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2026-06-26

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Abstract

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Full Text

DOI:10.11931/guihaia.gxzw202505008

Effects of Mycorrhizal Fungal Inoculation Methods on the Growth and Physiological Characteristics of Blueberry Seedlings

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Abstract: To explore effective methods for inoculating blueberry with mycorrhizal fungi, this study used one-year-old tissue-cultured blueberry seedlings and one strain of mycorrhizal fungus (*Talaromyces aculeatus*) as materials. Five inoculation methods were designed for a pot experiment: immersion of roots in a fungal suspension (F1), mixing the fungal suspension with the substrate (F2), inoculation with solid fungal blocks (F3), immersion of roots in a fungal suspension after root pruning (F4), and irrigation with a fungal suspension (F5). Potted seedlings immersed in sterile water and not inoculated with mycorrhizal fungi served as the control (CK). After inoculation, root colonization of the seedlings was observed, and growth and physiological indices were measured. The results showed that: (1) the root colonization rate of blueberry seedlings was highest under the F2 treatment, followed by the F5 treatment; the root colonization rates under the five inoculation methods were 2.6, 3.7, 3.4, 3.2, and 3.6 times that of CK, respectively. (2) Except for leaf biomass and root biomass, the growth indices of blueberry seedlings inoculated with mycorrhizal fungi were generally higher than those of CK. Compared with CK, under the five inoculation methods, seedling height, ground-diameter growth, and total biomass increased by 3.21%–30.47%, 16.37%–37.43%, and 9.69%–39.79%, respectively; total root length, total root surface area, total root volume, and number of root tips increased by 38.63%–118.43%, 5.08%–94.89%, 11.97%–65.14%, and 28.90%–92.44%, respectively. (3) The physiological characteristics of blueberry seedlings differed markedly among inoculation methods. The maximum electron transport rate of leaves, minimum saturated light intensity, chlorophyll b content, and total chlorophyll content were higher under the F3 treatment; chlorophyll a content and root activity were higher under the F2 treatment; and the initial slope under CK was higher than those under the inoculation treatments. (4) A comprehensive evaluation of the inoculation treatments using the membership-function method ranked them as follows: F3>F2>F5>F4>F1>CK. The inoculation method significantly affected the growth-promoting effect of mycorrhizal fungi on blueberry. Suitable inoculation methods were inoculation with solid fungal blocks, mixing the fungal suspension with the substrate, and irrigation with the fungal suspension, providing an important reference for efficient cultivation of mycorrhiza-inoculated blueberry seedlings.

Keywords: mycorrhizal fungi; blueberry seedlings; inoculation method; colonization rate; growth-promoting effect; membership function

CLC number: Q945 **Document identification code:** A **Article number:**

Effects of inoculation ways of mycorrhizal fungi on growth and physiological characteristics of blueberry seedlings

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Abstract: To explore effective methods for inoculating blueberry with mycorrhizal fungi, a pot experiment was conducted using one-year-old tissue-cultured blueberry seedlings and a strain of mycorrhizal fungus (*Talaromyces aculeatus*). Five inoculation ways were designed, as root immersion in fungal suspension(F1), mixing fungal suspension with substrate(F2), inoculating with solid fungal blocks(F3), root immersion in fungal suspension after root pruning(F4), and irrigation with fungal suspension(F5). Seedlings immersed in sterile water without inoculation served as the control (CK). The root infection status after inoculation was statistically analyzed, and several growth- and physiology-related indices were determined. The results were as follows: (1) The root infection rates of blueberry differed significantly among inoculation methods. Seedlings inoculated by F2 showed a higher infection rate than those treated by other methods, and the infection rate of seedlings treated by F5 was the second highest. The root infection rates of treatments F1, F2, F3, F4, and F5 were 2.6, 3.7, 3.4, 3.2, and 3.6 times higher than that of CK, respectively. (2) Except for leaf biomass and root biomass, all other growth parameters of blueberry seedlings inoculated with mycorrhizal fungi were significantly higher than those of CK. Compared with CK, seedling

Fund projects: National Modern Agricultural Industrial Technology System of the Ministry of Finance and the Ministry of Agriculture and Rural Affairs (CARS-29-24); Major Science and Technology Special Project of Guizhou Province (Qian Ke He Major Special Project [2024] No. 028); Guizhou Provincial Science and Technology Program Project (Qian Ke He Support [2019] No. 2267; Qian Ke He [2020] No. 4Y012).

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height, ground diameter, and total biomass under the five inoculation methods increased by 3.21%–30.47%, 16.37%–37.43%, and 9.69%–39.79%, respectively. Total root length, total root surface area, total root volume, and number of root tips increased by 38.63%–118.43%, 5.08%–94.89%, 11.97%–65.14%, and 28.90%–92.44%, respectively. (3) The physiological characteristics of blueberry seedlings differed significantly among inoculation methods. The electron transport rate, light saturation point, chlorophyll b, and total chlorophyll under F3 were higher than those under the other treatments; chlorophyll a and root activity were higher under F2, whereas CK had a higher initial slope than the treatments inoculated with mycorrhizal fungi. (4) Based on membership function analysis, the comprehensive evaluation result was F3 > F2 > F5 > F4 > F1 > CK. The inoculation method significantly affected the growth-promoting effect of mycorrhizal fungi. The suitable inoculation methods were solid fungal blocks, mixing fungal suspension with substrate, and irrigation with fungal suspension, providing important insights for the efficient cultivation of mycorrhizal blueberry seedlings.

Key words: mycorrhizal fungi; blueberry seedlings; inoculation methods; infection rate; growth promotion; membership function

Blueberry (*Vaccinium* ssp.), also known as bilberry, belongs to the genus *Vaccinium* of the family Ericaceae and is a shrub-type small berry plant (Wu Wenyong, 2008). Its fruit is rich in nutrients such as vitamin C, fructose, and anthocyanins, and has extremely high nutritional value and health-promoting functions. It has been selected by the Food and Agriculture Organization of the United Nations as one of the five major foods beneficial to human health (Norberto et al., 2013). Guizhou began cultivating blueberries at the end of the 20th century, and by 2024 its promoted cultivation area ranked first in China (Li Yadong et al., 2025). With increasing planting demand, requirements for seedling cultivation have become increasingly high. At present, blueberry seedling production mainly relies on aseptic tissue culture and cutting propagation. However, because blueberry root systems are underdeveloped, their ability to absorb soil nutrients and adapt to the soil ecological environment is relatively weak; therefore, symbiosis with mycorrhizal fungi is needed to promote the growth and development of blueberry plants (Luo Xiaohui, 2017; You Shibei et al., 2020). Numerous studies have shown that inoculation with mycorrhizal fungi significantly promotes the growth of blueberry plants (Liu Fenghong et al., 2014; An Changrong et al., 2022; Yang Weiping et al., 2025), increases fruit yield (Liu Jing et al., 2016; Xiao Longhai et al., 2021), and enhances plant stress resistance (Xu Qinglong et al., 2016; Yang et al., 2020). Some studies have also shown that inoculation with mycorrhizal fungi does not promote blueberry growth (Liu Xiaoyan et al., 2012; Qiao Jie et al., 2019). Thus, whether inoculation produces a growth-promoting effect on plants is influenced by many factors, and it is necessary to identify the key links through which mycorrhizal fungi exert their effects.

Invasion by mycorrhizal fungi and colonization within plant tissues are prereq-

uisites for their functioning. In recent years, studies on mycorrhizal fungal inoculation technologies have been reported one after another and have become increasingly mature. For ectomycorrhizae, Yuan Guiyun (2023) selected three inoculation methods—root pruning plus inoculation, direct inoculation, and mixing fungal inoculum with substrate—while Song Ruiqing et al. (2008) used two inoculation methods, root pruning with root dipping and furrow application, to inoculate ectomycorrhizal fungi; these studies showed that appropriate inoculation methods significantly promoted plant growth and development. For endophytic fungi, Wang Jiawei et al. (2024) used root irrigation with a hyphal-fragment suspension and root wrapping with hyphal pellets; Bi Yinli et al. (2023) used root soaking and simulated soil-culture inoculation; and Cui Lihong et al. (2017) used soil application and foliar spraying for inoculation. The results all indicated that inoculation method is a key factor in the growth-promoting function of endophytic fungi. For arbuscular mycorrhizae, Zhu Fangao et al. (2025) used mixing inoculum with substrate and separate inoculum application, and Zhang Yuyin

et al. (2025) used three inoculation methods—mixing the inoculant with the substrate, dipping roots in the inoculant, and applying solid fungal blocks—and showed that an appropriate inoculation method is key to promoting plant growth and biomass accumulation. For mycorrhization by fungi of the family Duclauxiaceae, Yu Fang et al. (2008), in their study on true fungal inoculation techniques for mycorrhizae of *Rhododendron fortunei*, adopted three inoculation methods; the results showed that inoculation with a solid inoculant was conducive to the growth of the fungal strain and the plant root system. In addition, regarding methods for true fungal inoculation of blueberry mycorrhizae, Liu Fenghong et al. (2014) used three methods—basal inoculation, soaking inoculation, and spray inoculation—to inoculate mycorrhizal fungi; the results showed that, under spray inoculation, the infection rate, growth, and physiological characteristic indices of the roots of blueberry cutting seedlings were lower than those under basal inoculation and soaking inoculation. Gong Ying et al. (2015) used three inoculation methods—fungal blocks, irrigating with inoculant, and root soaking—to inoculate different types of seedlings, and showed that the use of an appropriate inoculation method significantly promoted blueberry plant growth. In summary, selecting an appropriate inoculation method is an important link in bringing the effects of mycorrhizal fungi into play. However, there are marked differences among fungal strain types, plant types, and inoculation methods, resulting in difficulties in production application; meanwhile, there have been relatively few systematic studies on methods for inoculating blueberry mycorrhizae. Therefore, studying the growth-promoting effects of different mycorrhizal fungal inoculation methods on blueberry seedlings, screening out the optimal inoculation method, is of great significance for improving the effectiveness of blueberry mycorrhizal infection and promoting plant growth.

In this study, one-year-old blueberry plug seedlings were used as the research material. One mycorrhizal fungus, *Talaromyces aculeatus*, previously isolated, identified, and verified to have a relatively good growth-promoting effect, was

selected as the test strain. Five inoculation methods were used in the pot experiment: soaking the root system in a fungal suspension, mixing the fungal suspension with the substrate, applying selected solid fungal blocks, soaking the root system after pruning it, and irrigating with fungal suspension. Pot seedlings soaked in sterile water and those not inoculated were used as controls. The aim was to explore the effects of different mycorrhizal fungal inoculation methods on root infection and the resulting growth-promoting effects in blueberry seedlings. Through comprehensive evaluation, a suitable method for inoculating blueberry seedlings with mycorrhizal fungi was identified, providing a theoretical basis and technical guidance for artificial inoculation with mycorrhizal agents in blueberry seedling cultivation, improving inoculation efficiency, and enhancing seedling quality.

1 Materials and Methods

1.1 Experimental Materials

Test strain: The genus of the test strain was *Talaromyces*, and the most similar species was *Talaromyces aculeatus*, with a similarity of 99.82%. After purification on PDA medium, part of the strain was transferred to PDB liquid medium and cultured on a shaker at 25 °C for about 7 d to collect the fungal suspension for later use.

Test material: The inoculation material used in the experiment was one-year-old blueberry plug seedlings, cultivar 'Legacy' . After the blueberry seedlings had produced 5–6 cm embryonic roots and 6–8 leaves, they were hardened outdoors for one week. Seedlings with average seedling height and ground diameter of 8.0 cm $\pm$ 0.4 cm and 7.80 mm $\pm$ 0.39 mm, respectively, were selected to begin the experimental treatments.

Cultivation substrate and containers: Peat, coir, and perlite were mixed in equal amounts as the cultivation substrate. Before use, the substrate was sterilized in a high-pressure sterilizer for 2 h. The measured substrate properties were: pH 4.86, total nitrogen 2.59 g · kg, total phosphorus 1.51 g · kg, total potassium 5.89 g · kg, organic matter 23.19%, alkali-hydrolyzable nitrogen 55.33 mg · kg, available phosphorus 0.25 mg · kg, and readily available potassium 89.00 mg · kg. The cultivation container size was 25.0 cm $\times$ 16.5 cm $\times$ 14.5 cm. The containers were soaked in 0.1% potassium permanganate solution for disinfection for half an hour and then rinsed repeatedly with distilled water.

1.2 Experimental Methods

Five inoculation methods were designed and carried out simultaneously in mid-May 2024. The specific inoculation methods were as follows. Root-system soaking with fungal suspension: after hardening, the blueberry seedlings were removed from the culture substrate and cleaned; the roots were completely im-

mersed in the fungal suspension, soaked for 1 h, and then transplanted, denoted as F1. Mixing the fungal suspension with the substrate: the fungal suspension and sterile water were mixed at a 1:1 ratio and thoroughly mixed and stirred with the sterilized cultivation substrate, after which the seedlings were transplanted, denoted as F2. Solid fungal-block inoculation: the hardened blueberry seedlings were first transplanted, and then solid fungal blocks with a diameter of 1 cm were selected with tweezers and applied around and covering the seedling root zone; each seedling was inoculated with 7–8 fungal blocks, denoted as F3. Root-system pruning plus fungal-suspension soaking: the roots of the hardened blueberry seedlings were appropriately pruned by 2–3 cm, soaked in the fungal suspension for 1 h, and then transplanted, denoted as F4. Irrigation with fungal suspension: the hardened blueberry seedlings were first transplanted, and then the fungal suspension was irrigated around the base of the seedling roots; each seedling received 50 mL, denoted as F5. Blank control: the roots of the hardened blueberry seedlings were completely immersed in sterile water, soaked for 1 h, and then transplanted; subsequently only sterile water was irrigated as needed to maintain water, denoted as CK.

Each of the above inoculation methods had 4 replicates, with 10 seedlings per replicate. To prevent contamination from the external environment, the surface of each flowerpot was covered with plastic wrap. The seedlings were grown in a greenhouse with an average temperature of about 25 °C and light at about 75% of full illumination. Sterile Hoagland nutrient solution or sterile water was irrigated regularly, no fertilizer was applied during the period, and the management practices for seedlings after treatment were consistent. Seedling height and ground diameter were measured for the first time at the time of treatment, and thereafter once every 1 month, until monitoring was stopped at the end of the seedling growth period (late October). After 60 d of treatment, the root systems were collected to observe infection of the plant roots. At the same time, during the vigorous growth period of the seedlings (mid-August), collected

blueberry plants were measured for their physiological and biochemical indicators; at the end of the growth period, indicators such as plant biomass and root morphology were collected and measured.

1.3 Indicator Measurement

1.3.1 Statistics of Root Infection Root segments were processed and stained with reference to the method of Zhang Chunying (2013). Root systems were observed and photographed using a phmias microscope and imaging system, with 50 root segments observed for each treatment. During observation, root segments in which hyphae could be observed were recorded as infected root segments. The infection rate (R) was calculated according to the number of infected root segments and the number of observed root segments, and infection grades were classified with reference to the method of Liu Runjin and Chen Yinglong (2007).

1.3.2 Measurement of Growth Indicators Seedling height was measured with a tape measure (accuracy to 0.1 cm), and ground diameter was measured with a vernier caliper (accuracy to 0.01 mm).

Biomass measurement: After the seedling growth period ended (late October), biomass was measured. Ten standard plants were selected according to average ground diameter and average seedling height ± 5%. The leaves, stems, and roots of each plant were separately placed in different kraft paper envelopes, then dried at 105 °C for 30 min to deactivate enzymes, and subsequently dried at 80 °C to constant weight and weighed.

Measurement of root morphological parameters: The samples used for root morphological parameter determination were the root systems used for biomass measurement; that is, root analysis was performed first, followed by biomass measurement. The root systems to be tested were scanned and analyzed using the Wanshen LA-S root analysis system (Wanshen LA-S, Hangzhou Wanshen Testing Technology Co., Ltd., China) to obtain total root length, total root surface area, total root volume, and number of root tips.

1.3.3 Measurement of Physiological Indicators Measurement of leaf photosynthetic response parameters: On a continuously clear day (mid-August), 10 standard plants were randomly selected from each treatment. Leaves were measured using a chlorophyll fluorometer (Junior-PAM, Shanghai Zealquest Scientific Technology Co., Ltd., Germany) for PAR (photosynthetically active radiation) and ETR (relative electron transport rate), etc. After fitting the curves, three main parameters were obtained: initial slope, maximum electron transport rate, and minimum saturation irradiance.

Chlorophyll content and root activity were determined with reference to the methods of Hao Jianjun et al. (2006). During the vigorous seedling growth period (mid-August), fresh leaves and roots of blueberry plants were collected for measurement. Chlorophyll content was determined by extraction with an acetone–ethanol mixed solution (acetone : ethanol = 1 : 1); root activity was determined using the TTC method.

1.4 Data Processing

Data are expressed as mean ± standard deviation. Excel 2010 software was used for statistical data processing, and Origin pro 2021 software was used for plotting. SPSS 23.0 software was used to conduct one-way analysis of variance and Pearson correlation analysis. Duncan' s multiple comparison was used for analysis of significant differences. The membership function method in fuzzy mathematics was used to comprehensively evaluate the growth and physiological characteristics of blueberry seedlings under the five inoculation-method treatments. The calculation formula of the membership function method was $R(X_i) = (X_i - X_{\min})/(X_{\max} - X_{\min})$.

2 Results and Analysis

2.1 Comparison of the Effects of Different Inoculation Methods on Root Infection Rate of Blueberry Seedlings

As shown in Table 1, the root infection rate of blueberry seedlings under the F1 inoculation treatment differed significantly from those under the F2, F3, and F4 inoculation methods ($P < 0.05$), while differences among the other inoculation methods were not significant ($P > 0.05$). Among them, the infection rate of blueberry seedling roots under the F2 treatment was relatively high, followed by the F5 inoculation treatment, while the F1 treatment had the lowest root infection rate. The root infection rates of blueberry seedlings under the five inoculation-method treatments were 2.6, 3.7, 3.4, 3.2, and 3.6 times that of CK, respectively. In addition, in terms of infection grade, the infection grade of the F1 treatment was grade 4, while those of the other inoculation methods were all grade 5.

Table 1 Comparison of the effects of different inoculation methods on the root infection rate of blueberry seedlings

Inoculation method	Number of infected root segments	Infection rate (%)	Infection grade
F1	26	52.00$±3.98$b$\|4\|\|F2\|37\|74.00±3.50a\|5\|\|F3\|34\|68.00±9.32a|5||F4|32|64.00	

[[header not visible]]	[[header not visible]]	[[header not visible]]	[[header not visible]]
F5	36	72.00$±4.18$a$|5||CK|10|20.00±$3.33c	2

Note: Different lowercase letters in the same column indicate that Duncan's test showed significant differences among different inoculation methods ($P < 0.05$). The same below.

2.2 Effects of Different Inoculation Methods on the Growth of Blueberry Seedlings

2.2.1 Seedling Height and Ground Diameter Growth

As shown in Figure 1, the differences in seedling height growth among blueberry seedlings treated with different inoculation methods were significant ($P < 0.05$), whereas the differences in ground diameter growth were not significant ($P > 0.05$). Among them, the seedling height growth was greatest under treatment F5, and the ground diameter growth was greatest under treatment F3. Seedling height growth was lower under treatment F4, and ground diameter growth was

lower under treatment F1. In addition, inoculation with mycorrhizal fungi significantly promoted the early growth of blueberry seedlings. Compared with CK, the increases in seedling height growth and ground diameter growth under the five inoculation treatments were 3.21%–30.47% and 16.37%–37.43%, respectively.

Note: Different lowercase letters indicate that Duncan' s test among different inoculation methods reached a significant level ($P < 0.05$). The same below.

Figure 1. Effects of different inoculation methods on the growth of height and ground diameter in blueberry seedlings

2.2.2 Biomass

As shown in Table 2, the biomass and its allocation in blueberry seedlings treated with different inoculation methods differed significantly from CK ($P < 0.05$). Among them, leaf biomass was greater under treatment F3, followed by treatment F5, while leaf biomass under treatments F1 and F2 was lower than CK; stem biomass was highest under treatment F2, and the lower stem biomass occurred under treatment F1. The stem biomass of inoculated blueberry seedlings was higher than that of CK. Root biomass was higher under treatment F2, followed by treatment F5, while the root biomass of the remaining treatments was lower than CK. The total biomass per plant under the five inoculation treatments was greater than that of CK, being 1.10, 1.40, 1.31, 1.22, and 1.27 times that of CK, respectively. In terms of biomass allocation, the aboveground biomass of blueberry seedlings under each inoculation treatment was greater than the belowground biomass. For allocation among different plant parts, stem biomass under treatments F2, F3, and F4 was greater than leaf biomass and root biomass; root biomass under treatments F1, F5, and CK was greater than leaf biomass and stem biomass.

Table 2. Effects of different inoculation methods on biomass and distribution of blueberry seedlings

Inoculation way	Leaf (g)	Stem (g)	Root (g)	Total biomass (g)
F1	1.50$±0.12*bc*	1.85±0.34*b*	1.97±0.45*ab*	5.32±0.76*bc*
F2	1.42±0.49*c*	2.84±0.20*a*	2.52±0.51*a*	6.78±0
F4	1.89$±0.34*abc*	2.31±0.37*ab*	1.74±0.34*c*	5.94±0.12*ab*
F5	2.01±0.19*ab*	2.07±0.22*b*	2.11±0.37*ab*	6.1

2.2.3 Root morphology

As can be seen from Figure 2, the root-morphology indices of blueberry seedlings treated with different inoculation methods differed significantly ($P < 0.05$). Among them, seedlings under the F5 treatment performed best in total root

length and total root volume; the F3 treatment performed best in total root surface area; the F2 treatment had the highest number of root tips; whereas under the F1 treatment, all root-morphology indices of the seedlings were relatively poor. Compared with CK, the root-morphology indices of inoculated blueberry seedlings increased to varying degrees. The increases in total root length, total root surface area, total root volume, and number of root tips were 22.05%–118.43%, 5.08%–94.89%, 11.97%–65.14%, and 28.90%–92.44%, respectively. Among the different inoculation treatments, F2 significantly promoted an increase in the number of root tips, F3 significantly enlarged the root surface area of the plants, and F5 significantly promoted total root-length growth and expanded the overall root volume.

Figure 2. Effects of different inoculation methods on the root morphology of blueberry seedlings

Visible panel labels in Figure 2: total root length (cm), total root surface area (cm^2), total root volume (cm^3), and root tip number; x-axis: inoculation method (F1, F2, F3, F4, F5, CK).

2.3 Effects of different inoculation methods on the light-response parameters of blueberry seedlings

As can be seen from Table 3, the initial slopes of leaves of blueberry seedlings treated with different inoculation methods did not differ significantly ($P > 0.05$). At the same time, the initial slopes of leaves in all inoculated-strain treatments were lower than that of CK, indicating that during the initial stage of light-energy utilization, the relative light-energy-use efficiency of seedling leaves treated with inoculated strains was lower than that of CK. Under different inoculation methods, the maximum electron transport rates of blueberry seedling leaves differed significantly ($P < 0.05$). Among them, the maximum electron transport rate of blueberry seedling leaves was highest under the F3 treatment, followed by the F2 treatment, while the relatively lower electron transport rate occurred under the F1 treatment. Meanwhile, the maximum electron transport rates of blueberry seedling leaves under the various inoculation treatments increased by 21.412%–59.08% compared with CK, indicating that blueberry seedlings inoculated with strains had stronger adaptability to high light than CK. Similar to the pattern of change in the maximum electron transport rate, the minimum saturating light intensity of blueberry seedling leaves was highest under the F3 treatment, followed by the F2 treatment, while it was relatively lower under the F1 treatment. The minimum photosynthetic light intensities of blueberry seedling leaves under the different inoculation treatments were all higher than CK, with their increases

The increase ranged from 24.80% to 98.04%. In summary, the leaves of blueberry seedlings under F2, F3, and F5 treatments had lower efficiency in using light energy at the initial stage of light-energy utilization, faster electron transfer rates under light saturation, and stronger tolerance to intense light.

Table 3 Effects of different inoculation ways on optical response parameters of blueberry seedlings

Inoculation way	Initial slope	Maximum electron transfer rate (μmol · m^{-2} · s^{-1})	Minimum saturated light intensity (μmol · m^{-2} · s^{-1})
F1	0.236$±0.021*ab*	103.301±13.105*bc*	441.848±82.789*cd*
F2	0.208±0.011*ab*	124.085±14.187*ab*	59

2.4 Effects of different inoculation methods on chlorophyll content and root activity in blueberry seedlings

As shown in Table 4, the chlorophyll contents in blueberry seedling leaves differed significantly among the different inoculation treatments ($P < 0.05$). Among them, the chlorophyll a contents were relatively high in the F2, F4, and F5 treatments, while the chlorophyll b content and total chlorophyll content were highest in the F3 treatment. Inoculation with bacterial strains significantly promoted the accumulation of chlorophyll in blueberry seedling leaves. Compared with CK, the increases in chlorophyll a content, chlorophyll b content, and total chlorophyll content were 14.74%-33.33%, 23.91%-152.17%, and 27.72%-52.48%, respectively. The root activity of blueberry seedlings also differed significantly among inoculation treatments ($P < 0.05$). The F2 treatment had the highest root activity, followed by F3, while the lowest root activity was observed in the F1 treatment. Compared with CK, root activity in seedlings under the different inoculation treatments increased by 19.58%-95.46%.

Table 4 Effects of different inoculation ways on chlorophyll content and root activity of blueberry seedlings

Inoculation way	Content of chlorophyll a (mg · g^{-1})	Content of chlorophyll b (mg · g^{-1})	Total content of chlorophyll (mg · g^{-1})	Root activity (μg · min^{-1} · g^{-1})
F1	1.79$±0.23*bc*	0.79±0.22*b*	2.58±0.27*b*	87.39±15.32*bc*
F2	2.08±0.16*a*	0.75±0.18*b*	2.83±0.07*ab*	142.

2.5 Correlation analysis of various indicators of blueberry seedlings under different inoculation treatments

As shown in Table 5, under different inoculation treatments, there were many correlations among the various indicators of blueberry seedlings. Among all indicators, there were 71 indicators showing significant or extremely significant correlations, including 26 extremely significant correlations. Among all significant and extremely significant correlations, 61 were positive and 10 were negative. There were 82 correlations among the indicators that were nonsignificant or [[unclear: described as "extremely significant" in the visible text]].

Table 5 Correlation analysis of different inoculation ways on all indicators of blueberry seedlings

Index	IR	SHG	GDI	LB	SB	RB	TB	TRL	TRSA	TRV	TN	Chla	Chlb	Chl	IS	METR	MSLI	RA
IR	1																	
SHG	0.739	1																
GDI	0.920**	0.752	1															
LB	0.377	0.373	0.365	1														
SB	0.886*	0.506	0.867*	0.041	1													
RB	0.295	0.537	0.452	-0.449	0.481	1												
TB	0.920**	0.723	0.959**	0.293	0.930**	0.524	1											
TRL	0.939**	0.860*	0.879*	0.589	0.735	0.264	0.886*	1										
TRSA	0.746	0.330	0.791	0.536	0.778	0.054	0.824*	0.723	1									
TRV	0.876*	0.908*	0.838*	0.603	0.661	0.322	0.858*	0.986**	0.665	1								
TN	0.887*	0.606	0.960**	0.189	0.957**	0.529	0.979**	0.797	0.827*	0.747	1							
Chla	0.946**	0.617	0.792	0.341	0.886*	0.206	0.872*	0.883*	0.773	0.818*	0.822*	1						
Chlb	0.532	0.443	0.740	0.468	0.378	0.137	0.529	0.528	0.494	0.490	0.581	0.263	1					
Chl	0.916**	0.668	0.961**	0.513	0.774	0.216	0.868*	0.877*	0.781	0.815*	0.870*	0.768	0.820*	1				
IS	0.797	0.697	0.954**	0.523	0.710	0.383	0.885*	0.838*	0.792	0.820*	0.876*	0.640	0.829*	0.930**	1			
METR	0.868*	0.669	0.980**	0.498	0.779	0.325	0.905*	0.856*	0.821*	0.816*	0.909**	0.720	0.829*	0.977**	0.984**	1		
MSLI	0.793	0.656	0.952**	0.526	0.708	0.342	0.870*	0.814*	0.801	0.792	0.872*	0.631	0.855*	0.941**	0.997**	0.989**	1	
RA	0.870*	0.733	0.976**	0.375	0.856*	0.519	0.979**	0.872*	0.823*	0.858*	0.962**	0.776	0.657	0.896*	0.957**	0.950**	0.944**	1

Note: **IR**. Infection rate; **SHG**. Seedling height growth; **GDI**. Ground diameter increment; **LB**. Leaf biomass; **SB**. Stem biomass; **RB**. Root biomass; **TB**. Total biomass; **TRL**. Total root length; **TRSA**. Total root surface area; **TRV**. Total root volume; **TN**. Tip number; **Chla**. Chlorophyll a content; **Chlb**. Chlorophyll b content; **Chl**. Chlorophyll content; **IS**. Initial slope; **METR**. Electron transfer rate; **MSLI**. Minimum saturated light intensity; **RA**. Root activity. * indicates a significant correlation at the 0.05 level (two-tailed); ** indicates an extremely significant correlation at the 0.01 level (two-tailed).

Note: **IR**. Infection rate; **SHG**. Seedling height growth; **GDI**. Ground diameter increment; **AGB**. Above-ground biomass; **LB**. Leaf biomass; **SB**. Stem biomass; **RB**. Root biomass; **TB**. Total biomass; **TRL**. Total root length; **TRSA**. Total root surface area; **TRV**. Total root volume; **TN**. Tip number; **Chla**. Chlorophyll a content; **Chlb**. Chlorophyll b content; **Chl**. Chlorophyll content; **IS**. Initial slope; **METR**. Maximum electron transfer rate; **MSLI**. Minimum saturated light intensity; **RA**. Root activity. * indicates significant correlations

at the 0.05 level (bilateral); ** indicates significant correlation at the 0.01 level (bilateral).

2.6 Comprehensive evaluation of the effects of different inoculation methods on blueberry seedling inoculation

As shown in Table 6, the comprehensive evaluation values for infection rate, growth, and physiological characteristics of blueberry cutting seedlings treated with the five inoculation methods were all greater than that of CK. The ranking was F3, F2, F5, F4, F1, and CK, indicating that the infection and growth-promoting effects of different inoculation methods on blueberry seedlings differed. Therefore, in the cultivation of mycorrhizal blueberry seedlings, inoculation methods that can improve infection effectiveness, provide better growth promotion, and enhance seedling quality may include inoculation with solid fungal blocks, mixing fungal suspension with the substrate, and drench inoculation with fungal suspension.

Table 6 Comprehensive evaluation of different inoculation ways on growth-promotion of blueberry seedlings

Index	F1	F2	F3	F4	F5	CK
Infection rate	0.59	1.00	0.89	0.81	0.96	0.00
Seedling height growth	0.24	0.65	0.58	0.11	1.00	0.00
Ground diameter increment	0.44	0.98	1.00	0.55	0.72	0.00
Leaf biomass	0.11	0.00	1.00	0.64	0.80	0.20
Stem biomass	0.37	1.00	0.59	0.66	0.51	0.00
Root biomass	0.29	1.00	0.35	0.00	0.47	0.36
Total biomass	0.24	1.00	0.79	0.56	0.69	0.00
Total root length	0.33	0.76	0.84	0.62	1.00	0.00

Index	F1	F2	F3	F4	F5	CK
Total root surface area	0.06	0.79	1.00	0.93	0.46	0.00
Total root volume	0.18	0.69	0.78	0.48	1.00	0.00
Tip number	0.31	1.00	0.78	0.55	0.53	0.00
Chlorophyll a content	0.44	1.00	0.71	1.00	0.96	0.00
Chlorophyll b content	0.47	0.41	1.00	0.16	0.33	0.00
Chlorophyll content	0.53	0.76	1.00	0.59	0.70	0.00
Initial slope	0.78	0.27	0.00	0.65	0.44	1.00
Maximum electron transfer rate	0.36	0.78	1.00	0.48	0.60	0.00
Minimum saturated light intensity	0.25	0.70	1.00	0.37	0.52	0.00
Root activity	0.21	1.00	0.96	0.48	0.68	0.00
Average	0.34	0.77	0.79	0.54	0.69	0.09
Rank	5	2	1	4	3	6

3 Discussion and Conclusions

Blueberry roots have no root hairs and need to form a mutually beneficial symbiotic relationship with mycorrhizal fungi. After a symbiotic relationship is established with the host plant, the absorption of water and nutrients by plant roots is significantly promoted, which is ultimately reflected in plant growth (Yuan Jixin and Hou Zhixia, 2012). The mycorrhizal infection rate is a direct indicator of whether mycorrhizal fungi can successfully infect roots, and the level of infection is related to plant species, fungal strain type, environmental conditions, and other factors (Li Chenyuan, 2015). The results of this study show that different inoculation methods produced clear differences in the root infection rate of blueberry seedlings, indicating that the degree of symbiosis between blueberry seedlings and mycorrhizal fungi is affected by the inoculation method. Among them, the root infection rate of blueberry seedlings treated with the F2 inoculation method was higher than that under the other inoculation methods, which is contrary to the results reported by Yuan Guiyun (2023). The reason for this difference may be that the microbial inoculant used in this study was a strain that had been favorably screened in the early stage; at the same time, during treatment, the fungal suspension and sterile water were mixed in equal proportions, resulting in a relatively high concentration of the inoculant in the cultivation substrate and less influence from contaminating microorganisms over a short period, thereby providing conditions for the establishment of symbiotic

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provided sufficient conditions for the symbiotic relationship. Second, the infection rate under the F5 treatment was relatively high, possibly because the seedling root system could come into direct contact with the inoculum and rapidly recognize it, thereby producing a better infection effect (Liu Fenghong, 2014). The root infection rate under the F2 treatment was intermediate; this may be because, after inoculation with solid fungal blocks, contact between the root system and the inoculant was insufficient during the early stage of seedling growth, but became adequate as the root system developed. For the F1 and F4 inoculation methods, the root infection rates were lower than those under the other inoculation methods. A possible reason is that the underdeveloped blueberry root system had only a short infection period when contacting the inoculant; however, root-pruning treatment markedly increased the root infection rate, which is similar to the findings of Zhu Jianghua (2016) and Chen Meng (2017). In addition, this study also found that the control roots soaked in sterile water and not inoculated with mycorrhizae were infected. This may have occurred because, during cultivation of blueberry seedlings in the greenhouse, the influence of airborne or surrounding miscellaneous microorganisms was unavoidable, and one or more mixed fungi may have existed in the cultivation substrate, resulting in root infection; however, the root infection rate was significantly lower than that of the inoculated treatments.

After mycorrhizal fungi form a symbiotic relationship with the host plant, the effect is ultimately reflected in plant growth. Among growth traits, seedling

height, ground diameter, and biomass are important morphological indicators of plant growth. Studies have shown that inoculation with mycorrhizal fungi significantly promotes plant growth and biomass accumulation, and that the growth-promoting effect differs with inoculation method (Yu Fang et al., 2008; Liu Fenghong et al., 2014; Wang Jiawei et al., 2024). The results of this study showed that blueberry seedlings treated with different inoculation methods differed markedly in seedling-height increment, ground-diameter increment, and biomass accumulation, and that the affected plant parts also differed. The F5 inoculation treatment significantly promoted seedling height growth; the F3 inoculation treatment significantly promoted ground diameter and leaf biomass accumulation; and the F2 inoculation treatment significantly promoted root and stem biomass accumulation as well as total biomass accumulation. The reasons for these differences may be as follows: first, the contact mode and duration between the inoculant and the plant root system, as well as the inoculant concentration, differed; second, the peak period of the growth-promoting effect produced by the inoculant differed, and in the later stage after treatment the growth-promoting effect of the target fungus was not obvious or weakened; third, during greenhouse cultivation, one or more mixed fungi may have existed in the cultivation substrate and formed synergistic or antagonistic interactions with the target fungus, thereby affecting plant growth.

Root morphology is an indicator reflecting the capacity of plants to absorb water and nutrients. Among root traits, root tips are the most active parts of the root system and are related to the ability to absorb water and nutrients. Studies have shown that an increase in the number of root tips promotes root elongation on the one hand and increases root surface area and volume on the other (Li Zhu, 2024). The results of this study showed that the root morphology of blueberry seedlings differed significantly among inoculation methods, and that the promoted root traits also differed. Plants under the F5 treatment performed best in total root length and total root volume; plants under the F3 treatment performed best in total root surface area; and plants under the F2 treatment had the highest number of root tips. The possible reasons are as follows: under the F5 treatment, the root system directly contacted the liquid inoculant and rapidly established a symbiotic relationship, promoting an increase in root-tip number and increasing total root length and total root volume, after which the mycorrhizal effect weakened; under the F3 treatment, because the inoculated fungal blocks were distributed in the middle layer of the cultivation substrate, after the root system formed a symbiotic relationship with the inoculant, root growth toward the surrounding rhizosphere was promoted, thereby expanding the total root surface area of the plants; in the early stage of the F2 treatment, the underdeveloped root system came into close contact with the inoculant and the number of root tips increased, but because of the influence of environmental temperature and humidity, the ability of root tips to absorb water and nutrients from the soil weakened, thereby limiting root growth. Overall, the root-morphology indices of blueberry seedlings treated with the F1 and F4 inoculation methods were lower than those under the other inoculation

methods, which differs from the results of Liu Fenghong et al. (2014) and Zhang Yuyin et al. (2025). The reason for this difference may be that, after seedling transplanting, only a small amount of inoculant was present in the substrate and the mycorrhizal effect was weak; meanwhile, the miscellaneous fungi present in the substrate after root-pruning treatment could also infect the root system. In addition, root activity can indirectly reflect root growth and the level of nutrient absorption, and is usually closely related to plant root growth (Yuan Lanfang, 2022). The results of this study showed that the root activity of blueberry seedlings inoculated with mycorrhizae was higher than that of the uninoculated treatment, and that root activity differed markedly among inoculation methods. This may be because the degree of infection caused by contact between the seedling root system and the inoculant differed, and because the metabolism generated by the root system itself differed (Liu Jiangdong, 2020).

Efficient cultivation is reflected not only in seedling morphology, but also in seedling physiological characteristics. The contents of relevant indicators reflect, to a certain extent, the plant' s photosynthetic capacity and its ability to adapt to the light environment. Studies have shown that inoculation with mycorrhizal fungi can significantly promote chlorophyll accumulation and photosynthetic characteristics in plants (An Changrong et al., 2022; Fu Xiang et al., 2023; Li Zhu et al., 2024). The results of this study showed that, under different inoculation methods, the chlorophyll a and b contents, chlorophyll content, maximum electron transport rate, and minimum saturating light intensity of blueberry seedlings were higher than those of the CK treatment, whereas the initial slope of the CK treatment was higher than that of the inoculated treatments. The reasons for these differences may be as follows: first, under inoculation treatment, contact between the root system and the inoculant expanded the root absorption range, increased the transport of relevant elements, and promoted chlorophyll synthesis; at the same time, symbiosis enhanced leaf enzyme activity and maintained chlorophyll stability. Second, inoculation treatment enhanced PSII activity, which was beneficial for photosynthesis in the plants. However, the chlorophyll content and light-response parameters of blueberry seedlings differed markedly under different inoculation methods, and these differences were related to the type of inoculated agent, the degree of infection, and the environmental factors at the time of monitoring.

This study conducted correlation analysis and comprehensive evaluation among indicators under different inoculation treatments. The correlation analysis showed that the growth-promoting effects of inoculating blueberry seedlings with mycorrhizal fungi were significantly or extremely significantly positively correlated with the level of root colonization, and that growth indicators and physiological indicators were significantly or extremely significantly positively correlated. This indicates that the inoculation method is a key factor in enabling mycorrhizal fungi to exert growth-promoting effects. Meanwhile, the comprehensive evaluation showed that inoculation using solid fungal blocks, mixing fungal suspension with substrate, and drenching with fungal suspension were relatively suitable inoculation methods. It should be noted

that, in practical application, suitable inoculation methods should be selected according to the symbiotic characteristics of the fungal strains and seedlings, with preference given to methods that can act synergistically and achieve the best effects. Drenching with mycorrhizal inoculant, selecting fungal cakes or fungal blocks, mixing fungal suspension with substrate, and soaking after pruning seedling roots are suitable inoculation methods for enhancing the growth-promoting effect of strains while also reducing costs.

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