# Growth-Promoting Characteristics and Mechanisms of Arbuscular Mycorrhizal Fungi (AMF) in Ammopiptanthus mongolicus Seedlings: Postprint

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## Abstract

To address the technical challenges of underdeveloped root systems, poor regeneration, and low survival rates of transplanted Mongolian Ammopiptanthus mongolicus seedlings, as well as for effective propagation and conservation of this resource, this study employed different inoculation methods of arbuscular mycorrhizal fungi (AMF) to treat the root systems of A. mongolicus seedlings. The analysis focused on AMF colonization status, changes in plant growth and biomass, root and leaf physiological characteristics, rhizosphere soil enzyme activity changes, and their correlations with seedling growth and biomass variations. The study aimed to investigate the growth-promoting effects and mechanisms of root mycorrhization on A. mongolicus seedlings. Results indicated that arbuscular mycorrhizal fungi (Rhizophagus intraradices, Funneliformis mosseae) could effectively colonize A. mongolicus seedling roots and establish mutualistic symbioses, with varying affinity levels between different fungal species and the host plant. (1) Both single-inoculation and mixed-inoculation treatments showed improved growth compared to the non-inoculated control (CK), primarily manifested as significantly greater plant height (34.7%~47.3%) and root length (32.7%~72.9%). Mixed inoculation significantly increased total root projection area, total root volume, root-to-shoot ratio, root dry weight, root surface area, total plant biomass, and seedling quality index compared to single-inoculation treatments. (2) AMF inoculation, particularly the R. i + F. m treatment, significantly enhanced root activity, root cation exchange capacity, and root soluble protein content by 338.7%, 177.2%, and 240.4% compared to the control, respectively. Root nitrate reductase and alkaline phosphatase activities were also significantly higher than those in single-inoculation (R. i, F. m) and non-inoculated treatments. Under this treatment, leaf net photosynthetic rate and soluble protein content of seedlings were also significantly increased by 237.5% and 54.3% compared to the control. (3) All three inoculation methods significantly enhanced rhizosphere soil urease, alkaline phosphatase, and sucrase activities in A. mongolicus seedlings, with the most pronounced increases observed under mixed inoculation, which surged by 564.7%, 145.8%, and 154.3% compared to the control, respectively. Comprehensive analysis of these indicators revealed that AMF promotes seedling growth and biomass accumulation in A. mongolicus by colonizing the root system, stimulating the production and secretion of rhizosphere soil enzymes, enhancing root cation exchange capacity and activity, facilitating enzymatic processes in root metabolism and accumulation of nutrients such as proteins, while simultaneously influencing and improving photosynthetic metabolism and nutrient storage in seedlings.

## Full Text

## Abstract

To address the technical challenges of underdeveloped root systems, poor regeneration, and low transplant survival rates in *Ammopiptanthus mongolicus* seedlings, as well as to enable effective propagation and conservation of this resource, we inoculated *A. mongolicus* seedlings with arbuscular mycorrhizal fungi (AMF) using different methods. We analyzed AMF colonization status, changes in plant growth and biomass, root and leaf physiological characteristics, rhizosphere soil enzyme activities, and their relationships with seedling growth and biomass. The study aimed to investigate the growth-promoting effects and mechanisms of root mycorrhization on *A. mongolicus* seedlings. The results showed that AMF (*Rhiaophagus intraradice*, *Funneliformis mosseae*) could effectively colonize *A. mongolicus* seedling roots and establish mutualistic symbioses. The degree of compatibility between different fungal strains and the host plant varied. (1) Both single and mixed inoculation treatments increased growth compared with the non-inoculated control, primarily manifested in greater plant height (34.7%-47.3%) and root length (32.7%-72.9%). Mixed inoculation significantly increased total root projection area, total root volume, root-shoot ratio, root dry weight, root surface area, total plant biomass, and seedling quality index compared with single inoculation. (2) Inoculation significantly enhanced root activity, cation exchange capacity, and root soluble protein content, with the mixed inoculation (*R. i* + *F. m*) showing increases of 338.7%, 177.2%, and 240.4% over the control, respectively. Root nitrate reductase and alkaline phosphatase activities were also significantly higher than in single-inoculation treatments. Meanwhile, leaf net photosynthetic rate and soluble protein content under this treatment increased by 237.5% and 54.3% compared with the control. (3) All three inoculation methods significantly increased rhizosphere soil urease, alkaline phosphatase, and invertase activities, with the most pronounced enhancement observed under mixed inoculation, which surged by 564.7%, 145.8%, and 154.3% over the control, respectively. Comprehensive analysis of these indicators indicates that AMF colonization stimulates enzyme production and secretion in the seedling rhizosphere, promotes root cation exchange and activity, enhances enzymatic processes and nutrient accumulation (such as proteins) during root metabolism, and improves photosynthetic metabolism and nutrient storage, thereby promoting root and seedling growth and biomass accumulation in *A. mongolicus*.

**Keywords:** Arbuscular Mycorrhizal Fungi; root mycorrhization; seedling growth; *Ammopiptanthus mongolicus*

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## 1.2 Sowing and Seedling Cultivation Experiment

The experiment included three inoculation treatments, each with 30 replicates: single inoculation with *R. intraradice* (*R. i*), single inoculation with *F. mosseae* (*F. m*), mixed inoculation with *R. i* + *F. m* (mixed inoculant), and a non-inoculated control (*CK*). Pots (13 cm × 12 cm) were filled with sterilized soil substrate and inoculant (volume ratio 9:1) to approximately 3/4 of the pot height. After disinfecting the seeds, 10 *A. mongolicus* seeds were evenly sown in each pot, covered with approximately 2 cm of sterilized substrate, with each pot maintained at 1.0 kg. The pots were cultured in a growth chamber at 25°C with a light intensity of 2500 lx. During the experiment, water loss was replenished by weighing to maintain water content at 60% of field capacity. After 120 days of cultivation, various indicators were measured.

### 1.3.1 Determination of Root Mycorrhizal Infection Rate and Mycorrhizal Dependency

Inoculated *A. mongolicus* roots were washed with deionized water, cut into 1 cm segments, placed in 50 mL tubes, and treated with 30 mL of 10% KOH solution in a water bath at 90°C for 30 minutes. After rinsing three times with distilled water, 2% HCl was added for 5 minutes, followed by 15 mL of 0.05% trypan blue staining solution. The samples were heated in a water bath for 10 minutes, decolorized at room temperature for 24 hours, and then mounted in glycerin for microscopic observation (Olympus BX51). Mycorrhizal colonization was assessed using the gridline intersection method by Phillips and Hayman (1970) to calculate infection rate. Mycorrhizal dependency was calculated based on shoot and root dry weights: (dry mass of inoculated plants - dry mass of non-inoculated plants) / dry mass of inoculated plants × 100%.

### 1.3.2 Determination of Seedling Growth and Physiological Indices

Growth and physiological indices were measured after cultivation. Plant height and ground diameter were measured with digital calipers. Shoot and root parts were separated, and fresh weights were measured with a Sartorius BS124S electronic balance (0.1 mg precision). Root parameters including total projection area, surface area, average diameter, and total volume were analyzed using a

WinRHIZO root scanning system. Fresh samples were used to determine soluble protein content in roots and leaves by Coomassie brilliant blue G-250 staining, chlorophyll content by ethanol extraction, root phosphatase activity by the method of Tisserant et al. (1993), nitrate reductase activity by the method of Li (2000), root activity by TTC reduction, and photosynthetic characteristics using a Li-6400 system. Remaining samples were oven-dried at 105°C for 15 minutes and then at 75°C to constant weight to determine dry weights of roots, stems, and leaves. Dried samples were ground and passed through a 100-mesh sieve for determination of root cation exchange capacity. Seedling quality index was calculated as: seedling quality index = stem biomass (g) / (seedling height (cm) / ground diameter (mm) + shoot dry weight (g) / root dry weight (g)).

#### 1.3.3 Determination of Rhizosphere Soil Enzyme Activities

Indophenol blue and disodium phenyl phosphate colorimetric methods were used to determine urease and alkaline phosphatase activities, reflecting nitrogen and phosphorus transformation and utilization. The 3,5-dinitrosalicylic acid colorimetric method was used for invertase activity to reflect soil biological activity.

### 1.4 Data Processing

Data were analyzed using SPSS 22.0 software with one-way ANOVA. Significance was tested using Duncan' s method. Pearson correlation analysis was conducted between rhizosphere soil enzyme activities and seedling growth indices. Origin 9.0 software was used for graphing.

## 2.1 Mycorrhizal Infection Rate and Mycorrhizal Dependency of *A. mongolicus* Seedlings

The mycorrhizal colonization status of *A. mongolicus* seedlings is shown in [Figure 1: see original paper]. No arbuscules or aseptate hyphae were observed in root cortex cells of the non-inoculated control (*CK*), indicating that *A. mongolicus* roots do not contain endogenous AMF. After inoculation, both AMF strains could enter root endodermal cells and form vesicles, colonizing plant roots to varying degrees. Analysis of mycorrhizal infection rates under different inoculation methods () showed that *R. i* + *F. m* mixed inoculation achieved the highest colonization rate (39.8%), significantly higher than single inoculation treatments. Single inoculation with *R. i* or *F. m* also significantly increased colonization rates compared with the control. Mycorrhizal dependency reflects the relationship between plants and mycorrhizal fungi. shows that *A. mongolicus* seedlings exhibited different dependencies on different inoculants, with mixed inoculation (*R. i* + *F. m*) showing significantly higher dependency (32.3%) than single inoculation (12.8% and 15.4%). This indicates that mixed inoculation creates a stronger mycorrhizal symbiotic association, and seedling growth is more dependent on mycorrhization under this treatment.

## 2.2 Effects of AMF Inoculation on Seedling Growth and Biomass of *A. mongolicus*

The growth status of *A. mongolicus* seedlings after inoculation is shown in [Figure 2: see original paper]. Both mixed and single inoculation treatments showed differences in shoot height and root length compared with the control. indicates that inoculation significantly increased seedling height and root length, with *R. i* + *F. m* treatment showing the fastest growth—seedling height increased by 47.3% and root length by 72.9% compared with the control, with significant differences between mixed and single inoculation treatments. Total root projection area, total root volume, shoot fresh weight, root-shoot ratio, root and leaf dry weights, and total plant biomass were all significantly greater in inoculated treatments than in the control, with increases ranging from 147.3% to 412.5% ($P < 0.05$). The *R. i* + *F. m* treatment was most effective, significantly outperforming single inoculation treatments. Root average diameter and root fresh weight showed no significant differences among inoculation treatments but were significantly greater than the control. Significant differences in root surface area and seedling quality index were observed among treatments, with *R. i* + *F. m* mixed inoculation most effectively expanding root surface area (increased by 145.8% over control) and improving seedling quality index.

### 2.3.1 Root Activity and Cation Exchange Capacity

Root activity and cation exchange capacity (CEC) are key indicators of root absorption capacity and indirectly reflect plant physiological metabolism and growth status. [Figure 3: see original paper] shows that different AMF inoculation treatments significantly increased root activity and CEC compared with the non-inoculated control, with significant differences among inoculation treatments. Mixed inoculation (*R. i* + *F. m*) was most effective in enhancing root activity (increased by 338.7% over control, $P < 0.05$) and promoting CEC (increased by 177.2% over control), significantly outperforming single inoculation treatments. This demonstrates that inoculation enhances root absorption capacity in *A. mongolicus* seedlings, thereby indirectly affecting physiological metabolism and growth of both root and shoot organs.

### 2.3.2 Root Soluble Protein Content, Nitrate Reductase (NR), and Alkaline Phosphatase (ALP) Activities

As shown in [Figure 4: see original paper], root soluble protein content in inoculated seedlings was higher than in the control, with mixed inoculation most effectively promoting soluble protein synthesis (increased by 240.4% over control, $P < 0.05$). Single inoculation treatments also significantly increased root soluble protein content (by 161.5% and 136.5% over control), with no significant difference between the two single treatments. Changes in root soluble protein reflect seedling responses to nitrogen and phosphorus metabolism and their availability, which are closely related to N and P absorption. Further analysis of key enzymes for N and P absorption showed that root nitrate reductase (NR) and alkaline phosphatase (ALP) activities were significantly higher in all inoculated treatments compared with the control. Mixed inoculation was most effective, significantly outperforming single inoculation treatments (increased by 109.4% and 66.0% over control, respectively, $P < 0.05$), while single inoculation treatments increased NR and ALP activities by 39.5% and 36.7% over control, respectively.

### 2.4.2 Leaf Soluble Protein Content

[Figure 5: see original paper] shows that leaf soluble protein content was significantly higher in inoculated seedlings than in the non-inoculated control. Mixed inoculation was most effective, increasing leaf soluble protein by 54.3% over control ($P < 0.05$). Single inoculation treatments also significantly increased leaf soluble protein content, but their relative increases (42.1% and 12.6% over control) were lower than that of mixed inoculation. As an important indicator of overall plant metabolism, the significant increase in leaf soluble protein content, combined with changes in root soluble protein, indicates that AMF inoculation promotes root mycorrhization in *A. mongolicus* seedlings, enhancing not only root physiological metabolism but also leaf metabolic activity. Inoculation with AMF thus affects seedling growth and biomass formation by influencing physiological characteristics.

## 2.5 Effects of Inoculation on Rhizosphere Soil Enzyme Activities of *A. mongolicus*

[Figure 6: see original paper] shows changes in rhizosphere soil urease, alkaline phosphatase, and invertase activities after AMF inoculation. All three inoculation methods significantly increased these enzyme activities, with the most pronounced enhancement under mixed inoculation. For soil urease activity, the surge under mixed inoculation (increased by 564.7% over control) was significantly greater than under single inoculation (increased by 198.0% and 193.0% over control). For alkaline phosphatase, mixed inoculation increased activity by 145.8% over control, significantly greater than single inoculation treatments. Both mixed and single inoculation significantly increased invertase activity by 237.5% and 88.6% over control, respectively, with no significant difference between the two single treatments. This indicates that AMF inoculation can induce *A. mongolicus* to effectively release soil enzymes, which is beneficial for activating soil mineral nutrients and increasing their available concentrations, potentially underlying root mycorrhization formation and its effects on seedling root physiology and growth.

## 2.6 Correlation Analysis Between Rhizosphere Soil Enzyme Activities and Seedling Growth and Physiological Indices

Correlation analysis between seedling growth, root and leaf physiological characteristics, and rhizosphere soil enzyme activities () showed that total biomass was extremely significantly positively correlated with soil urease and alkaline phosphatase activities ($P < 0.01$) and significantly correlated with invertase activity ($P < 0.05$). Root-shoot ratio was extremely significantly positively correlated with all three soil enzymes. Root activity, CEC, root NR and ALP activities, leaf net photosynthetic rate, leaf soluble protein, and root soluble protein were all extremely significantly positively correlated with soil enzyme activities. Leaf net photosynthetic rate was significantly positively correlated with root activity ($P < 0.05$) and extremely significantly correlated with root CEC. Leaf soluble protein was extremely significantly correlated with both root activity and CEC. These results indicate that increased rhizosphere soil enzyme activity can promote root CEC and activity, enhance enzymatic processes and nutrient accumulation (such as proteins) during root metabolism, and simultaneously affect and improve shoot metabolism and nutrient absorption.

# 3 Discussion

### 3.1 Effects of AMF Inoculation on *A. mongolicus* Seedling Growth

AMF affect plant growth by colonizing host plants and producing mycorrhizal effects. Mycorrhizal infection rate and dependency are important indicators for measuring the degree of root mycorrhization and plant reliance on mycorrhizae. Higher infection rates indicate greater root mycorrhization, while stronger dependency means greater mycorrhizal effects and influence on plant growth. In this study, both single and mixed inoculation with two AMF species could colonize *A. mongolicus* seedlings, with mixed inoculation achieving the highest colonization rate, indicating the most extensive mycorrhization. Mycorrhizal dependency under $R.\ i + F.\ m$ treatment was 212.2% and 23.3% higher than under single inoculation treatments, respectively, suggesting that seedling growth was most influenced by mycorrhization under mixed inoculation.

Analysis of growth indices showed that mixed inoculation most significantly improved seedling height, ground diameter, root length, root surface area, total root volume, root-shoot ratio, and seedling quality index. These results are consistent with studies on lavender, *Vitex negundo*, and lily. Gu et al. (2020) also confirmed that mixed inoculation produced the best mycorrhizal effects and optimal growth in *Aucklandia lappa*. When different AMF simultaneously colonize the same plant, the host exhibits greater selectivity for AMF, and this selectivity determines the extent of mycorrhizal growth and function. In this experiment, the two AMF species acted synergistically on *A. mongolicus* seedlings, with mixed inoculation producing more comprehensive and complementary effects than single inoculation, creating non-overlapping ecological niches that enabled seedlings to acquire more nutrients.

### 3.2 Effects of AMF Inoculation on Physiological Metabolism of *A. mongolicus* Seedlings

The growth-promoting effects of AMF are not limited to root growth and physiological metabolism but also directly affect shoot physiological processes. This study found that AMF inoculation significantly increased net photosynthetic rate, chlorophyll content, and leaf soluble protein content in *A. mongolicus* seedlings. Wang et al. (2017) reported that AMF inoculation significantly improved net photosynthetic rate and biomass of plants in mining areas. Chang et al. (2016) found that inoculating *Diospyros lotus* seedlings with AMF improved net photosynthetic rate and light adaptability, significantly promoting growth. This may occur because AMF colonization and growth require photosynthates from the host, altering carbohydrate formation and export, thereby promoting increased net photosynthetic rate, improving chlorophyll synthesis and leaf nitrogen metabolism, and accumulating more soluble proteins in leaves.

Inoculation with *R. i + F. m* significantly enhanced root activity, CEC, alkaline phosphatase and nitrate reductase activities, and soluble protein content. This indicates that AMF inoculation can induce changes in root physiological metabolism, promoting nutrient absorption and accumulation of nutritional substances (soluble proteins), thereby enhancing root physiological processes and expanding the spatial capacity for soil nutrient absorption.

### 3.3 Effects of AMF Inoculation on Rhizosphere Soil Enzyme Activities of *A. mongolicus*

Soil enzymes drive soil biochemical processes, and their activity reflects soil fertility, quality, and environmental evolution. Studies show that AMF inoculation can increase rhizosphere soil enzyme activities. Luo et al. (2015) and Jia et al. (2015) reported that AMF significantly increased urease and invertase activities in upland rice rhizosphere. Ren et al. (2016) found that AMF significantly improved urease and invertase activities in continuous cropping red pepper soil, while Jia et al. (2020) observed increased phosphatase and urease activities in *Salvia miltiorrhiza* rhizosphere.

This study demonstrated that AMF inoculation increased urease, alkaline phosphatase, and invertase activities in *A. mongolicus* rhizosphere. AMF induce plants to secrete related enzymes and stimulate rhizosphere soil enzyme production and secretion, thereby activating soil enzymes. By influencing rhizosphere soil enzyme activities, AMF can indirectly balance soil nutrient levels. Enhanced enzyme activity strengthens enzymatic processes, accelerating the transformation of total nitrogen and phosphorus into organic forms and increasing seedling absorption capacity for soil N and P.

## 4 Conclusion

Arbuscular mycorrhizal fungi (*Funneliformis mosseae* and *Rhiaophagus intraradice*) can effectively colonize *Ammopiptanthus mongolicus* seedling roots and establish mutualistic symbioses. The growth-promoting effects of AMF on *A. mongolicus* seedlings first manifest as stimulation of enzyme production and secretion in the seedling rhizosphere, significantly increasing urease, alkaline phosphatase, and invertase activities. Through enzymatic action, this promotes soil nutrient activation and increases available nutrient concentrations, thereby enhancing soil fertility. Simultaneously, AMF increase root surface area and volume, induce root absorption-related enzyme activities (nitrate reductase and alkaline phosphatase), promote root cation exchange and activity, enhance nutrient accumulation (such as soluble proteins) during root metabolic processes, and improve shoot photosynthetic metabolism and nutrient storage. These mechanisms collectively promote root and seedling growth and biomass accumulation in *A. mongolicus*. These findings are significant for addressing the challenges of underdeveloped root systems, poor regeneration, and low transplant survival in *A. mongolicus*, and provide a theoretical basis for developing mycorrhizal afforestation technologies for this species.

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*Note: Figure translations are in progress. See original paper for figures.*