

## Mycorrhiza-induced changes in root growth and nutrient absorption of tea plants

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### ABSTRACT

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Tea plants grown in acidic soils are strongly dependent on arbuscular mycorrhizas, whereas it is not clear whether soil arbuscular mycorrhizal fungi (AMF) improve plant growth, root development, and nutrient absorption in tea plants. A potted study was conducted to determine the effects of *Claroideoglossum etunicatum*, *Diversispora spurca*, *D. versiformis* and a mixture of the three AMF species on plant growth, root morphology, root-hair growth, and leaf nutrient status in *Camellia sinensis* cv. Fuding Dabaicha in Jingzhou, China. After 12 weeks of AMF inoculation, root mycorrhizal colonization ranged from 15.12% to 40.23%. AMF inoculation heavily increased plant height, shoot and root biomass, and total leaf area, whilst the increased effect was ranked as *C. etunicatum* > *D. spurca* > mixed-AMF > *D. versiformis* in the decreasing order. Mycorrhizal inoculation also considerably increased total root length and volume, whereas obviously inhibited root-hair length and number, in company with an increment in root-hair diameter. Leaf N, P, K, Ca, Mg, Zn, and Mn contents were significantly higher in AMF-inoculated plants than in non-AMF-inoculated plants, regardless of AMF species. It concludes that AMF inoculation had positive effects on plant growth performance, root morphology, and leaf nutrient levels in cv. Fuding Dabaicha seedlings, whilst *C. etunicatum* performed the best effects.

**Keywords:** root hair; soil microorganism; symbiotic fungi; white tea

Tea (*Camellia sinensis* (L.) O. Kuntze) is the popular beverage consumed in the world (Kahneh et al. 2006). In general, tea plants are known to

grow in acidic soils, where the availability of soil nutrients is often deficient marginal (Singh et al. 2010). As a result, tea plants in acidic soils often

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encounter nutrient-deficient stress. A study in the past observed that cultivated and natural tea bushes existed in a kind of soil symbiotic fungi, namely arbuscular mycorrhizal fungi (AMF), dominated by *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* (Singh et al. 2008). Inoculation with AMF could heavily stimulate plant growth parameters and quality parameters (amino acid, total protein, total polyphenols, caffeine, and sugars) in tea plants (Kahneh et al. 2006, Singh et al. 2008, 2010). Therefore, arbuscular mycorrhizal (AM) symbiosis is the key factor in tea growth.

In tea cultivation, fine roots play a vital function in determining a plant's ability to compete for soil nutrients (Razaq et al. 2017). Root morphology is often affected by phytohormones, soil pH, and soil microbes (Osmont et al. 2007). Past studies showed that AM fungal inoculation was able to effectively regulate root morphological parameters, for stimulating plant growth and accelerating nutrient absorption (Wu et al. 2011, 2017). Poplar plants inoculated with two *Glomus* species significantly increased the root length and the number of 2<sup>nd</sup>- and 3<sup>rd</sup>-order lateral roots, but not 1<sup>st</sup>-order lateral roots (Hooker et al. 1992). In peach, *Funneliformis mosseae* (former name is *Glomus mosseae*) and *Diversispora versiformis* (former name is *Glomus versiforme*), but not *Paraglomus occultum*, significantly increased root length, projected area, surface area, and volume, relative to non-AM fungal control (Wu et al. 2011). In trifoliolate orange seedlings, *D. versiformis* considerably increased root hair density under ample water and drought stress and root hair length under drought stress, while dramatically decreased root hair length under ample water, but not drought stress (Zou et al. 2017). Berta et al. (1995) reported that inoculation with *F. mosseae* and *Glomus intraradices* increased root length and the intensity of branching in all root orders of *Prunus cerasifera* plants. Root diameter of *Prunus cerasifera* was increased by *G. intraradices*, but not *F. mosseae*. However, in maize plants, inoculation with *F. mosseae* strongly decreased root dry weight, root length, and root hair density and length, compared to the non-inoculated control (Kothari et al. 1990). These results indicated that AM fungal inoculation strongly regulated root morphology, dependent on AM fungal species, soil water status, and host species. Topological analysis indicated that in the early stages of plant growth, AM and

non-AM plants possessed a similar pattern of root branching (Schellenbaum et al. 1991). After 8 weeks, non-AM plant roots adopted a more herringbone pattern, whereas AM plants retained a more dichotomous pattern with repeated bifurcation, which is a more economical root developed pattern. Until now, the information regarding AM effects on root morphology of tea plants is scarce.

*C. sinensis* cv. Fuding Dabaicha, belonging to series of white tea, possesses an excellent comprehensive character in tea cultivars, including budding rate, shoot-keeping tenderness, drought tolerance, and cold resistance (Liu et al. 2008). However, the tea plant possesses a relatively inferior root system. In this background, the objective of the present study was to evaluate effects of AMF on root morphology, root-hair growth, and leaf nutrient levels of the tea plant.

## MATERIAL AND METHODS

**Plant culture.** Seeds of tea (cv. Fuding Dabaicha), which were provided by the Tea Research Institute, Guizhou Academy of Agricultural Science, were sterilized by 75% of alcohol solutions, rinsed with ddH<sub>2</sub>O, and sown into autoclaved (121°C, 0.1 MPa, 1 h) sands for germination under the conditions of 28/20°C day/night temperature. After approximate one month, a two-leaf-old tea seedling was transplanted into a pot with 18 cm upper diameter, 11 cm bottom diameter, and 15 cm height, where 2300 g of autoclaved soils was supplied. The soil used here was collected from a 20-year-old tea plantation (31°15'N, 111°05'E) with Yichang Dayecha cultivars, which was located in the Yiling District, Yichang City, Hubei, China.

At transplanting, three AM fungal species, namely, *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler, *Diversispora spurca* (C.M. Pfeiff., C. Walker & Bloss) C. Walker & A. Schüßler, and *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd, were applied into the potted soils. In addition, a mixture of the three AM fungal species was used as a mycorrhizal treatment. For non-AM fungal treatment, same accounts of autoclaved inoculums were applied. The monospecific spores of these AMF species were propagated on white clover in the disinfested mixture of soils and sands using pot culture for 12 weeks. Mycorrhizal inoculums included spores,

infected root segments, and growth substrates. For AMF inoculations, 1200 spores of mycorrhizal inoculum in each AM fungal species were applied.

The AMF- and non-AMF-colonized plants were grown in a greenhouse in the Yangtze University campus for 12 weeks, where photosynthetic photon flux density was 948  $\mu\text{mol}/\text{m}^2/\text{s}$ , day/night temperature 28/23°C, and relative air humidity 82%.

**Experimental design.** The experiment was arranged in an absolutely randomized blocked design with five AMF treatments: (1) inoculation with *C. etunicatum*; (2) inoculation with *D. spurca*; (3) inoculation with *D. versiformis*; (4) mixed inoculation with *C. etunicatum*, *D. spurca* and *D. versiformis*, and (5) inoculation without AMF (non-AMF). Each treatment replicated four times, for a total of 20 pots.

**Variable determinations.** At harvesting, plant height and total leaf area were determined. Subsequently, the seedlings were divided into the shoot and the root, whose fresh biomass was measured. All the roots were scanned with the Epson Perfection V700 Photo Dual Lens System (J221A, Jakarta Selatan, Indonesia), and root morphological traits of the figures including length and volume were evaluated by a professional WinRHIZO 2007b software (Regent Instruments Inc., Quebec, Canada).

Root mycorrhizas were stained as per the protocol of Phillips and Hayman (1970). Root mycorrhizal colonization was calculated as the percentage of mycorrhizal colonized root lengths versus observed total root lengths.

Root hairs were measured with a Scanning Electron Microscope (JSM-6391LV, JEOL Co., Tokyo, Japan) after fixed by 2.5% glutaric dialdehyde, dehydrated by ethanol with increasing

concentrations, and dried with the critical-point (Wu et al. 2016). Root-hair length, density, and diameter were analyzed by the Image J software (National Institute of Health, Bethesda, USA). Root hair number = root hair density  $\times$  root surface area.

The concentrations of P, K, Ca, Mg, Fe, Cu, Mn, and Zn in leaves were measured by the inductively coupled plasma-atomic emission spectrometry (ICP-AES, IRIS Advantage, Waltham, USA). Leaf nitrogen (N) concentration was determined by the Smartchem 200 (Westco Scientific Instruments Inc., Brookfield, USA).

**Statistical analysis.** All the data were analyzed with one way ANOVA (SAS 8.1v, SAS Institute, Inc., Cary, USA) to determine significant effects, and the Duncan's multiple test at the 5% level was used to compare significant differences between treatments.

## RESULTS AND DISCUSSION

**Root mycorrhizal colonization.** Cv. Fuding Dabaicha plants could be colonized by the AMF species used, varied from 15.12% to 40.23% (Table 1). Significantly higher root mycorrhizal colonization was ranked as the trend of *C. etunicatum* > mixed-AMF > *D. spurca* > *D. versiformis* in the decreasing order. The variation in mycorrhizal colonization may be due to the compatibility between AMF species and host plant species (Tian et al. 2004).

**Changes in plant growth.** Generally, mycorrhizal inoculation could increase growth of host plants (Carretero et al. 2009), which is in accordance with the findings in our work (Table 1). Compared to the non-AMF-inoculation seedling, plant height, total leaf area, shoot and root biomass were signifi-

Table 1. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on growth of *Camellia sinensis* cv. Fuding Dabaicha seedlings

Treatment	Root mycorrhizal colonization (%)	Plant height (cm)	Biomass (g FW/plant)		Total leaf area (cm <sup>2</sup> )
			shoot	root	
<i>Claroideoglossum etunicatum</i>	40.23 $\pm$ 1.65 <sup>a</sup>	18.50 $\pm$ 0.58 <sup>a</sup>	3.54 $\pm$ 0.13 <sup>a</sup>	2.48 $\pm$ 0.05 <sup>a</sup>	23.95 $\pm$ 1.17 <sup>a</sup>
<i>Diversispora spurca</i>	24.94 $\pm$ 1.55 <sup>c</sup>	18.25 $\pm$ 0.96 <sup>a</sup>	3.11 $\pm$ 0.05 <sup>b</sup>	2.00 $\pm$ 0.03 <sup>c</sup>	22.87 $\pm$ 1.29 <sup>a</sup>
<i>D. versiformis</i>	15.12 $\pm$ 1.06 <sup>d</sup>	13.00 $\pm$ 0.82 <sup>b</sup>	2.20 $\pm$ 0.11 <sup>d</sup>	1.82 $\pm$ 0.08 <sup>d</sup>	16.18 $\pm$ 1.23 <sup>c</sup>
Mixed-AMF	32.37 $\pm$ 1.18 <sup>b</sup>	17.75 $\pm$ 1.26 <sup>a</sup>	2.88 $\pm$ 0.04 <sup>c</sup>	2.19 $\pm$ 0.06 <sup>b</sup>	18.22 $\pm$ 1.01 <sup>b</sup>
Non-AMF	0 $\pm$ 0 <sup>e</sup>	12.75 $\pm$ 0.50 <sup>b</sup>	1.71 $\pm$ 0.09 <sup>e</sup>	1.76 $\pm$ 0.10 <sup>d</sup>	14.97 $\pm$ 1.17 <sup>c</sup>

Data (means  $\pm$  standard deviation,  $n = 4$ ) followed by different letters in same column are significantly different at  $P < 0.05$ ; FW – fresh weight

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Table 2. Effects of arbuscular mycorrhizal fungi (AMF)-inoculation on root of *Camellia sinensis* cv. Fuding Dabaicha seedlings

Treatment	Total root length (cm)	Root volume (cm <sup>3</sup> )	Root-hair length (μm)	Root-hair diameter (μm)	Root-hair number (× 10 <sup>4</sup> /plant)
<i>Claroideoglossum etunicatum</i>	176.48 ± 3.61 <sup>a</sup>	1.17 ± 0.04 <sup>a</sup>	49.52 ± 3.55 <sup>b</sup>	10.77 ± 0.42 <sup>b</sup>	53.52 ± 2.95 <sup>b</sup>
<i>Diversispora spurca</i>	153.54 ± 2.97 <sup>b</sup>	0.84 ± 0.02 <sup>b</sup>	39.03 ± 2.19 <sup>c</sup>	10.83 ± 0.16 <sup>b</sup>	25.35 ± 1.15 <sup>d</sup>
<i>D. versiformis</i>	136.02 ± 1.36 <sup>c</sup>	0.73 ± 0.02 <sup>c</sup>	55.92 ± 3.60 <sup>a</sup>	11.65 ± 0.30 <sup>a</sup>	45.04 ± 1.64 <sup>c</sup>
Mixed-AMF	177.31 ± 5.46 <sup>a</sup>	1.15 ± 0.04 <sup>a</sup>	55.99 ± 3.51 <sup>a</sup>	9.81 ± 0.11 <sup>c</sup>	52.74 ± 2.10 <sup>b</sup>
Non-AMF	114.80 ± 5.24 <sup>d</sup>	0.69 ± 0.03 <sup>c</sup>	55.79 ± 4.44 <sup>a</sup>	10.25 ± 0.45 <sup>c</sup>	74.45 ± 3.36 <sup>a</sup>

Data (means ± standard deviation,  $n = 4$ ) followed by different letters in same column are significantly different at  $P < 0.05$

cantly increased by 45.1, 60.0, 107.0 and 40.9% after inoculated with *C. etunicatum*, by 43.1, 52.8, 81.9 and 13.6% with *D. spurca*, and by 39.2, 21.7, 68.4 and 24.4% with mixed-AMF, respectively (Table 1). *D. versiformis* did not alter plant growth traits, with exception of shoot biomass, relative to the non-AMF treatment. In addition, amongst mycorrhizal treatments, *C. etunicatum* exhibited greater effects on plant growth performance than mixed-AMF inoculation, suggesting that the effect of *C. etunicatum* was partly neutralized by other AMF species in the mixed AMF treatment. The mycorrhizal effect on plant growth parameters including plant height ( $r = 0.81$ ,  $P < 0.01$ ), shoot biomass ( $r = 0.84$ ,  $P < 0.01$ ), root biomass ( $r = 0.79$ ,  $P < 0.01$ ), and total leaf area ( $r = 0.76$ ,  $P < 0.01$ ) was in line with the root mycorrhizal colonization, indicating the positive effects of AMF on tea plant growth.

**Changes in root morphology.** Root morphology, the spatial configuration of a root system in soil,

plays an important role in below-ground resource acquisitions, because it determines the ability of a plant to exploit soil resources (Ho et al. 2004). In the present work, mycorrhizal inoculation notably improved root morphological traits of cv. Fuding Dabaicha seedlings (Table 2; Figure 1). Compared with the non-AMF treatment, AMF inoculations significantly increased root total length and volume by 53.7% and 69.6% under *C. etunicatum*, 33.7% and 21.7% under *D. spurca*, 18.5% and 5.8% under *D. versiformis*, 54.5% and 66.7% under mixed-AMF, respectively (Table 2). The present results also showed the significantly positive correlation between root mycorrhizal colonization and root volume ( $r = 0.93$ ,  $P < 0.01$ ) and total root length ( $r = 0.90$ ,  $P < 0.01$ ), which is in agreement with a previous study in red tangerine conducted by Wu et al. (2012). There were no differences in root morphological traits between *C. etunicatum* and mixed-AMF treatments, indicating no counteraction between *C. etunicatum*

Table 3. Effects of arbuscular mycorrhizal fungi (AMF)-inoculation on leaf nutrient element content (mg/plant dry weight) of *Camellia sinensis* cv. Fuding Dabaicha seedlings

Treatment	N	P	K	Ca	Mg	Cu (× 10 <sup>-3</sup> )	Zn (× 10 <sup>-3</sup> )	Mn	Fe
<i>Claroideoglossum etunicatum</i>	20.12 ± 0.47 <sup>a</sup>	0.852 ± 0.010 <sup>b</sup>	8.72 ± 0.38 <sup>b</sup>	6.95 ± 0.28 <sup>a</sup>	1.48 ± 0.04 <sup>a</sup>	4.94 ± 0.28 <sup>a</sup>	10.47 ± 0.49 <sup>b</sup>	1.52 ± 0.04 <sup>a</sup>	0.153 ± 0.003 <sup>a</sup>
<i>Diversispora spurca</i>	17.78 ± 0.39 <sup>b</sup>	1.088 ± 0.012 <sup>a</sup>	9.57 ± 0.41 <sup>a</sup>	4.32 ± 0.18 <sup>c</sup>	1.27 ± 0.03 <sup>c</sup>	4.30 ± 0.24 <sup>b</sup>	12.98 ± 0.60 <sup>a</sup>	0.99 ± 0.01 <sup>c</sup>	0.110 ± 0.002 <sup>d</sup>
<i>D. versiformis</i>	12.80 ± 0.29 <sup>c</sup>	0.535 ± 0.006 <sup>c</sup>	5.35 ± 0.23 <sup>c</sup>	4.17 ± 0.17 <sup>c</sup>	1.03 ± 0.03 <sup>d</sup>	3.36 ± 0.19 <sup>c</sup>	6.92 ± 0.32 <sup>c</sup>	0.83 ± 0.03 <sup>d</sup>	0.146 ± 0.003 <sup>b</sup>
Mixed-AMF	17.46 ± 0.39 <sup>b</sup>	0.542 ± 0.006 <sup>c</sup>	8.17 ± 0.37 <sup>b</sup>	5.57 ± 0.23 <sup>b</sup>	1.34 ± 0.03 <sup>b</sup>	5.24 ± 0.30 <sup>a</sup>	9.15 ± 0.43 <sup>d</sup>	1.16 ± 0.03 <sup>b</sup>	0.097 ± 0.002 <sup>e</sup>
Non-AMF	10.16 ± 0.23 <sup>d</sup>	0.464 ± 0.005 <sup>d</sup>	4.10 ± 0.18 <sup>d</sup>	1.64 ± 0.07 <sup>d</sup>	0.66 ± 0.02 <sup>e</sup>	2.93 ± 0.17 <sup>c</sup>	6.05 ± 0.28 <sup>e</sup>	0.74 ± 0.01 <sup>e</sup>	0.134 ± 0.002 <sup>c</sup>

Data (means ± standard deviation,  $n = 4$ ) followed by different letters in same column are significantly different at  $P < 0.05$

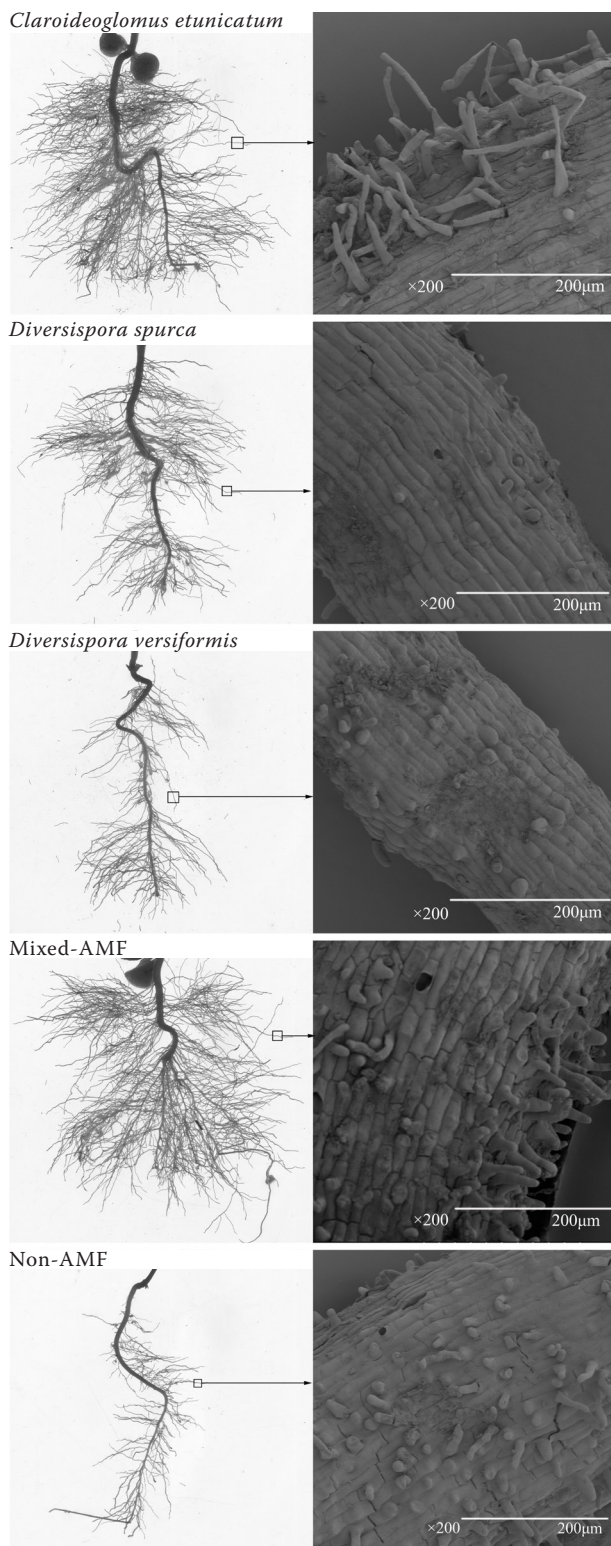


Figure 1. Whole root morphology and root hairs in *Camellia sinensis* cv. Fuding Dabaicha seedlings colonized by *Claroideoglomus etunicatum*, *Diversispora spurca*, *Diversispora versiformis* and a mixture of the three arbuscular mycorrhizal fungi (AMF) species

and the other two AMF species in the mixed-AMF treatment (Table 2). Earlier studies had shown that mycorrhizal effects on root morphology were closely associated with mycorrhiza-modulated polyamines (PAs) and indole-3-acetic acid levels of roots (Upreti et al. 2016, Wu et al. 2016).

**Changes in root hairs.** In the present work, mycorrhizal inoculation notably affected the root-hair growth of cv. Fuding Dabaicha seedlings (Table 2; Figure 1). Compared with non-AMF treatment, the number of root hairs was significantly decreased by 28.1, 66.0, 39.5 and 29.2% in *C. etunicatum*, *D. spurca*, *D. versiformis* and mixed-AMF treatments, respectively (Table 2). In addition, root mycorrhizal colonization was significantly negatively correlated with root-hair number ( $r = 0.45$ ,  $P < 0.05$ ). Sun and Tang (2013) reported that inoculation with *F. mosseae* and *G. intraradices* decreased root-hair incidence of *Sorghum bicolor*. However, Orfanoudakis et al. (2010) discovered that inoculation with *Gigaspora rosea* resulted in an increase in the total number of root hairs but a substantial reduction in root-hair density of *Alnus glutinosa*. Our study also found that compared with non-AMF, inoculation with *D. spurca* and *C. etunicatum* significantly decreased root-hair length by 30.0% and 11.2%, which is in line with the result reported by Sun and Tang (2013) in sorghum plants colonized by *F. mosseae* and *G. intraradices*. On the other hand, *D. versiformis* and mixed-AMF treatments produced no changes in root-hair length in comparison with non-AMF-inoculation (Table 2). This indicates the diverse effects of AMF on root-hair length. A study conducted by Wu et al. (2016) also reported that inoculation with *C. etunicatum*, *D. versiformis*, *F. mosseae*, and *Rhizoglossum intraradices* induced diverse responses in root-hair length of trifoliolate orange. In this study, mycorrhizal colonization evidently promoted root-hair diameter by 5.1, 5.7 and 13.7% in the presence of *C. etunicatum*, *D. spurca* and *D. versiformis* (Table 2). It is well known that mycorrhizal extraradical hyphae and root hairs collectively take part in water and nutrient absorption in root surface (Morgan and Connolly 2013). After inoculated with AMF, mycorrhizal extraradical hyphae possibly replace root hairs to perform the absorbed functioning, thereby, resulting in the reduction of root hairs.

**Changes in leaf nutrient levels.** Compared to the non-AMF inoculation, leaf N, P and K contents were significantly increased by 98.0, 112.7 and

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Table 4. Correlation coefficients between leaf nutrient element content and root mycorrhizal colonization, root volume, or total root length in *Camellia sinensis* cv. Fuding Dabaicha seedlings

Root variables	N	P	K	Ca	Mg	Cu	Zn	Mn	Fe
Root mycorrhizal colonization	0.97**	0.52*	0.85**	0.97**	0.98**	0.92**	0.67**	0.92**	-0.12
Root volume	0.84**	0.23	0.67**	0.87**	0.84**	0.93**	0.44	0.92**	-0.19
Total root length	0.93**	0.41	0.83**	0.93**	0.95**	0.97**	0.63**	0.87**	-0.27

\* $P < 0.05$ ; \*\* $P < 0.01$

112.7% with the inoculation of *C. etunicatum*, by 75.0, 134.5 and 133.4% with *D. spurca*, by 26.0, 15.3 and 30.5% with the inoculation of *D. versiformis*, and by 71.9, 16.8 and 99.3% with mixed-AMF, respectively (Table 3). It is in agreement with previous results reported by Krishnan and Sharavanan (2016) in black gram (*Vigna mungo* L.) colonized by *F. mosseae* under  $\text{CdCl}_2$  stress. Compared to the non-AMF-inoculation, leaf Ca, Mg, Cu, Zn and Mn contents were significantly increased by 323.8, 125.5, 68.6, 73.1 and 104.2% with the inoculation of *C. etunicatum*, by 163.4, 93.6, 46.8, 114.5 and 33.3% with *D. spurca*, by 154.3, 57.6, 14.7, 14.4 and 11.6% with the inoculation of *D. versiformis*, and by 239.6, 104.9, 78.8, 51.2 and 56.7% with mixed-AMF-inoculation, respectively (Table 3). It is in accordance with previous studies in *Melilotus albus* inoculated with *Glomus* Zac-19 (Hernández-Ortega et al. 2012) and black gram inoculated with *F. mosseae* (Krishnan and Sharavanan 2016). In general, AMF can generate the developed hyphae network, which can help host plants to absorb soil nutrient elements. AMF could stimulate the development of root morphology which enhanced the ability to absorb water and nutrients in plants (Upreti et al. 2016, Wu et al. 2012, 2016). Moreover, root mycorrhizal colonization, root length, and root volume was significantly positively correlated with leaf nutrient levels, except Fe and P (Table 4). It concludes that mycorrhizal formation helps the tea plant to absorb N, P, K, Ca, Mg, Zn, and Mn by means of extraradical hyphae.

In short, inoculation with AMF represented the positive effects on plant growth performance (plant height, leaf area, and shoot and root biomass), root morphology (root total length, root volume, and root-hair diameter), and leaf nutrient levels in the cv. Fuding Dabaicha seedlings. Hereinto, *C. etunicatum* conferred the best effects.

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