Ectomycorrhizal synthesis between four Bolete species and two kinds of trees

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Abstract

The cultivation of Bolete has very high economic value and ecological value. In order to select suitable symbionts for cultivation, we conducted greenhouse-based mycorrhizal experiments on four boletes(*Suillus bovinus*, *Suillus luteus*, *Suillus grevillei* and *Retiboletus sinensis*) and two plants(*Pinus thunbergii* and *Quercus acutissima*). All plant seedlings were either inoculated or not inoculated with the four Bolete liquid strains. Three months after inoculation, *Suillus bovinus*, *Suillus luteus*, *Suillus grevillei* and *Retiboletus sinensis* ectomycorrhizae were successfully formed on these two tree species, as evidenced by both morphological and molecular analyses. The mycorrhizal infection rate of all plants reached 40–55%, and the morphology of mycorrhiza is determined by the type of host plant and has nothing to do with the species of Bolete. Subsequently, plant growth, photosynthesis and endogenous hormone secretion were evaluated, mainly affected by host species. The infection of four boletes significantly promoted the growth and photosynthesis rate of host plants, the secretion of IAA, ZT and GA increased, and ABA decreased significantly. In addition, we found IAA in the fermentation broth of four kinds of Bolete. And *Retiboletus sinensis* can differentiate fruiting bodies by artificial pure culture.

Introduction

Ectomycorrhizal fungi (ECMF) have a 125 million-year history of origin. Although they are widespread in forest systems, they form ectomycorrhizas with only 3% of vascular plants. Nevertheless, they have effectively boosted the growth of various tree species, such as pines and oaks, contributing to overall forest health[1]. Edible mycorrhizal fungi (EMF) are a subset of ectomycorrhizal fungi, characterized by their edible sporocarps. These fungi play a crucial role in ecological restoration and support local economies[2]. In some regions of China with abundant forest resources, sales of these valuable wild edible fungi may account for more than half of local residents' income. In fact, in some cases, mushroom-based ecosystems provide far greater economic benefits than traditional wood industries[3]. Examples include *Bolete edulis* and others[4]. EMF are not only a delicious food source but also an important provider of minerals and non-meat amino acids[5, 6].

ECMF enhance plant resistance to heavy metal and drought stress. In soils contaminated with heavy metals, plant species are increasing as fungal species adapt to the environment[7]. Interestingly, in some cases, the presence of heavy metals is not only harmless but also beneficial to fungal growth, increasing fungal diversity and promoting plant growth[8]. ECMF exhibit strong ecological adaptability and plasticity, tolerating drought and high-temperature environments. Since the 1960s, researchers have found that ECMF play a role in promoting the drought resistance of host plants. *Cenococcum geophilum*, for instance, can form ECMF with various trees under extreme drought conditions. *Pisolithus arhizus* also possesses strong drought resistance, and ectomycorrhizal fungi can tolerate the low water potential of the culture substrate[9].

The enhanced performance of mycorrhizal plants under drought stress is often linked to the improvement of root water absorption by regulating plant aquaporins and hormones through fungi [10]. Plant hormones refer to trace organic compounds synthesized by plants themselves, which produce physiological effects at their synthesis sites or are transported to other parts, playing a crucial role in regulating plant growth and development. The infection of host plant roots and establishment of symbiotic relationships directly or indirectly induce the synthesis of multiple hormones[11], including gibberellins, auxins, cytokinins, ethylene, abscisic acid, brassinosteroids, jasmonic acid, and strigolactones. Due to the synergistic and antagonistic effects among various hormones, hormones also exhibit a complex mechanism for establishing mycorrhizal symbiosis, with each hormone in a dynamic equilibrium state to jointly regulate the normal growth and development of plants.

Earlier studies discovered that ectomycorrhizal fungi can secrete indole compounds[12]. Moreover, ectomycorrhizal fungi such as *Laccaria bicolor*, *Tuber borchii*, and *Tuber melanosporum* can produce a significant amount of auxin IAA, causing morphological changes in symbiotic plant roots. This change results from direct contact between ectomycorrhizal fungi and plant roots or indirect diffusion signals of fungi[13, 14]. In mycorrhizal symbionts of poplar, it was observed that fungal hyphae inhibited the growth of the main roots of symbiotic plants and increased the growth of lateral roots. This effect was similar to that of exogenous auxin treatment on plant roots[13, 14]. In addition to auxin, *L. bicolor* can also produce ethylene, which activates the plant auxin synthesis pathway. Ethylene production by ectomycorrhizal fungi may induce auxin production in their symbiotic plants, thereby enhancing the effect of auxin on root development, particularly promoting lateral root formation. The formation of new lateral roots can lead to the development of more mycorrhizas.

Mycorrhizal synthesis is the first step in developing cultivation programs and exploring the potential for different combinations of host plants and EMF[15]. The traditional and most popular method of raising seedlings in China involves adding fruiting body isolates (mixed, dumped, or injected) to the substrate before or after sowing[16, 17]. In this study, we determined the hormone contents in the pure culture of four types of mushrooms, namely *Suillus bovinus, Suillus luteus, Retiboletus sinensis*, and *Suillus grevillei*. Simultaneously, we discussed the effects of their mycorrhizal synthesis with pine and oak on plant growth and development.

Materials and methods

Mycelia culture

Four strains were isolated from fruiting bodies using tissue isolation method(Table 1) and maintained by subculturing every 2–3 months on Modified Nutrient Catabolite agar (MNC) or modified Potato Glucose agar (PDA)[18]. The modified PDA medium(potato 200 g/L, glucose 20 g/L, peptone 7.88 g/L, K_2HPO_4 1 g/L, $MgSO_4$ 0.9 g/L, $CaCl_2$ 0.05 g/L, NaCl 0.075 g/L, β-cyclodextrin 16.77 g/L, ascorbic acid 0.0055 g/L). The modified MNC medium:(KH_2PO_4 1 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, 0.2% $ZnSO_4$ 0.5 mL, ammonium

tartrate 0.5 g/L, 1%ferric citrate 0.5 mL, thiamine 50 μg /L, casein hydrolysate 0.23 g/L, yeast extract 0.5 g/L, glucose 10 g/L)Removal of agar from modified PDA or MNC medium to prepare liquid spawn, incubated at 22℃ thermostatic oscillator for 20 days at a speed of 150 r/min.

Table 1
Four strains tested in this study

Strain number in laboratory	Species	Location	GeBank number
S1	*Suillus bovinus*	Kunyushan	OM846602
S2	*Suillus luteus*	Kunyushan	OM846601
S3	*Suillus grevillei*	Xiaoyuanmiao	OM865366
R1	*Retiboletus sinensis*	Xiaoyuanmiao	OL339344

Preparation of host plant seedlings

Fruits of *Pinus thunbergii* and *Quercus acutissima* were collected from the Ludong University inner mountain. Seeds were extracted from ripe fruits and rinsed with sterile water for 4 hours. Next, seeds were soaked in a 1 mg/L GA3 solution for 2 days, with the water solution changed every 8 hours in a refrigerator to break dormancy. These seeds were used immediately after treatment to prevent damage from long-term storage. After soaking, non-dormant seeds were transferred to an antiseptic solution containing sodium hypochlorite (2% available chlorine) and 0.01% polyoxyethylene sorbitan monooleate (Tween 80) (*P. thunbergii* seeds soaked for 0.5 h and *Q. acutissima* seeds soaked for 2 h). After being thoroughly rinsed in sterile water, the two types of seeds were sown separately in autoclaved substrate composed of vermiculite and water (1:1 by volume) in large sterilized glass jars in April 2021.

Inoculation methods

After one month, a selection of healthy, vigorous seedlings was transferred to the bottom of straight-sided, polycarbonate-based, 500 mL wide-mouth transparent autoclaved jars. These jars contained a substrate composed of vermiculite, perlite, and vermiculite (2:1:1 by volume) and were inoculated with fungi. For each tree species, seedlings were inoculated with 100 mL of liquid spawn per plant. All the jars were fitted with four aeration holes sealed with a fluorocarbon membrane filter (pore size, 0.45 μm).

Seedlings were axenically given 10–20 mL of deionized water per month to compensate for water loss due to evaporation.

Determination of plant morphology and physiology

Mycorrhizal infection rate

The mycorrhizal infection rate was determined by the grid crossing method after six months of cultivation using three inoculation methods. The mycorrhizal length was denoted as 'a', root length as 'b', and the mycorrhizal infection rate was calculated as c = a / b.

Determination of photosynthetic parameters

Twelve months after mycorrhizal synthesis, photosynthetic rates and photosynthetic pigment of all seedlings were measured in June 2022 using a photosynthetic rate instrument.

Determination of fermentation broth of 4 liquid strains and host plant hormone

Fermentation Broth of Mushrooms: 20 mL of fermentation broth from each of the four types of bolete was accurately extracted using a pipette, and an 80% methanol aqueous solution stored at 3°C was added. The mixture was then stored in a refrigerator at 3°C for 8 hours.

Plant Samples: The roots, stems, and leaves of *Pinus thunbergii* and *Quercus acutissima* were washed in running water for 30 minutes, and then washed three times with pure water to remove any residue. Two grams of *Pinus thunbergii* and *Quercus acutissima* were accurately weighed using an analytical balance and ground thoroughly in a mortar under a liquid nitrogen environment. An 80% methanol aqueous solution stored at 3°C was added and the mixture was refrigerated at 3°C for 8 hours.

The pretreated samples were placed in a low-temperature centrifuge at 4°C and 9,000 rpm. After centrifugation for 10 minutes, the supernatant was collected in a new centrifuge tube and stored in a refrigerator at 3°C. The remaining precipitate was further extracted with 80% methanol aqueous solution for 4 hours, followed by centrifugation at 4°C and 9,000 rpm for 10 minutes. The supernatants from both centrifugations were combined. After removing the methanol in the supernatant using a nitrogen blow dryer, the remaining liquid was combined with petroleum ether in a separation funnel for extraction and decolorization three times. The pH of the solution was adjusted to 3 using 1 mol/L citric acid, and ethyl acetate was added for three extractions. The organic extracts from the three extractions were combined. Ethyl acetate was dried using a nitrogen blow dryer, dissolved in 1 mL methanol, and filtered through a 0.22 μm needle organic filter.

The chromatographic column used was a ZORBAX SB-C18 (4.6 mm, 250 mm, 5 μm), and the mobile phase was methanol-formic acid gradient elution. The flow rate was 1.0 mL/min, the column temperature was 25°C, the injection volume was 20 μL, and the detection wavelengths were 260 nm and 220 nm. HPLC gradient elution system are shown in Table 2.

Table 2
HPLC gradient elution system

Time	Methanol	0.06%Formic
0–15 min	48.7%	51.3%
15–20 min	50.7%	49.3%
20-30min	50.8%	49.2%

Results

The results of ITS sequence alignment and pure culture of four kinds of Bolete

The mycelia of four bolete species were obtained through tissue isolation and purification. After two months of mycelium culture, the ITS sequences were acquired by amplifying the DNA of the mycelium, followed by BLAST alignment in GenBank. The ITS sequences of strains S1, S2, and R1 (OM846602, OM846601, OL339344) showed 99.69%, 99.32%, and 100% homology with the Chinese specimens of *S. bovinus*, *S. luteus*, and *R. sinensis* [19–21], respectively. The ITS sequence of S3 (OM865366) showed 99.03% homology with the Japanese *S. grevillei*[22]. The original fruiting bodies were sampled from coniferous and broad-leaved mixed forests in Yantai, China, at Xiaoyuanmiao Forest Farm and Trapped Mountain Forest Farm (Table 1). One representative strain was registered in the China General Microbiological Culture Collection Center (CGMCC No. 23887).

The three Bolete species have similar mycelial morphology. The mycelia are white and dense, primarily comprising creeping mycelium, and secreting brown substances during the later stages of culture (Fig. 1a-c). The mycelia of *Retiboletus sinensis* are light yellow, mainly consisting of erect mycelium, and also secreting brown substances during the later stages of culture. During the pure culture process, the mycelium was stimulated by temperature and scattered light. It could produce primordium and fruiting bodies independently from symbiotic plants. The primordium of the fruiting body was light yellow and millet grain-shaped, the pileus was light yellow during the early stage of fruiting body differentiation, and the stipe was white with villi (Fig. 1d-f).

Observation of mycorrhizal appearance morphology

The appearance of Bolete mycorrhiza was observed using a stereoscope. The mycorrhiza formed by the combination of four types of mushrooms and black pine (Fig. 2a-d) was predominantly binary branching structure, with a small amount of rod-like structure mycorrhiza. This rod-like structure was likely an unbranched or soon-to-be bifurcated binary branching structure. A small amount of hyphae were seen on the surface of the mycorrhiza, and there was no apparent fungal sheath. Mycorrhizal branch length ranged from 0.6 to 1.23 mm, with a thickness of 0.22 to 0.29 mm.

Observing the mycorrhizal fungi combined with *Q. acutissima* (Fig. 2e-h), it was found that they were all rod-like structures, and no two-branched structure similar to *Q. acutissima* mycorrhizal was found. The hyphae on the surface of mycorrhizal were visibly entangled. The length of mycorrhiza ranged from 0.33 to 0.96 mm, with a thickness of 0.05 to 0.18 mm. From an appearance perspective, the rod-shaped mycorrhizal size of *Q. acutissima* was significantly smaller than that of *P. thunbergii*, but the mycorrhizal density of *Q. acutissima* was much higher than that of *P. thunbergii* (Fig. 3, Fig. 4). The mycorrhizal morphology is not related to the species of boletes, but is determined by the species of symbiotic plants.

The mycorrhizal infection rates of four types of mushrooms (*Suillus bovinus*, *S. luteus*, *S. grevillei*, and *Retiboletus sinensis*) using liquid inoculation aseptic culture method were as follows: *Pinus thunbergii* – 49.7%, 54.97%, 52.3%, and 43.4%; *Quercus acutissima* – 54.83%, 59.6%, 48.43%, and 55.37%.

Plant appearance and biomass

The appearance of the two plants changed after inoculation. The roots of *Pinus thunbergii* and *Quercus acutissima* were significantly more developed than those of uninoculated seedlings, and there were more lateral roots. By measuring their biomass, it was found that the biomass of *Pinus thunbergii* and *Quercus acutissima* increased significantly (Fig. 5), the biomass of *Pinus thunbergii* increased by 25%, and the biomass of *Quercus acutissima* increased by 32%.

Determination of photosynthetic parameters

By measuring the photosynthetic rate of *Pinus thunbergii* and *Quercus acutissima*, we found that the photosynthetic rate of plants inoculated with bolete was significantly increased ($P < 0.05$). The photosynthetic rate of *Pinus thunbergii* increased by 51%-93% compared with the control group CK without inoculation. The photosynthetic rate of *Pinus thunbergii* increased by 93% after inoculation with *R. sinensis*, and the lowest increase was 51% in *S. grevillei*. After the same inoculation, the photosynthetic rate of *Q.acutissima* was also significantly increased by 35%-72%, of which *S. bovinus* increased the highest by 72%, and *R. sinensis* was the lowest by 35% (Fig. 6).

Hormone content in Bolete fermentation broth

After 20 days of liquid fermentation of the mycelia, the fermentation broth of the four mushrooms was brown to dark brown (Fig. 7), with a strong aroma. High-performance liquid chromatography revealed that the fermentation broth contained a large amount of IAA, while the other three hormones (ZT, GA, ABA) were not detected. The IAA content of Suillus luteus was the highest, reaching 455.77 μg/L; the contents of the other three species were relatively low, which were *Suillus bovinus* 246.67 μg/L, *Suillus grevillei* 215.39 μg/L, and *Retiboletus sinensis* 157.25 μg/L, respectively. There was no significant difference in IAA content in the three fermentation broths by one-way ANOVA (Fig. 8). The root changes of *Q. acutissima* seedlings after irrigation with fermentation broth were equivalent to the effect of IAA in tissue culture (Fig. 9).

Hormone contents of Seedling

Compared to the uninoculated *Quercus acutissima* seedlings, the total IAA content in seedlings inoculated with four types of mushrooms (*Suillus bovinus*, *Suillus luteus*, *Suillus grevillei*, and *Retiboletus sinensis*) increased by 27.1%, 29.5%, 25.2%, and 30%, respectively(Fig. 10). Similarly, the total ZT content in inoculated seedlings increased by 25.4%, 34.8%, 34.6%, and 36.7%, respectively. Among various plant hormones, GA content exhibited the most significant change, with total GA content increasing by 86.3%, 95.1%, 95.7%, and 83.1%, respectively. In contrast to the changes in IAA, ZT, and GA, ABA content in inoculated seedlings was much lower than that in control seedlings, with total ABA content in roots, stems, and leaves decreasing by 22.7%, 23.3%, 23.1%, and 26.8%, respectively.

The ratio of total GA to total ABA (GA/ABA) in mycorrhizal seedlings was significantly higher than that in sterile root seedlings ($p < 0.05$). The GA/ABA ratio for sterile root seedlings was 0.31, whereas the GA/ABA ratios for mycorrhizal seedlings of *Suillus bovinus*, *Suillus luteus*, *Suillus grevillei*, and *Retiboletus sinensis* were 0.74, 0.78, 0.78, and 0.76, respectively, with no significant difference. The IAA/ABA ratios for mycorrhizal seedlings were 5.50, 5.64, 5.44, and 5.98, respectively, which were significantly higher than the 3.34 ratio observed in sterile seedlings. The ZT/ABA ratios for mycorrhizal seedlings were 4.55, 4.93, 4.91, and 5.24, respectively, which were significantly higher than the 2.81 ratio observed in sterile seedlings. The IAA/GA ratios for mycorrhizal seedlings were 7.46, 7.36, 6.99, and 7.83, respectively, which were significantly lower than the 10.95 ratio observed in aseptic seedlings. The IAA/ZT ratios for mycorrhizal seedlings were 1.21, 1.44, 1.11, and 1.14, with no significant difference compared to the 1.19 ratio observed in sterile root seedlings.

The contents of IAA, ZT and GA in *Pinus thunbergii* seedlings inoculated with 4 kinds of bolete increased, and the content of ABA was inhibited(Fig. 11). Compared with CK, the total content of IAA in seedlings inoculated with four kinds of bolete increased by 28.7%, 30.8%, 29.8% and 33% respectively. The total ZT content increased by 28.1%, 28.05%, 38.5% and 42.5%, respectively. The total GA content increased by 98.1%, 90%, 102.8% and 102%, respectively. On the contrary, the ABA content of *Pinus thunbergii* seedlings inoculated with four kinds of boletes was significantly lower than that of the control group, and the total content was reduced by 24.1%, 26.9%, 22.6% and 29%, respectively.

The ratio of total GA to total ABA (GA/ABA) in mycorrhizal seedlings was significantly higher than that in sterile root seedlings ($p < 0.05$). The ratio of GA / ABA in sterile root seedlings was 0.14, and the ratio of GA/ABA in mycorrhizal seedlings of *Suillus bovinus, Suillus luteus, Suillus grevillei, Retiboletus sinensis* was 0.38, and there was no significant difference. The IAA/ABA of mycorrhizal seedlings were 3.43,3.62,3.39,3.53, respectively, which were significantly higher than 2.02 of aseptic seedlings. The ZT/ABA of mycorrhizal seedlings were 2.56,2.66,2.55 and 2.84, respectively, which were significantly higher than 1.52 of sterile seedlings. The IAA/GA of mycorrhizal seedlings were 8.42,9.52,8.79 and 8.31, respectively, which were significantly lower than 15.96 of aseptic seedlings. The IAA/ZT of mycorrhizal seedlings were 1.34,1.36,1.32,1.24, and the sterile root seedlings were 1.33, with no significant difference.

Bolete mushrooms have a growth-promoting effect on plant seedlings (*Quercus acutissima* and *Pinus thunbergii*). After inoculation with bolete mushrooms, the seedlings' growth hormone levels undergo significant changes.

After inoculation with bolete mushrooms, the seedlings' indoleacetic acid (IAA), zeatin (ZT), and gibberellic acid (GA) levels generally increase, while abscisic acid (ABA) levels decrease. This suggests that bolete mushrooms form a symbiotic relationship with the plant seedlings, promoting plant growth. The seedlings' GA/ABA, IAA/ABA, and ZT/ABA ratios significantly increase. This implies that the ratio of growth-promoting hormones (such as GA, IAA, and ZT) to growth-inhibiting hormones (such as ABA) increases, potentially enhancing the growth rate of plant seedlings.

For mycorrhizal seedlings, the IAA/GA ratio significantly decreases, which may help regulate plant growth and maintain a balance between growth rate and structural development. However, there is no significant difference in the IAA/ZT ratio between the two types of seedlings, indicating that the relative proportion between growth hormone and cytokinin remains relatively stable.

Discussion

This study represents the first report on the secretion of indole compounds in the fermentation broth of four bolete species and the use of sterile *Pinus thunbergii* and *Quercus acutissima* seedlings to form mycorrhiza. We describe the appearance of mycorrhiza in detail and examine the physiological changes in symbiotic plants. We also discovered that *Retiboletus sinensis* can produce fruiting bodies under pure culture conditions, which is a significant finding in the field of bolete cultivation, providing valuable material for studying the artificial cultivation of boletes. Our future work will further investigate this species.

Previous studies have found that ectomycorrhizal fungi (ECMF) can secrete indole compounds, which strengthen the fungi's effect on root development, particularly promoting the formation of lateral roots and the creation of more mycorrhizas. Additionally, IAA secreted by ECMF participates in the formation of the Hartig network as a diffusion signal[23, 24]. In this study, we measured the presence of IAA in the fermentation broth of the four boletes, confirming our hypothesis that these boletes can secrete IAA to participate in the mycorrhizal colonization process, similar to other reported ECMF. Many microorganisms capable of secreting IAA and IAA precursors form beneficial relationships with plants, altering host root development. Furthermore, other signals produced by microorganisms can affect hormone content in host plants[25].

In this study, four bolete species formed mycorrhizal symbionts with *Pinus thunbergii* and *Quercus acutissima*. *Quercus acutissima* mycorrhiza is predominantly rod-shaped, while *Pinus thunbergii* mycorrhiza mostly exhibits a binary branching structure (Fig. 5). This binary branching structure appears to be an inherent feature of pine plants[26–28], likely resulting from changes in plant hormones caused by fungal secretion of hormones or other signals[29, 30]. However, the specific reasons for this change remain

unclear. This might explain why the roots of inoculated seedlings were more developed than those of the control group (Fig. 6, Fig. 7).

ECMF holds significant ecological importance, particularly in areas with poor site conditions and fragile ecological environments. Seedling growth quality directly determines the success of afforestation, and growth indicators more intuitively reflect seedling quality. Studies have found that most woody plants in northern temperate forests form mutually beneficial symbionts with ECMF, exchanging nutrients and water for carbon (C) fixed by photosynthesis [1]. Due to the prevalence of this symbiotic interaction, ECMF potentially plays a critical role in ecosystem restoration and regulation[31]. However, the improvement of plant nutrition caused by this interaction comes at a cost. Mycorrhizas absorb approximately half of photosynthetic products, and plants meet the high carbohydrate requirements of mycorrhizal symbionts by enhancing their photosynthetic intensity[32]. In this study, four bolete species increased the photosynthetic rate of *Pinus thunbergii* and *Quercus acutissima* seedlings by 51% − 93%, consistent with previous findings. We determined the contents of IAA, ZT, GA, and ABA in the roots, stems, and leaves of plants. IAA, primarily indolebutyric acid, promotes robust plant growth[33, 34]. In this study, the IAA content increased in the roots, stems, and leaves of plants, with the highest concentration in the roots. When fungi promote plant growth, they initially help plants build more developed roots. As previously mentioned, IAA secreted by fungi also assists plants in forming more lateral roots, which can better help plants absorb more nutrients and water. ZT regulates the opening and closing of plant stomata and controls the rate of photosynthesis[35]. In this study, the ZT content in the roots, stems, and leaves of inoculated plants increased to varying degrees. Although the ZT content in some stems or leaves was not significantly different from the control group, it sufficiently demonstrated that fungal invasion affected the ZT content in their symbiosis, thus affecting the synthesis of photosynthesis-related organic matter. This may be one of the reasons for the changes in plant photosynthetic rate mentioned above.

There is a synergistic effect between GA and IAA, which can regulate IAA content, prevent organ detachment, and break seed dormancy[36]. GA is involved in regulating various essential processes in plant growth and development, including photosynthesis[37]. In this study, the content of GA in leaves of the two plants changed the most, while the content in roots and stems changed relatively little, which also led to the increased photosynthetic rate of plants inoculated with fungi. ABA can promote plant root growth, establish a solid underground root system for plants to overcome survival challenges under environmental stress, and provide a guarantee for plants to increase the root absorption area[38, 39]. In this study, the ABA content in the roots, stems, and leaves of the two plants inoculated with the four boletes was lower than that of the control group, and most of them reached a significant difference level. The above results showed that the hormone content in plants changed significantly after inoculation with 4 kinds of boletes, which directly or indirectly led to the changes of morphology and biomass of symbiotic plants.

In this paper, we studied the physiological effects of four boletes on *Pinus thunbergii* and *Quercus acutissima* from the perspective of plant growth regulators. In the future, we will strengthen research on

related gene expression and signal transduction. In summary, this study provides data support for further research in the future and a theoretical basis for the application of ectomycorrhizal science.

Declarations

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Author contributions

TAN Qianwen and YOU Lunhe wrote the main manuscipt text and they contributed equally to this work and should be considered co-first authors.

WANG Jianrui and HAO Chen prepared figures 1-11.

Liu Yu provided the research idea for this study.

All authors reviewed the manuscript

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Availability of data and materials

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Ethics approval and consent to participate

Not applicable.

Consent for publication

N applicable.

Competing interests

The authors declare that they have no competing interest.

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Figures

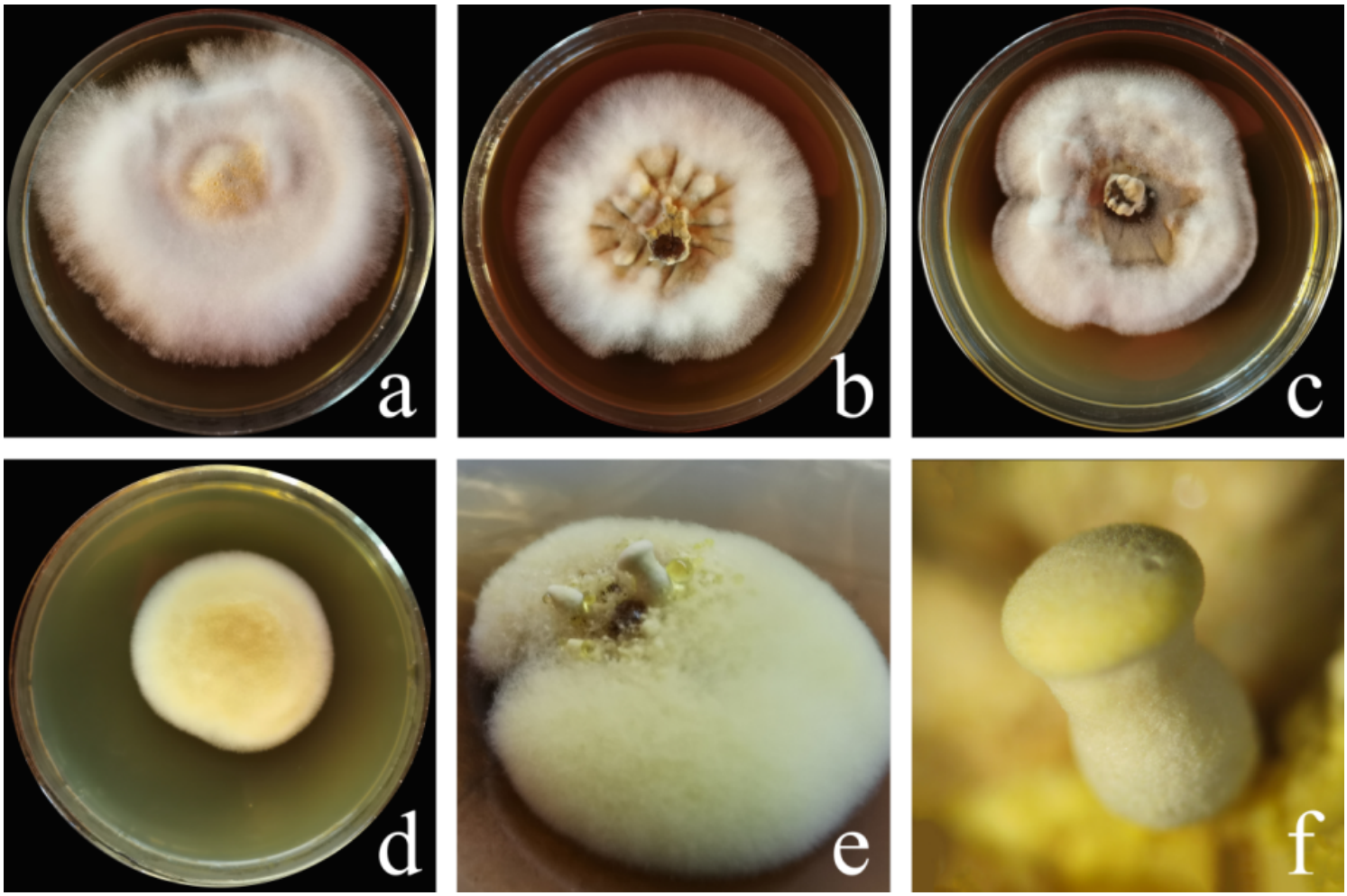

Figure 1

Mycelia of 4 kinds of Bolete (a. *Suillus bovinus*. b. *Suillus luteus*. c. *Suillus grevillei*. d. *Retiboletus sinensis*. e-f. Fruiting body of *Retiboletus sinensis*.)

Figure 2

Mycorrhiza morphology diagram (a-d. *Pinus thunbergii* mycorrhizal. a. *suillus bovinus*. b. *Suillus luteus*. c. *Suillus grevillei*. d. *Retiboletus sinensis*. e-h. *Quercus acutissima*mycorrhiza. e. *suillus bovinus*. f. *Suillus luteus*. g. *Suillus grevillei*. h. *Retiboletus sinensis*)

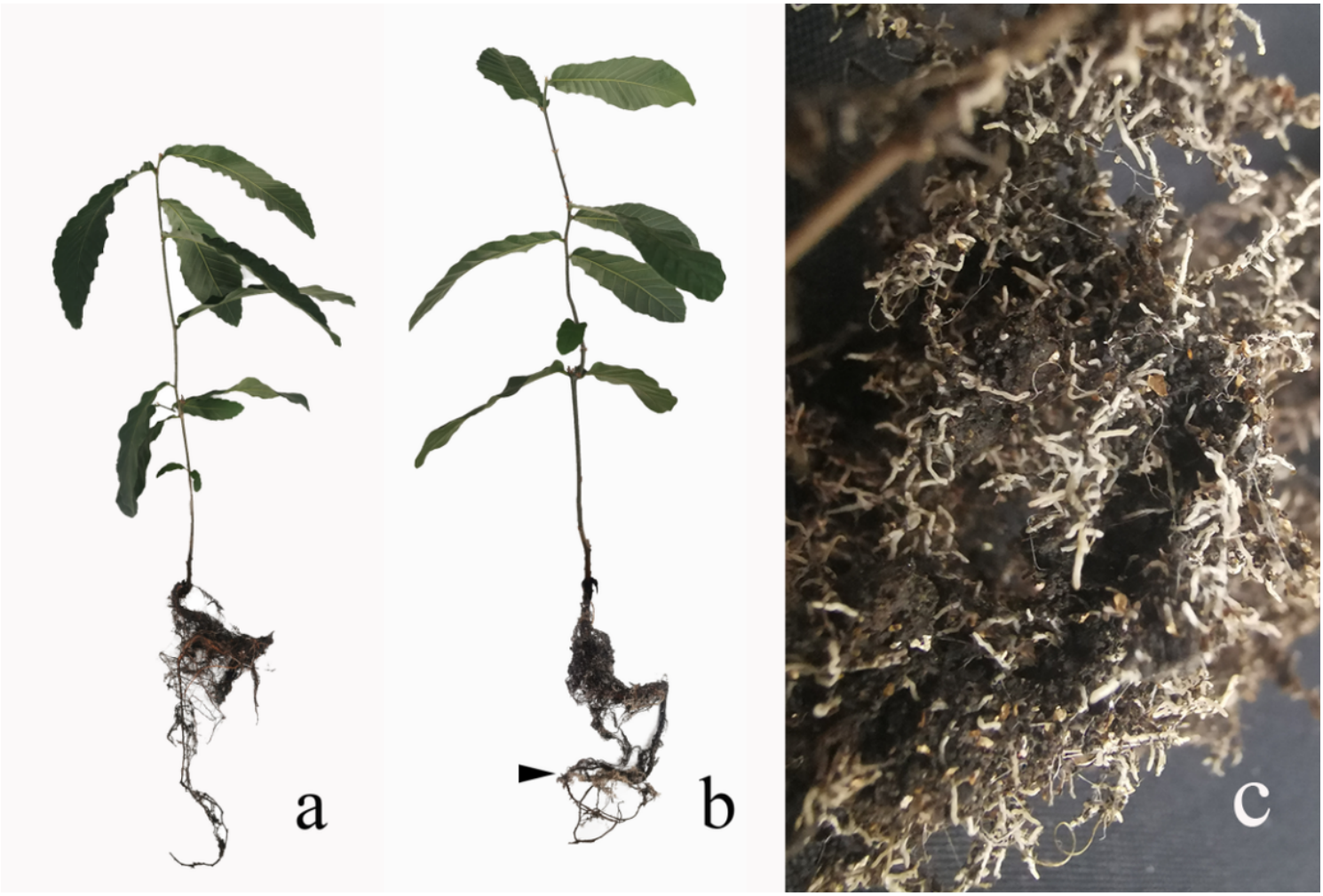

Figure 3

Quercus acutissima seedling shape picture (a. Arbuscular mycorrhizal *Quercus acutissima.* b.Mycorrhizal *Quercus acutissima.* c. Mycorrhizal *Quercus acutissima*)

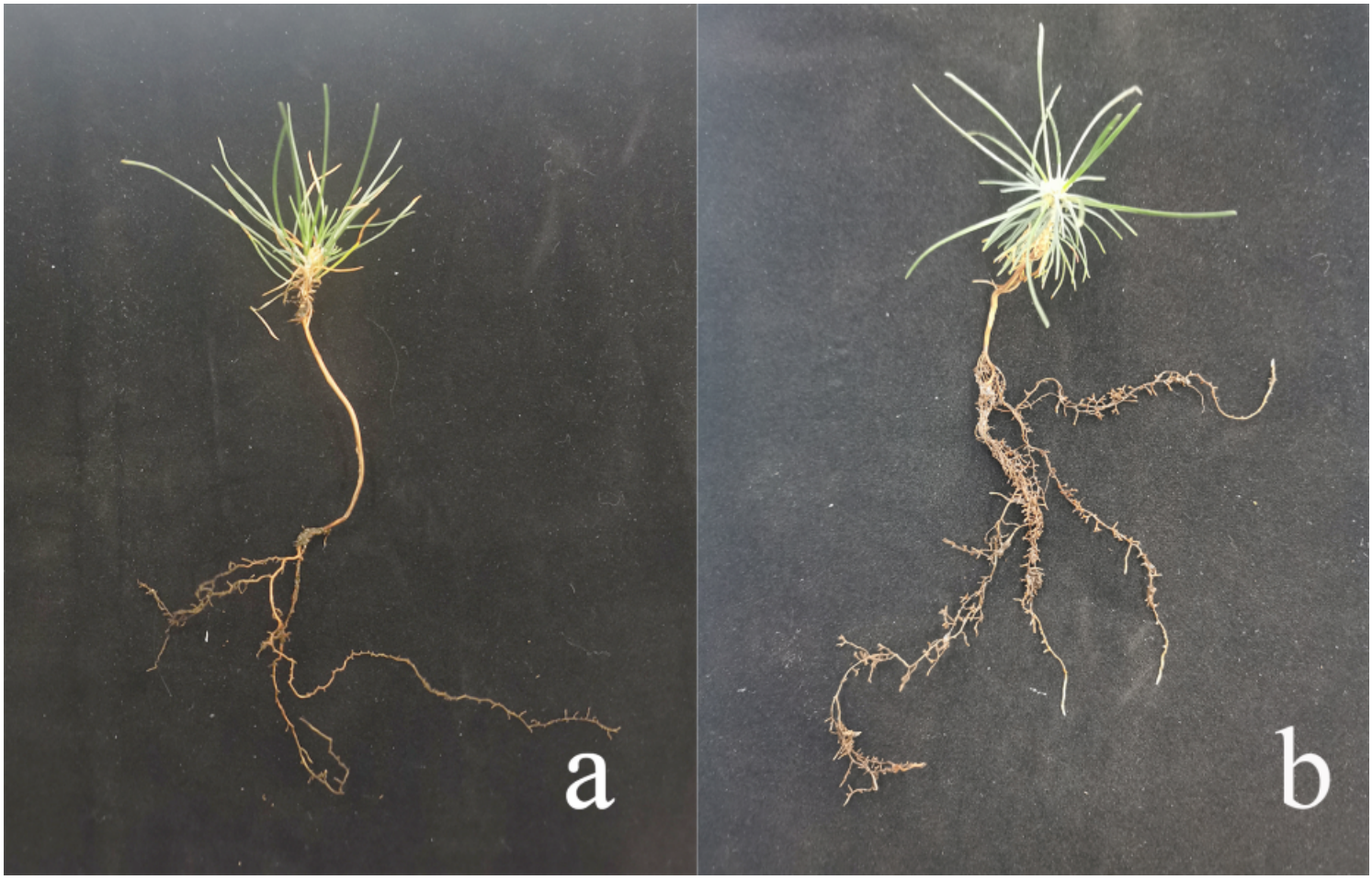

Figure 4

Pinus thunbergii seedling shape map (a: Non-mycorrhizal *Pinus thunbergii* , b: Mycorrhizal *Pinus thunbergii*)

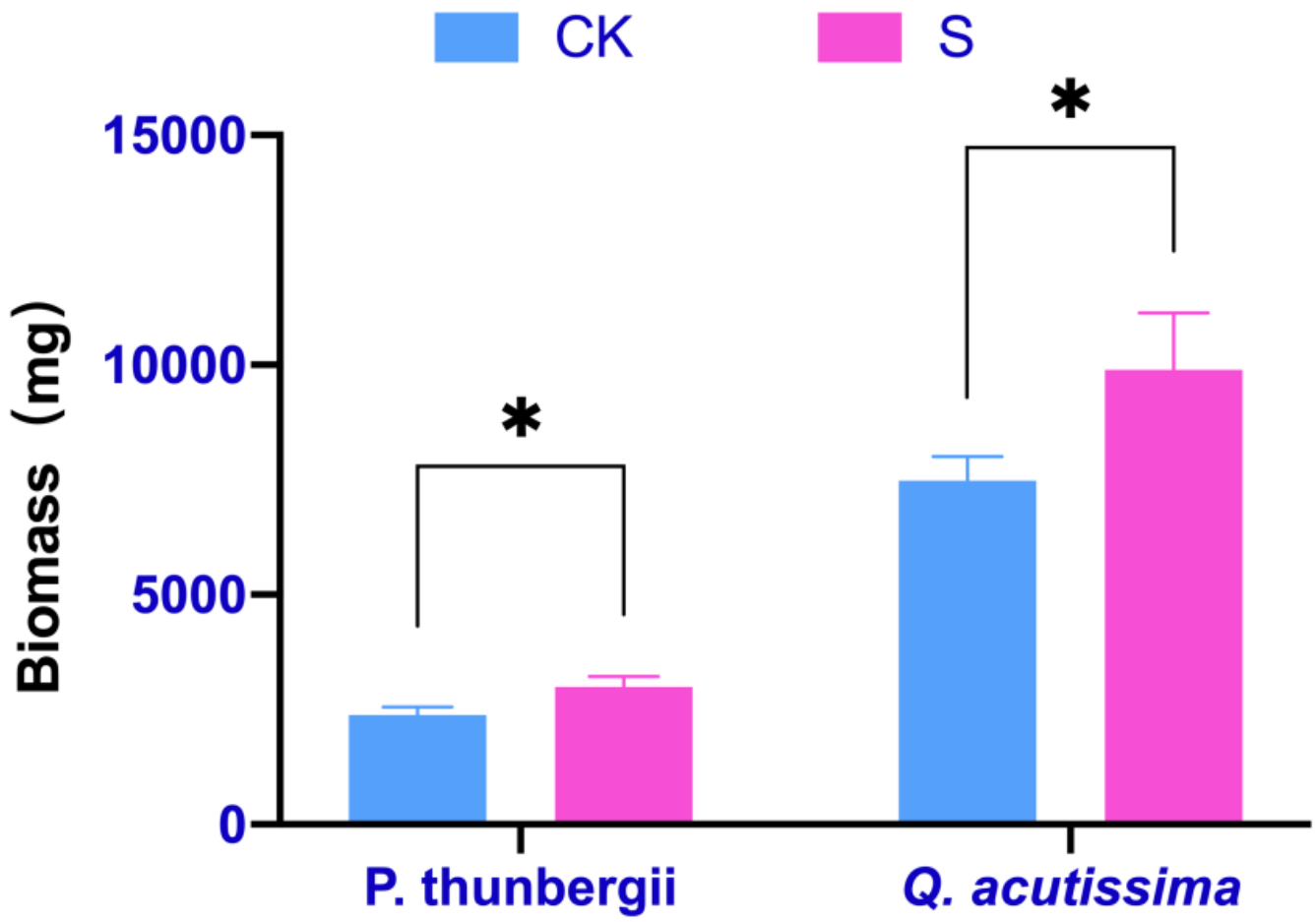

Figure 5

Biomass of plants CK.Arbuscular mycorrhizal plants. S.Mycorrhizal plants. * p<0.05

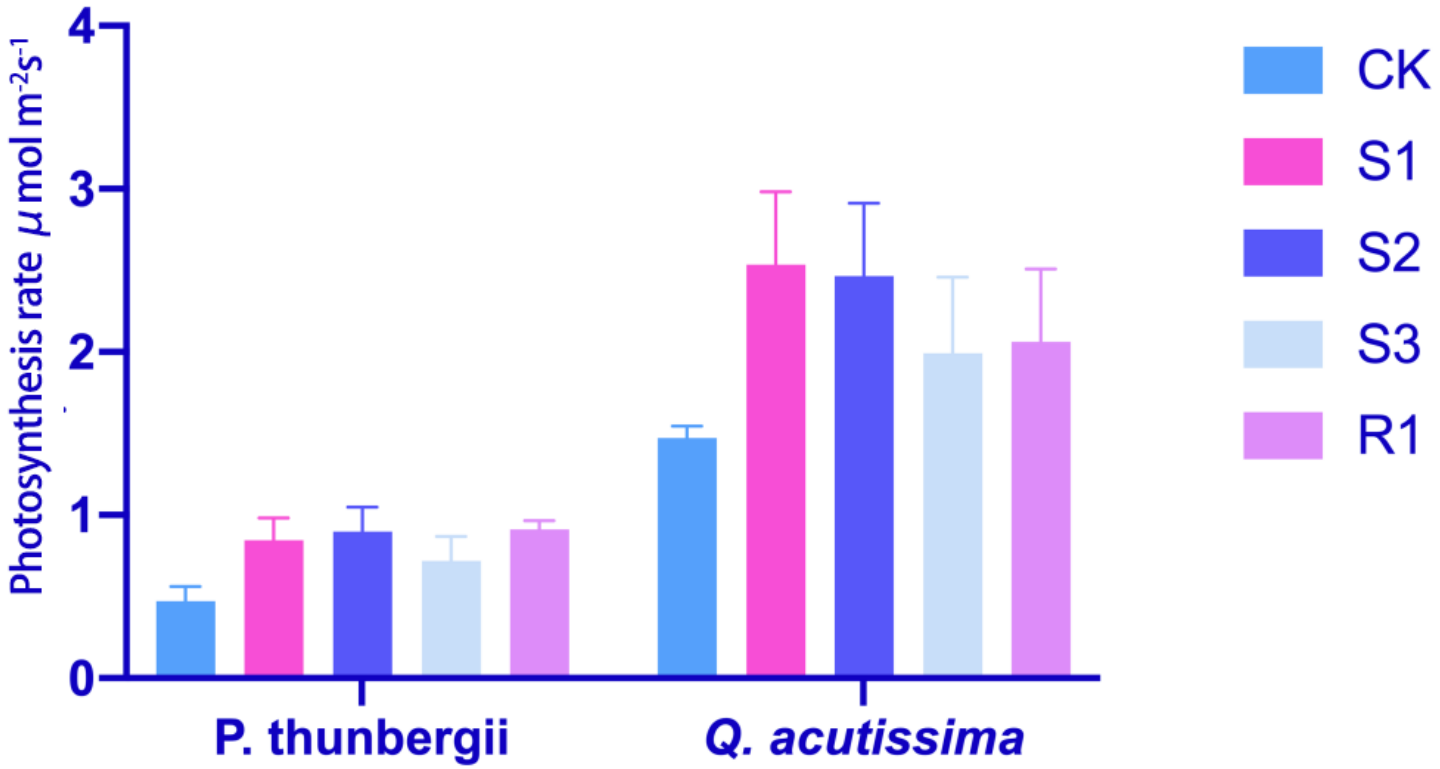

Figure 6

Photosynthetic rate of *Pinus thunbergii* and*Quercus acutissima* (CK.Arbuscular mycorrhizal*Pinus thunbergii* and *Quercus acutissima.* S1 .*Suillus bovinus*. S2.*Suillus luteus*. S3.*Suillus grevillei*. R1. *Retiboletus sinensis*)

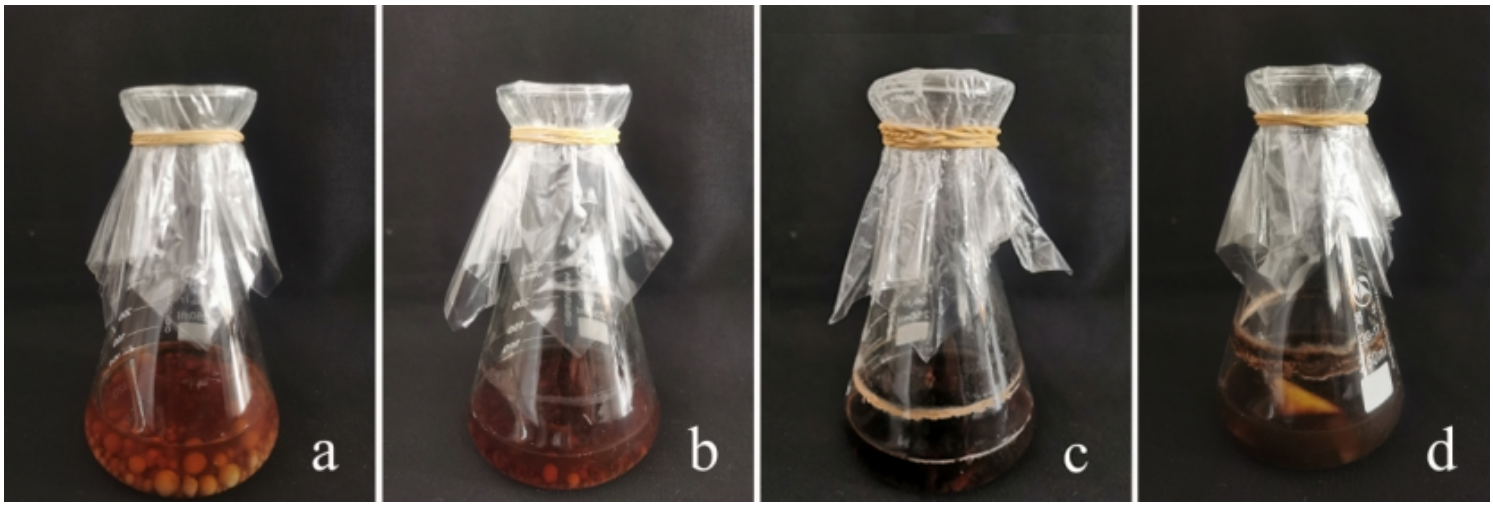

Figure 7

Liquid strain (a.*Suillus bovinus*. b.*Suillus luteus*. c.*Suillus greville*. d.*Retiboletus sinensis)*

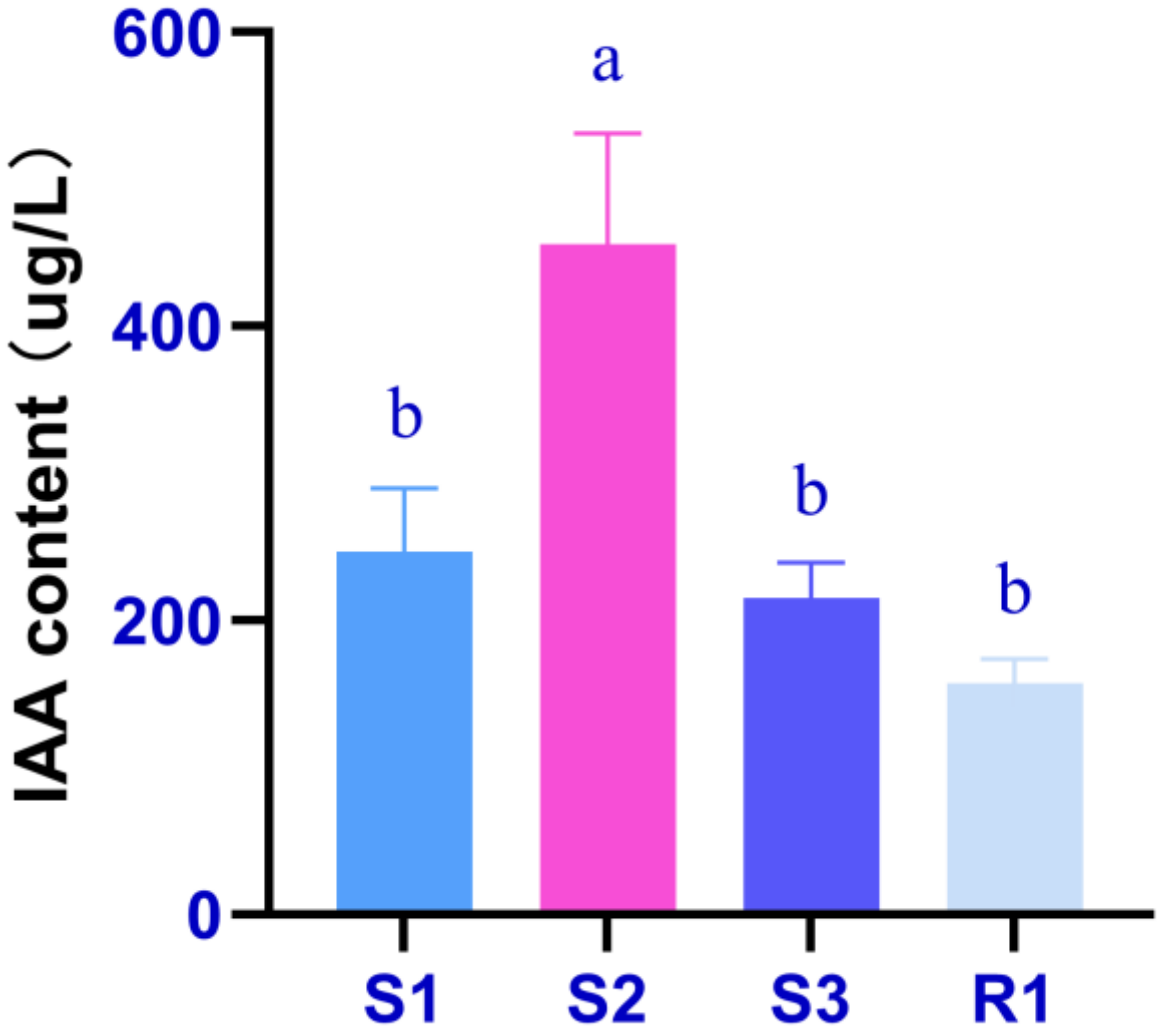

Figure 8

IAA content in fermentation broth

Figure 9

Seedlings of *Pinus thunbergii* without mycorrhizal formation after inoculation

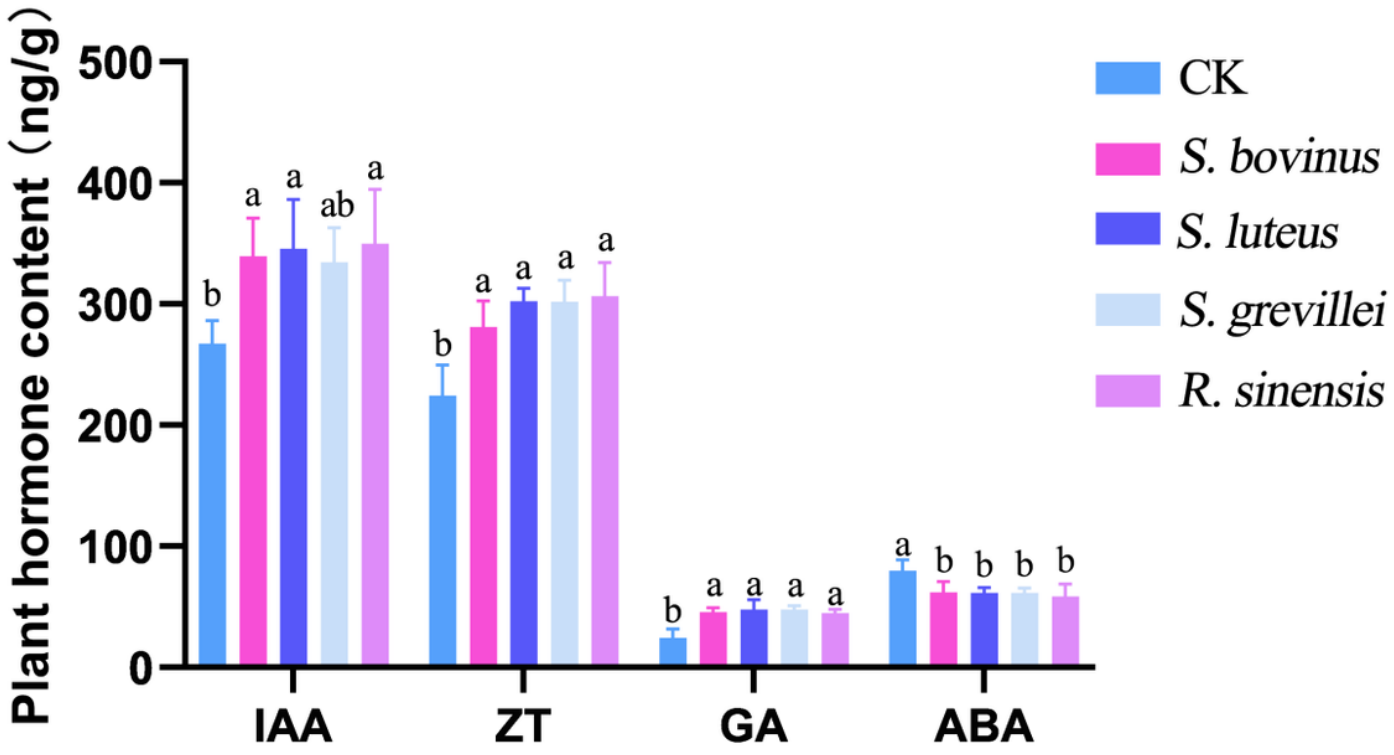

Figure 10

The hornone content of *Quercus acutissima.* (CK. Arbuscular mycorrhizal *Quercus acutissima.*)

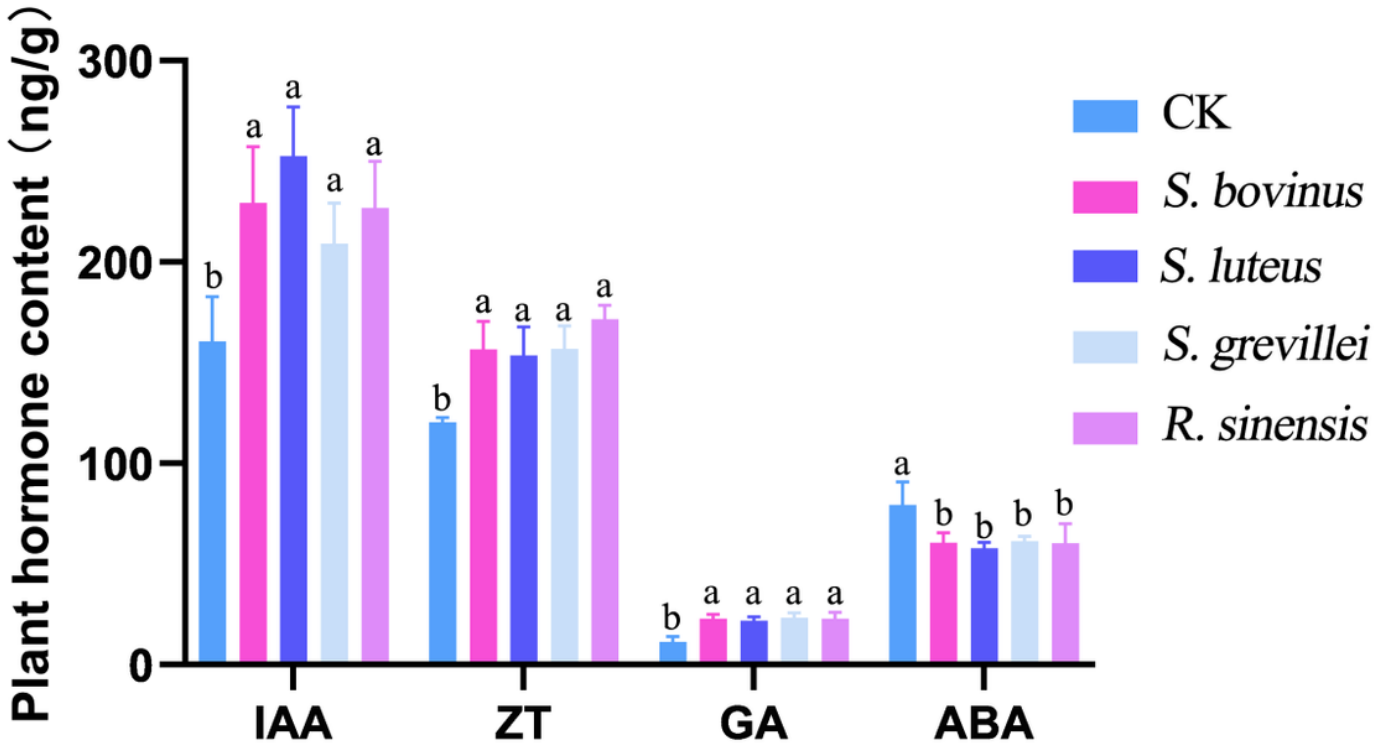

Figure 11

The hornone content of *Pinus thunbergii.* (CK. Arbuscular mycorrhizal *Pinus thunbergii.*)