroxidase activity in root increases, otherwise the polyphenol oxidase activity decreases. The peroxidase activity value determined in the peak growth period of seedlings is smaller than the other two periods, but the peroxidase activity in inoculated

treatments is much higher than control treatment ($P < 0.05$) and has a larger improvement due to the mycorrhizal effect. The peroxidase activity of seedlings root in treatment inoculating with GM is 0.35 degrees higher than that in control treatment and change rate is 10.12%. On account of the mycorrhizal influence, the change rate in treatment inoculating with AD is above 9%. As mycorrhizal effects on seedlings are large, photosynthesis and respiration are relatively strong, peroxidase activity as well increases, bringing benefit for resisting other pathogens infection.

In general, the peroxidase activity determined in the later period is higher than that in the peak growth period, but lower than that in control treatment, showing a negative correlation with mycorrhizal infection rate ($r = -0.9523$, $P < 0.05$). The activity of peroxidase in aging tissues is generally stronger, while weaker in young tissues, for the reason that the peroxidase can turn C3-C8 compounds into lignin, enhancing tissue lignification, which is a physiological and biochemical index indicating root aging. According to determinations of peroxidase activity in all the treatments, arbuscular mycorrhiza delay the senescence of root and enhance root vigor and active

absorption area in the later growth period; on the other hand, arbuscular mycorrhiza relatively prolong the growth period of seedlings, effectively promote the biomass accumulation of seedlings as well.

At the end of seedlings growth, peroxidase activity of seedlings in each treatment increased, particularly, the peroxidase activity of mycorrhizal seedlings roots was higher than control group significantly. It suggests that higher peroxidase activity of inoculated group roots reflects seedlings adaption to environment. As temperature decreases, peroxidase activity in root is increased by mycorrhizal fungi stimulation, causing active C3-C8 in root tissue converted to lignin which will accelerate root lignification and enhance cold resistance of seedlings for overwintering. Due to the influence of mycorrhizal, the polyphenol oxidase activities of inoculated roots were always significant higher than control group ($P < 0.05$) from the peak growth period to the end of growth. In different stages, the various between two inoculated groups from mycorrhizal influence were not significant. Although the activities of enzymes for each treatment were decreasing from the peak growth period to the end, the difference was not large, which indicated that effect of time was not the major factor to enzyme activity.

Discussion: After the symbiotic relationship formed between AM fungi and seedlings, the roots of seedlings will connect with the hyphae of AM fungi in the form of network, which will help plant to absorb and take advantages of nutrients, likewise phosphorus, from poor phosphorus area of soil. This effect has been widely studied [19,20]. The phosphorus absorption rate of mycorrhiza was 6 times to non-mycorrhiza [21-23]. Therefore, the outside hyphae of mycorrhiza increases the absorption surface area of roots and volume of roots. Meanwhile, inside hyphae of roots, arbuscular and vesicle will enhance active absorption area of plant root which will be better for water and nutrients absorption of plant, satisfying the material and energy requirements needed by plant cell elongation, division and differentiation during growing [24]. The research indicates that AM fungi enhance the active adsorption area and absorption ability of *Elaeagnus mollis* Diels seedling roots greatly. One important factor for growing plants in poor soil is the strengthened absorption ability of arbuscular mycorrhizal root [25,26], which is also significant for phytocoenosium succession. Arbuscular mycorrhizal which is benefit to subsequent afforestation successfully plays a predominant role in forming secondary vegetation at abandoned land [27-29].

Polyphenol oxidase with higher activity has a self-protection mechanism that phenolic compounds caused by the tree metabolism in bad environment are oxidized to toxic quinone, which can efficiently prevent microorganisms invasion, thus keep plant growing and developing normally [30]. The influence of AM fungi on enzyme system of host plant helps plant to resist to all kinds of stress [31,32]. This is one of the reasons why AM are used to prevent and control plant diseases by many plant pathologists [33-35]. Botanists are conducting some extensive and further researches on enhancing the stress resistance of host plant inoculating with AM fungi. This study illustrated that AM fungi increased the activity of polyphenol oxidase of *Elaeagnus mollis* Diels seedling roots which was important to prevent *Elaeagnus mollis* Diels seedlings from other pathogenic microorganism invasion and keep seedlings growing well. Specific regularity occurred in the change of roots peroxidase activity which was impacted by mycorrhiza. This characteristic is good to enhance the disease and cold resistance of seedlings which reflects the mutual relationship between mycorrhizal plants and environment ecology. As a consequence, enhancing the defense capability of host plant by AM fungi is significant to expand the distribution range of *Elaeagnus mollis* Diels plants to improve its endangered status.

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