

Inoculation with *Clariodeoglossum etunicatum* improves leaf food quality of tea exposed to P stress

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Abstract

The present study aimed to evaluate the effect of an arbuscular mycorrhizal fungus (AMF), *Clariodeoglossum etunicatum*, on leaf food quality and relevant gene expression levels of tea (*Camellia sinensis* cv. 'Fuding Dabaicha') seedlings exposed to 0.5 μM P ($P_{0.5}$) and 50 μM P (P_{50}) levels. Twenty-four weeks later, the seedlings recorded higher root mycorrhizal fungal colonization in P_{50} than in $P_{0.5}$. AMF-inoculated tea plants represented significantly higher leaf fructose and glucose contents and lower sucrose content than non-inoculated plants, irrespective of substrate P levels. AMF treatment also increased total amino acids content in $P_{0.5}$ and P_{50} , accompanied with higher expression of *glutamate dehydrogenase* (*CsGDH*) and lower expression of *glutamine synthetase* (*CsGS*) and *glutamine oxoglutarate aminotransferase* (*CsGOGAT*). The total flavonoid content was higher in mycorrhizal versus non-mycorrhizal plants under $P_{0.5}$ and P_{50} , together with induced expression of *phenylalanine ammonia-lyase* (*CsPAL*) and *cinnamic acid 4-hydroxylase* (*CsC4H*). Mycorrhizal fungal inoculation improved catechins content, which is due to the up-regulated expression of *flavanone 3-hydroxylase* (*CsF3H*), *flavonoid 3'-hydroxylase* (*CsF3'H*), *dihydroflavonol 4-reductase* (*CsDFR*), *leucoanthocyanidin reductase* (*CsLAR*), *anthocyanidin reductase* (*CsANR*), and *chalcone isomerase* (*CsCHI*) under $P_{0.5}$. However, under P_{50} , the gene involved in catechins synthesis was not affected or down-regulated by mycorrhization, implying a complex mechanism (e.g. nutrient improvement). AMF also inhibited the *tea caffeine synthase 1* (*CsTCSI*) expression regardless of P levels. Therefore, the results of this study concluded that inoculation with *C. etunicatum* improves leaf food quality of tea exposed to P stress, but the improved mechanisms were different between $P_{0.5}$ and P_{50} .

Keywords: catechins; flavonoid; mycorrhiza; secondary metabolites; white tea

Introduction

Tea (*Camellia sinensis* L. O. Kuntze), an important economic crop, contains a variety of natural active ingredients, which can improve human immunity and have the effects of anti-oxidation, anti-tumor, anti-

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cancer and lowering blood pressure. It is well known that tea plants grow in acidic soils subjected to seasonal changes, topography, soil, temperature, light, average annual rainfall and other conditions. Acidic soils also cause low nutrient utilization, in which nutrient deficiency such as phosphorus (P) stress is inevitable (Singh *et al.*, 2010; Ahmed *et al.*, 2019). To improve the acquisition and utilization efficiency of P in response to the effectiveness of P, the plants dissolve soil insoluble P through gene signal transmission and regulation of organic acid and phosphatase secretion. In addition, the plants can also establish a mutualistic symbiotic relationship with arbuscular mycorrhizal fungi (AMF) to uptake minerals from soil.

Plant rhizosphere is inhabited by various microorganisms, including beneficial endophytic fungi (Yang *et al.*, 2021). Among them, AMF in soil can form symbiotic arbuscular mycorrhizas (AMs) with most terrestrial plants. In AM symbiotic system, AM fungi can not only directly improve the absorption of soil nutrients through external hyphae (Mei *et al.*, 2019; He *et al.*, 2020a; Wu *et al.*, 2020), enhance the ability of plants to resist abiotic stresses (Wu *et al.*, 2019a; He *et al.*, 2020b; Zhang *et al.*, 2020; Zou *et al.*, 2020, 2021), but also improve the disease resistance of plants (Zhang *et al.*, 2019b), thus improving the yield and quality of plants (Wu *et al.*, 2020). In sustainable agricultural development, the role of AMF in improving plant growth and quality has been widely concerned (Wu *et al.*, 2020). AMF are commonly found in the rhizosphere of tea trees, whilst *Glomus* and *Claroideoglossum* species are common in the roots and soil of tea (Wu *et al.*, 2019b). Studies have confirmed the effect of AMF on the growth of tea plants (Singh *et al.*, 2010). Under the condition of the greenhouse, inoculation of AMF in acidic soil significantly increased root length and root dry weight of tea trees, accompanied with a significant increase in sugar, amino acid, total protein, tea polyphenol, and caffeine content (Singh *et al.*, 2010). Huang *et al.* (2006) found that under low P stress, inoculation with *Glomus mosseae* and *G. versiforme* could promote the vegetative growth of maize plants in the acidic red soil. In addition, Mei *et al.* (2019) found that AMF significantly increased the soil available P concentration and promoted the uptake of P by plants in temperate meadow ecosystem, thus reducing the nitrogen and phosphorus ratio of plants to alleviate the restriction of P. At present, the AMF application into tea trees provides the potential for improving the quality of tea trees, while it is not clear whether AMF improve the tea quality in P stress.

The secondary metabolites produced by sugars and other organic substances in tea are largely regulated by genes (Xiong *et al.*, 2013). Li *et al.* (2015) identified a total of 1719 genes involved in the secondary metabolic pathways of tea trees. It is well known that P is a major source of secondary metabolites, and the colonization of AMF increased the concentration of secondary metabolites in plants (Zhang *et al.*, 2020). AMF had a positive effect on leaf quality of tea seedlings. The present study was to evaluate effects of AMF on plant growth, leaf food quality, and relevant gene expression levels of tea grown in 0.5 μM and 50 μM P levels of substrates, in order to elucidate mycorrhizal fungal role on food quality of tea under P deficiency conditions.

Materials and Methods

Plant culture

The tea cultivar, *Camellia sinensis* cv. 'Fuding Dabaicha', was selected and provided by the Tea Research Institute of Guizhou Academy of Agricultural Sciences, Guiyang, China. The tea seed was sterilized with 75% ethanol, rinsed with distilled water, and sown into autoclaved (121 °C, 0.11 MPa, 2 h) sands for germination at 28/20 °C day/night temperature and 80% relative air humidity. A month later, a two-leaf age seedling was transplanted into a plastic pot (18 cm upper diameter \times 11 cm bottom diameter \times 15 cm height) that containing 2.3 kg of autoclaved (121 °C, 0.11 MPa, 2 h) river sand, which was removed substrate P by soaked in 15% of sulphuric acid solution for one week and rinsed with distilled water.

An arbuscular mycorrhizal fungus, *Clariodeoglossum etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler, was selected, based on a positive effect on growth performance of *C. sinensis* cv. 'Fuding Dabaicha' in our previous study (Shao *et al.*, 2018). This fungal strain was propagated by white clover (*Trifolium repens*

L.) for three months in pots. The fungal inoculum consisted of fungus-colonized root segments, soil hyphae, spores, and substrates. At the time of transplantation, a tea seedling was inoculated with 1200 spores per pot as the AMF treatment. The non-AMF inoculation received equivalent sterilized inoculants. The treated tea seedlings were cultured in the glass greenhouse for 24 weeks with 948 $\mu\text{mol}/\text{m}^2/\text{s}$ photon flux density, 28/23 °C day/night temperature, and 68% relative humidity.

P treatments

P treatments began one week after transplanting the seedlings. The selection of P concentrations (0.5 μM and 50 μM P) was based on the result of Salehi and Hajiboland (2008). Each pot was irrigated with 100 mL Hoagland solutions with 0.5 μM and 50 μM P levels at an interval of 3 days. To avoid P accumulation of the potted substrate, 100 mL ddH₂O per pot was used to remove the residual P in the sand of pots every two days. The P concentration in Hoagland solutions was controlled by KH₂PO₄ and K₂SO₄ was added into the 0.5 μM P treatment to maintain a uniform K concentration, relative to 50 μM P.

Experimental design

The experiment was designed in a 2² factory completely randomized blocked design: inoculation with or without *C. etunicatum*, and P treatments with 0.5 μM (P_{0.5}, a low P concentration) and 50 μM (P₅₀, an optimum P concentration). There are four treatments in the study, and each treatment was replicated six times.

Measurements of root AMF colonization

Root samples of tea were rinsed with tap water, cut into 1-cm long, stained by 0.05% trypan blue in lactoglycerol according to Phillips and Hayman (1970). The root mycorrhiza was observed under light microscope to visualize fungal colonization. The root AMF colonization was calculated using the formula: AMF colonization (%) = 100 × root length infected by AMF / total root length observed.

Determinations of food quality in leaf

Sucrose, glucose and fructose concentrations in leaves were determined as per the protocol described by Wu *et al.* (2010). The concentration of total soluble protein in leaves was evaluated by Bradford (1970) with bovine serum albumin as the standard. Total flavonoid content was estimated according to the method followed by Cheng *et al.* (2004). The concentration of total amino acid in leaves was estimated using the ninhydrin reaction following the method outlined by Tchameni *et al.* (2012). Tea polyphenol concentration was assayed by de La Rosa *et al.* (2011). The concentration of catechuic acid was determined by the vanillin method using the catechuic acid as the standard (Zhao, 2010).

Gene expression analysis

A total of fourteen genes including glutamate dehydrogenase gene (*CsGDH*), glutamine synthetase gene (*CsGS*), glutamine oxoglutarate aminotransferase gene (*CsGOGAT*), tea caffeine synthase 1 gene (*CsTCSI*), phenylalanine ammonia-lyase gene (*CsPAL*), cinnamic acid 4-hydroxylase gene (*CsC4H*), flavanone 3-hydroxylase gene (*CsF3H*), flavonoid 3'-hydroxylase gene (*CsF3'H*), dihydroflavonol 4-reductase gene (*CsDFR*), leucoanthocyanidin reductase gene (*CsLAR*), anthocyanidin reductase gene (*CsANR*), chalcone synthase gene (*CsCHS*), chalcone isomerase gene (*CsCHI*) and flavonoid 3',5'-hydroxylase gene (*CsF3'5'H*) were selected to analyze the relative expression levels (Xiong *et al.*, 2013). Total RNA was extracted from leaves using a TaKaRa MiniBEST Plant RNA Extraction Kit (9769, Takara Bio. Inc, Japan). RNA samples were reverse-transcribed using the PrimeScript™ RT reagent kit with gDNA eraser (PK02006, Takara Bio. Inc, Japan). qRT-PCR was run on a CFX96 Real Time PCR Detection System (BIO-RAD, USA). These primers for selected genes were designed based on the Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>) and a previous study (Xiong *et al.*, 2013), and shown in Table 1. qRT-PCR reactions were carried out in the following compositions: 8.8 μL ddH₂O, 0.4 μL cDNA, 10 μL AceQ qPCR SYBR Green Master Mix, 0.4 μL forward

prime, and 0.4 μ L reverse prime. qRT-PCR determinations were conducted on three independent biological samples with three technical replications for each sample. Quantification of the gene expression was done with the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2002) in which the housekeeping gene (*GADPH*) acted as the control. The measured transcript was normalized to the relative expression value in non-AMF-colonized plants grown in P₅₀ levels.

Statistical analysis

All the data were analyzed using SAS 8.1 (SAS Institute, Cary, NC, USA) to determine the variance, and the Duncan's multiple tests were used to compare the significant differences among treatments at the 0.05 level.

Table 1. The primers of relevant genes designed of *Camellia sinensis* cv. 'Fuding Dabaicha' for real time quantitative PCR amplification

Gene name	GenBank accession No.	Sequence forward (5'-3')	Sequence reverse (5'-3')
<i>GADPH</i>	XM_002263109	TTGGCATCGTTGAGGGTCT	CAGTGGGAACACGGAAAGC
<i>CsGDH</i>	JN602371	AAGCGGCAAATCATCCTACTGA	TCGTCCCACATGAAACCTTGA
<i>CsGS</i>	JN602372	CCTCAGAAGCAAAGCAAGGACT	AACATCAGGGTGGCTGAAAATC
<i>CsGOGAT</i>	JN602373	TGCTTCAGGACGTTTTGGTGT	CATGATGTGGAGGTGGGGATAT
<i>CsTCSI</i>	AB031280	TTCCGTGTTATGTGATGGGAGTA	TGAGTCCTTTTGGTGCCTGA
<i>CsPAL</i>	D26596	ATGACTTCTACAACAATGG	GGAGTTCTGAGCAATAAG
<i>CsC4H</i>	AY641731	CAATGGCAATGACTTTAG	CTCAGCAGTATCAATCTT
<i>CsF3H</i>	AY641730	GCGACAGTATACCCCTGAA	AGTATGGCAAAGGCACATCC
<i>CsF3'H</i>	GQ438849	ACCTTTCGACTTCACCCATCAAC	TAACGGACCATACGCAACCCTA
<i>CsDFR</i>	AB018685	AGTTTGTCGTTTCTCATC	GTATCAATGGCTCCTCTG
<i>CsLAR</i>	AY169404	GGGGCATCCTGTATCAAAGA	CCGCATACCTTTCAGTCCAT
<i>CsANR</i>	AY641729	GCGAAGTTGATCCTCTCGTC	AACCACATCGTCAAGTGAACA
<i>CsCHS</i>	AY656677	TTACTAATAGCGAGCATAAGGT	CTAGCATCAAGCGAAGGT
<i>CsCHI</i>	DQ904329	GTGATGGATGAAGTTGTG	AAGAGAGAAAGCAGAGTC
<i>CsF3'5'H</i>	AY945842	AATCCTGGTGAAGAGAAG	TCTATTATGCTTGATGATGTG

Results and Discussion

Changes in root mycorrhizal colonization

The root mycorrhiza was found in the AMF-inoculated tea plants, but not the non-AMF-inoculated plants. The root fungal colonization rate varied from 15.7 \pm 2.8% in P_{0.5} to 56.0 \pm 8.4% in P₅₀, respectively. There was a significant interaction for root AMF colonization between the seedlings grown in P_{0.5} and P₅₀ conditions (Table 2). Our study indicated the dramatical reduction of root AMF colonization in P_{0.5} treatment versus P₅₀ treatment. In general, low P stress and mycorrhizal fungi increased auxins to trigger more formation of root hairs (Zhang *et al.*, 2019a; Ceasar, 2020). Shao *et al.* (2021) reported that low P conditions induced more changes in the number and length of root hairs of tea plants to absorb P than mycorrhizal P absorption.

Changes in leaf sucrose, fructose and glucose

Sugar in leaves of tea accounts for 20%-25% of the dry matter, among which monosaccharides (fructose and glucose) and disaccharides (sucrose) are the major components of soluble sugar (Wu *et al.*, 2015). In our study, under the conditions of P_{0.5} (or P₅₀), the contents of fructose and glucose increased in AMF-treated plants (Table 2). It suggested that AMF stimulated the accumulation of fructose and glucose in leaf to regulate sugar levels. Additionally, the growth of AMF requires hexoses to maintain its growth, and higher hexoses originated from sucrose cleavage also provides more carbon source for mycorrhizal fungi (Wu *et al.*, 2015). Under P_{0.5} and P₅₀ conditions, leaf sucrose content was substantially decreased by 32.2% and 15.0%, respectively, in AMF-treated plants versus non-AMF-treated plants. The reduced accumulation of sucrose

content in leaves of tea after AMF inoculation may be caused by the low P stress that leads to the carbon transfer from leaves to roots as mycorrhizal-C sources for its growth, as well as sucrose cleavage into hexoses.

Table 2. Effects of *Clariodeoglomus etunicatum* and P treatments on root mycorrhizal colonization and sugar content of *Camellia sinensis* cv. 'Fuding Dabaicha' seedlings

Treatments	Root AMF colonization (%)	Sucrose (mg/plant DW)	Fructose (mg/plant DW)	Glucose (mg/plant DW)
P _{0.5} -AMF	0±0c	482.9±10.0a	5.5±0.01d	32.2±3.5c
P _{0.5} +AMF	15.7±2.8b	327.4±12.8c	5.8±0.06c	47.6±0.5b
P ₅₀ -AMF	0±0c	473.5±24.5a	5.9±0.02b	42.5±2.6b
P ₅₀ +AMF	56.0±8.4a	402.4±31.6b	6.8±0.03a	64.3±8.8a
Significance				
AMF	<0.0001	0.0302	<0.0001	0.0014
P	<0.0001	<0.0001	<0.0001	0.0002
AMF×P	<0.0001	0.0095	<0.0001	0.2918

Data (means ± SD, n = 4) followed by different letters in the same column indicate significant ($P < 0.05$) difference between treatments.

Changes in leaf total amino acids and expression of CsGDH, CsGS, and CsGOGAT

Amino acids are the main components of tea, which regulates and balances the bitterness and viscosity of catechins and caffeine in tea drinks. GDH is one of the key enzymes regulated the conversion of ammonium nitrogen to amino acids; GS and GOGAT are involved in GS/GOGAT cycle for glutamic acid (Duan *et al.*, 2020). In our study, amino acids were increased by AMF inoculation, regardless of P_{0.5} and P₅₀ levels (Figure 1a), accompanied with down-regulation or no expression of *CsGDH*, *CsGS*, and *CsGOGAT*, except an induced expression of *CsGDH* under P₅₀ conditions (Figure 1b-d). Salvioli *et al.* (2012) reported the increase of total amino acids (especially glutamine and asparagine) in tomato after inoculated with *Glomus mosseae*. In fact, early studies have shown that there were a large number of amino acids in mycorrhizal hyphae, such as aspartic acid, glutamic acid, glutamate, aspartic ester, ornithine, serine and aminoacetic acid (Johansen *et al.*, 1996), which can transfer into roots, thus, resulting in the increase of total amino acids. It concluded that AMF-induced increase of total amino acids is not due to the induced expression of *CsGDH*, *CsGS*, and *CsGOGAT*, but the synthesis of amino acids by mycorrhizal hyphae.

Changes in leaf tea polyphenols

Tea polyphenol is the main extract of tea leaves, which determines the color, aroma, taste and efficacy of tea leaves. Tea polyphenols are divided into catechins, flavonoids, anthocyanins and phenolic acids. The present study showed that AMF inoculation improved the contents of tea polyphenols in leaves of tea grown in the condition of P_{0.5} or P₅₀ by 19.43% and 26.96%, respectively, indicating that AMF has a positive effect on tea polyphenols production (Figure 2). Thokchom *et al.* (2020) found that AMF colonization in roots increased the content of polyphenols in leaves of green tea. Zhao *et al.* (2014) also reported that in tea plants, inoculation with *G. mosseae* dramatically increased tea polyphenols contents under different soil N levels. The increase of tea polyphenols is comparable to the effect of applying N fertilizer (Zhao *et al.*, 2014). Mycorrhizal fungi can strongly promote nitrogen uptake by plants (Wu *et al.*, 2020). In the study of Wang *et al.* (2002), it was found that inoculation of AMF significantly promoted the growth of asexual tea seedlings and the absorption of nutrient elements (especially P, Ca and Mg). Thus, we concluded that mycorrhizas improved nutrient acquisition of tea leave, thus, further accelerating tea polyphenols accumulation.

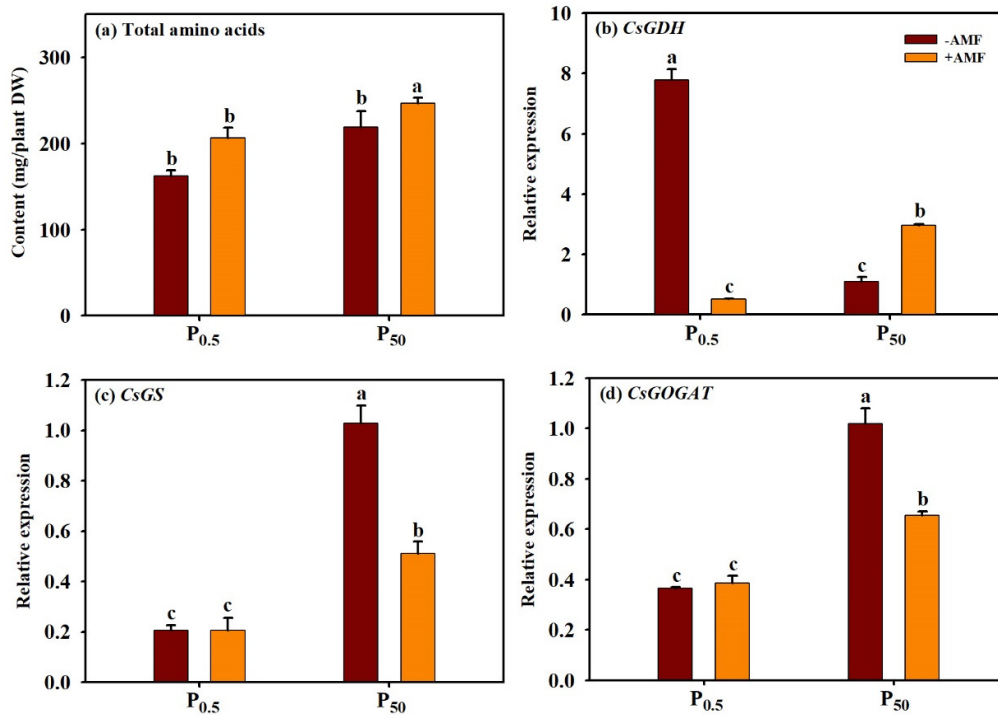


Figure 1. Effects of *Clariodeoglossum etunicatum* and P treatments on the contents of total amino acids (a) and relative expression of leaf *CsGDH*(b), *CsGS*(c) and *CsGOGAT*(d) in *Camellia sinensis* cv. ‘Fuding Dabaicha’ seedlings. Data (means ± SD, n = 3) are significantly (P < 0.05) different followed by different letters above the bars.

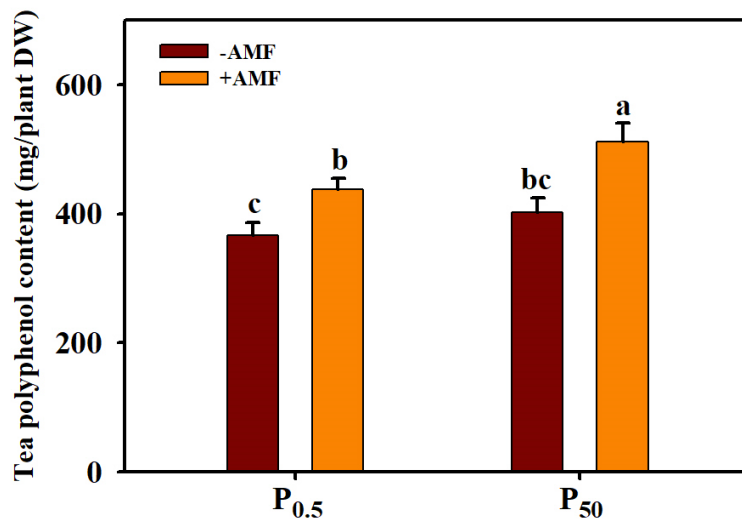


Figure 2. Effects of *Clariodeoglossum etunicatum* and P treatments on content of tea polyphenol in *Camellia sinensis* cv. ‘Fuding Dabaicha’ seedlings. Data (means ± SD, n = 3) are significantly (P < 0.05) different followed by different letters above the bars.

Changes in leaf total flavonoid and expression of CsPAL, CsC4H, and CsCHS

The secondary metabolism of plants needs the involvement of enzymes. Chalcone synthase (CHS) is the primary key enzyme in the flavonoid pathway. Phenylalanine ammonia-lyase (PAL) and cinnamic acid 4-hydroxylase (C4H) are involved in chalcone synthesis. In our study, compared with non-AMF-inoculated plants, under P_{0.5} and P₅₀ conditions, the contents of flavonoids in leaves of AMF-inoculated plants were

increased by 52.9% and 18.8%, respectively (Figure 3a). In green tea, AMF inoculation significantly elevated the content of flavonoids (Thokchom *et al.*, 2020), which could increase antioxidant properties of essential oils. Zhang *et al.* (2020) also reported an increase in total flavonoids of citrus after colonized by *G. epigaeum*. qRT-PCR analysis also revealed that compared with non-AMF-inoculated plants, the expression level of *CsPAL* and *CsC4H* genes were significantly up-regulated after inoculating AMF under $P_{0.5}$ condition, and under the condition of P_{50} , the expression level of *CsPAL*, *CsC4H*, and *CsCHS* genes was significantly up-regulated after inoculation of AMF (Figure 3b-d). The upregulation of related genes in the flavonoid synthesis indicated that mycorrhizal fungi promoted the accumulation of flavonoids in leaves by inducing the expression of related genes.

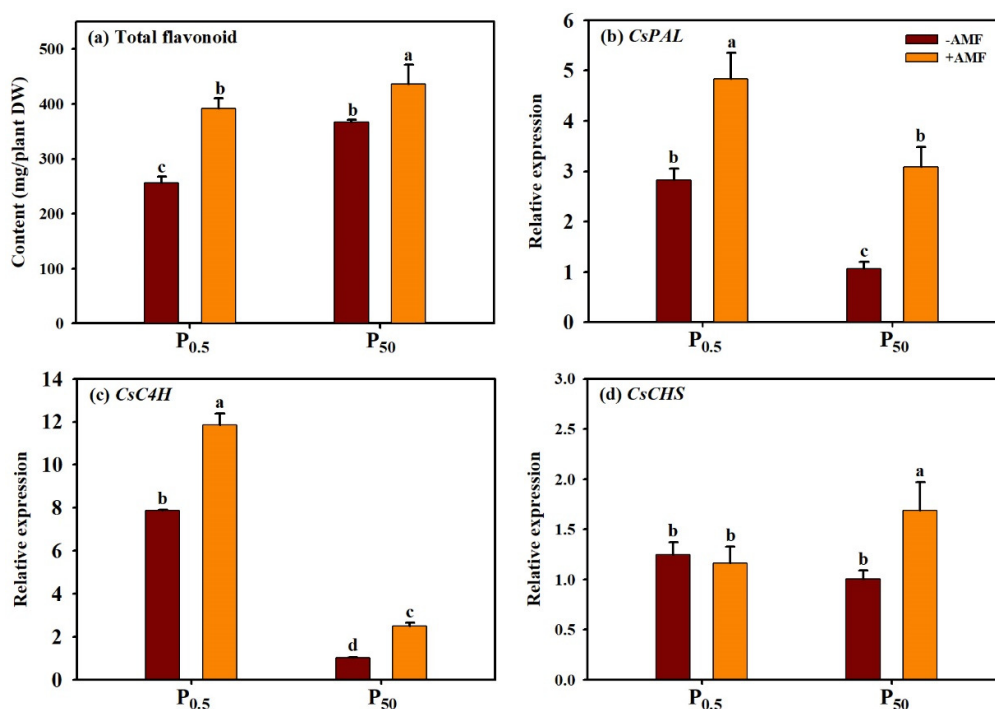


Figure 3. Effects of *Clariodeoglomus etunicatum* and P treatments on content of total flavonoid (a) and relative expression of leaf *CsPAL* (b), *CsC4H* (c) and *CsCHS* (d) in *Camellia sinensis* cv. 'Fuding Dabaicha' seedlings. Data (means \pm SD, $n = 3$) are significantly ($P < 0.05$) different followed by different letters above the bars.

Changes in leaf catechins and relevant gene expression

In tea, catechins account for about 70%~80% of the total tea polyphenols, and they play an important role in the formation of the color, aroma and taste quality of tea leaves. Many enzymes such as F3H, F3'H, F3'5'H, LAR, DFR, ANR, and CHI are involved in catechin synthesis (Xiong *et al.*, 2013). Our study indicated that under $P_{0.5}$ and P_{50} conditions, AMF inoculation significantly increased leaf catechins content by 35.1% and 39.0%, respectively, compared with non-AMF treatment (Figure 4a), compared with non-AMF-inoculated plants. Similar results are reported by Tomar *et al.* (2012) in tea after inoculated with AMF. In addition, AMF colonization also induced the expression of *CsF3H*, *CsF3'H*, *CsDFR*, *CsLAR*, *CsANR* and *CsCHI* genes under $P_{0.5}$ condition and the expression level of *CsF3H* under $P_{0.5}$ condition (Figure 4b-f, h). However, the fungal inoculation also down-regulated the expression level of *CsF3'5'H* genes under $P_{0.5}$ condition and the expression level of *CsF3'H*, *CsLAR* and *CsCHI* genes under P_{50} condition (Figure 4c, e, g, h). These results here indicated that in low P condition ($P_{0.5}$), AMF accelerated leaf catechins content of tea, due to the up-regulated expression of the relevant genes involved in catechin synthesis. However, in an appropriate P level (P_{50}) condition, AMF inoculation almostly induced the expression of the relevant genes involved in catechin

synthesis, though the leaf catechin was increased after AMF treatment. This indicated that mycorrhizas promoted catechin synthesis through different mechanisms, dependent on altered substrate P levels. We speculated that mycorrhizal improvement of plant nutrient uptake at an appropriate P level (Wu *et al.*, 2020) may be an important reason for promoting catechin accumulation, which needs further studies.

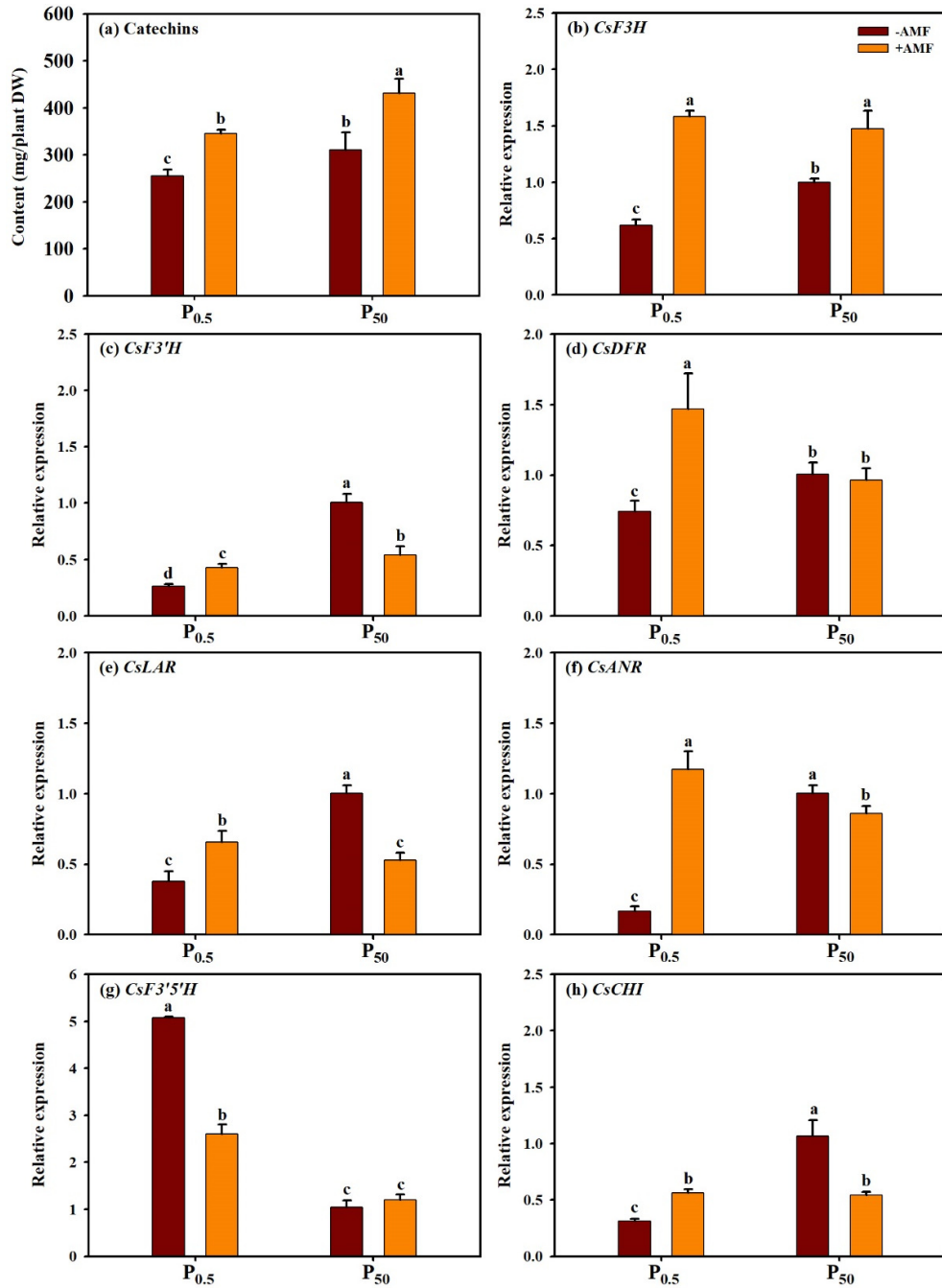


Figure 4. Effects of *Clariodeoglomus etunicatum* and P treatments on content of catechins (a) and relative expression of leaf *CsF3H* (b), *CsF3'H* (c), *CsDFR* (d), *CsLAR* (e), *CsANR* (f), *CsF3'5'H* (g) and *CsCHI* (h) in *Camellia sinensis* cv. 'Fuding Dabaicha' seedlings. Data (means \pm SD, $n = 3$) are significantly ($P < 0.05$) different followed by different letters above the bars.

Changes in CsTCSI expression levels in leaf

Coffeine is the principal biochemical active substance in tea, and theobromine synthase (TCS) is the key enzyme in the caffeine biosynthesis pathway of tea tree, among which TCS1 has the dual function of synthesizing theobromine and coffeine (Kato *et al.*, 2000). In this study, under the condition of P_{0.5} or P₅₀, the *CsTCSI* gene in tea leaves was significantly down-regulated after the inoculation of AMF (Figure 5), indicating that the inoculation of AMF had a significant inhibitory effect on the expression of *CsTCSI* gene in tea. Liu *et al.* (2019) found that TCS1a expressed protein in tea could convert most theobromine to coffeine. However, the application of AM fungi may provide the possibility for low or no coffeine tea and tea products to maintain the unique flavor and nutritional value of tea leaves.

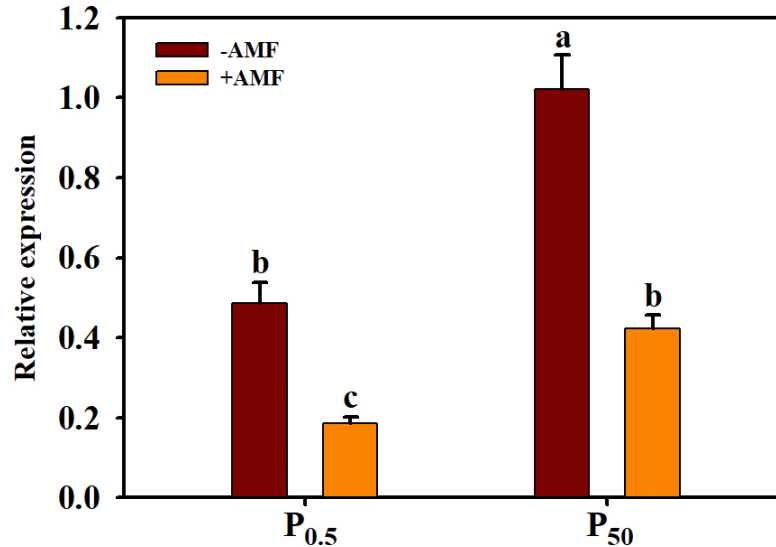


Figure 5. Effects of *Clariodeoglossum etunicatum* and P treatments on relative expression of leaf *CsTCSI* in *Camellia sinensis* cv. 'Fuding Dabaicha' seedlings
Data (means \pm SD, $n = 3$) are significantly ($P < 0.05$) different followed by different letters above the bars.

Conclusions

AMF, such as *C. etunicatum*, residing in the rhizosphere of tea trees significantly improved the content of glucose, fructose, total amino acids, total flavonoids, tea polyphenols and catechins in leaves, thus, resulting in great leaf food quality of tea. The positive improvement was associated with AMF-induced up-regulation of related gene expression. However, the associated regulatory network is not clear. In addition, such results also suggest that future studies should pay attention to promoting mycorrhizal fungal colonization in tea trees in field management and intervening in mycorrhizalization in tea nursery.

Authors' Contributions

Conceptualization: JLC and QSW; Data curation: YDS, QSW and JLC. Formal analysis: JLC, YNZ, KK and QSW; Funding acquisition: QSW; Investigation: JLC and YDS; Methodology: JLC and YDS; Project administration: QSW and TYY; Supervision: QSW; Writing - original draft: JLC and YDS; Writing - review and editing: QSW, TYY and KK.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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