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Arbuscular Mycorrhizal Fungi, Especially *Rhizophagus intraradices* as a Biostimulant, Improve Plant Growth and Root Columbin Levels in *Tinospora sagittata*

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Abstract: *Tinospora sagittata* is a columbin-rich medicinal plant, but its columbin levels are reduced under artificial cultivation conditions. The objective of this study was to analyze the effects of inoculations with *Diversispora versiformis* (*Dv*), *Funneliformis mosseae* (*Fm*), *Rhizophagus intraradices* (*Ri*), and mixed inoculation (*Dv* + *Fm* + *Ri*) (Mix) on growth performance, root morphology, leaf photosynthetic physiology, and root columbin levels in *T. sagittata*. These arbuscular mycorrhizal fungi (AMF) were able to colonize the roots, as evidenced by a root mycorrhizal colonization rate ranging from 17% to 48% and soil hyphal lengths ranging from 17.51 cm/g to 32.02 cm/g, with the Mix treatment being the greatest. AMF inoculations improved plant height (16–151%), leaf number (119–283%), shoot (37–211%), and root biomass (22–318%) to varying extents, with *Ri* and Mix treatments being the most prominent. AMF-treated plants presented relatively greater root total length, projected area, surface area, volume, and average diameter, especially those treated with *Ri* and Mix. AMF inoculations also significantly improved the leaf nitrogen balance index, transpiration rate, and stomatal conductance, while the photosynthesis rate and chlorophyll index varied by AMF species, along with a decrease in intercellular CO_2 levels. Root columbin levels ranged from 0.524 mg/g to 5.389 mg/g, and AMF inoculation significantly increased root columbin levels by 228–928%, with *Ri* being the most significant. Root columbin levels were significantly positively correlated with soil hyphal length, root total length, root projected area, root surface area, root volume, and root average diameter, but not root AMF colonization rate. This study demonstrates for the first time that AMF, especially *Ri*, can be employed as a biostimulant to promote growth as well as root columbin levels in *T. sagittata*, where AMF-triggered improvement in root morphology is an important reason for promoting root columbin levels.

Keywords: arbuscular mycorrhizal fungi; columbin; gas exchange; medicinal plant; secondary metabolite

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1. Introduction

Tinospora sagittata (Oliv.) Gagnep. is an ancient and valuable medicinal plant, whose tuberous roots are used in medicine for the treatment of inflammation and have anti-inflammatory and antimicrobial properties [1,2]. *T. sagittata* contains a large number of secondary metabolites, including alkaloids, terpenoids, and flavonoids [3–5]. Among them, columbin is one of the plant's active constituents [6–8], and it belongs to the phenolic acid family, with a benzene ring and an alcohol group in its chemical structure; it has a bitter taste and antioxidant activity, and is thought to have a variety of biological activities, such as anti-inflammatory, antimicrobial, hepatoprotective, and anti-tumor effects [9,10]. Columbin,

primarily in the roots of *T. sagittata*, exhibits analgesic, antiviral, and antipathogenic microbial pharmacological properties [3,11]. As a result of *T. sagittata* being a key source of columbin, its demand has increased, resulting in overexploitation of wild resources and deterioration of the soil environment, as well as a decrease in biomass production and columbin levels in *T. sagittata* [12]. Therefore, artificial cultivation is an important way to achieve sustainable and high-quality development. It is urgent to improve plant growth and increase columbin levels in *T. sagittata*.

Many medicinal plants inhabit arbuscular mycorrhizal fungi (AMF) in their rhizosphere [13,14]. Zubek and Błaszkowski investigated the mycorrhizal colonization and communities of 31 medicinal plants at Jagiellonian University, Poland, and found that 30 plants could form arbuscular mycorrhizae (23 arum-type, 5 paris-type, and 2 intermediate-type), and 15 AMF species were identified [15]. In the southern part of Shannxi, China, six AMF species (*Acaulospora scrobiculata*, *Glomus constrictum*, *G. fasciculatum*, *G. albidum*, *G. multicaule*, and *Gigaspora gigantean*) were found in eight medicinal plants [16]. These fungal communities contribute to host growth and adaptation to the native environment and are thus important for understanding the soil microbial environment [16]. AMF are obligate biotrophic fungi capable of colonizing approximately 80% of vascular plant roots to form mycorrhizal symbioses, allowing nutrients and water to be transported from AMF to the host plant and carbohydrates to be transported from the host plant to AMF [17,18]. The extraradical mycelial network generated by AMF on the surface of host roots aids in nutrient exchange and signaling communication between plants [19]. At the same time, they can influence the growth, stress resistance, and levels of medicinal constituents in medicinal plants [14]. AMF are widely inhabited in a variety of medicinal plants and thus play an important role in the growth and development of medicinal plants through the mycelium network, secrete organic acids into the rhizosphere, increase nutrient acquisition, and enhance photosynthetic capacity [20]. Huang et al. [21] reported that inoculation with *Glomus mosseae* and *G. versiforme* distinctly increased biomass production, leaf gas exchange, nutrient (N, P, and K) acquisition, as well as artemisinin concentration in stems, branches, and leaves of potted *Artemisia annu*. AMF colonization dramatically boosted root glycyrrhizic acid and glycyrrhizin levels in *Glycyrrhiza uralensia* under soil drought conditions [22]. Inoculation with AMF also significantly increased root biomass and polydatin and resveratrol levels in *Polygonum cuspidatum* plants, which was associated with up-regulated expression of relevant genes in the resveratrol synthesis pathway [23,24]. This promoting effect was observed at low phosphorus levels in soil [25]. Therefore, AMF are essential for promoting the growth and secondary metabolite levels of medicinal plants, especially under unfavorable environmental conditions. Other studies, however, have reported a decrease in aromatic alcohol concentration in basil [26], as well as ursolic acid and oleanolic acid in *Ocimum gratissimum* after AMF inoculation [27]. Interestingly, early inoculation of AMF significantly increased camptothecin content in *Camptotheca acuminata* plants, but later inoculation of AMF promoted biomass accumulation but not camptothecin content [28]. This suggests that the effect of AMF on plant secondary metabolites is quite complex.

Since AMF are able to act as a fungal stimulant, affecting the growth and secondary metabolites of medicinal plants, we therefore hypothesized that AMF have a regulatory effect on the growth, physiological activity, and columbin of *T. sagittata* plants. Three AMF species were applied to potted *T. sagittata* plants to analyze changes in growth, leaf photosynthetic physiology, and root columbin levels, thus revealing the potential role of AMF in *T. sagittata*.

2. Materials and Methods

2.1. AMF Inoculum Preparation

The experiment consisted of three AMF species used in this study: *Funneliformis mosseae* (Nicol. & Gerd.) *Gerdemann* & Trappe, *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler, and *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd. These fungi were provided by the Institute of Root Biology, Yangtze University.

These AMF strains were trapped in potted *Trifolium repens* for 11 weeks, and the roots and growth substrates were harvested as fungal inoculum containing mycorrhizalized root segments and spores, with approximately 25, 22, and 20 spores/g for *F. mosseae*, *R. intraradices*, and *D. versiformis*, respectively.

2.2. Plant Culture

Seeds of *T. sagittata* were provided by the Shiyan Academy of Agricultural Sciences (Shiyan, China). The seeds were surface sterilized with 75% ethanol and germinated in autoclaved sands at 26–28 °C on 15 April 2022. Six weeks later, 4-leaf-old *T. sagittata* seedlings with consistent growth were transplanted in plastic containers (upper diameter 20.5 cm, down diameter 10.7 cm, and height 12.7 cm). The pots were pre-filled with an autoclaved (121 °C, 0.11 MPa, 2 h) mixture of soil and sand at a ratio of 2:1 (*v*:*v*) to eliminate indigenous AMF spores. AMF inoculation was carried out at a dose of 150 g inoculum/pot at the time of seedling transplanting. The non-AMF treatment also received 150 g of autoclaved inoculum plus 2 mL of 25 μm fungal filtrates. All the plants were grown under environmentally controlled conditions, with a light flux density of 907 μmol/m^2/s, day and night temperatures of 28 °C/23 °C (16 h/8 h), and a relative humidity of 64%. The plants received no additional chemical fertilizers during the experiment. The plants were grown from 28 May 2022 to 12 September 2022 under these conditions.

2.3. Experimental Design

The experiment consisted of five treatments: (i) inoculation with *D. versiformis* (*Dv*); (ii) inoculation with *F. mosseae* (*Fm*); (iii) inoculation with *R. intraradices* (*Ri*); (iv) inoculation with a mixture of *Ri*, *Fm*, and *Dv* (1:1:1, m/m) (Mix); and (v) inoculation without AMF (non-AMF). Each treatment was replicated eight times, for a total of 40 pots.

2.4. Determination of Plant Growth and Root Mycorrhizal Colonization Rate

Before harvesting the plants, the height and number of leaves were determined. The treated plants were then divided into shoots and roots, and their fresh weights were weighed. All the roots were scanned with an Epson's scanner (V700), and the scanned root figures were analyzed with WinRHIZO software (version 2007b) to determine root length, surface area, volume, projected area, and average diameter.

Root segments of 1–2 cm length were collected and stained for root mycorrhizae using trypan blue (0.05%, *w*/*v*) staining [29] before being examined microscopically. Approximately 2 cm long root segments were incubated in a 10% KOH solution at 90 °C for 80 min, decolorized for 3 min in a 10% hydrogen peroxide solution, acidified in 2% hydrochloric acid for 5 min, and finally stained in 0.05% trypan blue in a lactophenol solution for 2 min. Mycorrhizal colonization rate (%) was the percentage of mycorrhizal colonization root segment lengths versus total observed root segment lengths. Soil hyphal density was determined by the method described by Bethlenfalvay and Ames [30], staining the hyphae with trypan blue (0.05%, *w*/*v*).

2.5. Determination of Leaf Photosynthetic Properties

On the day of harvest, the chlorophyll index (Chl) and nitrogen balance index (Nbi) were determined in the 4th leaf at the top using a portable plant polyphenol chlorophyll meter (Dualex Scientific+). Leaf gas exchange parameters, including net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci), were measured in the 4th leaf at the top at 9:00 and 10:00 a.m. by using a Li-6400 portable photosynthesizer (Li-COR).

2.6. Determination of Columbin Levels in Roots

High-performance liquid chromatography (HPLC) was used to determine the columbin level in the roots. The 0.2 g of dried samples (1 mm in size) was mixed with 20 mL of 70% methanol under ultrasonication for 20 min, and the solution was then fixed to 25 mL

using 70% methanol. The fixed solution was filtered via a 0.22 µm membrane for HPLC analysis. This HPLC was a Shimadzu LC-20AT (Tokyo, Japan) with a Venusil XBP C18 column (4.6 × 250 mm, 5 µm). The mobile phase consisted of acetonitrile and ddH_2O (40:60, *v*/*v*), the detection wavelength was 210 nm, the flow rate was 1.0 mL/min, and the column temperature was 30 °C. The linear gradient elution was as follows: 0–10 min, 15–20%; 10–23 min, 20–25%; 23–27 min, 25–43%; 27–36 min, 43–50%; 36–47 min, 50–68%; 47–65 min, 68–78%.

2.7. Statistical Analysis

One-way analysis of variance, multiple comparisons, and correlations was performed using SAS® software (9.1.3v) (SAS Institute Inc., Cary, NC, USA). Origin Software (2021v) was used for plotting.

3. Results

3.1. Changes in Mycorrhizal Status

No mycorrhizal colonization or soil mycelium was seen in the roots of plants inoculated with non-AMF, while mycorrhizal colonization was observed in the roots of all inoculated plants, with root AMF colonization rates ranging from 17% to 48% and soil hyphal length ranging from 17.51 cm/g to 32.02 cm/g (Figure 1a,b). Of these, the root mycorrhizal colonization and soil hyphal length of the Mix-inoculated plants were the highest among inoculated treatments, whereas the *Dv*-inoculated plants had the lowest.

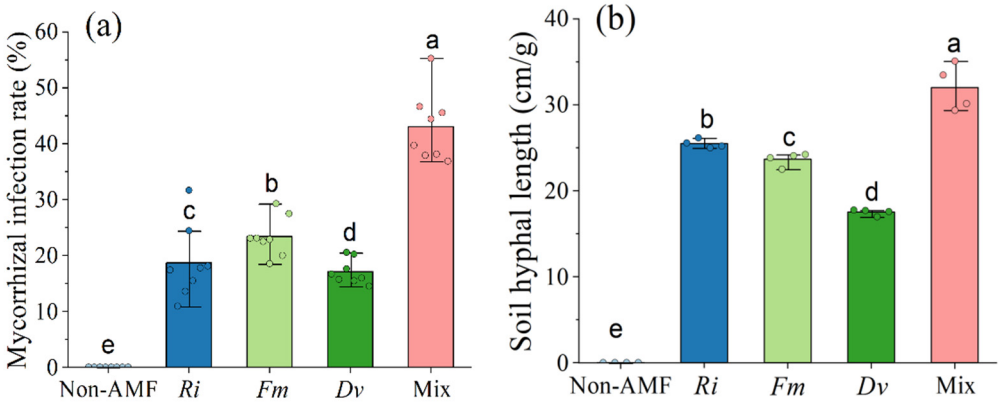

Figure 1. Changes in root mycorrhizal colonization rate (**a**) and soil hyphal length (**b**) in *Tinospora sagittata* plants after inoculation with different arbuscular mycorrhizal fungi. Different letters on the bar indicate significant ($p < 0.05$) differences. Abbreviations: *Ri*: inoculation with *Rhizophagus intraradices*; *Fm*: inoculation with *Funneliformis mosseae*; *Dv*: inoculation with *Diversispora versiformis*; Mix: inoculation with a mixture of *R. intraradices*, *F. mosseae*, and *D. versiformis*.

3.2. Changes in Plant Growth Behavior

Inoculation with AMF showed different changes in plant growth performance (Figure 2a–e). Compared with the non-AMF inoculation, inoculation with *Ri*, *Fm*, *Dv*, and Mix significantly increased plant growth variables by 151%, 73%, 16%, and 136% in plant height (Figure 2b), 210%, 130%, 37%, and 211% in shoot biomass (Figure 2d), and 74%, 100%, 22%, and 318% in root biomass (Figure 2e). In addition, *Ri*, *Fm*, and Mix, but not *Dv*, also significantly increased leaf number by 246%, 119%, and 283%, respectively, compared with the non-AMF (Figure 2c).

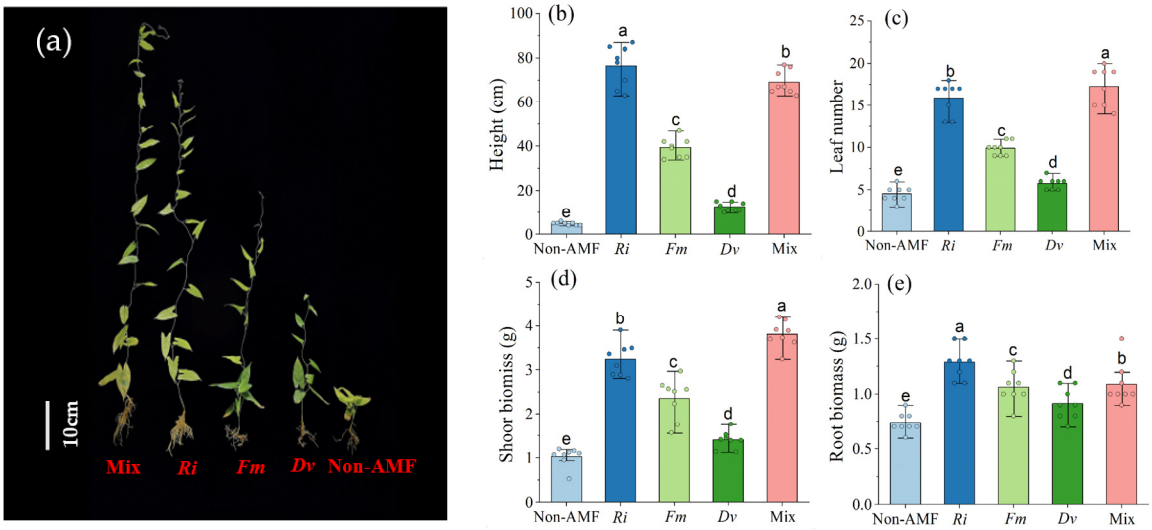

Figure 2. Changes in growth performance (**a**), height (**b**), leaf number (**c**), shoot biomass (**d**), and root biomass (**e**) in *Tinospora sagittata* plants after inoculation with different arbuscular mycorrhizal fungi. Different letters on the bar indicate significant ($p < 0.05$) differences.

3.3. Changes in Root Morphological Variables

AMF treatments significantly affected root morphology variables (Figure 3a), as evidenced by an increase in root total length (Figure 3b), projected area (Figure 3c), surface area (Figure 3d), average diameter (Figure 3e), and volume (Figure 3f) by 100%, 38%, 39%, 43%, and 184% in *Ri* versus non-AMF conditions, and by 28%, 25%, 19%, 40%, and 97% under mix versus non-AMF conditions, respectively. *Fm* treatment also significantly increased root length, surface area, average diameter, and volume by 20%, 18%, 22%, and 146%, respectively, compared with non-AMF treatment. Dv treatment only significantly increased root volume and average diameter by 83% and 28%, respectively, compared to non-AMF treatment.

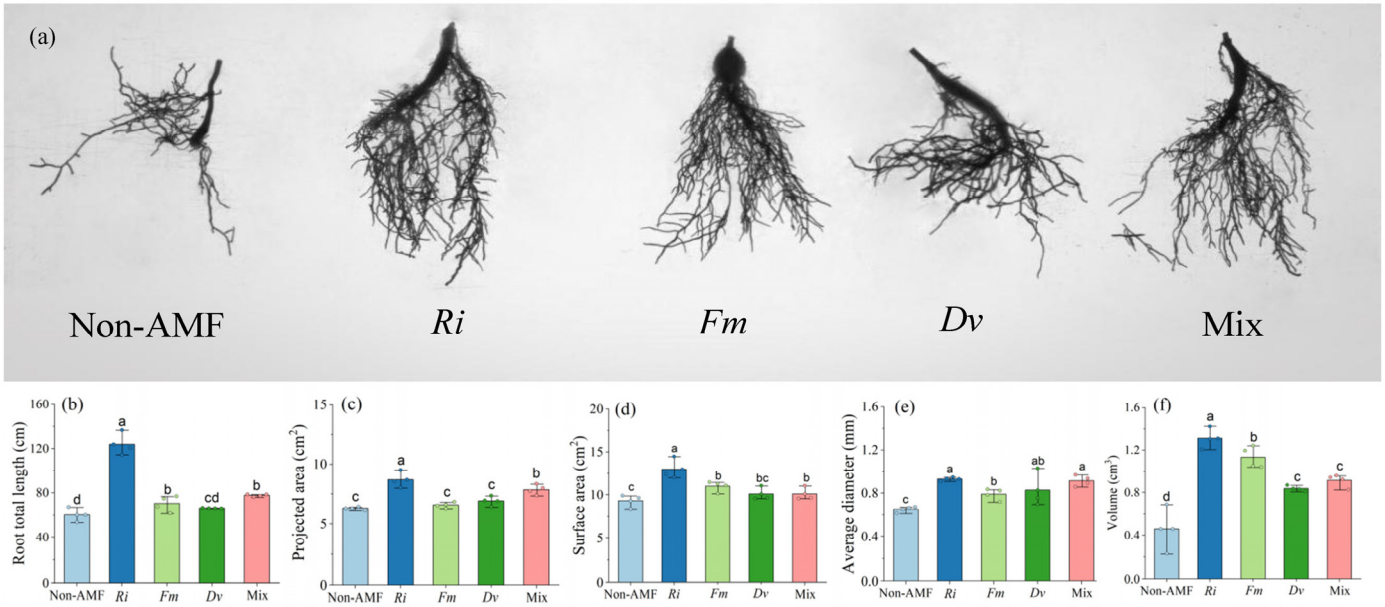

Figure 3. Changes in root growth (**a**), root total length (**b**), projected area (**c**), surface area (**d**), average diameter (**e**), and volume (**f**) in *Tinospora sagittata* plants after inoculation with different arbuscular mycorrhizal fungi. Different letters on the bar indicate significant ($p < 0.05$) differences.

3.4. Changes in Chlorophyll Index, Nitrogen Balance Index, and Gas Exchange in Leaves

Ri, *Fm*, *Dv*, and Mix inoculation significantly increased Nbi by 183%, 110%, 108%, and 148%, respectively, compared with non-AMF inoculation (Figure 4a). Except for *Dv* inoculation, which did not significantly affect Chl, *Ri*, *Fm*, and Mix inoculation significantly increased Chl by 22%, 20%, and 55%, respectively, compared with non-AMF treatment (Figure 4b).

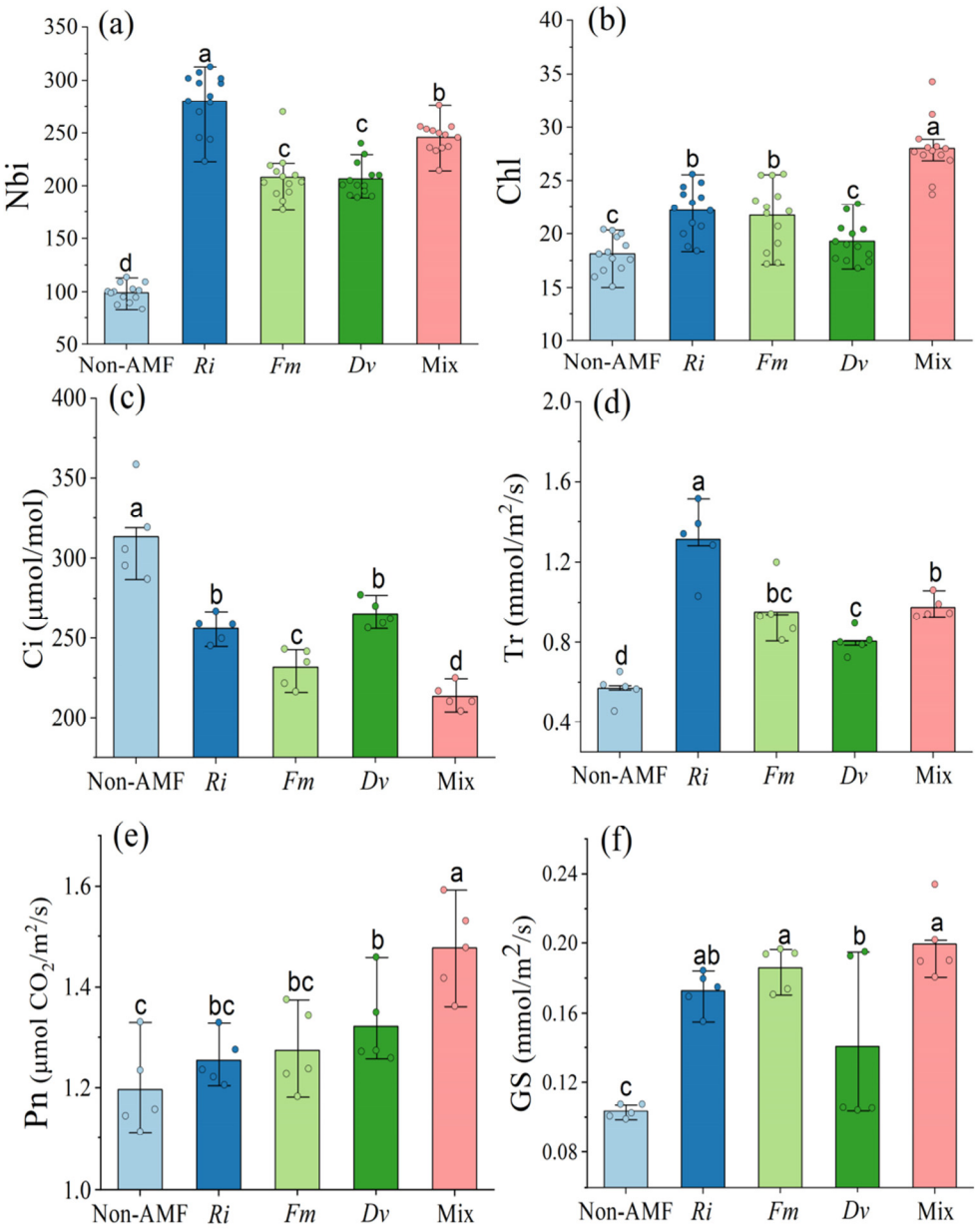

Figure 4. Changes in leaf nitrogen balance index (Nbi) (**a**), chlorophyll index (Chl) (**b**), intercellular CO_2 concentration (Ci) (**c**), transpiration rate (Tr) (**d**), photosynthetic rate (Pn) (**e**), and stomatal conductance (Gs) (**f**) in *Tinospora sagittata* plants after inoculation with different arbuscular mycorrhizal fungi. Different letters on the bar indicate significant ($p < 0.05$) differences.

Leaf gas exchange was also affected by AMF inoculations. *Ri*, *Fm*, *Dv*, and Mix inoculation significantly increased leaf Pn by 4%, 6%, 10%, and 23%, respectively, at the same time as leaf Tr by 129%, 66%, 40%, and 70%, respectively, as well as leaf Gs by 70%, 90%, 40%, and 100%, respectively, compared with non-AMF treatment (Figure 4d–f). *Dv* and Mix inoculation significantly increased leaf Pn by 10% and 23%, respectively, compared with non-AMF

treatment (Figure 4e). In addition, *Ri*, *Fm*, *Dv*, and Mix inoculation significantly reduced leaf Ci by 18%, 26%, 15%, and 32%, respectively, compared with non-AMF inoculation (Figure 4c).

3.5. Changes in Columbin Concentrations in Roots

Root columbin was found in both standard (Figure 5a) and root (Figure 5b) samples using HPLC. *Ri*, *Fm*, *Dv*, and Mix treatments significantly increased columbin concentrations in roots by 928%, 467%, 228%, and 320%, respectively, compared with non-AMF treatments (Figure 6a). In addition, soil hyphal length, but not root mycorrhizal colonization rate, significantly ($p < 0.01$) and positively correlated with root columbin concentrations (Figure 6b). Root columbin concentrations were also significantly ($p < 0.01$) positively correlated with root total length, root projected area, root surface area, root average diameter, and root volume, respectively (Table 1).

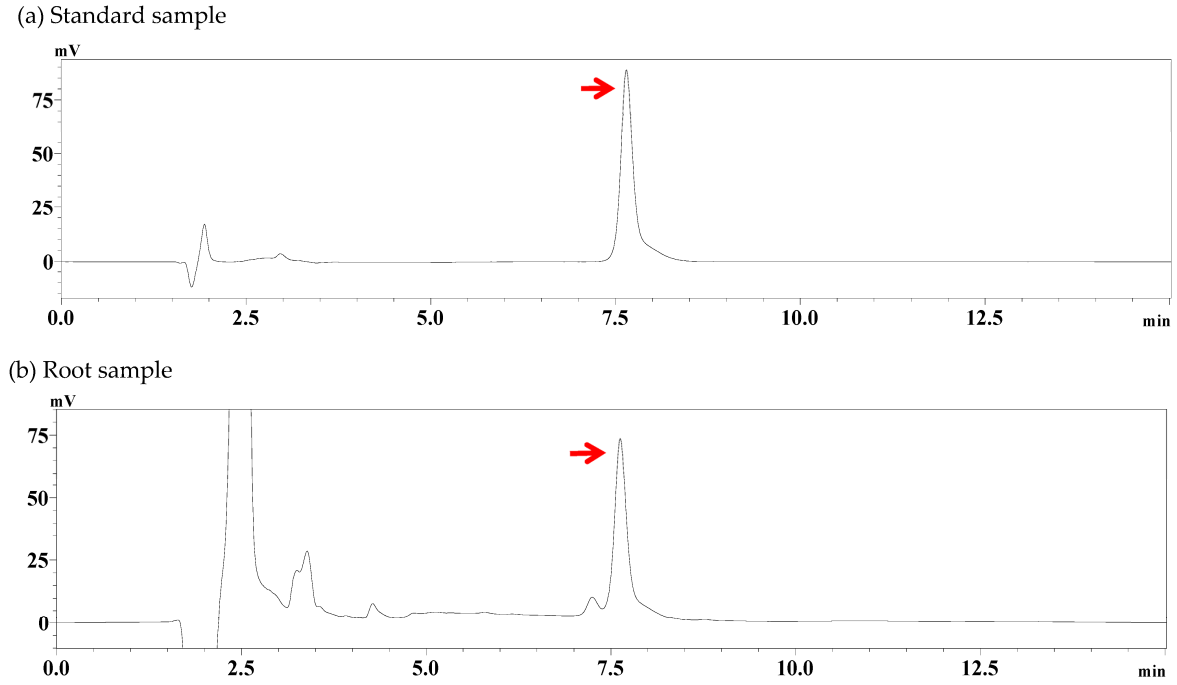

Figure 5. HPLC chromatogram of the standard sample (**a**) and root sample (**b**). Here, the red arrow represents the columbin.

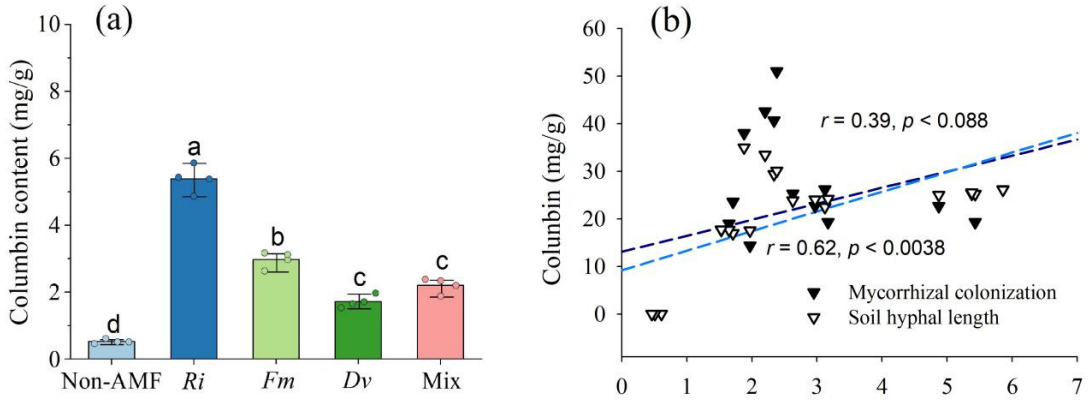

Figure 6. Changes in root columbin concentrations in *Tinospora sagittata* plants after inoculation with different arbuscular mycorrhizal fungi (**a**) and their correlation with mycorrhizal status (**b**). Different letters on the bar indicate significant ($p < 0.05$) differences.

Table 1. The correlation coefficient between root columbin concentrations and root morphological variables.

	Root Total Length	Root Projected Area	Root Surface Area	Root Volume	Root Average Diameter
Root columbin	0.92 **	0.73 **	0.87 **	0.89 **	0.64 **

** $p < 0.01$.

4. Discussion

This study showed that several AMF species colonized the roots of *T. sagittata* plants, with Mix inoculation having the highest root mycorrhizal colonization rate and soil hyphal length among the inoculated treatments and *Dv* treatment having the lowest. This suggests that mycorrhizal status in the roots and soil of *T. sagittata* plants is dependent on the AMF species, indicating compatibility between AMF and the host plant [31–33]. This result has also been reported on other plants, such as *Polygonum cuspidatum*, *Citrus sinensis*, *Camellia oleifera*, and *Vicia villosa* [21,34–36]. Indeed, differences in mycorrhizal status reveal a high degree of specificity and complex phenotypic characterization among mycorrhizal fungi [37].

The improvement of plant growth by AMF is partly attributed to the improved root morphology of the host, thus favoring nutrient and water uptake by the host [38]. In the present study, the root morphology of *T. sagittata* plants underwent significant improvement after AMF inoculation, as evidenced by the fact that both *Ri* and Mix treatments significantly increased root total length, projected area, surface area, volume, and average diameter, whereas *Fm* treatment significantly increased root surface area, average diameter, and volume, along with an increase in root volume and average diameter under *Dv* treatment. A similar result was also reported on *Polygonum cuspidatum* inoculated with *Fm*, *Serendipita indica*, and *Fm* + *S. indica*, as well as *Vicia villosa* inoculated with *Diversispora spurca*, *Fm*, *S. indica*, and *Ri* [21,36]. The growth performance of *T. sagittata* plants also showed differential changes after different AMF inoculations, with the *Ri* and Mix treatments showing significant improvement in growth and the *Dv* treatment being the worst. The response in root morphology and plant growth to AMF inoculation was almost identical to their mycorrhizal status. Sometimes, along with the increase in root mycorrhizal colonization, the growth of host plants such as *Panax notaginseng* showed a trend of first increasing and then gradually decreasing [39]. In fact, AMF improves plant growth and root morphology by increasing the levels of auxins, cytokinins, and polyamines in the host [40,41]. In addition, the function of AMF on the host is not exclusively measured in terms of improved plant growth and nutrient acquisition but should be determined by the phenotypic response of AMF and the host together with the symbiotic function of AMF [37].

AMF establish a well-developed mycorrhizal extraradical hyphal network on plant roots, thus increasing contact areas between roots and the soil [24]. It would promote nutrient (e.g., Fe and Mg) uptake in mycorrhizal plants, therefore increasing chlorophyll synthesis and photosynthetic efficiency in plants [42]. AMF also govern the efficiency of light energy utilization [36]. This study showed that, with the exception of *Dv*, all AMF inoculations significantly increased Chl, demonstrating that AMF may increase chlorophyll synthesis by promoting the levels of mineral elements, but this is dependent on the AMF species. As a result, AMF-inoculated plants can produce more photosynthetic products that can be allocated to various pools, including the mycorrhizal carbon pool, which continues to sustain mycorrhizal growth and functions [43]. Such a virtuous cycle provides for the functionality of arbuscular mycorrhizae. All AMF treatments increased Nbi, suggesting that AMF promote N accumulation in the host because mycorrhizal hyphae take up N directly [44], and AMF accelerate the glutamine synthase/glutamate synthase cycle for amino acid accumulation [45]. In addition, AMF inoculation significantly altered leaf gas exchange in *T. sagittata* plants, as evidenced by an increase in Tr and Gs as well as a decrease in Ci after all AMF inoculations, plus a significant increase in Pn triggered by *Dv* and Mix.

It demonstrated that AMF-modulated improvement in leaf gas exchange is dependent on the AMF species. AMF increased host plants' Pn, which is attributed to an increase in chlorophyll levels, photosynthetic activity of the chloroplasts, and the efficiency of light energy conversion [46].

Earlier studies have shown that AMF affect plant secondary metabolism and the accumulation of its products [47,48]. Mandal et al. [49] reported that AMF inoculation promoted artemisinin accumulation by up-regulating *1-deoxyxylulose 5-phosphate synthase* (*DXS*) and *1-deoxyxylulouse 5-phosphate reductoisomerase* (*DXR*) gene expression in *Artemisia annua* leaves and increasing glandular hair density and structure for the reservoir capacity. The inoculated patchouli plants had higher levels of total phenols and flavonoids than the uninoculated group [50]. The present study showed that AMF inoculation significantly increased root columbin concentrations in *T. sagittata* plants by 228% to 928%. This is in agreement with the findings of Liang et al. [51], who observed that the levels of hinesol and β-eudesmol increased in *Atractylodes lancea* plants inoculated with *Glomus etunicatum*, *G. tortuosum*, and *G. mosseae*. Among the tested AMF species, the effect of *Ri* was significantly greater than that of other AMF inoculations, implying that *Ri* is superior to mixed AMF treatment. However, *Ri*-inoculated *T. sagittata* plants did not present the highest root AMF colonization rate, but they had the highest root total length, projected area, surface area, average diameter, and volume. In contrast, mixed AMF-treated plants showed the highest root AMF colonization rate, but their root morphological variables were relatively lower than *Ri*-treated plants. We also observed that root columbin levels did not show a significantly positive correlation with root AMF colonization rate, but they were significantly ($p < 0.01$) positively correlated with root morphological variables (e.g., total length, projected area, surface area, volume, and diameter). This implies that it is not the root AMF colonization rate but the improvement of root morphology modulated by AMF that determines root columbin levels. The combination of *Fm* and *Piriformospora indica* raised root polydatin in *Polygonum cuspidatum* more than their single inoculation [24]. In addition, Zhou et al. [52] reported the diverse changes in alkaloid content of amur cork (*Phellodendron amurense*) seedlings after inoculation with *G. mosseae*, *G. etunicatum*, and *G. intraradices*, with *G. mosseae* having the most prominent effect on berberine and jatrorrhizine levels and *G. intraradices* having the most prominent effect on palmatine levels. This suggested the variable effects of single versus combined AMF treatments on plant secondary metabolite levels. Wagg et al. [53] proposed that combinations of AMF with great functional variability can play a more mycorrhizal role than a single arbuscular mycorrhizal fungus with a single function. However, it is unclear how AMF increased root columbin concentrations. Columbin is a furanolactone diterpene, and its synthesis pathway is still unclear. However, AMF can affect terpenoid synthesis by regulating C partitioning and gene expression of key enzymes in the terpene synthesis pathway [54]. At the same time, AMF are also involved in the transport and accumulation of terpenoids. Mycorrhiza-triggered changes in secondary metabolite levels may be due to mycorrhiza's improved uptake of plant mineral elements such as P and the regulation of carbohydrate distribution, as well as the expression of genes associated with the pathway of secondary metabolite synthesis [55]. Inoculation with AMF under field conditions is more effective in increasing plant growth and thus terpene content than potted experiments that allowed plant root growth and nutrients to be limited [56]. Therefore, AMF are more valuable for *T. sagittata* plants in the field.

5. Conclusions

This study confirmed that AMF inoculations improved the growth, root morphology, and leaf photosynthetic physiology of *T. sagittata* plants to varying degrees and also significantly increased root columbin levels, especially by 928% following *Ri* inoculation. This demonstrated the role of *Ri* as a dominant AMF strain in increasing root columbin levels. AMF inoculation, especially *Ri*, provides a feasible way to increase root columbin levels in *T. sagittata* plants in artificial cultivation. As medicinal plants are authentic in nature, it is also necessary to focus on screening the indigenous dominant AMF strains in their growing

environment, in combination with soil and climate conditions, to comprehensively carry out AMF inoculation in medicinal plants.

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