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# EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON BIOMASS AND DISEASE-RESISTANCE ENZYME ACTIVITIES OF *ACTINIDIA ARGUTA* AGAINST CANKER

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## Abstract

In this study, the seedlings of *Actinidia arguta* were used as the experimental materials. Arbuscular mycorrhizal fungi (AMF), *Rhizophagus irregularis*, and the canker pathogen *Dothiorella gregaria* were inoculated. The effects of AMF on the biomass of roots, stems and leaves of *Actinidia arguta*, the contents of malondialdehyde (MDA), proline, and stem cellulose, as well as the activities of disease-resistant enzymes such as superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO), chitinase, and polygalacturonase were determined. The results showed that the inoculation of AMF could increase the biomass of roots and stems of *Actinidia arguta* to enhance the tree vigor and reduce the incidence of canker disease. The inoculation of AMF could significantly reduce the MDA content in the stems and roots of *Actinidia arguta* infected with the disease, increase the proline content in the leaves and roots, and improve the PPO activity in roots, stems and leaves, the SOD activity in stems, and the CAT activity in leaves. In the early stage of AMF inoculation, the activities of chitinase and β-1,3-glucanase in stems and roots could be increased. It is concluded that AMF enhances the resistance of *Actinidia arguta* to canker disease and its disease resistance by accumulating proline, reducing the MDA content, and increasing the activities of SOD, CAT, and PPO.

## Introduction

Arbuscular mycorrhizal fungi (AMF) are widely distributed in different types of ecological environments. They can form mycorrhizae with most plants. Through their powerful mycorrhizal and hyphal networks, they promote the absorption of soil water and nutrients by the roots of host plants, enhance the disease resistance of plants, promote plant growth, and to a certain extent, alleviate the damage caused by adverse stress. They play an important role in the vegetation restoration of ecosystems (Weng *et al.* 2022, Ruiz-Lozano *et al.* 2023, Li *et al.* 2024). At the same time, AMF have a wide range of biological activities and can inhibit the infection or growth of plant pathogenic microorganisms such as fungi, bacteria, and viruses, thus playing a role in improving the disease resistance of plants (Guo *et al.* 2023).

*Actinidia arguta* (Siebold and Zucc.) Planch. ex Miq. is a perennial deciduous liana plant belonging to the genus *Actinidia* of the family *Actinidiaceae* (Zhao *et al.* 2020, Yang 2023). Its fruits are rich in functional compounds and are one of the fruits with extremely high nutritional value and health care functions. It is widely cultivated in large areas in Sichuan, Chongqing, and Hunan. It has been reported that fungal diseases cause the greatest harm to kiwifruit, mainly including canker disease, root rot, and gray spot disease, etc. Among them, kiwifruit canker disease is one of the most important diseases (Ma *et al.* 2021, Zhang *et al.* 2024).

*Actinidia arguta* is a typical plant that can form arbuscular mycorrhizae (Yang 2023). The formation of AMF can promote the growth of *Actinidia arguta* and improve its nutritional status.

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However, the impact of AMF on the disease resistance of *A. arguta* is not fully understood. Especially, there are few reports on the study of the resistance of *A. arguta* to canker disease by AMF (Yang *et al.* 2023, Zhang *et al.* 2024). In this study, *A. arguta* was taken as the research object to study the effects of AMF inoculation on the biomass of roots, stems, and leaves and the activities of disease resistance-related enzymes of *A. arguta* infected with canker disease, providing a reference for further revealing the mechanism by which AMF improves the resistance of *A. arguta* to canker disease.

## Materials and Methods

The experiment was conducted in the Plant Tissue Culture Center of Loudi Vocational and Technical College. The test materials were one-year-old *Actinidia arguta* seedlings. The sand was passed through a 2-mm sieve, washed, and sterilized. The sand and vermiculite were mixed in equal volumes (1:1) and filled into pots (12.5 cm × 11.6 cm). The pathogenic bacteria used was *Dothiorella gregaria* (CXY160). After being activated on LB medium，it was purified twice, cultured at 28°C for one week, and then inoculated for standby. The AMF fungus used was *Rhizophagus irregularis.* A mixture of spores (26 spores per gram), hyphae, and infected root segments was obtained as the tested inoculum.

A two-factor interaction design was adopted. Factor one was the AMF inoculation treatment (inoculation with *R. irregularis*, AM); Factor two was the canker pathogen inoculation treatment (inoculation with *D. gregaria*). Each treatment had 3 replicates, with 3 pots in each replicate, totaling 36 pots.

The robust *A. arguta* seedlings were disinfected with 70% alcohol and washed with distilled water, and then planted in pots filled with 800 g of the tested substrate. Each pot was inoculated with 20 g of AMF inoculum, and for the non-inoculation treatment, each flowerpot was added with 20 g of sterilized inoculum (170°C, 3 hrs). The potted plants were cultured for 12 weeks, and during this period, each plant was watered with 100 ml of water every 2 days. After 12 weeks, the pathogenic bacteria were inoculated: after disinfecting the stem epidermis of the seedlings with 75% ethanol, a hole was punched in the middle part of the stem with a hole puncher with a diameter of 5 mm. At the same time, a fungal disc was taken from the edge of the pathogenic bacteria colony, and the side with hyphae was attached to the punched part. The sterilized LB medium was used as the treatment without inoculating the pathogenic bacteria. Harvesting was carried out after continuing to culture for 1 week. All treatments were placed in a greenhouse with a temperature of 25°C, a relative humidity of 40%, a light duration of 16 h/d, and a light intensity of 2,000 lx.

Canker disease occurrence in *Actinidia arguta* seedlings was monitored daily. The number of lesions per seedling was recorded to calculate the disease incidence rate and disease index. Based on the *A. arguta* canker disease classification standard, the disease index was converted into corresponding disease resistance grades (Table 1).

**Table 1. Grade of canker resistance of *Actinidia arguta* after AMF inoculation.**

| Infection index | Resistance | Representative symbol |
|---|---|---|
| 0.01–27.00 | High resistance | ++ |
| 27.01–52.00 | Moderate resistance | + |
| 52.01–72.00 | Low resistance or low susceptibility | 0 |
| 72.01–87.00 | Moderate susceptibility | - |
| 87.01–100.00 | High susceptibility | -- |

After cultivation, the sealing film was removed, and the roots, stems, and leaves of *Actinidia arguta* were collected separately. The fresh weight of each organ was recorded. Roots were washed, blotted dry, and the length of the main root was measured with a ruler. A portion of the roots was stained following the method of Phillips and Hayman (1970), and the mycorrhizal infection rate was determined using the gridline intersect method. The remaining root, stem, and leaf samples were immediately placed into pre-labeled aluminum foil bags, rapidly frozen in liquid nitrogen, and stored at -80°C for subsequent determination of disease resistance–related enzyme activities and resistance substance contents. Leaves stored at -80°C were thawed, veins removed, and cut into small pieces. Approximately 0.1-0.2 g of leaf tissue was placed into a 50 mL centrifuge tube, and chlorophyll content was determined by the acetone extraction method. Stems were dried to a constant weight at -80°C, and 0.3 g of stem tissue was used to determine cellulose content using the colorimetric method. Fresh samples stored at -80°C were ground into fine powder with liquid nitrogen. Malondialdehyde (MDA) content was determined by the thiobarbituric acid method, and proline content was measured using the ninhydrin colorimetric method (Zhao *et al.* 2020). For the determination of disease resistance-related enzyme activities, 0.1 g of frozen tissue was ground in liquid nitrogen. Superoxide dismutase (SOD) activity was assayed by the nitroblue tetrazolium (NBT) method, and peroxidase (POD) activity by the guaiacol method. The activities of catalase (CAT), phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO) were determined according to Gao (2006). Chitinase and β-1,3-glucanase activities were measured using the dinitrosalicylic acid (DNS) method. Experimental data were organized using Microsoft Excel, and statistical analyses were performed using IBM SPSS Statistics 28.0. Significant differences among treatments were evaluated using LSD tests at $P < 0.05$.

**Results and Discussion**

Eight days after the pathogenic bacteria treatment, the occurrence of canker disease in *Actinidia arguta* tended to be stable, and the incidence rate and disease index of *A. arguta* were calculated. The results showed that regardless of whether AMF was inoculated or not, the all *A. arguta* seedlings developed the disease eight days after the inoculation of pathogenic bacteria. AMF significantly reduced the incidence rate and disease index of canker disease in *A. arguta*. Compared with the plants without AMF inoculation, the incidence rate of the seedlings after AMF inoculation under disease stress decreased by 14.32%, and the disease index decreased by 28.14%. The seedlings inoculated with AMF showed low susceptibility, while the seedlings without AMF inoculation showed moderate susceptibility (Table 2). This indicates that AMF inoculation can reduce the incidence rate of canker disease, increase the disease resistance of *A. arguta*, and alleviate the damage of canker pathogens to *A. arguta*.

**Table 2. Effects of AMF inoculation on *Actinidia arguta* canker caused by *Dothiorella gregaria* (Dg).**

| Treatment | Infection rate (%) | Infection index | Resistance |
|---|---|---|---|
| -Dg+NM | 0.00 | 0.00 | -- |
| -Dg+AM | 0.00 | 0.00 | -- |
| +Dg+NM | 74.64 | 85.45 | - |
| +Dg+AM | 60.32 | 57.31 | 0 |

+Dg: Dg inoculation, -Dg: Dg uninoculation, AM: AMF inoculation, NM: Non-AMF inoculation.

According to the observation results of the roots of the tested *A. arguta* seedlings, the arbuscular mycorrhizal structure was observed in the roots of *A. arguta* inoculated with AMF, while no mycorrhizal structures such as arbuscules were observed in the roots of *A. arguta* without AMF inoculation. The statistical results showed that there was no significant difference in hyphae, vesicles, arbuscules and infection rate between *A. arguta* inoculated with pathogenic bacteria and those not inoculated. For *A. arguta* without pathogenic bacteria inoculation but inoculated with AMF, the mycorrhizal infection rate was 78.8%, and for *A. arguta* inoculated with pathogenic bacteria, the mycorrhizal infection rate was 78.4%. However, the spores in the mycorrhizal roots without pathogenic bacteria inoculation were larger, while the spores in the roots inoculated with pathogenic bacteria were smaller.

The treatment of inoculating pathogenic bacteria significantly reduced the fresh weights of roots, stem segments and leaves of *A. arguta*. Under the stress of pathogenic bacteria, AMF inoculation significantly increased the root weight and stem weight (Table 3). This indicates that AMF inoculation can increase the biomass of *A. arguta*, promote the growth of *A. arguta*, enhance the tree vigor, and improve the ability to resist canker disease.

**Table 3. Effects of AMF on biomass of *Actinidia arguta.***

| Treatments | Leaf weight (g) | Stem weight (g) | Root weight (g) |
|---|---|---|---|
| -Dg+NM | 6.88b | 2.61a | 3.23a |
| -Dg+AM | 7.32b | 3.62a | 5.11b |
| +Dg+NM | 5.42a | 2.29a | 2.89a |
| +Dg+AM | 7.18b | 3.28b | 4.38b |

Different lowercase letters indicate significant differences among treatments at $P < 0.05$.

Compared with the treatment without AMF inoculation, the inoculation of AMF significantly increased the cellulose content (by 52.16%) and root length (by 25.74%) of *A. arguta*. Under the stress of the disease, the contents of chlorophyll and cellulose and the root length of *A. arguta* inoculated with AMF were significantly higher than those of the treatment without AMF inoculation, increasing by 72.72, 58.76 and 44.49%, respectively. This indicates that inoculating *A. arguta* with AMF under disease stress can increase the contents of chlorophyll and cellulose and the root length of *A. arguta*, and help resist the damage caused by pathogenic bacteria to plants. The stress of pathogenic bacteria had no significant effect on the mycorrhizal infection rate of *A. arguta* (Table 4).

**Table 4. Effects of AMF on chlorophyll, cellulose content, root length and colonization rate of *Dothiorella gregaria.***

| Treatment | Chlorophyll content (mg/g FW) | Cellulose content (μg) | Root length (cm) | Colonization rate (%) |
|---|---|---|---|---|
| -Dg + NM | 0.72b | 18.04b | 18.26a | - |
| -Dg + AM | 0.83bc | 27.45c | 22.96b | 78.8 |
| +Dg + NM | 0.55a | 11.88a | 16.97a | - |
| +Dg + AM | 0.95c | 28.81c | 24.52b | 78.4 |

Different lowercase letters indicate significant differences among treatments at $P < 0.05$.

After *A. arguta* was inoculated with AMF, the activities of SOD and CAT increased significantly, while the activity of POD decreased. When the pathogenic bacteria were not inoculated, for the seedlings inoculated with AMF, the activity of SOD in the roots increased most significantly, reaching 63.39%, the activity of CAT in the leaves increased the most, reaching 54.19%, and the activity of POD in the stems decreased most significantly, by 70.25%.

Under the stress of the disease, for the seedlings inoculated with AMF, there were significant differences in the activities of SOD in the leaves and stems, which increased by 16.21 and 16.19%, respectively, while there was no significant difference in the activity of SOD in the roots. Under the same treatment, the activity of CAT in the leaves increased the most, reaching 41.42%, and there was no significant difference in the activities of CAT between the roots and the stem segments. The activities of POD in the roots and leaves decreased significantly, reaching 45.76 and 38.82%, respectively. The activity of POD in the stems also decreased, but there was no significant difference.

This indicates that under disease stress, AMF can increase the activities of SOD and CAT in the leaves and the activity of SOD in the stems of *A. arguta*, and improve the disease resistance (Table 5).

**Table 5. Effects of AMF on SOD, CAT and POD activity of *Actinidia arguta*.**

| Treatments | POD | | | CAT | | | SOD | | |
|---|---|---|---|---|---|---|---|---|---|
| | Root | Stem | Leaf | Root | Stem | Leaf | Root | Stem | Leaf |
| -Dg+NM | 649.55c | 2990.21b | 1864.19ab | 266.52a | 510.48a | 477.42a | 72.54a | 129.92b | 125.35b |
| -Dg+AM | 531.85c | 889.55a | 2455.16b | 383.08b | 529.67ab | 736.18bc | 118.53c | 136.92c | 146.87d |
| +Dg+NM | 324.87b | 1220.13a | 2378.96b | 508.54c | 618.75ab | 559.24ab | 84.87ab | 108.83a | 115.63a |
| +Dg+AM | 176.21a | 504.21a | 1455.27a | 453.25c | 679.85b | 790.86c | 97.65b | 126.45b | 134.38c |

Different lowercase letters indicate significant differences among treatments at $P < 0.05$.

After *A. arguta* was inoculated with AMF, the content of MDA decreased. Under the treatment of inoculating pathogenic bacteria, AMF significantly reduced the contents of MDA in the roots and stems. Compared with the seedlings without AMF inoculation, the contents of MDA in the roots and stems decreased by 31.71 and 47.84%. respectively. The decrease in the content of MDA in the roots after AMF inoculation indicates that the oxidative damage to the roots of *A. arguta* caused by MDA is minimal after AMF inoculation.

After *A. arguta* was inoculated with AMF, the content of proline increased in the roots and leaves. When the pathogenic bacteria were not inoculated, the content of proline in the leaves increased most significantly, by 517.29%. Under the stress of pathogenic bacteria, the content of proline in the roots increased most significantly, by 217.27%. However, in the stems, the content of proline decreased after AMF inoculation. The content of proline decreased by 63.35% in the treatment without pathogenic bacteria inoculation, and decreased by 46.54% after the pathogenic bacteria treatment (Table 6).

After *A. arguta* was inoculated with AMF, the activity of PPO in *A. arguta* was increased. Compared with the situation without pathogenic bacteria inoculation, the activity of PPO increased more significantly under disease stress, with the activities in the roots, stems and leaves increasing by 105.59, 188.42 and 225.93%, respectively. From this, it can be seen that under the interactive effect of AMF and the disease, AMF can play a better role and increase the activity of PPO.

**Table 6. Effects of AMF on MDA and proline content of *Actinidia arguta.***

| Treatments | Proline content | | | MDA content | | |
|---|---|---|---|---|---|---|
| | Root | Stem | Leaf | Root | Stem | Leaf |
| -Dg+NM | 2.14a | 7.45bc | 2.14a | 35.38a | 30.77a | 63.63b |
| -Dg+AM | 3.11a | 2.73a | 13.21b | 32.31a | 24.61a | 31.82a |
| +Dg+NM | 1.39a | 8.81c | 2.49a | 47.28b | 53.07b | 67.27b |
| +Dg+AM | 4.41b | 4.71ab | 5.71a | 32.29a | 27.68a | 49.78ab |

Different lowercase letters indicate significant differences among treatments at $P < 0.05$.

After *A. arguta* was inoculated with AMF, the activity of PAL could also be increased. Although the activities of PAL in the stems and leaves increased under disease stress, there was no significant difference, while the activity of PAL in the roots decreased, by 12.26% (Table 7).

**Table 7. Effects of AMF on PAL and PPO activity of *Actinidia arguta*.**

| Treatment | PPO activity | | | PAL activity | | |
|---|---|---|---|---|---|---|
| | Root | Stem | Leaf | Root | Stem | Leaf |
| -Dg+NM | 29.28b | 62.17b | 33.88bc | 6210.53a | 11769.23b | 12975.34a |
| -Dg+AM | 32.85c | 77.93c | 41.12c | 6631.57a | 13153.85b | 13846.22a |
| +Dg+NM | 17.86a | 22.03a | 8.87a | 7209.52b | 11423.07a | 12467.14a |
| +Dg+AM | 36.72c | 63.54b | 28.91b | 6315.78a | 12230.77b | 13198.51a |

Different lowercase letters indicate significant differences among treatments at $P < 0.05$.

The inoculation of *A. arguta* with AMF reduced the activity of chitinase. Compared with those not inoculated with AMF, the chitinase activities in the roots, stems and leaves of Actinidia arguta inoculated with AMF decreased by 68.11, 440.75 and 24.49%, respectively. Under disease stress, the chitinase activities in the roots, stems and leaves of *A. arguta* inoculated with AMF decreased by 99.97, 71.82 and 88.03%, respectively. This indicates that the decrease in chitinase activity in *Actinidia arguta* inoculated with AMF under pathogen treatment is less severe than that without pathogen inoculation, and the effect of AMF on chitinase is more obvious in the presence of pathogens. Chitinase and β-1,3-glucanase have a synergistic effect. Under pathogen stress, there were significant differences in the activities of β-1,3-glucanase in the stems and leaves of *A. arguta* inoculated with AMF compared with those not inoculated with AMF. The enzyme activity in the stems decreased by 77.78% and that in the leaves decreased by 28.64%. However, in the roots, the enzyme activity increased by 29.67% (Table 8).

Inoculation with AMF can enhance the disease resistance of plants (vander der Heijden *et al.* 2015, Lutz *et al.* 2023, Zhang *et al.* 2023). The research results show that the incidence rate and disease index of *A. arguta* inoculated with AMF are both lower than those of the plants not inoculated with AMF. Under the stress of pathogenic bacteria, the biomass, chlorophyll content and cellulose content of mycorrhizal *A. arguta* all increased to varying degrees. This indicates that mycorrhizal *A. arguta* seedlings can make up for the reduction in biomass caused by the invasion of pathogenic bacteria, and indirectly enhance the disease resistance of *A. arguta*.

Plants can resist the infection of pathogenic microorganisms by increasing the activities of resistance-related enzymes (Thakur and Sohal 2013, Kaur *et al*. 2022, Dai *et al*. 2024). During the disease resistance process, POD and PPO play a major role. In addition, SOD and CAT form an effective reactive oxygen species scavenging system to remove the excessive reactive oxygen species in the plants (Kaur *et al*. 2013).

**Table 8. Effects of AMF on chitinase and β-1,3-glucanase activity of *Actinidia arguta*.**

| Treatment | β-1,3-glucanase activity | | | Chitinase activity | | |
|---|---|---|---|---|---|---|
| | Root | Stem | Leaf | Root | Stem | Leaf |
| -Dg+NM | 7.96b | 7.52b | 1.07a | 154.17c | 75.11c | 74.87b |
| -Dg+AM | 9.75c | 1.35a | 0.98a | 91.71b | 13.89a | 60.14a |
| +Dg+NM | 5.73a | 12.51c | 2.07b | 66.67a | 71.94c | 170.64c |
| +Dg+AM | 7.43b | 2.78a | 1.48a | 33.34a | 41.87b | 90.75b |

Different lowercase letters indicate significant differences among treatments at $P < 0.05$.

This study shows that under disease stress, the activities of PPO in the whole plant, SOD in the stems and leaves, and CAT in the leaves of *A. arguta* inoculated with AMF increased significantly, while the activity of POD decreased. This indicates that in the disease resistance process of *A. arguta*, PPO is mainly involved in scavenging free radicals in *A. arguta* to protect *A. arguta* from damage. Proline accumulation is more affected by adverse stress and less affected by AMF.

In this study, the proline content in the stems of *A. arguta* increased significantly under disease stress. The reason may be that the stems of diseased *A. arguta* are more affected by pathogenic bacteria, resulting in a surge in the proline content induced by the pathogenic bacteria. While the leaves and roots are affected by AMF, and the increase in proline content helps to resist the harm caused by pathogenic bacteria to *A. arguta* and maintain the normal physiological metabolism of *A. arguta*. This indicates that *A. arguta* inoculated with AMF is less damaged by pathogenic bacteria. Chitinase will be transiently induced in the early stage of AMF infection, but then it will gradually decrease. The activity of chitinase in poplar trees inoculated with AMF shows a trend of first increasing and then slowly decreasing (Haw *et al.* 2013, Ye *et al.* 2020).

In this study, the activity of chitinase in *A. arguta* inoculated with AMF is lower than that of the control. This result may be that in the early stage of pathogenic bacteria inoculation, the activities of chitinase and β-1,3-glucanase increased sharply. By the 8th day after inoculation, the two enzymes had completed the decomposition of chitin in the cell wall of the pathogenic bacteria. After the pathogenic bacteria lost their infectivity, the enzyme activities gradually decreased.

The effects of AMF inoculation on canker disease of *A. arguta* are manifested in three aspects: (1) Increasing the biomass of *A. arguta*, improving the chlorophyll and cellulose contents of *A. arguta*, reducing the incidence rate, enhancing the self-resistance ability of *A. arguta*, and indirectly enhancing the disease resistance; (2) Increasing the activities of SOD in the stems and leaves, the activity of CAT in the leaves and the activity of PPO in the whole plant, reducing the MDA contents in the roots and stems, increasing the proline contents in the roots and leaves, and improving the antioxidant capacity of the plants themselves to resist the damage caused by pathogenic bacteria to the plants, directly enhancing the disease resistance of *A. arguta*; (3) Increasing the activities of chitinase and β-1,3-glucanase, and increasing the activities of polygalacturonase and pectin methylesterase to inhibit the growth and reproduction of the mycelia of canker pathogens, weakening the expansion ability of pathogenic bacteria, and reducing the damage of pathogenic bacteria to *A. arguta*.

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