Pak. J. Bot., 49(4): 1531-1540, 2017.

INTERACTIVE EFFECTS OF COPPER STRESS AND ARBUSCULAR MYCORRHIZAL FUNGI ON PHOTOSYNTHETIC CHARACTERISTICS AND CHLOROPHYLL FLUORESCENCE PARAMETERS OF *ELSHOLTZIA SPLENDENS*

YUELING LI[1,2], ZEXIN JIN[1,2] AND JUNMIN LI[1,2*]

[1]*Zhejiang Provincial Key Laboratory of Plant Evolutionary and Conservation, Taizhou University, Taizhou 318000, China*
[2]*Institute of Ecology, Taizhou University, Taizhou 318000, China*
[*] *-mail: lijmtzc@126.com (J Li); Tel (Fax): +86 576 88660336*

Abstract

To determine interactive effects of added copper (Cu) and arbuscular mycorrhizal fungi (AMF) inoculation on the photosynthesis of *Elsholtzia splendens*, a greenhouse pot experiment was conducted. Four treatments were used, including -Cu-AMF (no Cu addition and no AMF inoculation), +Cu-AMF (Cu addition but no AMF inoculation), -Cu+AMF (no Cu addition and AMF inoculation), and +Cu+AMF (Cu addition and AMF inoculation). Cu addition did not change diurnal variation curves of the net photosynthetic rate(P_N), the intercellular CO_2 concentration (C_i), the stomatal conductance $_s$), or the transpiration rate (E); however, it significantly decreased the daily mean P_N, g_s, E, light-use efficiency (LUE), and carboxylation efficiency (CE). Furthermore, AMF inoculation significantly increased the daily mean P_N, g_s, LUE, and CE of *E. splendens.* In response to light, Cu addition significantly decreased the light-saturated net photosynthetic rate (P_{Nmax}), the light saturation point (LSP), the light compensation point (LCP), and the apparent quantum yield (AQY), while AMF inoculation significantly increased P_{Nmax} and AQY. In response to the CO_2 concentration, Cu addition significantly decreased P_{Nmax} and the CO_2saturation point (CSP), while AMF inoculation significantly increased P_{Nmax}. Both Cu addition and AMF inoculation significantly decreased the relative chlorophyll content. Compared to the negative control treatment (-Cu-AMF), Cu addition significantly increased the minimal fluorescence, but significantly decreased maximal fluorescence, variable fluorescence,and maximum photochemical efficiency of PSII. These results suggest that AMF inoculations alleviate the inhibitory effect of copper stress on *E. splendens* plants by weakening its toxic effects on the photosynthetic apparatus and pigments.

Key words: AMF, Cu stress, Gas exchange, , Light response, CO_2 response, Chlorophyllfluorescence

Introduction

Copper (Cu) is a redox-active transition metal, essential for many metabolic pathways, such as respiratory and photosynthetic electron transport, antioxidant activity, and protein and cell wall metabolism (Kamali *et al*., 2012); however, an excess of Cu can potentially cause complete disruption of plant growth and development (Cook *et al*., 1997, Wang *et al*., 2012, Arunakumara *et al*., 2013). Cu toxicity is very severe in agriculture due to the use of agrochemicals containing Cu as an active component (Chen *et al*., 2013), in the electrolytically generated Cu to restrain algae and diseases (Zheng *et al*., 2004), and in mine-waste tailings of Cu ores due to the residue of Cu particles in mine slurry (Kabata-Pendias & Pendias, 2001). Remediation techniques are urgently required to reduce the concentrations of this metal in the soil and to avoid its absorption by crop plants (Arunakumara *et al*., 2013).

Arbuscular mycorrhizal fungi (AMF) are common root symbionts of terrestrial plants. AMF can significantly enhance the heavy metal tolerance of plants, including Cu stress (Carvalho *et al*., 2006, Malekzadeh *et al*., 2007, Hildebrandt *et al*., 2007, Ferrol *et al*., 2009; Meier *et al*., 2011; 2012; 2015). The alleviative effect of AMF inoculation on Cu stress might be due to increasing water and nutrient absorption, particularly phosphate (Andrade *et al*., 2009; Helgason & Fitter 2009; Smith & Smith, 2013), thus reducing the transfer of toxic metals into the shoots (Andrade *et al*., 2009; Amir *et al*., 2013), while increasing heavy-metal accumulation in plant tissues (Orlowska *et al*., 2012). However, few of these studies have investigated the physiological changes within plants under Cu stress induced by AMF colonization.

Photosynthesis is one of the central physiological processes in plants contributing to their growth (Cheng *et al*., 2000). It is well documented that Cu exerts direct toxicity on photosynthesis (Lidon, 1999; Maksymiec, 1997) by disturbing lipid peroxidation of thylakoid membranes as well as the interaction between lipids and proteins in the chloroplast membrane (Szalontai *et al*., 1999), thus severely affecting the photosynthetic electron transport chain -Kurdziel & Strazalka, 2002). Cu causes indirect activities or with net CO_2 assimilation (Prasad & Strzalka, 1999), ultimately inhibiting plant growth (Vinit-Dunand *et al*., 2002; Qian *et al*., 2005). Mycorrhizal symbiosis can alter the photosynthesis of the host plant to aid against adverse environmental conditions (Zhu *et al.*, 2010). Several reports revealed that AMF inoculation could also affect photosynthesis in strawberries (Borkowska, 2002) and citrus fruits (Wu & Xia, 2006) under drought stress. Aloui *et al.* (2011) demonstrated that inoculation with the AMF *Glomus irregulare* resulted in positive effects on photosynthesis in the presence of Cd, while increasing photosynthesis-related proteins. However, the effects on photosynthesis of AM symbiosis are related to the species of fungus, soil nutrient condition, and to the particular plant involved. Bittman *et al.* (2006) detected poor photosynthetic response to AMF inoculation under high nutrient condition. Syvertsen & Grahanm (1990) reported no influence of AMF infection on the net gas exchange characteristics of citrus leaves on plants. To our knowledge, no report exists that focuses on the

effect of AMF inoculation on the photosynthesis of plants under Cu stress.

Elsholtzia splendens is an annual herb from the Lamiaceae family and is a Cu-tolerant plant used as ametal hyperaccumulator (Jiang *et al*., 2008). *E. Splendens* is widely distributed on Cu-polluted soils and Cu-mining wastes (Tang *et al*., 1999, Lou *et al*., 2004) and is reported to be an obligate symbiont with AMF (Yang *et al*., 2010). AMF in turn plays a central role in plantuptake and accumulation of heavy metals (Wang *et al*., 2006). In high concentrations, Cu significantly inhibits photosynthetic parameters (Ke *et al*., 2007); however, inoculation with soil microbes can significantly increase the photosynthetic ability of *E. splendens* (Li *et al*., 2015). We conducted a pot experiment and found significant interactions between mycorrhizal inoculation and Cu addition on the total seed number, vegetative biomass, and inflorescence number of *E. splendens* (Jin *et al*., 2015). Here, we were using the same experimental system to explore interactions between AMF and Cu, affecting the photosynthetic capability of *E. splendens*to ascertain the following: 1) How are Cu and AMF interactively affecting the daily photosynthetic process of *E. splendens*? 2) How are Cu and AMF interactively affecting the photosynthetic capability of *E.splendens*? These results provide a basic reference for the application of hyperaccumulators in the phytoremediation and ecological restoration of Cu polluted soils.

Materials and Methods

Soil preparation: Theculture medium that was used for the pot experiment consisted of vermiculite, sand, and peat soil (1:3:6, v/v/v). The soil medium was autoclaved under pressure (0.11 MPa) at 121°C for 2 h to neutralize all native microbial populations (Andrade *et al*., 2009). Subsequent to autoclaving, each kilogram of soil had the following properties: 20.16±0.26 gorganic matter, 14.61±0.53 mgtotal N, 17.86±0.49 mg available P, and 56.67±0.16mgavailable K. The pH (in water) was 5.73±0.04.

Seed germination: On the 20th of December 2012, seedsof *E. splendens* were obtained from clean soilin the Tainan

(31°30.632 N, 114°32.620 E; altitude of 118 m) after which, they were transferred to an incubator at room temperature. On the 5th of May 2013, seeds were surface disinfectedin a 0.5% solution of hypochlorite and thoroughlyrinsed with sterileredistilled water.Then, they were sowed into the autoclaved soil mixture within 4×8 trays for germination in a greenhouse at the Taizhou University in Zhejiang Province

Treatments: On the 1st of May 2013, plastic pots (15 cm deep, round, and with an inner diameter of 19 cm)were filled with 1.7 kg of autoclaved soil mixture, after sterilization via 75% ethanol. All pots were randomized and placed into the greenhouseunder a relative humidity of 70% ± 10.5% and a temperature of 30.0 ± 5 °C during the days and 18.0 ± 2°C during nights. Plants were illuminated with natural light. The experiment consisted of four treatments, including (1) -Cu+AMF (no Cu addition and AMF inoculation), (2) +Cu+AMF (both Cu addition and AMF inoculation), (3) -Cu-AMF (no Cu addition and no AMF inoculation), and (4) +Cu-AMF (Cu addition but no AMF inoculation). A total of 60 pots were used with 15 repetitions per treatment. On the 5th of May 2013, 50 mL aliquot of $CuSO_4 \cdot 5H_2O$ solution (34 mg mL^{-1}) were added to each pot of the treatment groups +Cu+AMF and +Cu-AMF. The available Cu content at the start of experiment in the soil of all four treatments was 18.90 ± 2.05 $mgkg^{-1}$.

On the 21st of December 2012, bulk sandy clay soilwas collected from the top layer (0-20 cm) at a Cu mine tailing, which was located within the Chimashan Mountains, Yangxin County, Hubei Province, China (29°59.776 N, 115°05.856 E; altitude 138 m). The accompanying plants were *Xanthium sibiricum*,*Cynodon dactylon*, *Commelina communis*, *Artemisia capillaries*,and *Silene fortunei*. The soil was sieved with a 2-mm sieve to remove all litter and vegetation, subsequently stored at -20°C until further use as a resource of soil microbes. On the 6h of May 2013, soil obtained from a Cu mine tailing was taken out of the refrigerator and incubated at room temperature for 48 h. TheAMF were inoculated, following a previously published procedure (Jin *et al*., 2015). In the no AMF inoculation treatments, 50 mL filtrate was applied to each of the pots to compensate for the microbe treatment of the other groups. On the 5th of June 2013, one 12-cm-tall seedling was transplanted into each pot. All pots were well watered and the soil moisture content wasmonitored via weight.

Gas exchange measurement:

$^{-2}s^{-1}$. The resulting light response curves were analyzed via the revised exponential equation (Ye, 2007);

$$P(I) = \alpha \frac{1-\beta I}{1+\gamma I}(I - I_c),$$

$P(I)$ isthe net photosynthetic rate, I is the incidentPAR, and I_c is the light saturation point. The maximumleaf light-saturated net photosynthetic rate (P_{Nmax}), light saturation point (LSP), light compensation point(LCP), andthe apparent quantum yield (AQY) were calculated via the above equation (Ye, 2007).

CO_2 response curves: The CO_2 response curves were measured between 09:30 and 11:00 h (Beijing time) on fully expanded leaves fromeach plant with a leaf temperature of 25°C, a light saturating intensity of 1,500 $^{-2}s^{-1}$ (LI6400-02B; LED red/blue light source), and a relative humidity of 70 ± 5%. $CO_2$was supplied from a small portable cylinder, filled to a specified CO_2 pressure. Prior to the measurements, the leaves were equilibrated at thelight saturating intensityfor at least15 min to reach steady-state photosynthesis. Once stable, the photosynthetic capacity of the leaves was measuredat aseries of CO_2 concentrations of 1,500, 1,200, 1,000, 800, 600,400, 200, 150, 120, 100, $^{-1}$. The interval between each CO_2 concentration was 300 s and the entire CO_2-response curves were analyzed via the rectangular hyperbolic equation (Ye & Yu, 2009).

$$P(C_a) = a\frac{1-C_a}{1+C_a}C_a - R_p,$$

where a is a coefficient, C_a is the concentration of atmospheric CO_2, $P(C_a)$ isthe net photosynthetic rate, and R_p is the light respiration rate. The maximumleaf light-saturated photosynthetic rate (P_{Nmax}), the CO_2-saturation point (CSP), the CO_2-compensation point(CCP),and the apparent carboxyl efficiency (CE) were calculated via the above equation (Ye & Yu, 2009).

Chlorophyll content determination: The leaf chlorophyllvalues were obtained, using a CCM-200 plus chlorophyll content meter (Opti-Science Inc., Hudson, NH, USA). The third adult leaf counted from the apex of a plant was tested.

:

Chlorophyll fluorescence was measured between 08:00 and 11:00 h (Beijing time) usinga OS30P portable fluorometer (Opti-Science Inc., Hudson, NH,USA) (Li *et al*., 2012). The thirdhealthy and mature leaf from the apex of a plant was tested afterdark-adaptatation for 30 min with dark leaf chips. The variable fluorescence (F_v), the minimal fluorescence yield (F_0), and the maximal flurescence yield (F_m) were measured of dark-adapted leaf tissues. The maximum photochemical efficiency of PSIIwas defined asF_v/F_m to express the maximum PSII photochemical efficiency.

Rate determination of AMF colonization: Subsequent to the measurements, the fine roots of the plants were collected, and AMF infection was verified via mycorrhizal colonization rate. Analyses of AMF colonization of host plants were performed according to previously described staining methods (Kormanik *et al*., 1980; Jin *et al*., 2015), and observed via light microscopy. Vesicles, arbuscules, and intercellular hyphalwere observed in root segments that were considered to be mycorrhizal. The rate of AMF colonization was calculated, using the following formula: Colonization (%)= (length of root infected / total length of root observed) × 100% (Graham & Syvertsen, 1985). In both the +Cu+AMF and -Cu+AMF treatments, the AMF colonization rates were 42.50% and 52.78%, respectively; however, they were zero in both non-inoculated plant treatments. These results suggest that AMF treatments were successfully colonized.

Statistical analysis: The differences of AMF inoculation, Cu addition, and their interactive effects on plant photosynthetic characteristic parameterswere determined via two-way (ANOVA).Means among four treatments were compared $p<0.05$. Data are expressed as means with standard deviations (SD). SigmaPlot (version 13.0) was utilized to create all figures, and the SPSS software package (version 17.0) was used for all statistical analyses.

Results

Interactive effects of the daily photosynthetic process of *E. splendens*: The P_N, g_s, and E diurnal variation curves of *E. splendens*in all four different treatments showed similar single peaks without a midday depression, while the diurnal C_i variation curves showed a V-type curve (Fig. 1). Cu addition significantly decreased the daily mean P_N, g_s, LUE, and CE, while AMF inoculation significantly increased these parameters (Table 1). Cu addition significantly decreased the daily mean E, however, significantly increased C_i, while AMF inoculation had no significant effect (Table 1). Cu addition had no significant effect on WUE, while AMF inoculation increased it significantly. The interactive effect of AMF inoculation and Cu addition significantly affected the daily mean C_i but had no significant effect on the remaining three parameters (Table 1).

Interactive effect of light and CO_2 response curves in *E. splendens*: The light and CO_2 photosynthetic rate response curves of all four different treatments are shown in Figure 2. AMF-inoculated plants exhibited a significantly higher photosynthetic rate, while plants that were subjected to Cu-addition exhibited a significantly lower rate. In response to light, Cu addition significantly decreased P_{Nmax}, LSP, LCP, and AQY; however, AMF inoculation significantly increased P_{Nmax} and AQY in *E. splendens*. The interactive effect of AMF inoculation and Cu addition significantly affected P_{Nmax} and AQY in *E. splendens* (Fig. 3). In response to CO_2 concentration, Cu addition significantly decreased P_{Nmax} and CSP in *E. splendens*; however, AMF inoculation significantly increased P_{Nmax} only. Two-way *ANOVA* revealed that Cu had a significant effect on P_{Nmax} and CSP, while AMF inoculation had a significant effect on P_{Nmax}, CSP, and CCP. The interactive effect of AMF inoculation and Cu addition on P_{Nmax} was significant for *E. splendens* (see Fig. 4).

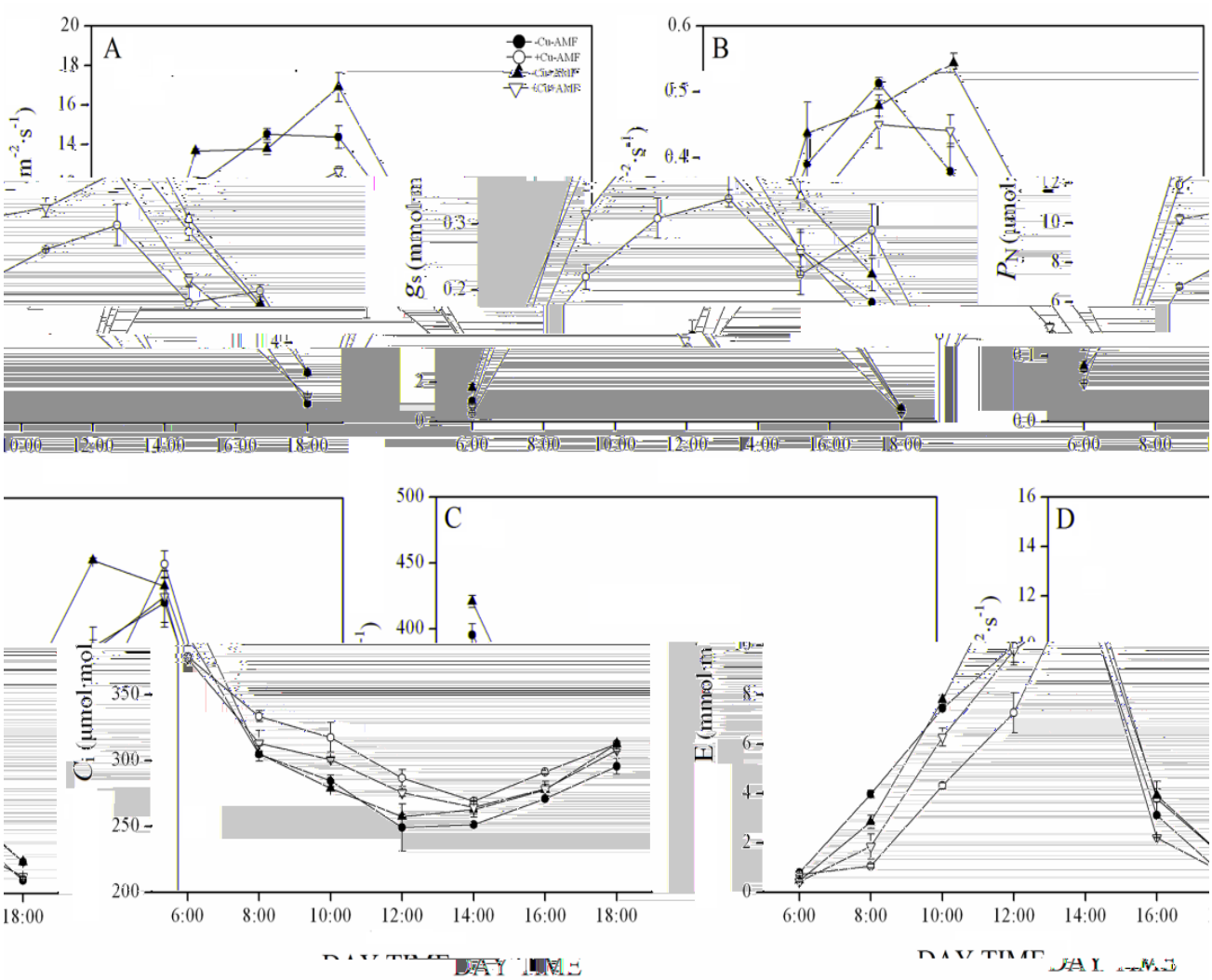

Fig. 1. Diurnal variation curves in *E. splendens*in four different treatments. Data points represent average results of three plants per treatment ± standard deviation.-Cu-AMF, +Cu-AMF, -Cu+AMF, and +Cu+AMF indicate no Cu addition and no AMF inoculation, Cu addition but no AMF inoculation, AMF inoculation but no Cu addition, Cu addition and AMF inoculation, respectively.

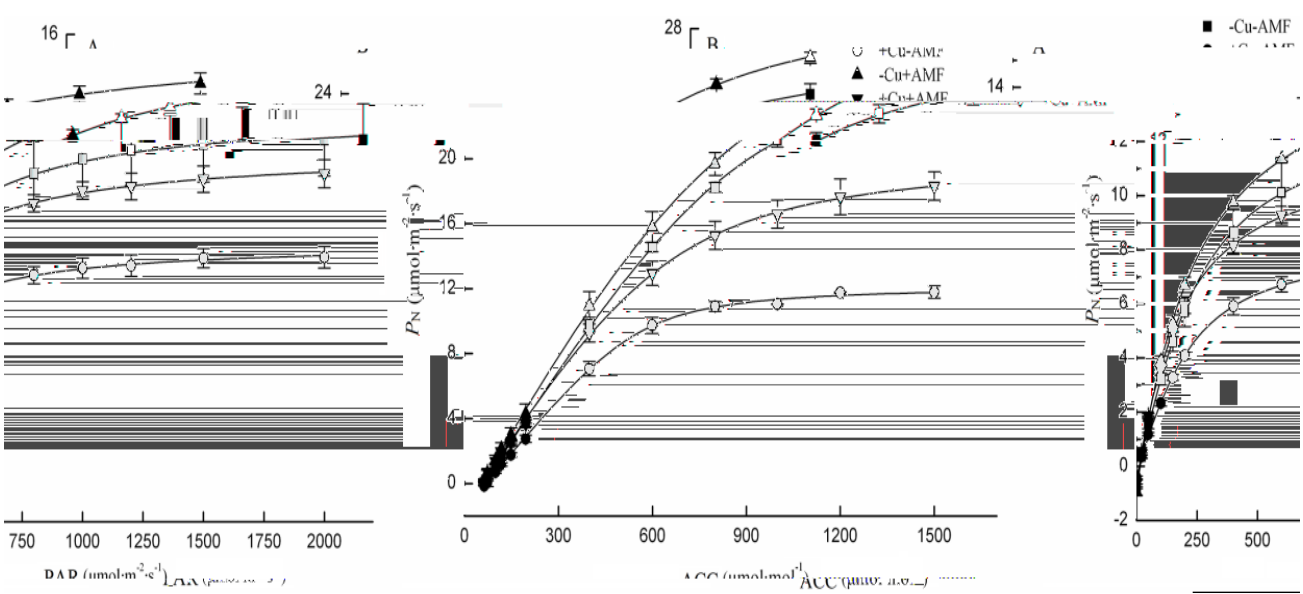

Fig. 2. Response of the net photosynthetic rate (P_N) on photosynthetically active radiation (A) and atmospheric CO_2 concentration (B) in *E. splendens*. Data points represent average results of three plants per treatment ± standard deviation.-Cu-AMF, +Cu-AMF, -Cu+AMF, and +Cu+AMF indicate no Cu addition and no AMF inoculation, Cu addition but no AMF inoculation, AMF inoculation but no Cu addition, Cu addition and AMF inoculation, respectively.

Table 1. Interactive effects of Cu addition and AMF inoculation on P_N, g_s, C_i, E, LUE, WUE and CE in *E. splendens* in four different treatments, and the two-way ANOVA results.

Treatments	P_N/ $^{-2}\cdot s^{-1}$)	g_s/ (mmol·m^{-2}·s^{-1})

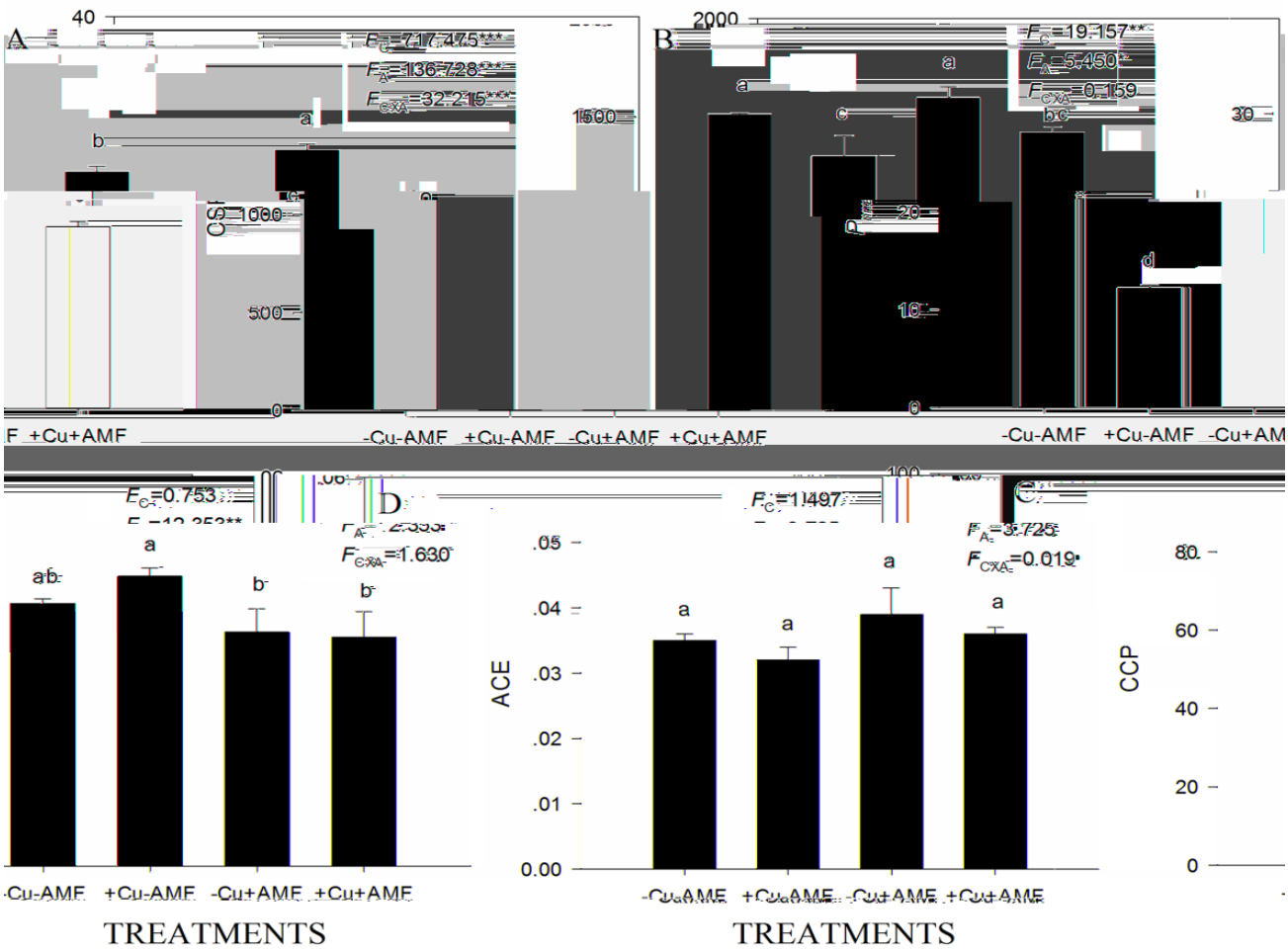

Fig. 4. Interactive effects of Cu addition and AMF on the maximum net photosynthetic rate (P_{Nmax}, A), CO_2 saturation point (CSP, B), CO_2 compensation point (CP, C), and apparent carboxylation efficiency (ACE, D). -Cu-AMF, +Cu-AMF, -Cu+AMF, and +Cu+AMF indicate no Cu addition and no AMF inoculation, Cu addition but no AMF inoculation, AMF inoculation but no Cu addition, Cu addition and AMF inoculation, respectively. Different small letters indicate significant differences among the different treatments at $p<0.05$. F_C indicates the effect of Cu addition. *F*-value and significance levels: *, **, and *** indicate significant differences at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

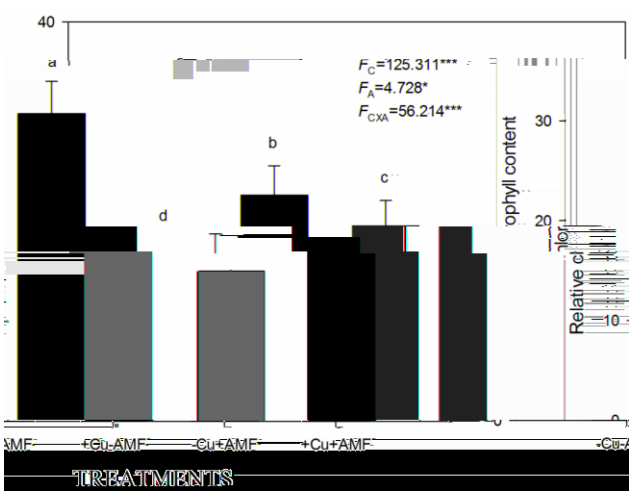

Fig. 5. Interactive effects of Cu addition and AMF on relative chlorophyll content. -Cu-AMF, +Cu-AMF, -Cu+AMF, and +Cu+AMF indicate no Cu addition and no AMF inoculation, Cu addition but no AMF inoculation, AMF inoculation but no Cu addition, Cu addition and AMF inoculation, respectively. Different small letters indicate significant differences among different treatments at $p<0.05$. F_C indicates the effect of Cu addition. *F*-value and significance levels: *, **, and *** indicate significant differences at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Interactive effects on relative chlorophyll contents: Both AMF inoculation and Cu addition significantly decreased the relative chlorophyll content of *E. splendens*, while their interaction significantly affected it (Fig. 5).

Interactive effect of the chlorophyll fluorescence parameters: Compared to the -Cu-AMF treatment, Cu addition significantly increased F_0 but significantly decreased F_m, F_v, and F_v/F_m. Compared to the -Cu-AMF treatment, AMF inoculation did not significantly affect any parameters (Fig. 6). Two-way *ANOVA* revealed that Cu addition had a significant effect on F_0, F_m, F_v, and F_v/F_m, while AMF inoculation significantly affected F_0, F_v, and F_v/F_m. The interaction between AMF inoculation and Cu addition significantly affected F_0 and F_v/F_m (Fig. 6).

Discussion

significantly increased the daily mean P_N, g_s, LUE, and CE in *E. splendens.* These results indicate that Cu addition inhibits photosynthesis in *E. splendens* via an alteration of gas exchange capability and a weakening of the light utilization and carboxylation efficiency, while AMF inoculation could alleviate these inhibitory effects. A similar inhibitory effect of Cu stress has been reported for *E. splendens* (Ke *et al*., 2007) and *Limoniastrum monopetalum* (Cambrollé *et al*., 2013), and a similar enhancement effect of AMF inoculation has been reported for *Zea mays* (Zhu *et al*., 2011). No interactions between AMF and Cu were observed in the above photosynthetic parameters, indicating that both factors might separately influence photosynthesis in *E. splendens*. Further study is required to verify these differences.

The observed decline in P_N might be ascribed to stomatal and/or non-stomatal limitations (Flexas & Medrano, 2002 Akhkha *et al*., 2017). Cambrollé *et al*. (2013) reported that excessive Cu reduced P_N and g_s but had no effect on C_i and the authors thus suggested that the observed reduction of photosynthetic activity might be a non-stomatal limitation. The significant C_i increase that accompanied the increase of P_N and g_s in *E. splendens* under Cu stress also indicated that the inhibition of photosynthesis in this species via excessive Cu might be a non-stomatal limitation; however, it might possibly be related to the inactivation of Rubisco and the limitation of its regeneration via photosynthetic electron transport (Cornejo *et al*., 2008, Zhu *et al*., 2011). This explanation is in agreement with previous studies on *Cucumis sativus* seedlings (Vinit-Dunand *et al*., 2002) as well as rice (Lidon *et al*., 1999).

In this study, light and CO_2 response curves were used to further evaluate the photosynthetic capability of *E. splendens*, treated with the addition of Cu and inoculation of AMF. In response to light and CO_2, Cu stress significantly decreased P_{Nmax}, while AMF inoculation significantly increased it. This indicates that Cu stress weakens the photosynthetic efficiency due to toxicity for the photosynthetic apparatus (Danilov & Ekelund, 2001), while AMF could recover this efficiency. Furthermore, the interactive effect was significant. Cu stress significantly decreased LSP, LCP, AQY, and CSP, while AMF inoculation had no significant effect on these parameters, indicating that *E. splendens* requires greater light intensity to reach the saturation and compensation points (Ögren & Evans, 1993) as well as greater CO_2 concentration to reach the saturation point.

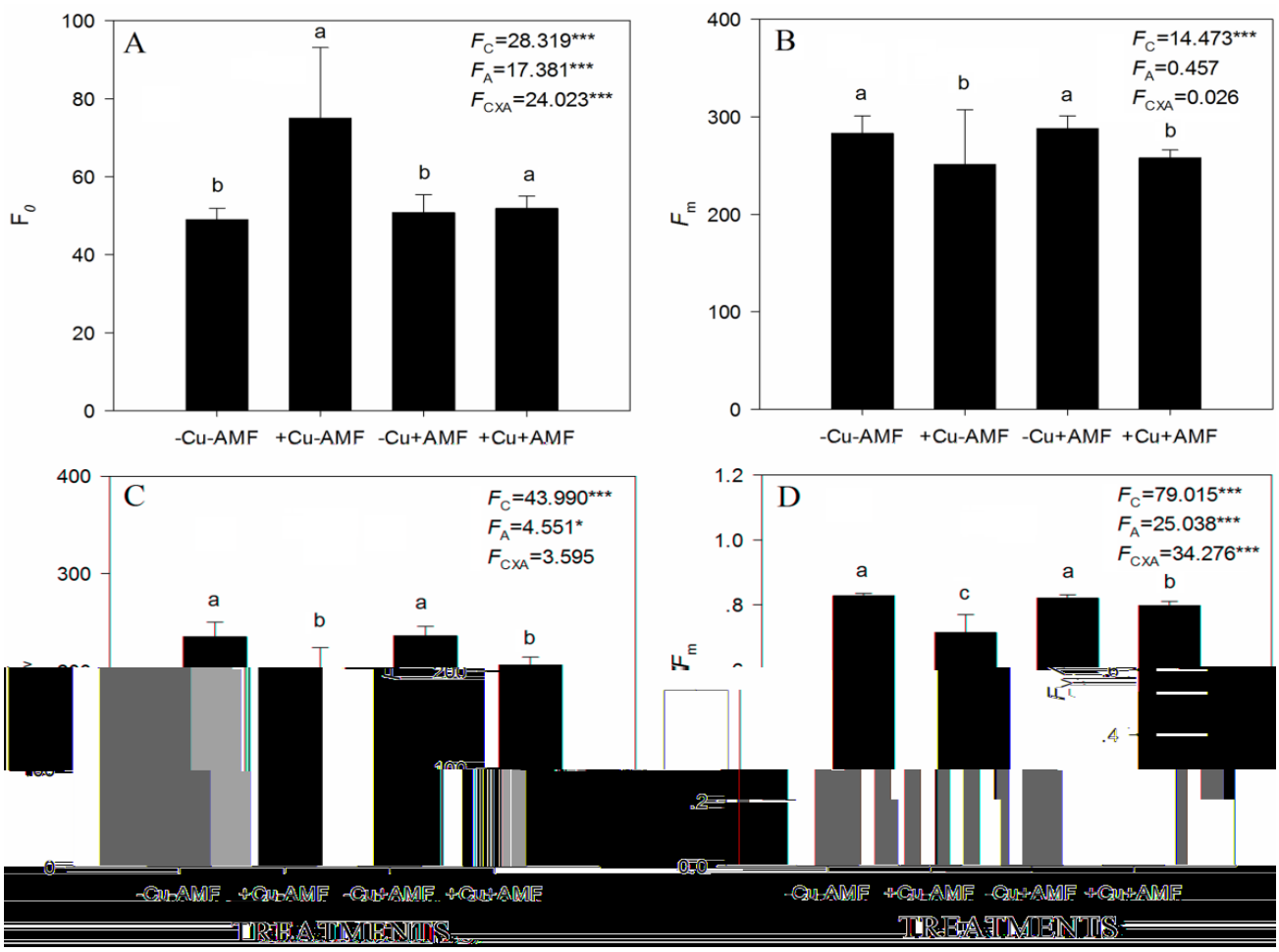

Fig. 6. Interactive effects of Cu addition and AMF on F_0 (A), F_m (B), F_v (C), and F_v/F_m (D). -Cu-AMF, +Cu-AMF, -Cu+AMF, and +Cu+AMF indicate no Cu addition and no AMF inoculation, Cu addition but no AMF inoculation, AMF inoculation but no Cu addition, Cu addition and AMF inoculation, respectively. Different small letters indicate significant differences among different treatments at $p<0.05$. F_C indicates the effect of Cu addition. *F*-value and significance levels: *, **, and *** indicate significant differences at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Three target sites of heavy metal interaction exist in photosynthesis, including photosynthetic pigments, photosynthetic enzymes, and photosystems (Aggarwal *et al*.,

Cambrollé, J., J.M. Mancilla-Leytón, S. Muñoz-Vallés, E. Figueroa-Luque, T. Luque and M.E. Figueroa. 2013. Effects of copper sulfate on growth and physiological responses of *Limoniastrum monopetalum*. *Environ. Sci. Pollut. R.*, 20: 8839-8847.

Cambrollé, J., J.M. Mancilla-Leytón, S. Muñoz-Vallés, T. Luque and M.E. Figueroa. 2012. Zinc tolerance and accumulation in the salt-marsh shrub *Halimione portulacoides*. *Chemosphere*., 86: 867-874.

Carvalho, L., I. Cacador and M. Martinis-Loucao. 2006. Arbuscular mycorrhizal fungi enhance root cadmium and copper accumlation in the roots of the salt marsh plant *Aster tripolium* L. *Plant Soil*, 285: 161-169.

Chen, B.C., P.C. Hoand K.W. Juang. 2013. Alleviation effects of magnesium on copper toxcity and accumulation in grape vine roots evaluated with biotic ligand models. *Ecotoxicol.*, 22: 174-183.

Cheng, W.X., D.A. Sims, Y.Q. Luo, J.S. Coleman and D.W. Johnson. 2000. Photosynthesis, respiration, and net primary production of sunflower stands in ambient and elevated atmospheric CO_2 concentrations: an invariant NPP: GPP ratio? *Glob. Change Biol.*, 6: 931-941.

Cook, C.M., A. Kostidou, E. VardakaandT. Lanaras. 1997. Effects of copper on the growth, photosynthesis and nutrient concentrations of Phaseolus plants. *Photosyn.*, 34: 179-193.

Cornejo, P., S. Meiera, G, Borie, M.C. Rilling and F. Borie. 2008. Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. *Sci. Total Environ.*, 406: 154-160.

Danilov, R.A. and N.G.A. Ekelund. 2001. Responses of photosynthetic efficiency, cell shape and motility in *Euglena gracilis* (Euglenophyceae) to short-term exposure to heavy metals and pentachlorophenol. *Water. Air. Soil. Poll*., 132: 61-73.

Ferrol, N., M. González-Guerrero, A. Valderas, K. Benabdellah and C. Azcón-Aguilar. 2009. Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. *Phytochem. Rev.*, 8: 551-559.

Flexas, J. and H. Medrano. 2002. Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Ann. Bot*., 89: 183-189.

, S. Jonasson, H. Medrano and M. Mus. 2001. Seasonal patterns and control of gas exchange in local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L. *Acta Oecol.*,22: 33-43.

Graham, J.H. and J.P. Syvertsen. 1985. Host determinants of mycorhizal dependency of citrus rootstock seedlings. *New Phytol.*, 101: 667-676.

Hamid, M.A., W. Agata and Y. Kawamitsu. 1990. Photosynthesis, transpiration and water use efficiency in four cultivars of mungbean, *Vigna radiata* (L.) Wilczek.. *Photosyn.*, 24: 96-101.

Helgason, T. and A.H. Fitter. 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). *J. Exp. Bot.*, 60: 2465-2480.

Hildebrandt, U., M. Regvar and H. Bothe. 2007. Arbuscular mycorrhiza and heavy metal tolerance. *Phytochem.*, 68: 139-146.

Hussein, M., A. mbiale, A. Husen,I.M. Aref and M. Iqbal. Salinity-induced modulation of plant growth and photosynthetic parameters in faba bean (*Vicia faba*) cultivars. *Pak. J. Bot*., 49: 867-877.

Jiang, L.Y., X.E. Yang and J.M. Chen. 2008. Copper tolerance and accumulation of *Elsholtzia splendens* Nakai in a pot environment. *J. Plant Nutr.*, 31: 1382-1392.

Jin, Z.X., J.M. Li. and Y.L. Li. 2015. Interactive Effects of Arbuscular mycorrhizal fungi and copper stress on flowering phenology and reproduction of *Elsholtzia splendens*. *PLoS ONE*, 10: e0145793.

Kabata-Pendias, A.andH. Pendias. 2001. Trace elements in soils and plants. CRCPress, Boca Raton, Florida, USA.

Kamali, M., M.S. Pourand A.A.M. Moud. 2012. Copper effects on growth parameters of hollyhock (*Althaea rosea* L.). *J. Ornam. Hortic. Plant.*, 2: 95-101.

Kaschuk,G., T.W. Kuyoer, P.A. Leffelaar, M, Hungria and K.E. Giller.2009. Are the rates of photosynthesis stimulated by the carbon sink strengthof rhizobial and arbuscular mycorrhizal symbioses? *Soil. Biol. Biochem*., doi: 10.1016.

Katz, J.J., J.R. Norris and L.L. Shipman. 1978. Chlorophyll function in the photosynthetic reaction centrer. *Ann. Rev. Biophys. Bio.*, **7**: 393-434.

Ke, W.S., Z.T. Neng and S.S. Ke. 2007. Effects of copper toxicity on photosynthesis and transpiration of three *Elsholtzia splendens* Nakai ex F. Maekawa populations. *Acta Ecol. Sin.*, 27: 1368-1375.

Kormanik, P.P., W.C. Bryon and R.C. Schultz. 1980. Procedures and equipment for staining large numbers of plant root samples fo endomycorrhizal assay. *Can. J. Microbiol*., 26: 536-538.

Krause, G.H. and E. Weis. 1991. Chlorophyll fluorescence and photoysnthesis: the basics annual review in plant physiology. *Plant Mol. Biol.*, 42: 313-349.

Lagriffoul, A., B. Mocquot, M. Mench and J. Vangronsveld. 1998. Cadmium toxicity effects on growth mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). *Plant Soil*, 200: 241-250.

Li, J.M., J.J. Liao, M. Guan, E.F. Wang and J. Zhang. 2012. Salt tolerance of *Hibiscus hamabo* seedlings: a candidate halophyte for reclamation areas. *Acta Physiol. Plant.*, 34: 1747-1755.

Li, Y.L., Z.X. Jin and J.M. Li. 2015. Effects of soil microbe inoculation on the growth and photosynthetic physiology of *Elsholtzia splendens* under copper stress.*Acta Ecol. Sin.*, 35: 3926-3937.

Lidon, F.C. 1999. An overview of the effects of excess copper on the photosynthesis of rice plants. *Agron. Lusit.*, 47: 69-88.

Long, S.P., N.R. Baker and C.A. Rains. 1993. Analyzing the responses of photosynthetic CO_2 assimilation to long-term elevation of atmospheric CO_2 concentration. *Vegetation*, 104: 33-45.,

Lou, L.Q., Z.G. Shen and X.D. Li. 2004. The copper tolerance mechanisms of *Elsholtizia haichowensis*, a plant from copper-enriched soils. *Environ. Exp. Bot.*, 51: 111-120.

Maksymiec, W. 1997. Effects of copper on cellular processes in higher plants. *Photosyn.*, 34: 321-342.

Malekzadeh, P, J. Khara and S. Farshian. 2007. Copper toxicity influence onantioxidant enzymes activity in tomato plants and role of arbuscular mycorrhizal fungus, *Glomus etunicatum* in the tolerance of toxicity. *Pak. J. Biol. Sci.*, 10: 2008-2013.

Meier, S., F. Borie, G. Curaqueo, N. Bolan and P. Cornejo. 2012. Effects of arbuscular mycorrhizal inoculation on metallophyte and agricultural plants growing at increasing copper levels. *Appl. Soil Ecol.* 61: 280-287.

Meier, S., P. Cornejo, P. Cartes, F. Borie, J. Medina and R. Azcón. 2015. Interactive effect between Cu-adapted arbuscular mycorrhizal fungi and biotreated agrowaste residue to improve the nutritional status of Oenothera picensis growing in Cu-polluted soils. *J. Plant Nut. Soil Sci.*, 178: 126-135.

Meier, S., R. Azcón, P. Cartes, F. Borie and P. Cornejo. 2011. Alleviation of Cu toxicity in *Oenothera picensis* by copper adapted arbuscular mycorrhizal fungi and treated agrowaste residue. *Appl. Soil Ecol.* 48:117-124.

-Kurdziel, B and K. Strzalka. 2002. Influence of metals on the biosynthesis of photosynthetic pigments. In: Prasad M.N.V., Strzalka K. (eds): *Physiology and biochemistry of metal toxicity and tolerance in plants*. Springer, Netherlands. 201-228.

Ögren, E. and J.R. Evans. 1993. Photosynthetic light-response curves. *Planta*,189:182-190.

Orlowska, E., B. Godzik and K. Turnau. 2012. Effect of different arbuscular mycorrhizal fungal isolates on growth and arsenic accumulation in *Plantago lanceolata* L. *Environ. Poll.*, 168:121-130.

Prasad, M.N.V and K. Strzalka. 1999. *Impact of heavy metals on photosynthesis.* In: Prasad M. N. V., Hagemeyer J., eds: Heavy Metal Stress in Plants. Springer Publ., Berlin. 117-138.

Qian, M., X. Li and Z. Shen. 2005. Adaptive copper tolerance in *Elsholtzia haichowensis* involves production of Cu-induced thiol peptides. *Plant Growth Regul.*, 47: 66-73.

Smith, F.A and S.E. Smith. 2013. How useful is the mutualism-parasitism continuum of arbuscular mycorrhizal functioning? *Plant Soil*,363: 7-18.

Smith, S.E. and D.J. Read. 2008. *Mycorrhizal symbiosis*. London: Academic Press, 1-20.

Syvertsen, J.P. andJ.H. Graham. 1990. Influence of vesiculararbuscular mycorrhizae and leaf age on net gas exchange of*Citrus*leaves. *Plant Physiol.*, 94: 1421-1428.

Szalontai. B., L.I. Horváth, M. Debreczeny., M. Droppa and G. Horvath. 1999. Molecular rearrangements of thylakoids after heavy metal poisoning, as seen byfouriertransform infrared (FTIR) and electron spin resonance (ESR) spectroscopy. *Photosyn. Res.*, 61: 241-252.

Tang, S.R., B.M. Wilke and C.Y. Huang. 1999. The uptake of copper by plants dominantly growing on copper mining spoils along the
China. *Plant Soil*, 209: 225-232.

Vinit-Dunand, F., D. Epron, B. Alaoui-Sossé and P.M. Badot. 2002 Effects of copper on growth and on photosynthesis of mature and expanding leaves in cucumber plants. *Plant Sci.*, 163: 53-58.

Wang, F.Y., X.G. Lin, R. Yin and L.H. Wu. 2006. Effects of arbuscular mycorrhizal inoculation on the growth of *Elsholtzia splendens* and *Zea mays* and the activities of phosphatase and urease in a multi-metal-contaminated soil under unsterilized conditions. *Appl. Soil Ecol.*, 31: 110-119.

Wang, P., N.W. Menzies, Y.M. Wang and D.M. Zhou. 2012. Identifying the species of copper that are toxic to plant roots in alkaline nutrient solutions. *Plant Soil*, 361: 317-327.

Wu, Q.S. and R.X. Xia. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *J. Plant Physiol.*, 163: 417-425.

Yang, R.Y., S.T. Zan, J.J. Tang, X. Chen and Q. Zhang. 2010. Variation in community structure of arbuscular mycorrhizal fungi associated with a Cu tolerant plant *Elsholtzia splendens*. *Appl. Soil Ecol.*, 44: 191-197.

Ye, Z.P. 2007. A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. *Photosyn.*,45: 637-640.

Ye, Z.P. and Q. Yu. 2009. A comparaison of response curves of winter wheat photosynthesis to flag leaf intercelluar and air CO_2 concentrations. *Chin. J. Ecol.* 28: 2233-2238.

Zheng, Y., L. Wang and M. Dixon. 2004. Response to copper toxicity for three ornamental crops in solution culture. *HortSci.*, 39: 1116-1120.

Zhu, X.C., F.B. Song and H.W. Xu. 2010. Arbuscular mycorrhizae improve low temperature stress in maize via alterations in host water status and pohotosynthesis. *Plant Soil*, 331: 129-137.

Zhu. X.C., F.B. Song, S.Q. Liu and T.D. Liu. 2011. Effects of arbuscular mycorrhizal fungus on photosynthesis and water status of maize under high temperature stress. *Plant Soil*, 346: 189-199.

(Received for publication 10 June 2016)