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RESEARCH ARTICLE

EFFECTS OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI ON *ELAEAGNUS MOLLIS DIELS* SEEDLING GROWTH UNDER DIFFERENT FERTILITY LEVEL

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A R T I C L E I N F O

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A B S T R A C T

In order to discuss the relationship between mycorrhizal infection rate and rhizosphere nutritional status, this paper studied the indexes (like seedling morphology, biomass, mycorrhizal infection rate, etc.) of *Elaeagnus mollis Diels* with inoculating or without inoculating AM fungi under different fertilizers and different fertility statuses. Results showed: AM fungi could increase absorption utilization of seedling on slightly soluble P fertilizer, calcium superphosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) (content of effective ingredient (P_2O_5) is 0.04 g/kg soil), and soluble P fertilizer, dipotassium phosphate (K_2HPO_4) (content of effective ingredient (P_2O_5) is 0.02 g/kg soil), which both had good promotion effect on seedling growth. With the increasing of soluble P concentration, however, AM fungi would inhibit the accumulation of seedling biomass. AM fungi also could promote the absorption of seedling on nitrate N fertilizer, potassium nitrate (KNO_3) (content of effective ingredient (NO_3^-) is 0.06 g/kg soil), and ammonia N fertilizer, ammonium sulfate ($(NH_4)_2SO_4$) (content of effective ingredient (NH_4^+) is 0.06 g/kg soil), which both were good to seedling growth. Therefore, it is concluded that rhizosphere nutritional status is one of important factors to mycorrhizal infection rate.

INTRODUCTION

Elacagnus mollis Diels which belongs to *Elaeagnus* of *Elaeagnaceae* is a two-tier protection of endangered plants in china. It is mainly distributed in southern of Lv-liang Mountain in Shanxi, western of Zhongtiao Mountain and Hu County of northern of Qinling Mountains in Shanxi and has significant economic value [1]. Arbuscular Mycorrhizal (AM) fungi has positive effect on arboreal growth, meanwhile, AM as "bio-fertilizer" plays an important role in the establishment, evolution and development process of forest ecosystem, even a decisive role sometimes [2-7]. Yuanli et al. [8]found the *Elacagnus mollis Diels* seedlings inoculated by *Glomus mosseae* (GM) and *Acaulospora delicate* (AD) had better invasion, and they also discussed the promotion effects of AM on *Elacagnus mollis Diels* seedlings. On this basis, research on the effect of AM fungi × fertilizer on seedling growth was conducted in order to reveal the influence of AM fungi on *Elacagnus mollis Diels.*

Material and Methods

Source of Fungi and Seeds

The inoculums of AM mycorrhizal fungi include Glomus mosseae GM and Acaulospora delicata(AD which were provided by Bank of Glomales in Chin (BGC), found by Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. Host plant was Elaeagnus mollis Diels seed from Yicheng, Shanxi province, China. Surface sterilizing seeds with 0.3% potassium permanganate solution were as the pretreatment measure and then sowing[9].

Seedling substrate

70% loess + 30% sand, sterilization before use; pH was 7.33; Nutrients content: total nitrogen(N) was 1.33 g/kg, total phosphorus(P) was 0.54 g/kg, total potassium(K) was 20.03 g/kg, hydrolyzable nitrogen was 145.76 mg/kg, effective phosphorus was 150.23 mg/kg, as well as effective was 90.01 mg/kg.

Fertility and usage

Phosphorus fertilizer : slightly soluble phosphorus fertilizer was $Ca(H_2PO_4)_2 \cdot H_2O$(P_1 content of active ingredients P_2O_5 was 0.04g/kg soil); soluble phosphorus fertilizer was K_2HPO_4, and three levels were set, content of active ingredients P_2O_5 were $P_{2\text{-}1}$ 0.02 g/kg soil, $P_{2\text{-}2}$ 0.04 g/kg soil and $P_{2\text{-}3}$ 0.06g/kg soil, respectively. Nitrogen fertilizer: nitrate nitrogen was KNO_3, N_1 content of active ingredients NO_3 was 0.06 g/kg soil; ammonium nitrogen was $(NH_4)_2SO_4$ and three levels were set, content of active ingredients NH_4^+ were $N_{2\text{-}1}$ 0.03 g/kg soil, $N_{2\text{-}2}$ 0.06 g/kg soil and $N_{2\text{-}3}$ 0.09g/kg soil, respectively.

Experimental design

Nutritional bowls were filled with the mixture of fertilizer and substrate in proportion first and surface inoculated with AM

mycorrhizal inoculums, then a thin substrate layer was distributed on the top. Each nutritional bowl had 2kg substrate and 20g mycorrhizal inoculums. After surface sterilizing the *Elacagnus mollis Diels* seeds, seeds were sowed at 15th April, 2014. Random blocks design was adopted: 5 repeat bowls in 2 groups, fertilization without inoculation and inoculation without fertilization were control groups, and routine management. After growing season, 5 standard strains were chose from each treatment group and were measured height of seedling, ground diameter and total dry biomass(65℃ 36 h). Experiments were conducted at greenhouse (daytime temperature was (26±2) ℃, evening temperature was (20±2) ℃, relative humility was 55%, illumination was 12h/d) in College of Life Science in Shanxi Normal University.

Data processing

Data was analyzed by GLM method in SAS software for variance analysis and followed by Dun-can method for multiple comparison testing. Mycorrhizal infection rate was tested by Phillip and Hayman's [10] method of KOH decoloration- acid fuchsin staining, mycorrhizal infection rate = (number of mycorrhiza root segments/measured root segments) ×100%.

RESULTS AND ANALYSIS

The height of seedling, ground diameter and total dry biomass of different treatments were analyzed by two-factor variance. Variance analysis resulted showed in Table 1, that different mycorrhizas, fertilizers, fertility levels, as well as their different combinations have significant influence(P<0.01) on *Elacagnus mollis Diels* seedling growth. It indicates that AM mycorrhizal fungi, different fertilizers, different fertility levels and different combinations have different effects on seedling growth. Therefore, further evaluation about the effects of different mycorrhizas, fertility and mycorrhizas × fertilizer on seedling is necessary.

Effects of inoculating AM mycorrhiza on Elacagnus mollis Diels seedling growth

Table 2 shows the effect of only using mycorrhizal fungi on seedling growth. According to the table, different AM mycorrhizal fungi have different effects on seedling growth. Thereinto, GM has significant promotion effect (P<0.01) on the height of seedlings, ground diameter and total dry biomass. And AD has significant promotion effect ($P<0.05$) on seedling growth. The difference effect of AM mycorrhizal fungi on seedlings might be affected by mycorrhizal infection rate, and is positively correlated with infection rate (Table 2).

Effect of fertilization on Elacagnus mollis Diels seedling growth

Fertility is one of the important factors to guarantee seedling growing healthy. Different fertilizers and fertility levels have different influence on seedling growth. In table 3, comparing P_1, N_1 with CK, although some differences have be showed, neither of them could compare with the effects from $P_{2\text{-}2}$ and $N_{2\text{-}2}$ which have the same effective fertility level to seedling growth. Because of the different fertility levels of P_2 and N_2, some desirable traits of seedlings will be more obvious with the increasing of fertilizer rate.

Effect analysis of slightly soluble P×AM fungi on Elacagnus mollis Diels seedling growth

After inoculating, AM mycorrhizal fungi all significantly promote the height of *Elacagnus mollis Diels* seedlings, ground diameter growth and accumulation of biomass, it is significant difference compared to control group($P<0.01$) Table 4 . All the inoculation effects were good. But different fungi have different effects on seedling growth. Accumulation of biomass in GM treatment is 3.93 times of control group. Meanwhile, AM mycorrhizal infection rate is also improved as the result of effect from slightly soluble P (compare to the infection rate in Table 2). For the treatment of slightly soluble P, AM mycorrhizal fungi can significantly promote *Elacagnus mollis Diels* seedlings growth. It indicates that AM mycorrhizal fungi can improve the effective absorption of slightly soluble inorganic phosphate for *Elacagnus mollis Diels.* This effect may be related to bigger root adsorption area with mycorrhizal, rich external hyphae through P deficiency area in rhizosphere, better rhizosphere phosphatase activity with mycorrhizal fungi, organic acid generated by mycorrhizal fungi activating slightly soluble P, as well as the adsorption of P and other nutrients of host to increase *Elacagnus mollis Diels* biomass significantly[11-14]. Therefore, inoculated with AM fungi in soil with low soluble P is necessary and beneficial.

Effect of soluble P ×AM fungi on Elacagnus mollis Diels seedling growth

Soluble P is better for seedling absorption and utilization. Three concentration levels (low, medium and high) of soluble P fertilizer were chose. Result indicates that the properties of *Elacagnus mollis Diels* seedling treated with mycorrhizal fungi become better and better as the increasing of fertilizer rate (Table 3). It represents that *Elacagnus mollis Diels* seedling growth needs P. But properties of seedling inoculated with mycorrhizal fungi don't become better with increasing of fertilizer rate. In other words, interaction effect of mycorrhizal and fertility does not equal to the sum of two single factors. Because biological properties of different mycorrhizal fungi are not the same, the effect on seedling will be different. All the mycorrhizal fungi treatments are significant difference from control group. And between 2 fungi also show significant difference sometimes. With the growing of fertilizer rate, the growth effect of seedling with fungi decreases, the same as the mycorrhizal infection rate (Table 5). Therefore, when cultivating *Elacagnus mollis Diels* seedling with soluble P, it is better to adopt the combination of GM and $P_{2\text{-}1.}$ Not only can fertilizer be saved, but also seedling's growth effect can be the best.

Effect of nitrate N ×AM fungi on Elacagnus mollis Diels seedling growth

Table 6 shows the effect of nitrate N (N_1) ×AM fungi on seedling growth. Mycorrhizal fungi treatment has significant difference compare to control group. Seedlings with GM treatment grow best; its total dry biomass is 2.73 times of control group. In the second place, total dry biomass of

Table 1 Variance analysis of two-factor experiment between the nursery stock and fertility

Difference source	Height of seedlings(cm)		Collar diameter(cm)		Whole plant dry weight (g)		$F_{0.05}$	$F_{0.01}$
	MS	*F*	*MS*	*F*	*MS*	*F*		
Treatment	87.82	46.11	0.0041	22.543	2.564	190.112	1.64	1.99
AM(A)	608.12	321.08	0.0064	118.001	14.001	899.768	2.53	3.97
Fertilizer (B)	110.90	56.31	0.0179	51.532	5.213	354.976	2.31	2.75
A×B	30.592	15.23	0.0019	6.241	0.659	60.011	1.51	2.01
Error	2.012		0.000365		0.016			

Note: $F>F_{0.05}$ Significant difference, $F>F_{0.01}$ Extremely striking contrast

Table 2 The growth effect of nursery stock with different processed without fertilization

Treatment	Height of seedlings(cm)	Collar diameter(cm)	Whole plant dry weight (g)	Colonization rate (%)
GM	22.50**	0.27**	2.63**	68.9
AD	15.20*	0.20*	1.67*	49.1
CK	10.4	0.16	0.87	

Note: *F* test , *Significant difference, $P<0.05$; ** Extremely striking contrast, $P<0.01$.

Table 3 The growth effect of nursery stock with different processed on different fertilization levels

性状	P_1	$P_{2\text{-}1}$	$P_{2\text{-}2}$	$P_{2\text{-}3}$	N_1	$N_{2\text{-}1}$	$N_{2\text{-}2}$	$N_{2\text{-}3}$	CK
Height of seedlings(cm)	25.6 B	27.32 B	26.10 B	30.7 A	32.42 A	27.40 B	27.55 B	40.11 A	20.32 C
Collar diameter(cm)	0.27 D	0.27 D	0.31 C	0.32C	0.30 C	0.37 B	0.39 B	0.43 A	0.24 D
Whole plant dry weight (g)	1.63 E	1.61 E	1.69 D	2.41 C	1.99 C	1.82 D	2.48 B	3.10 A	1.09 F

Note: the capital letters indicate the significant difference at 1% level, the same below.

Table 4 The growth effect of nursery stock with different processing methods on slightly soluble P fertilizer(P_1) level

Treatment	Height of seedlings(cm)	Collar diameter(cm)	Whole plant dry weight (g)	Colonization rate (%)
GM·P_1	42.8B	0.47B	4.21D	72.0
AD·P_1	31.0C	0.35C	3.12E	64.1
CK·P_1	18.05D	0.22D	1.07F	

Table 5 The growth effect of nursery stock with different processing methods on soluble P fertilizer(P_2) level

Treat-ment	$P_{2\text{-}1}$				$P_{2\text{-}2}$				$P_{2\text{-}3}$			
	Height of seedlings (cm)	Collar diameter (cm)	Whole plant dry weight (g)	Colonization rate (%)	Height of seedlings (cm)	Collar diameter (cm)	Whole plant dry weight (g)	Colonization rate (%)	Height of seedlings (cm)	Collar diameter (cm)	Whole plant dry weight (g)	Colonization rate (%)
GM	42.81B	0.47B	4.21D	58.22	39.00B	0.44B	3.71D	47.63	37.43B	0.41B	3.21D	41.82
AD	32.00C	0.36C	3.33E	48.22	36.02C	0.36C	3.45E	39.67	37.04C	0.36C	3.62E	26.65
CK	22.05D	0.27D	1.33F		21.35D	0.28D	1.39F		26.07D	0.30D	1.87F	

Table 6 The growth effect of nursery stock with different processing methods on nitrate N(N_1) level

Treatment	Height of seedlings(cm)	Collar diameter(cm)	Whole plant dry weight (g)	Colonization rate (%)
GM·N_1	43.27 A	0.44 A	5.09A	59.7
AD·N_1	37.76 B	0.37B	3.76B	43.1
CK·N_1	28.32 D	0.26 D	1.86D	

Table 7 The growth effect of nursery stock with different processing methods on ammonium N(N_2) level

Treat ment	$N_{2\text{-}1}$				$N_{2\text{-}2}$				$N_{2\text{-}3}$			
	Height of seedlings (cm)	Collar diameter (cm)	Whole plant dry weight (g)	Colonization rate (%)	Height of seedlings (cm)	Collar diameter (cm)	Whole plant dry weight (g)	Colonization rate (%)	Height of seedlings (cm)	Collar diameter (cm)	Whole plant dry weight (g)	Colonization rate (%)
GM	44.81A	0.46A	5.21A	57.21	47.00B	0.66A	5.34B	61.28	41.43B	0.44B	5.11B	54.16
AD	37.00B	0.43B	4.33B	53.32	41.02B	0.51B	4.85C	57.65	38.04B	0.45A	4.34B	44.88
CK	24.05C	0.37D	1.63D		24.35D	0.41C	2.39D		36.07C	0.40C	2.87C	

seedling with AD treatment is 2.03 times of control group. This result indicates AM mycorrhizal fungi enhancing the absorption and utilization efficiency of seedling to nitrate N and have significant promotion effect on seedling growth.

Effect of ammonia N ×AM fungi on Elacagnus mollis Diels seedling growth

Ammonia N is easy to adsorption and immobilization and low mobility in soil. Mycorrhizal can increase the absorption and utilization of ammonia N by increasing absorption area and contact points to soil. In 1983, Ames [15] proved AM mycorrhizal hypha could absorb NH_4^+ from few centimeters away soil and transported it to host plant by ^{15}N labeling experiment. According to the effect of different ammonia N concentrations on *Elacagnus mollis Diels* seedling growth, this experiment further verified absorption and utilization of ammonia N result from mycorrhizal (Table 7). Compare to the control group only with fertilization treatment (without inoculated fungi), interaction effect of different fungi and

ammonia N shows significant difference. Seedlings with mycorrihizal perform similar under three ammonia N concentration levels, and seedling properties of $N_{2\text{-}2}$ is the best (Table 7), its total dry biomass is 2.33 and 2.03 times of control group, mycorrhizal infection rate is also the highest for this level. For $N_{2\text{-}1}$ and $N_{2\text{-}3}$, there is no big difference on properties between them. Hence, when AM mycorrhizal fungi and ammonia N are used to cultivate *Elacagnus mollis Diels* seedling, $N_{2\text{-}2}$ combined with AD or GM fungi will obtain the ideal effect. Fertility level is one of the important constrains to mycorrihizal infection rate, especially for soluble P fertilizer in soil is more sensitive to mycorrihizal infection rate, and high concentration of ammonia N can inhibit mycorrihizal infection rate. However, it does not mean the more poor the soil is, the higher the mycorrihizal infection rate [Table 6, Table 7]. Therefore, substrate nutritional status and biological traits of mycorrhizal fungi should be considered while use mycorrhizal fungi in practice.

SUMMARIES

Mycorrhizal's absorption on nutrients has always been a hot spot for mycorrhizal scientist. Through studying the effect of AM fungi on *Elacagnus mollis Diels* seedling growth under different nutrient status, the result can be obtained that different AM fungi have significant difference on utilization of different fertilizers and fertility levels. AM can enhance seedling's absorption and utilization of slightly soluble P, particularly when GM is with slightly soluble P Ca $(H_2PO_4)_2 \cdot H_2O$ which has 0.04g/kg effective ingredient P_2O_5, seedling growth get good promotion. Addition of soluble P cannot fully compensate promotion effect of AM mycorrhizal on *Elacagnus mollis Diels* seedling. Only under low P, combination of mycorrhizal and soluble P fertilizer K_2HPO_4 whose effective ingredient P_2O_5 is 0.02g/kg can achieve the best promotion effect on seedling growth. When soluble P is high, however, AM mycorrhizal will inhibit biomass accumulation of seedling. AM mycorrhizal can also promote the absorption and utilization of nitrate N and ammonia N of seedling. Combination of AM mycorrhizal and KNO_3 whose effective ingredient NO_3^- is 0.06g/kg has good promotion effect on seedling growth. At the same time, experiments had proved that Rhizosphere nutrition was crucial for infected rate of fungi. AM mycorrhizal can fully improve absorption of host plant on environmental nutrients. It has significant meaning to substance cycle and energy flow in the whole system, as well as improving biodiversity and enhancing the adaptability of host plant.

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