

Effects of Arbuscular Mycorrhizal Fungi on Root Growth and Architecture of Tulip *Gesneriana*

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Abstract

Arbuscular mycorrhizal fungi (AMF) can promote the absorption of soil water and mineral nutrients, improve photosynthesis, and make host attain higher quality finally by establishing symbiotic relationship between AMF and host root. To improve *Tulip gesneriana* quality have practical meaning under no bad affect to cultivation soil, in the light of its economical and ecological values. However, some AMF may be diverse from others, the concrete function of AMF on commercial tulip varieties need to explore. Therefore, three different sets of arbuscular mycorrhizal fungi were inoculated into tulip rhizosphere soil, which were set as 4 (*Diversispora versiformis*), 7 (*Diversispora spurca*) and 1 + 3 + 4 (*Rhizophagus intraradias* + *Funneliformis mosseae* + *Diversispora versiformis*), respectively. The results showed that the activity of most of the measured indices increased, the average root diameter and sucrose content decreased in those three mycorrhizal treatments. Our research provide some theoretical basis for the application of AMF on *T. gesneriana* ecological cultivation in future.

Keywords: *Tulip gesneriana*, AMF, root architecture, host growth

1. Introduction

Fine root usually refers to the root with diameter less than 2mm, which has a huge absorption surface area. It is an important organ for plants to absorb, transport and store nutrients (Ren et al., 2014, pp. 2535-2543). However, fine roots are greatly affected by biological and abiotic factors, especially the activities of soil microorganisms. Among them, arbuscular mycorrhizal fungi (AMF), as a ubiquitous microorganism in soil, promotes the absorption of water and nutrients by plant roots and the growth of plants. Moreover, AMF colonization further increases the absorption area of fine roots, promotes host growth and development, and promotes the fine roots to play a more important role in plant function, energy flow and material cycle of ecosystem (Jackson et al., 1997, pp. 7362-7366; Wu et al., 2011, pp. 273-278). Many studies have shown that mycorrhizal colonization can affect the life span of the host plant root system, and AMF infection can induce significant changes in plant root morphology, which can promote the high branching of plant roots and induce the formation of higher secondary roots and fine roots (Yao et al., 2009, pp. 458-461). At the same time, the mycelial sheath on the surface of plant roots can play a good role in protecting the roots, reducing the damage of soil animals to the root system due to food intake. At the same time, the chemical substances produced can resist the invasion of pathogenic bacteria and enhance the resistance of plant roots (Boyle and Hellenbrand, 1991, pp. 1764-1771). At present, many researches mainly focus on the effects of soil and climate factors on the growth and death dynamics of plant roots, while few studies on the effects of AMF on plant root turnover are made.

Tulip belongs to tulip of Liliaceae. It is a world famous bulb flower and an excellent cut flower (Cui et al., 2020, pp. 5-7). The economic value and medicinal value of tulip can not be ignored. Tulip is a well-known flower that is regarded as the national flower by many countries such as Holland, Iran, Turkey and so on. The annual output of tulip in Holland ranks first in the world and has become one of the country's financial resources. The flower and leaf of tulip contain a kind of toxic alkaloid, its physiological function is similar to that of Veratrine Tulip ABC has inhibitory effect on *Bacillus subtilis* (Ge et al., 2020, pp. 31-35). After passing through cation and anion exchange resin, tulip juice still has antibacterial effect on *Staphylococcus aureus*. The active components of its stem and leaf alcohol extract contain a variety of amino acids, which has antibacterial effect on *Bacillus cereus* mycoides. The research on the effect of AMF on Tulip growth is not only improve its commercial quality, but also explore the green-cultivation technique, that is ecological cultivation which will be good at balancing flower quality and environment. In this experiment, tulip was used as the research material, and the effect of AMF on the

growth and root growth of tulip under different AMF inoculation was studied, which will provide theoretical basis for high quality cultivation and offer related experiment data that AMF has good effect on green-cultivation of tulip.

2. Materials and Methods

2.1 Test Materials

The tulip bulbs were from Zhejiang Hongyue Horticultural Company. AMF was provided by Professor Wu Qiangsheng, Institute of root biology, Yangtze University. The AMF applied in this experiment included *Diversispora versiformis*, *Diversispora spurca*, *Rhizophagus intraradialis* and *Funneliformis mosseae*. Plastic flowerpots (upper diameter 18cm, bottom diameter 12cm height 13cm) were used in the experiment. The soil that was sterilized by high pressure steam (121 °C, 2h) used in this experiment, which was the typical soil types in Jingzhou. Furthermore, the texture was moderate sticky with pH value from 6.5-7.0, and soil aggregates of 2mm, 1mm, 0.5mm and 0.25mm were 22.267g, 3.2g, 5.93g and 42.83g respectively per 100g dry soil.

2.2 Experimental Design

In this study, a single factor experiment design was used, with four treatments, namely, *Diversispora versiformis*, *Diversispora spurca*, *Rhizophagus intraradialis* + *Funneliformis mosseae* + *Diversispora versiformis*, and CK was set as the control group. Each treatment was repeated 4 times, 2 tulip plants were arranged randomly. The infection rate, root indices, carbohydrate, soil phosphatase activity and other indices were measured by three sampling times. The experiment had been carried out in the greenhouse and laboratory of Horticulture and Gardening College of Yangtze University.

2.3 Determination Index and Methods

2.3.1 Determine the Root Infection Rate

The roots of the samples were randomly selected and cut into 1cm long root segments. The roots were fixed in FAA solution (at least 24h at room temperature). Phillips & Hayman (1970) method was used for staining, decolorization and microscopic examination. Finally, the root infection rate was measured.

2.3.2 Determination of Glomus in Soil

The method of Wright (Wright, 2000, pp. 171-177) was used for the determination of glomus in soil with a slight change in dosage. 0.5g air-dried soil sample was put into a centrifuge tube with scale, and 4ml (pH = 7.0) 20 mmol·L⁻¹ sodium citrate extraction agent was added; sterilized for 30min at 121°C, 103 kPa, and centrifuged at 10000 rpm for 5min to obtain the supernatant; 0.25ml supernatant was added with 2.5ml Coomassie G-250, and then 0.25ml 50mmol·L⁻¹ sodium citrate extract was added; at the same time, 1mg·ml⁻¹ bovine serum albumin was accurately prepared. After 5min of color development, the OD values of samples and BSA standard solution were determined at 595nm with a spectrophotometer (Thermo multiskanfc, USA), and the amount of glomus in each gram of dry soil was calculated.

2.3.3 Determination of Total Sugar Content

Anthrone colorimetric method was used and the dosage was slightly modified (Wu et al., 2012, pp. 1554-1556). 0.02g dry root samples were treated with water bath, and the volume was 25 ml. After finishing those required treatments, the absorbance value was measured at 630nm.

2.3.4 Determination of Sucrose Content

According to the test method of Zhang et al., a slight modification is made (Zhang & Zhai, 2003, pp. 128-129). 50 mg dry root sample was weighed at a single time. During the preparation of standard curve, 150 µl alcohol extract was taken, 150 µl 2mol / L NaOH was added, boiled for 5min at 100 °C, cooled, and 2.1ml 30% hydrochloric acid and 0.6ml 0.1% resorcinol were added. After all treatments, colorimetric determination was performed at 480 nm.

2.3.5 Determination of Glucose Content

Glucose oxidase peroxidase colorimetric method was used with a slight change in dosage (Wang, 1997, pp. 13-19). In the preparation of enzyme preparation, 2.5ml glucose oxidase was added. Take 1.0ml of enzyme preparation and put it into a 30 % ethanol, stored in brown bottle of 30me solution rises to balance, add 0.5ml alcohol extract, shake well, keep temperature for 5min, add 2ml 10mol / L sulfuric acid, and determine the color at 460nm.

2.3.6 Determination of Root Architecture Parameters and Other Indicators

Sampling and testing plant appearance growth was directly measured by electronic scale, ruler and other measuring tools, including leaf length, width, plant height, weight of root ball, and wet weight of root after cleaning; root

scanner was used to scan root system after each sampling, and root length, diameter, area, volume and root tip counting were analyzed, and finally data processing was carried out.

2.3.7 Determination Acid Phosphatase in Rhizosphere Soil

The method was determined by sodium diphenyl phosphate (Zhao & Jiang, 1986, pp. 12-16).

2.4 Data Statistics and Analysis

The ANOVA process of SAS (8.1) software was used to test the difference between the treatments. Duncan's new complex range difference method was used for multiple comparative analysis ($P < 0.05$). Graphpad prim 8.0 were used for drawing.

3. Results and Analysis

3.1 Effect of AMF on Root Infection Rate and Growth of Tulip

Under different inoculation treatments, AMF had different effects on root infection rate and growth of tulip. The infection rate of group 1+3+4 is the highest, reaching 75%, and the group 4 and 7 are also more than 60% (Tab. 1). As for growth effect, group 7 and 1+3+4 have significant effects on plant height and bulb weight, but there is nothing positive influence to plant height and weight in group 4 except for ground diameter. In addition, group 7 has a little effect on the ground diameter of tulip (Tab. 2).

Table 1. Effect of AMF on root infection rate (mean \pm standard deviation)

Treatments	CK	4	7	1+3+4
Infection rate (%)	0	64.17 \pm 0.005b	64.47 \pm 0.0304b	75.03 \pm 0.0139a

Note: The different letters in the same column indicate significant differences at $P < 0.05$ level. The same below.

Table 2. Effect of AMF on tulip growth (mean \pm standard deviation)

Treatments	Plant height (cm)	Ground diameter (cm)	Bulb weight (g)
CK	38.65 \pm 1.3435ab	0.837 \pm 0.0184c	18.929 \pm 0.8676bc
4	37.65 \pm 1.2728b	0.918 \pm 0.0064a	17.544 \pm 4.5017c
7	41.98 \pm 2.0082a	0.873 \pm 0.0184bc	24.320 \pm 0.6693ab
1+3+4	43.18 \pm 3.4295a	0.912 \pm 0.0092ab	26.581 \pm 4.6507a

3.2 Effect of AMF on Glomeratin in Tulip Soil

The effect of inoculating different AMF strains on soil glomalin was different. The content of glomalin in three inoculation treatments was significantly higher than that in the CK. The content of glomus in group 4 and 7 had no significant difference, which was increased by 12% compared with the CK, while the effect of group 1+3+4 was the best one, up to 2.14 mg/g (Figure 1).

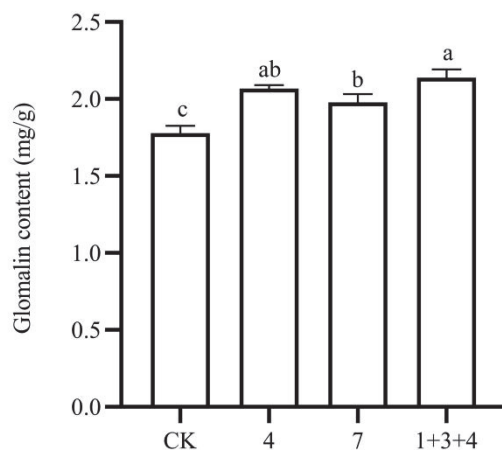


Figure 1. Effect of AMF on glomalin in soil

3.3 Effect of AMF on the Contents of Total Sugar, Sucrose and Glucose in Tulip

The results showed that different AMF treatments had different effects on the contents of total sugar, sucrose and glucose in tulip roots (Figure 2). The contents of soluble sugar and glucose in three AMF treatment groups were significantly increased, while sucrose was significantly decreased compared with the CK, and the group 1+3+4 being the most obvious, with a decrease of 26.3%. The contents of total sugar and glucose in tulip roots of group 1+3+4 were higher than another two inoculation groups. The effects of those three treatments on the contents of sucrose and glucose were different.

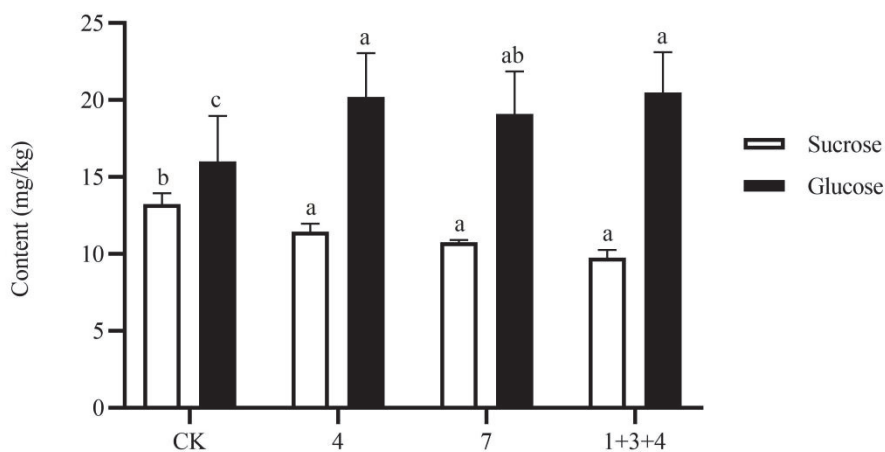


Figure 2. Effect of AMF on the contents of total sugar, sucrose and glucose in tulip

3.4 Effect of AMF on Root Architecture Parameters of Tulip

The average root diameter of three groups of AMF treatment was smaller than that of CK group, the most obvious was group 7, the average root diameter of tulip samples was significantly lower than that of other two strains treatment, but there was no significant difference between group 4 and 1+3+4 (Tab. 3). The root length, projected area, surface area and volume of tulip inoculated with AMF were significantly higher than control. The root length, projected area and surface area of group 1+3+4 were significantly higher than another two treatments, increased by 16.5%, 14.1% and 18.5%, respectively. The root volume of three treatments was 35% higher than that of control group, and there was no significant difference between group 1+3+4 and 4 (Figure 3).

Table 3. Effect of AMF on root architecture parameters of tulip (mean ± standard deviation)

Treatments	Length (cm)	Volume (cm ³)	Average diameter (cm)
CK	992.4797±26.0618a	4.369±0.2998a	0.8299±0.0194a
4	1115.5943±30.7225a	7.453±1.3195a	0.7696±0.0488a
7	1069.2454±12.8211a	5.8995±0.9751a	0.7095±0.094a
1+3+4	1245.1904±264.2475a	7.7035±3.2138a	0.7813±0.0446a

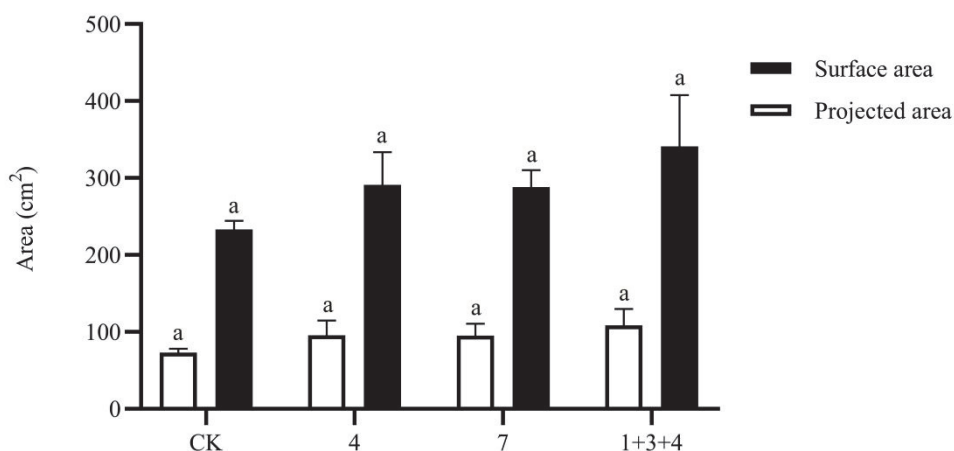


Figure 3. Effect of AMF on root architecture parameters of tulip

The total root length of tulip samples from high to low was 1+3+4 > 4 > 7 > CK (Figure 4). The root length of 0-1cm in AMF treatment group accounted for 85.7% - 86.9% of the total root length of tulip, which significantly increased the length of 0-1cm root of tulip, and had no significant influence on the length of root system at 1-2cm, 2-3cm, 3-4cm and > 4cm.

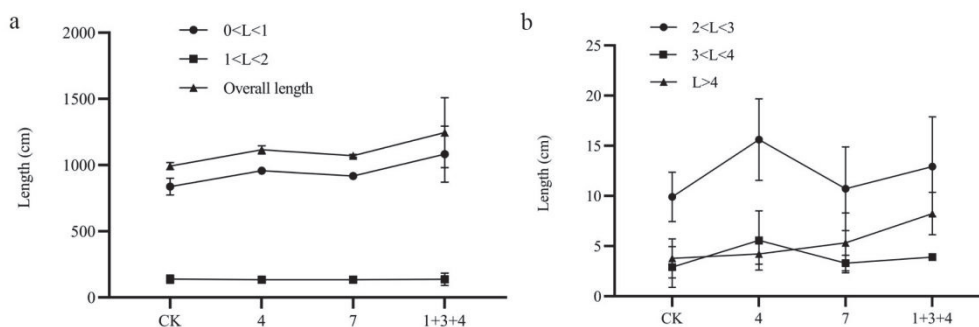


Figure 4. The effects of AMF on the root length of tulip

3.5 Effect of AMF on Phosphatase Activity of Tulip Soil Under Acid Condition

Under the same environment, the phosphatase activity of CK group under acid condition was significantly lower than that of three infection groups, the activity of phosphatase between group 7 and 4 had no significant difference under acid condition (Tab. 4), while the phosphatase activity of group 1+3+4 was significantly higher than that of other two infection groups. The results showed that the activity of acid phosphatase could reflect the infection rate and activity of AMF, and the infection rate was positively correlated with the activity of acid phosphatase.

Table 4. The relationship between soil phosphatase and infection rate in different AMF under the same habitat

Treatments	CK	4	7	1+3+4
Soil phosphatase [$\mu\text{g}/(\text{g}\cdot\text{h})$]	97.32	172.57	178.43	210.46
Infection rate (%)	0	64.17	64.47	75.03

4. Discussion

AMF can promote the vegetative growth of plant obviously after forming mycorrhizal symbionts with host roots (Chen et al., 2008, pp. 648-653). Root system is an important organ for plants to absorb water and mineral nutrients from soil. Good root system can promote plant growth and development, and root morphology can directly reflect the growth of root system (Willaume & Pagès, 2011, pp.653-662). AMF inoculation can induce more auxin production in the root system of host plants, thus inducing the formation of lateral root primordia (Kircher & Schopfer, 2012, pp.11217-11221). In this experiment, AMF significantly promoted the growth and morphological formation of tulip roots, and increased length and volume in mycorrhizal plants with a little decreasing in root diameter (Tab. 3). These results indicate that AMF have good effect on forming more fine roots. It is important that mycorrhizal plants keep fine roots architect and good function for providing guarantee for the growth of host by increasing the contact surface between roots and soil, and improving the absorption and utilization efficiency of water and nutrients in soil by roots.

Carbohydrate is one of the important products of plant photosynthesis. It directly participates in the growth and metabolism of plants. AM fungi, as beneficial microorganisms, can help plants absorb nutrients and water, but they need to absorb carbon sources from host plants to maintain their metabolism. Studies have shown that the carbon sources are mainly simple carbohydrates, such as glucose, fructose (Han et al., 2012, pp. 321-342) In this experiment, there were lower sucrose and more glucose in tulip roots inoculated by AMF, which had good effect on keeping host root growth and development by maintaining higher glucose content. Our results were consistent with Zou's research finding (Zou et al., 2014, pp. 1125-1129). This allocation of soluble sugar had better effect for tulip root length, surface area, volume and other related indicators (Tab. 3).

Soil enzyme plays an important guarantee for the smooth progress of various phytochemical processes in the soil environment. Being one of the active organic components in the soil, phosphatase is often regarded as an important standard to estimate the soil fertility by measuring its activity. It was reported that phosphatase affected soil organic components, and phosphatase had a close relationship with the colonization of mycorrhizal fungi (Tarafdar et al., 2015, pp. 279-282). The results of this experiment are consistent with above viewpoint in published literature. As for the relationship between phosphatase and AMF colonization, these are some interesting problem still need to be studied and verified by follow-up experiments.

5. Conclusion

In this experiment, AMF colonized successfully in the tulip roots with related high infection rate and had positive effect on plant growth. Furthermore, AMF played good influence on soil property that reflected in more glomalin, better root architecture, glucose in roots with higher soil acid phosphatase in rhizosphere. In conclusion, AMF had a positive impact on tulip growth and its root system architecture. Our research will offer some useful information for higher quality and lower environment effects in tulip cultivation in future.

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