

Arbuscular mycorrhizal fungi improve the antioxidant capacity of tea (*Camellia sinensis*) seedlings under drought stress

Chun-Yan LIU¹, Yu-Juan WANG¹, Qiang-Sheng WU^{1,2,3*},
Tian-Yuan YANG^{2*}, Kamil KUČA³

¹Yangtze University, College of Horticulture and Gardening, Jingzhou, Hubei, 434025,

China; 201573031@yangtzeu.edu.cn; 1278755411@qq.com; wuqiangsh@163.com (*corresponding author)

²Anhui Agricultural University, State Key Laboratory of Tea Plant Biology and Utilization, Hefei, Anhui 230036,
China; yangtianyuan2008@126.com (*corresponding author)

³University of Hradec Králové, Faculty of Science, Department of Chemistry, Hradec Králové 50003,
Czech Republic; kamil.kuca@uhk.cz

Abstract

A pot experiment was carried out to evaluate the response of leaf antioxidant enzyme systems to inoculation with arbuscular mycorrhizal fungus (AMF) *Clariodeoglossum etunicatum* in tea (*Camellia sinensis* cv. 'Fuding Dabaicha') seedlings under drought stress (DS). Root AMF colonization was significantly decreased after an eight-week soil drought treatment. Plant growth performance (plant height, stem diameter, leaf number and root biomass), leaf relative water content, and leaf water potential were notably decreased under DS conditions, whereas these variables exhibited significantly higher responses in mycorrhizal seedlings than in non-mycorrhizal seedlings. The DS treatment markedly increased leaf superoxide anion concentration but did not affect malondialdehyde content, whereas both were reasonably decreased by AMF colonization regardless of water status. The seedlings colonized by AMF showed substantially higher antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase, and ascorbate peroxidase than the non-AMF colonized seedlings under both well-watered and DS conditions. DS markedly upregulated the relative expression of *CsSOD* in both AMF and non-AMF seedlings and the relative expression of *CsCAT* in AMF seedlings. Meanwhile, AMF-colonized seedlings represent markedly higher relative expressions of *CsSOD* and *CsCAT* than non-AMF seedlings, irrespective of water status. It concludes that mycorrhizal tea plants had higher antioxidant enzyme activity and corresponding gene expression under DS, indicating a stronger ability to alleviate the oxidative damage of drought.

Keywords: antioxidant enzyme; drought; mycorrhiza; tea

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the oldest and most popular caffeinated nonalcoholic beverages in the world, consumed by more than 3 billion people in 160 countries (Ding *et al.*, 2017). Tea plants are rain-fed, evergreen perennial crops, mainly grown in tropical and subtropical regions of Asia (Wang *et al.*, 2017). Excessive soil moisture and water deficiency both can cause water stress (Gupta *et al.*, 2012). The most

common water stress encountered is moisture deficit, known as drought stress (DS), an adverse factor that severely impairs tea plant growth and development, and limits its distribution, performance, yield, and quality (Sharma *et al.*, 2005; Liu *et al.*, 2016). Recently, the frequency and intensity of seasonal DS has been increasing, with water deficit becoming an acute problem worldwide, especially in arid and semi-arid areas, seriously restricting the development of the tea industry (Gupta *et al.*, 2013).

DS conditions affect the root systems, photosynthesis and respiration, relative water content, membrane integrity, osmotic potential, antioxidant metabolism, nutrition content, and phytohormones in plants as well as the transcription levels of both regulatory and functional genes (Qian *et al.*, 2018). Generally, the most harmful effect of DS bring to plants is the massive accumulation of reactive oxygen species (ROS), which causes cell membrane damage (He *et al.*, 2020). Meanwhile, the antioxidant defense system is activated to remove excessive ROS effectively for reducing the damage of cell membrane caused by membrane lipid peroxidation under DS (Zhang *et al.*, 2020). Antioxidant enzymes, including superoxide dismutases (SODs), peroxidases (PODs), catalase (CAT), ascorbate peroxidase (APX), etc., are widely found in plant organelles, such as chloroplasts, mitochondria, peroxisomes, etc. (Liu *et al.*, 2014). SODs are a metal enzyme, that can break down the superoxide anion free radicals ($O_2^{\cdot-}$) produced in plant tissues into oxygen (O_2) and hydrogen peroxide (H_2O_2), and which is then disproportionated to water and O_2 in reactions catalyzed by CAT, POD, and APX, thereby maintaining a dynamic balance of the ROS in plants (Kapoor *et al.*, 2017). Furthermore, DS can stimulate the production of some non-enzymatic system substances such as vitamin A, vitamin E, and glutathione, etc. (Bellani *et al.*, 2012), in addition to inducing the expression of stress response genes coding for antioxidant enzymes and aquaporins to improve drought resistance of plants (He *et al.*, 2019; Zhang *et al.*, 2020).

Arbuscular mycorrhizal fungi (AMF) are a kind of beneficial soil microorganisms that enter into symbiosis structure with more than 80% of terrestrial plants (Xu *et al.*, 2016). The ability of AMF to improve plant nutrition, promote growth and enhance host plant resistance has remarkable effects, in tea plant as well as in others (Shao *et al.*, 2018). Under natural and cultivated conditions, a highly diverse array of AMF is ubiquitous in root systems of tea bushes (Nepolean *et al.*, 2012; Shao *et al.*, 2018; Wu *et al.*, 2019; Singh *et al.*, 2010). The mainly AMF species forming symbiosis with tea roots are *Acaulospora*, *Claroideoglossum*, *Gigaspora*, *Glomus* and *Scutellospora* (Singh *et al.*, 2008; Wu *et al.*, 2019). The symbiotic of AMF improved the productivity of tea plants and tea quality, and enhanced the ability of plants to adapt to adversity (Singh *et al.*, 2010; Liu *et al.*, 2013). Under salt stress, inoculation with *Glomus versiformis* promoted mineral nutrient absorption and plant growth performance, and enhanced the plants' tolerance to salt stress, improving their yield and quality (Liu *et al.*, 2013). In acidic soil, the tea quality traits, namely sugar, amino, protein, total polyphenol and caffeine content, were all notably improved by AMF inoculation (Singh *et al.*, 2010). Many previous studies have proved that AMF improved the resistance of host plants such as citrus (He *et al.*, 2019), bioenergy grass (Mirshad and Puthur, 2016), and wheat (Mustafa *et al.*, 2017), by increasing the activity of antioxidant enzymes. The information regarding the antioxidant enzyme activities of tea plants under mycorrhizal infection and DS is very scarce, and the underlying physiological mechanism of AMF in improving tea resistance needs to be further explored.

We hypothesized that mycorrhizal symbiosis plays a role in enhancing the drought tolerance of tea plants, which is related to the enhancement in antioxidant enzyme protected system. To test this hypothesis, we inoculated AMF into the rhizosphere of a drought sensitive plant, *C. sinensis* cv. 'Fuding Dabaicha', and analyzed the plant growth, leaf water potential, photosynthesis, oxidative metabolite content and antioxidant system activities, to evaluate the effects of AMF on the leaf antioxidant enzyme defense systems in tea seedlings exposed to well-watered (WW) and DS conditions.

Materials and Methods

Experimental materials

The seeds of *C. sinensis* cv. 'Fuding Dabaicha' were provided by the Tea Research Institute of Guizhou Academy of Agricultural Sciences. Tea seeds were surface-sterilized with 70% ethanol, and placed in river sand, which had been previously sterilized in autoclave (< 4 mm, 0.11 MPa, 121 °C, 2h), and germinated in dark at 28 °C and 80% relative humidity for 30 days. Then, 3-leaf-old tea seedlings in uniform size were transplanted into a plastic pot (15 cm upper diameter × 10 cm bottom diameter × 13 cm height) filled with 1.5 kg sterilized (0.11 MPa, 121 °C, 2h) mixture substance (soil: sand = 1:1, v/v).

The tested arbuscular mycorrhizal (AM) fungus was *Clariodeoglossum etunicatum* (Nicol. & Gerd.) Schüßler & Walker [BGC GZ03C], and the strain selection was based on the results of Shao *et al.* (2019) on tea seedlings. The AM fungus was provided by the Bank of Glomeromycota in China (BGC) and propagated with white clover for three months. The AMF treatment was inoculated with 80 g inoculum including spores (~ 1500 spores), the colonized root segments of white clover, and growth substance per pot at the time of seedlings transplanting. And the inoculum of *C. etunicatum* were placed around tea root below 5 cm of the soil surface. For non-AMF treatment, the same amount of sterilized inoculum was added and plus 2 mL of inoculated filtrate (25 µm filter) to minimize the differences in microbial populations expected for *C. etunicatum*. The AMF and non-AMF treated seedlings were exposed to conditions of 900 µmol m⁻² s⁻¹ photon flux density, 28/20 °C day/night temperature, and 68% relative humidity under greenhouse conditions at Yangtze University (Jingzhou, China).

Experimental design

The experiment consisted of four treatments including inoculation with or without *C. etunicatum* and soil water regimes with well-watered (WW) (corresponding to 75% of field maximum water capacity) and DS (corresponding to 55% of maximum water field capacity). Each treatment replicated six times, producing a total of 24 pots, and arranged in a complete randomized block design.

The soil water level of each experimental pot was maintained at WW level until the drought treatment began 4 weeks after seedlings transplanting (4 weeks after AMF inoculation). Half of the AMF and non-AMF seedlings were subjected to DS conditions until they were harvested, and the other half of the seedlings were also remained at WW level. In order to control the level of drought stress, the soil water content in each pot was measured daily, and the amount of water lost was resupplied to maintain the target soil water content. Eight weeks after drought stress treatment, all the seedlings were harvested to determine their growth and biochemical parameters.

Variables measurement

Before harvest, plant height, stem diameter and leaf numbers were recorded in all seedlings. Shoot and root fresh weights were measured immediately at harvesting. Sixty-one-cm-long fresh root segments (ten taproots, twenty 1st-order lateral roots, twenty 2nd-order lateral roots and ten 3rd-order lateral roots) per treatment were selected randomly. The root segments were cleared with 10% (w/v) KOH and stained with 0.05% (w/v) trypan blue in lactophenol for mycorrhizal status evaluation (Phillips and Hayman, 1970). Mycorrhizal colonization was calculated as the percentage of AM root length/total observed root length.

The fourth fully expanded top leaf of each seedling was sampled to determine the leaf relative water content (LRWC) and leaf water potential. The LRWC was assayed as described by the formula (Zou *et al.*, 2015): LRWC (%) = (FW-DW)/(SW-DW) × 100, where FW indicates fresh weight, DW dry weight (oven-dried at 70 °C for 48 h), and SW saturated weight (immersed in distilled water for 24 h). Leaf water potential was measured using a PSYPRO water potential system with a leaf hygrometer (L-51A-SF, WESCOR Inc.).

Leaf samples (0.2 g) were homogenized in 5 mL of 5% (w/v) trichloroacetic acid and centrifuged at $3000 \times g$ for 10 min. The extracts were used for malondialdehyde (MDA) assay according to the thiobarbituric acid reaction as described by Sudhakar *et al.* (2001).

Another 0.2 g fresh leaf sample was homogenized in 5 mL of 0.1 M phosphate buffer (pH 7.8) and centrifuged at $4,000 \times g$ for 10 min at 4°C . And the supernatant was used to assay the concentration of O_2^- and the activities of SOD, POD, and CAT. The O_2^- concentration was performed as described by Wang and Luo (1990) with minor modifications. Briefly, 1 mL supernatant was mixed with 0.1 mL 10 mM hydroxylamine chloride and 0.9 mL 65 mM phosphate buffer (pH 7.8) for 20 min at 25°C in room temperature. Subsequently, 1 mL of the above mixture was mixed with 1 mL 17 mM sulfanilic acid and 1 mL 7 mM α -naphthylamine for 20 min at 25°C , followed by determination of absorbance at 530 nm. SOD activity was determined by measuring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm according to Giannopolitis and Ries (1977). CAT activity was determined according to the method described by Goldblith and Proctor (1950). POD activity was measured by following the change in absorption at 470 nm due to guaiacol oxidation (Polle *et al.*, 1994).

APX was extracted with 5 mL of ice-cold 100 mM phosphate buffer (pH 7.0) containing 1 mM ascorbic acid and 1 mM EDTA and centrifuged at $15,000 \times g$ at 4°C for 15 min. APX activity was measured using the method described by Amako *et al.* (1994). Briefly, 1 mL reaction volume containing 50 mM K-phosphate buffer (pH 7.0), 0.1 mM H_2O_2 , and 0.5 mM ascorbate. The H_2O_2 reaction was initiated, and the decrease in absorbance at 290 nm was recorded for 1 min to determine the oxidation rate of ascorbate.

The relative expression of *CsCAT* and *CsSOD* was detected by real-time quantitative PCR (qRT-PCR) and calculated by the $2^{-\Delta\Delta\text{Ct}}$ method, according to Livak and Schmittgen (2001), with a housekeeping gene (*GADPH*) acting as control. The housekeeping gene selection was referred to the result of Shao *et al.* (2019) on tea. The specific primers for the antioxidant enzyme gene for qRT-PCR analysis were designed using Primer Premier 5 based on the Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>) and are shown in Table 1. Leaf total RNA was extracted in 0.1 g fresh sample using a TaKaRa MiniBEST Plant RNA Extraction Kit (9769, Takara Bio. Inc, Japan), and reverse transcription was done with PrimeScriptTM RT reagent kit with gDNA eraser (PK02006, Takara Bio. Inc, Japan) according to the manufacturer's instructions. The qRT-PCR system was as follows: 7.2 μL ddH₂O, 2 μL cDNA, 10 μL AceQ qPCR SYBR Green Master Mix, 0.4 μL forward primer, and 0.4 μL reverse primer. qRT-PCR was run on a CFX96 Real Time PCR Detection System (BIO-RAD, USA) under the following conditions: 95°C for 5 min, 40 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 30s. For each gene, reactions were conducted with three independent biological samples, and each sample comprised of three technical replicates.

Table 1. The specific primers of relevant genes designed for real time quantitative PCR amplification

Gene name	Accession	Sequence (5'-3')-forward	Sequence (5'-3')-reverse
<i>GADPH</i>	XM_002263109	TTGGCATCGTTGAGGGTCT	CAGTGGGAACACGGAAAGC
<i>CsCAT</i>	KR819178.1	TTTGATCTGGTGGAAACAA	TCCAATTCTCCTGGATGTGA
<i>CsSOD</i>	AY694187.1	CATTTCAATCCTGCTGGCAAAGA	GCATGGACAACAACGGCCCTACC

Statistical analysis

Data were analyzed by the SAS 8.1 software, and one-factor analysis of variance (ANOVA) was used to compare the significance of differences among treatments with the LSD test at the level of 0.05, and the CORR process was used to analyze the correlation coefficients between variables.

Results

Changes in plant growth performance

Roots of tea seedlings were colonized by *C. etunicatum* (Figure 1), whereas DS significantly restrained the mycorrhizal colonization by 41.95% (Table 2). Plant growth performance, including plant height, stem diameter, leaf number, and root biomass of 12 week-tea seedlings was markedly greater under WW than under DS conditions. Compared with non-AMF treatment, AMF inoculation substantially increased plant height, stem diameter, leaf number, and root biomass by 15.08%, 21.48%, 31.03% and 49.91% under WW and by 32.60%, 34.17%, 26.92%, and 13.77% under DS, respectively. Meanwhile, AMF seedlings also showed a significantly higher leaf biomass than non-AM seedlings under DS (Table 2).

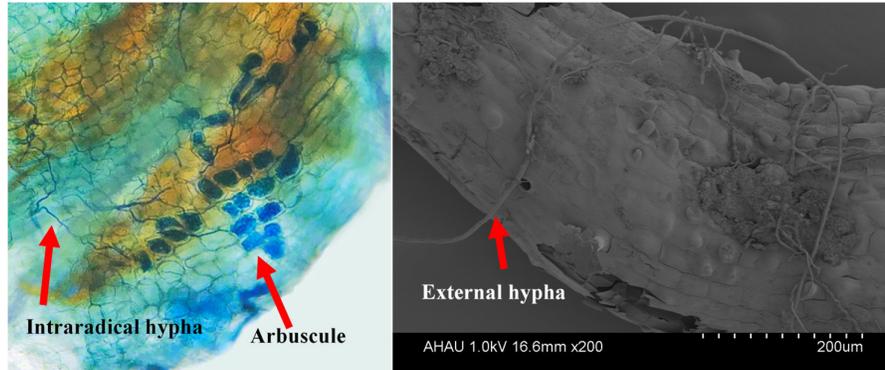


Figure 1. Root colonization of tea (*Camellia sinensis* ‘Fuding Dabaicha’) seedlings by *Clariodeoglossum etunicatum*. (left) intraradical hyphae and arbuscules; (right) external hyphae

Table 2. Effects of *Clariodeoglossum etunicatum* on the plant growth (per plant) and root AMF colonization of tea (*Camellia sinensis* ‘Fuding Dabaicha’) seedlings well-watered (WW) and drought stress (DS)

Treatments	Root AMF colonization (%)	Plant height (cm)	Stem diameter (mm)	Leaf number	Biomass (g/FW)	
					Leaf	Root
WW+AMF	24.67±2.53a	17.70±0.50a	0.328±0.014a	15±1a	1.18±0.08a	1.66±0.06a
WW-AMF	0±0c	16.92±1.71ab	0.322±0.028a	13±10b	1.10±0.06a	1.16±0.07b
DS+AMF	14.32±1.18b	15.38±1.44b	0.270±0.025b	12±2bc	1.12±0.03a	1.11±0.07b
DS-AMF	0±0c	12.76±0.59c	0.240±0.021b	10±1c	0.79±0.03b	1.02±0.07c
<i>Significance</i>						
DS	**	**	**	**	**	**
AMF	**	**	**	**	**	**
DS×AMF	**	NS	NS	*	*	NS

Note: Means ± SD ($n = 6$) followed by different letters meant significant difference within the same column at 0.05 level by LSD. * $P < 0.05$; ** $P < 0.01$; NS: no significant difference.

Changes in leaf moisture status

Compared with WW, DS significantly reduced the LRWC and leaf water potential, while markedly increased water saturation deficit in tea seedlings with or without AMF inoculation (Table 3). Mycorrhizal colonized seedlings presented a reasonably increased leaf relative water content and leaf water potential compared with those of non-AMF colonized seedlings under WW and DS: 3.2% and 2.9% higher in relative water content, and 12% and 22% in leaf water potential, respectively. Meanwhile, AMF inoculation dramatically decreased the water saturation deficit by 21.2% and 14.1% under WW and DS, respectively.

Changes in MDA and O₂^{•-} contents

DS dramatically increased the leaf O₂^{•-} concentration but did not alter the leaf MDA content regardless of AMF inoculation (Figure 2). Compared with non-AMF seedlings, the leaf MDA and O₂^{•-} concentrations of AMF seedlings were significantly lower by 29.29% and 31.81%, respectively, under WW, and by 37.62% and 31.13%, separately, under DS conditions.

Table 3. Effects of *Clariodeoglomus etunicatum* on the leaf water status of tea (*Camellia sinensis* 'Fuding Dabaicha') seedlings under WW and DS

Treatment	Leaf relative water content (%)	Water saturation deficit (%)	Leaf water potential (MPa)
WW+AMF	87.32±1.20a	12.68±1.19c	-0.36±0.02a
WW-AMF	84.63±1.61b	15.37±1.60b	-0.41±0.03b
DS+AMF	83.45±1.84b	16.55±0.84b	-0.43±0.02b
DS-AMF	81.12±1.90c	18.88±1.80a	-0.55±0.03c
<i>Significance</i>			
DS	**	**	**
AMF	**	**	**
DS×AMF	NS	NS	**

Note: Means ± SD (*n* = 6) followed by different letters meant significant difference within the same column at 0.05 level by LSD. ** *P* < 0.01; NS: no significant difference.

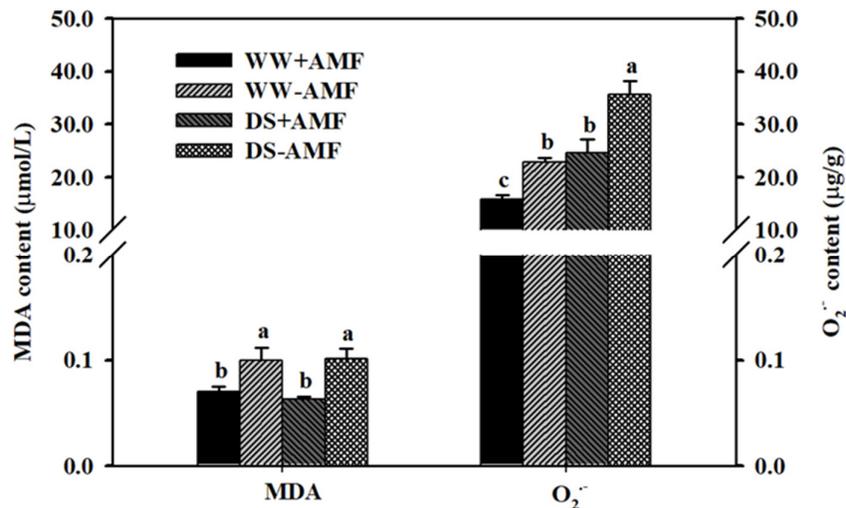


Figure 2. Effects of *Clariodeoglomus etunicatum* on the malondialdehyde and superoxide anion content of tea (*Camellia sinensis* 'Fuding Dabaicha') seedlings under well-watered (WW) and drought stress (DS) conditions

Data (means ± SD, *n* = 6) followed by different letters above the bars indicate significant differences (*P* < 0.05) between treatments.

Changes in antioxidant enzyme activities

Compared to WW treatment, DS significantly decreased the leaf CAT and SOD activities but markedly increased the leaf APX in AMF- and non-AMF-inoculated plants, whereas the POD activity was also decreased in non-AMF-inoculated plants (Figure 3). AMF treatment brought a notably increase in leaf antioxidant enzyme activities than non-AMF treatment. In detail, AMF inoculation triggered a significantly higher leaf CAT, SOD, POD, and APX activity by 41.40%, 12.05%, 25.28%, and 43.10% under WW and by 51.29%, 34.67%, 132.57%, and 11.70% under DS, respectively.

Changes in leaf CsSOD and CsCAT expression levels

DS markedly upregulated the relative expression levels of *CsSOD* by 1.39 and 2.39 times in AMF and non-AMF seedlings and *CsCAT* by 3.18 times in AMF seedlings, respectively (Figure 4). In addition, the expression levels of *CsSOD* and *CsCAT* were upregulated by 3.82 and 1.81 times under WW and by 2.22 and 5.71 times under DS after AMF inoculation, respectively.

Correlation analysis

Root AMF colonization and leaf water potential were markedly positively correlated with leaf relative water content but significantly negatively correlated with water saturation deficit (Table 4). MDA and O₂^{•-} were negatively correlated, and CAT, POD, and SOD activities were positively correlated with root AMF colonization, leaf relative water content, and leaf water potential. However, APX activity was markedly negatively correlated with leaf relative water content and leaf water potential.

Table 4. Pearson correlation coefficients between root AMF colonization and root antioxidant enzyme activities, leaf water status factor in tea (*Camellia sinensis* ‘Fuding Dabaicha’) seedlings under WW and DS conditions

Variables	Root AMF colonization	Leaf relative water content	Leaf water potential
Leaf relative water content	0.64**	1.00	0.76**
Water saturation deficit	-0.64**	-1.00	-0.76**
Leaf water potential	0.66**	0.76**	1.00
MDA	-0.78**	-0.36	-0.50*
O ₂ ^{•-}	-0.73**	-0.87**	-0.94**
CAT	0.89**	0.78**	0.80**
POD	0.62**	0.57*	0.77**
SOD	0.58**	0.81**	0.92**
APX	0.02	-0.58*	-0.55*

Note: *, *P* < 0.05; **, *P* < 0.01.

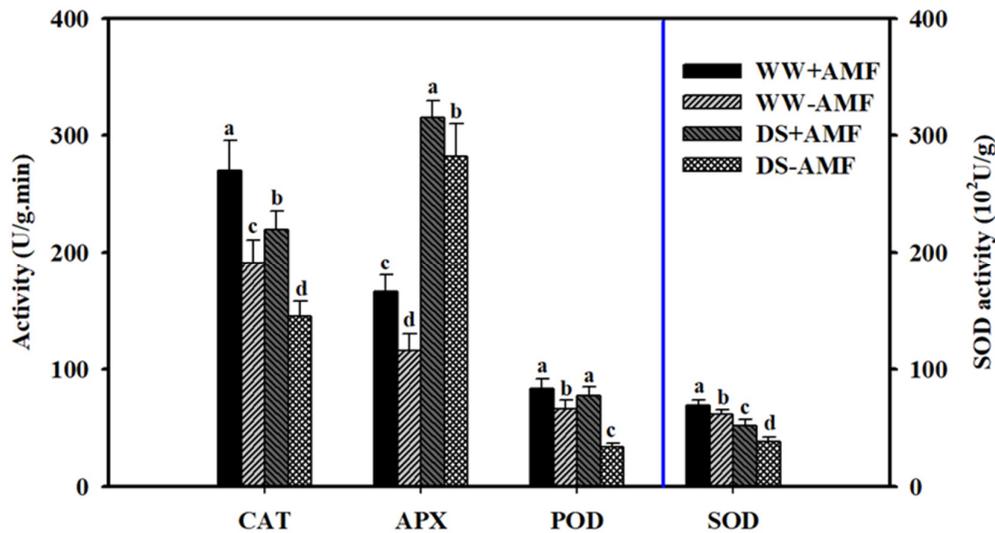


Figure 3. Effects of *Clariodeoglossum etunicatum* on the antioxidant enzyme activities of tea (*Camellia sinensis* ‘Fuding Dabaicha’) seedlings under well-watered (WW) and drought stress (DS) conditions. Data (means ± SD, *n* = 6) followed by different letters above the bars indicate significant differences (*P* < 0.05) between treatments.

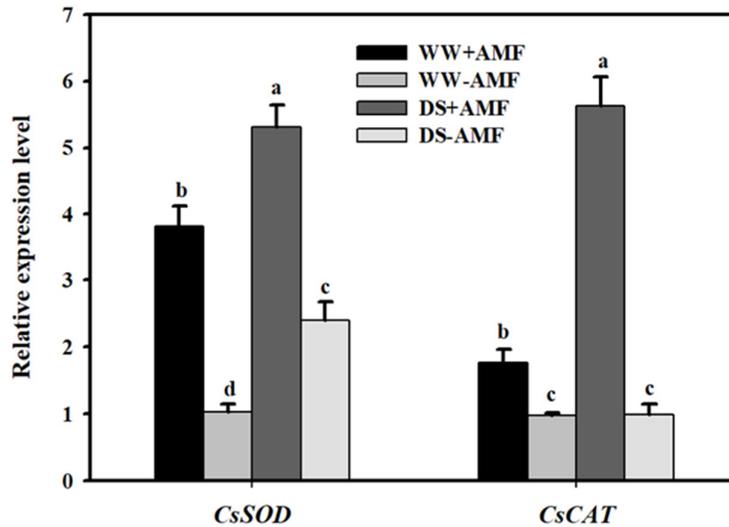


Figure 4. Effects of *Clariodeoglossum etunicatum* on the expression levels of *CsSOD* and *CsCAT* in tea (*Camellia sinensis* ‘Fuding Dabaicha’) seedlings under well-watered (WW) and drought stress (DS) conditions

Data (means \pm SD, $n = 6$) followed by different letters above the bars indicate significant differences ($P < 0.05$) between treatments

Discussion

The present study showed a dramatic reduction in root AMF colonization of tea plants under DS conditions as compared with tea plants under WW conditions, which is consistent with the results by Amiri *et al.* (2015), Tuo *et al.* (2017), and Zhang *et al.* (2020), who observed a significant decrease of mycorrhizal colonization in citrus, geranium, and white clover under water deficit conditions. Probably, water deficit inhibited the germination rate of spores, restricting the extension and colonization ability of AMF hyphae (Huang *et al.*, 2017).

DS is an important limiting factor for plant growth and development, crop yields, and quality (Liu *et al.*, 2016). The inhibitory effect of DS on the growth of tea plants was observed in the present study. Interestingly, inoculation with AMF dramatically promoted plant growth and total biomass of tea plants regardless of soil water regimes. This result is in accordance with previous studies on tea plants (Shao *et al.*, 2018, 2019; Quiroga *et al.*, 2020). Although DS treatment inhibited the development of the mycorrhizal association, the plant growth performance of tea plants was still improved by mycorrhization, indicating a positive role of AMF colonization in host plant. This positive effect is contributed to tenacious vitality of the mycorrhizal extraradical hyphae and the mycorrhizal network, which expanded the root absorption area underground (Zhang *et al.*, 2020).

LRWC reflects the degree of plant water deficit and the metabolic activity in plants exposed to DS conditions (Anjum *et al.*, 2011). The present study showed that DS markedly decreased the LRWC and leaf water potential, but significantly increased water saturation deficit in AMF and non-AMF seedlings, which was consistent with previous studies (Cheruiyot *et al.*, 2008; Upadhyaya and Panda, 2013; Liu *et al.*, 2015). Interestingly, the LRWC and leaf water potential were dramatically raised while the water saturation deficit was notably reduced in AMF seedlings under both WW and DS conditions. This result is in accordance with earlier studies on citrus (Wu *et al.*, 2017; He *et al.*, 2019) and snapdragon (Asrar *et al.*, 2012). Better leaf water status in the AMF seedlings was due to elevated water uptake imparted by the extraradical mycelium, who directly absorbs water in the hyphal tips from the soil matrix and then transfers it through the fungal cytoplasm

or inner wall layers into the root cortical cells, without any obstructions from the membrane (Allen, 2007; Zou *et al.*, 2015). Moreover, the efficiency of water absorption and transportation of external hyphae under soil water deficit was twice than under saturated soils (Zhang *et al.*, 2018). In addition, root AM colonization was significantly positively correlated with LRWC and leaf water potential, further confirming the role of mycorrhizal hyphae in water acquisition by their hosts.

ROS is one of the earliest biochemical responses in eukaryotic cells under biotic and abiotic stresses (Anjum *et al.*, 2011). DS induces ROS production and destroys the balance between ROS generation and quenching, resulting in the accumulation of MDA, which is a suitable marker for reflecting the degree of membrane lipid peroxidation (Qian *et al.*, 2018). In this work, DS promoted the accumulation of both $O_2^{\bullet-}$ and MDA accompanied with weakness of antioxidant enzymes system, such as CAT, SOD and POD, regardless of AMF inoculation, which then induced oxidative damage, resulting in different physiological and biochemical processes (Zhou *et al.*, 2014; Liu *et al.*, 2015; Wang *et al.*, 2016). Nevertheless, the present study showed that AMF inoculation stimulated leaf antioxidant enzyme activities, including those of CAT, SOD, POD, and APX for reducing ROS content, as compared with non-AMF treatment under WW and DS conditions. Similarly, studies by Zhu *et al.* (2011) on *Zea mays* and He *et al.* (2020) and Huang *et al.* (2014) on trifoliolate orange showed a notably higher SOD and CAT activities in AMF-inoculated plants than in non-AMF-inoculated plants, indicating that AMF maintain a dynamic balance between ROS production and elimination, thus reducing the damage from membrane lipid peroxidation. Furthermore, there was a significant positive correlation between root AM colonization and the activities of CAT, SOD, and POD, but a dramatically negative correlation with the concentration of $O_2^{\bullet-}$ and MDA. Probably, mycorrhizas preferentially stimulated the overexpression of antioxidant enzymes in host plants under DS, potentially leading to a low accumulation of ROS in the host plant (He *et al.*, 2020).

Conclusions

Mycorrhizal fungi could respond to drought and salt stress by regulating various antioxidant enzyme genes in host plants (Saxena *et al.*, 2017; Zou *et al.*, 2020). The RNA-sequence analysis further confirmed that AMF coped with oxidative stress via the induction of multiple metabolic processes, including antioxidant enzyme genes (Venice *et al.*, 2016). In the present study, DS sustainably up-regulated the expressions of *CsSOD* and *CsCAT* in mycorrhizal plants. Moreover, only the expression of *CsSOD* was markedly up-regulated by DS in non-mycorrhizal plants. The results of this study also showed that AMF treatment markedly up-regulated the expressions of *CsSOD* and *CsCAT* in leaves under both WW and DS conditions. These results are in agree with those reported by He *et al.* (2020) and Zhang *et al.* (2020) in citrus plants, indicating that AMF could induce the overexpression of SOD isoenzyme and CAT genes, thereby reducing oxidative stress and membrane lipid peroxidation damage. These results further confirmed that higher expression levels of genes related to antioxidant enzymes in AMF-inoculated plants could increase their capacity to eliminate ROS under DS conditions (He *et al.*, 2020; Zhang *et al.*, 2020). In addition to the role of antioxidant enzymes in ROS scavenging, fungal SOD is also a part of mycorrhizal establishment and plant defense response in AMF plant dialogue (González-Guerrero *et al.*, 2010). In *Gigaspora margarita*, the *CuZnSOD* gene was highly expressed in root cells containing mycorrhizas, followed by the inductions of H_2O_2 accumulation in the intracellular fungal structures, such as arbuscules (Lanfranco *et al.*, 2005), and a subsequent degradation during their life cycle (Fester and Hause, 2005). The AM fungus *Glomus intraradices*, a model arbuscular mycorrhizal organism, was observed to up-regulate the expression of *GintSOD*, thereby indicating that *GintSOD1* encodes SOD to maintain its function in ROS scavenging (González-Guerrero *et al.*, 2010). In tea plants, whether fungal antioxidant enzyme genes have similar functions to alleviate oxidative stress needs to be further studied, as the dialogue network between a plant and the AM fungal in alleviating ROS burst under drought stress is continuously complex.

Authors' Contributions

CYL, QSW, TYY and KK conceived and designed the study. CYL and YJW performed the experiments and interpreted the data. CYL and QSW wrote the manuscript, TYY and KK provided critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the Open Fund of State Key Laboratory of Tea Plant Biology and Utilization (SKLTOF20190105, SKLTOF20200122) and the University of Hradec Kralove (Faculty of Science, VT2019-2021).

Conflict of Interests

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

References

- Allen MF (2007). Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal* 6: 291-297. <https://doi.org/10.2136/vzj2006.0068>
- Amako K, Chen GX, Asada K (1994). Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. *Plant and Cell Physiology* 35:497-504.
- Amiri R, Nikbakht A, Etemadi N (2015). Alleviation of drought stress on rose geranium [*Pelargonium graveolens* (L.) Herit.] in terms of antioxidant activity and secondary metabolites by mycorrhizal inoculation. *Scientia Horticulturae* 197:373-380. <https://doi.org/10.1016/j.scienta.2015.09.062>
- Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM (2011). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *Journal of Agronomy and Crop Science* 197:177-185. <https://doi.org/10.1111/j.1439-037X.2010.00459.x>
- Asrar AA, Abdel-Fattah GM, Elhindi KM (2012). Improving growth, flower yield, and water relations of snapdragon (*Antirrhinum majus* L.) plants grown under well-watered and water-stress conditions using arbuscular mycorrhizal fungi. *Photosynthetica* 50:305-316. <https://doi.org/10.1007/s11099-012-0024-8>
- Bellani LM, Salvini L, Dell'Aquila A, Scialabba A (2012). Reactive oxygen species release, vitamin E, fatty acid and phytosterol contents of artificially aged radish (*Raphanus sativus* L.) seeds during germination. *Acta Physiologiae Plantarum* 34:1789-1799. <https://doi.org/10.1007/s11738-012-0976-0>
- Cheruiyot EK, Mumera LM, Ng'etich WK, Hassanali A, Wachira F, Wanyoko JK (2008). Shoot epicatechin and epigallocatechin contents respond to water stress in tea [*Camellia sinensis* (L.) O. Kuntze]. *Bioscience, Biotechnology and Biochemistry* 72:1219-1226. <https://doi.org/10.1271/bbb.70698>
- Ding ZT, Jia SS, Wang Y, Xiao J, Zhang YF (2017). Phosphate stresses affect ionome and metabolome in tea plants. *Plant Physiology and Biochemistry* 120:30-39. <https://doi.org/10.1016/j.plaphy.2017.09.007>
- Fester T, Hause G (2005). Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. *Mycorrhiza* 15:373-379. <https://doi.org/10.1007/s00572-005-0363-4>
- Giannopolitis CN, Ries SK (1977). Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiology* 59:309-314. <https://doi.org/10.1104/pp.59.2.309>
- Goldblith SA, Proctor BE (1950). Photometric determination of catalase activity. *Journal of Biological Chemistry* 187:70-709.
- González-Guerrero M, Oger E, Benabdellah K, Azcón-Aguilar C, Lanfranco L, Ferro N (2010). Characterization of a CuZn superoxide dismutase gene in the arbuscular mycorrhizal fungus *Glomus intraradices*. *Current Genetics* 56:265-274. <https://doi.org/10.1007/s00294-010-0298-y>

- Gupta S, Bharalee R, Bhorali P, Das SK, Bhagawati P, Bandyopadhyay T, ... Das S (2013). Molecular analysis of drought tolerance in tea by cDNA-AFLP based transcript profiling. *Molecular Biotechnology* 53:237-248. <https://doi.org/10.1007/s12033-012-9517-8>
- Gupta S, Bharalee R, Bhorali P, Bandyopadhyay T, Gohain B, Agarwal N, ... Das S (2012). Identification of drought tolerant progenies in tea by gene expression analysis. *Functional and Integrative Genomics* 12:543-563. <https://doi.org/10.1007/s10142-012-0277-0>
- He JD, Dong T, Wu HH, Zou YN, Wu QS, Kuča K (2019). Mycorrhizas induce diverse responses of root *TIP* aquaporin gene expression to drought stress in trifoliate orange. *Scientia Horticulturae* 243:64-69.
- He JD, Zou YN, Wu QS, Kuča K (2020). Mycorrhizas enhance drought tolerance of trifoliate orange by enhancing activities and gene expression of antioxidant enzymes. *Scientia Horticulturae* 262:108745. <https://doi.org/10.1016/j.scienta.2019.108745>
- Huang YM, Srivastava AK, Zou YN, Ni QD, Han Y, Wu QS (2014). Mycorrhizal induced calmodulin mediated changes in antioxidant enzymes and growth response of drought-stressed trifoliate orange. *Frontiers in Microbiology* 5:682. <https://doi.org/10.3389/fmicb.2014.00682>
- Huang YM, Zou YN, Wu QS (2017). Alleviation of drought stress by mycorrhizas is related to increased root H₂O₂ efflux in trifoliate orange. *Scientific Reports* 7:42335. <https://doi.org/10.1038/srep42335>
- Kapoor R, Singh N (2017). Arbuscular mycorrhiza and reactive oxygen species. In: Wu QS (Ed.), *Arbuscular mycorrhizas and stress tolerance of plants*. Springer Nature Singapore Pte Ltd., Singapore, pp 25-42. https://doi.org/10.1007/978-981-10-4115-0_10
- Lanfranco L, Novero M, Bonfante P (2005). The mycorrhizal fungus *Gigaspora margarita* possesses a CuZn superoxide dismutase that is up-regulated during symbiosis with legume hosts. *Plant Physiology* 137:1319-1330. <https://doi.org/10.1104/pp.104.050435>
- Liu J, Xiao B, Wang LX, Li J, Pu GT, Gao T, Liu W (2013). Influence of AM on the growth of tea plant and tea quality under salt stress. *Journal of Tea Science* 33:140-146. (in Chinese with English abstract)
- Liu SC, Jin JQ, Ma JQ, Yao MZ, Ma CL, Li CF, Ding ZT, Chen L (2016). Transcriptomic analysis of tea plant responding to drought stress and recovery. *PLoS One* 11:e0147306. <https://doi.org/10.1371/journal.pone.0147306>
- Liu SC, Yao MZ, Ma CL, Jin JQ, Ma JQ, Li CF, Chen L (2015). Physiological changes and differential gene expression of tea plant under dehydration and rehydration conditions. *Scientia Horticulturae* 184:129-141. <https://doi.org/10.1016/j.scienta.2014.12.036>
- Mirshad PP, Puthur JT (2016). Arbuscular mycorrhizal association enhances drought tolerance potential of promising bioenergy grass (*Saccharum arundinaceum* retz.). *Environment Monitoring and Assessment* 188:425. <https://doi.org/10.1007/s10661-016-5428-7>
- Mustafa G, Khong NG, Tisserant B, Randoux B, Fontaine J, Magnin-Robert M, Reignault P, Sahraoui ALH (2017). Defence mechanisms associated with mycorrhiza-induced resistance in wheat against powdery mildew. *Functional Plant Biology* 44:443-454. <https://doi.org/10.1071/fp16206>
- Nepolean P, Jayanthi R, Pallavi RV, Balamurugan A, Kuberan T, Beulah T, Premkumar R (2012). Role of biofertilizers in increasing tea productivity. *Asian Pacific Journal of Tropical Biomedicine* 2:S1443-S1445.
- Polle A, Otter T, Seifert F (1994). Apoplastic peroxidases and lignification in needles of Norway (*Picea abies* L.). *Plant Physiology* 106:53-60. <https://doi.org/10.1104/pp.106.1.53>
- Qian W, Hu J, Zhang X, Zhao L, Wang Y, Ding Z (2018). Response of tea plants to drought stress. In: Han WY (Ed.), *Stress physiology of tea in the face of climate change*, Springer, Singapore pp 63-81.
- Quiroga G, Ericea G, Aroca R, Zamarreño AM, García-Mina JM, Ruiz-Lozano JM (2020). Radial water transport in arbuscular mycorrhizal maize plants under drought stress conditions is affected by indole-acetic acid (IAA) application. *Journal of Plant Physiology* 246:153115. <https://doi.org/10.1016/j.jplph.2020.153115>
- Saxena B, Shukla K, Giri B (2017). Arbuscular mycorrhizal fungi and tolerance of salt stress in plants. In: Wu QS (Ed.), *Arbuscular mycorrhizas and stress tolerance of plants*. Springer Nature Singapore Pte Ltd., Singapore, pp 43-66.
- Shao YD, Zhang DJ, Hu XC, Wu QS, Jiang CJ, Gao XB, Kuca K (2019). Arbuscular mycorrhiza improves leaf food quality of tea plants. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 47:608-614. <https://doi.org/10.15835/nbha47311434>
- Shao YD, Zhang DJ, Hu XC, Wu QS, Jiang CJ, Xia TJ, ... Kuča K (2018). Mycorrhiza-induced changes in root growth and nutrient absorption of tea plants. *Plant, Soil and Environment* 64:283-289. <https://doi.org/10.17221/126/2018-PSE>

- Sharma P, Kumar S (2005). Differential display-mediated identification of three drought-responsive expressed sequence tags in tea [*Camellia sinensis* (L.) O. Kuntze]. *Journal of Bioscience* 30:231-235. <https://doi.org/10.1007/BF02703703>
- Singh S, Pandey A, Chaurasia B, Palni LMS (2008). Diversity of arbuscular mycorrhizal fungi associated with the rhizosphere of tea growing in 'natural' and 'cultivated' ecosystems. *Biology and Fertility of Soils* 44:491-500. <https://doi.org/10.1007/s00374-007-0231-9>
- Singh S, Pandey A, Kumar B, Palni LMS (2010). Enhancement in growth and quality parameters of tea [*Camellia sinensis* (L.) O. Kuntze] through inoculation with arbuscular mycorrhizal fungi in an acid soil. *Biology and Fertility of Soils* 46:427-433. <https://doi.org/10.1007/s00374-010-0448-x>
- Sudhakar C, Lakshmi A, Giridarakumar S (2001). Changes in the antioxidant enzymes efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Science* 161:613-619. [https://doi.org/10.1016/S0168-9452\(01\)00450-2](https://doi.org/10.1016/S0168-9452(01)00450-2)
- Tuo XQ, He L, Zou YN (2017). Alleviation of drought stress in white clover after inoculation with arbuscular mycorrhizal fungi. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 45:220-224. <https://doi.org/10.15835/nbha45110709>
- Upadhyaya H, Panda SK (2013). Abiotic stress responses in tea [*Camellia sinensis* L (O) Kuntze]: an overview. *Review of Agricultural Science* 1:1-10. <https://doi.org/10.7831/ras.1.1>
- Vannini C, Carpentieri A, Salvioli A, Novero M, Marsoni M, Testa L, ... Bonfante P (2016). An interdomain network: the endobacterium of a mycorrhizal fungus promotes antioxidative responses in both fungal and plant hosts. *New Phytologist* 211:265-75. <https://doi.org/10.1111/nph.13895>
- Wang AG, Luo GH (1990). Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. *Plant Physiology Communications* 26:55-57.
- Wang W, Xin H, Wang M, Ma Q, Wang L, Kaleri NA, Wang Y, Li X (2016). Transcriptomic analysis reveals the molecular mechanisms of drought-stress-induced decreases in *Camellia sinensis* leaf quality. *Frontiers in Plant Science* 7:385. <https://doi.org/10.3389/fpls.2016.00385>
- Wang Y, Fan K, Wang J, Ding ZT, Wang H, Bi CH, Zhang YW, Sun HW (2017). Proteomic analysis of *Camellia sinensis* (L.) reveals a synergistic network in the response to drought stress and recovery. *Journal of Plant Physiology* 219:91-99. <https://doi.org/10.1016/j.jplph.2017.10.001>
- Wu QS, Zou YN (2017). Arbuscular mycorrhizal fungi and tolerance of drought stress in plants. In: Wu QS (Ed.), *Arbuscular mycorrhizas and stress tolerance of plants*. Springer Nature Singapore Pte Ltd., Singapore, pp 25-42.
- Wu QS, Shao YD, Gao XB, Xia TJ, Kuča K (2019). Characterization of AMF-diversity of endosphere versus rhizosphere of tea (*Camellia sinensis*) crops. *Indian Journal of Agricultural Sciences* 89:348-352.
- Xu T, Veresoglou SD, Chen Y, Rillig MC, Xiang D, Daniel O (2016). Plant community, geographic distance and abiotic factors play different roles in predicting AMF biogeography at the regional scale in northern China. *Environmental Microbiology Reports* 8:1048-1057. <https://doi.org/10.1111/1758-2229.12485>
- Zhang F, Zou YN, Wu QS, Kuča K (2020). Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. *Environmental and Experimental Botany* 171:103962. <https://doi.org/10.1016/j.envexpbot.2019.103926>
- Zhang F, Zou YN, Wu QS (2018). Quantitative estimation of water uptake by mycorrhizal extraradical hyphae in citrus under drought stress. *Scientia Horticulturae* 229:132-136.
- Zhou L, Xu H, Mischke S, Meinhardt LW, Zhang D, Zhu X, Li X, Fang W (2014). Exogenous abscisic acid significantly affects proteome in tea plant (*Camellia sinensis*) exposed to drought stress. *Horticulture Research* 1:14029.
- Zhu X, Song F, Liu S (2011). Arbuscular mycorrhiza impacts on drought stress of maize plants by lipid peroxidation, proline content and activity of antioxidant system. *Journal of Food, Agriculture and Environment* 9:583-587.
- Zou YN, Huang YM, Wu QS, He XH (2015). Mycorrhiza-induced lower oxidative burst is related with higher antioxidant enzyme activities, net H₂O₂ effluxes, and Ca²⁺ influxes in trifoliate orange roots under drought stress. *Mycorrhiza* 25:143-152. <https://doi.org/10.1007/s00572-014-0598-z>
- Zou YN, Srivastava AK, Ni QD, Wu QS (2015). Disruption of mycorrhizal extraradical mycelium and changes in leaf water status and soil aggregate stability in root box-grown trifoliate orange. *Frontiers in Microbiology* 6:203. <https://doi.org/10.3389/fmicb.2015.00203>
- Zou YN, Wu QS, Kuča K (2020). Unravelling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress. *Plant Biology*. <https://doi.org/10.1111/plb.13161>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; UASVM, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.