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Full Length Article

Phosphorus Alleviates Aluminum Toxicity in *Camellia oleifera* Seedlings

Junqin Zhou[1], Zuozuo Ai[1], Hui Wang[1], Genhua Niu[1,2] and Jun Yuan[1*]
[1]*The Key Lab of Non-wood Forest Nurturing and Protection of the National Ministry of Education, Central South University of Forestry and Technology, PR China*
[2]*Texas A&M AgriLife Research, Texas A&M University System, USA*
[*]For correspondence: yuanjunchina@126.com

Abstract

Camellia oleifera Abel. (oiltea) is an edible oil tree and mostly distributed in red acid soil areas. High aluminium (Al) and low phosphorus (P) are often coexisting as two important factors limiting plant growth in acidic soils. This study evaluated plant growth, Chl *a* fluorescence, and P and Al contents of oiltea seedlings cultivated in sand with a toxic Al level at 4 mmol/L Al and P concentrations at 0, 0.5, 1.0 or 1.5 mmol/L in a pot experiment. Leaf photosynthesis and dry weights of shoots and roots of oiltea seedlings were increased linearly as P concentration increased. Contents of P in shoots and roots increased with P addition in the cultural solutions and peaked at 1.0 mmol/L, while Al contents in shoots and roots decreased. Under the toxic level of Al, the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased as P concentration increased. Twenty-eight, twenty, sixteen and nine organic compounds were identified by GC-MS in root exudates of plants subjected to 0, 0.5, 1.0 and 1.5 mmol/L P treatments, respectively. Hematoxylin staining showed that adding P increased root tip Al accumulation and transport. These results suggest that P treatment can partially alleviate Al toxicity. In addition, triethyl citrate in root exudates can serve as an indicator of Al stress.

Keywords: Oiltea; Root exudate; Antioxidant enzyme; Chl *a* fluorescence

Introduction

Approximately 40% arable land on earth are acidic, and most acidic soils are highly weathered with low natural soil fertility, high aluminium (Al) content and high phosphorus (P) fixation capacity (Ligaba *et al.*, 2004; Maejima *et al.*, 2014), which lead to Al toxicity and P deficiency in the soils hindering plant growth (Liao *et al.*, 2006; Chen *et al.*, 2011; Ward *et al.*, 2011). Plants can tolerate Al^{3+} toxicity and P deficiency through mechanisms such as organic acid secretion (Hocking, 2001; Khorassani *et al.*, 2011), physicochemical characteristics of root plasma membranes (Maejima *et al.*, 2014), environment modification in root zones (Ward *et al.*, 2011), relevant gene expression adjustment (Wang *et al.*, 2013), and chelation of Al and P between each other in soils (Iqbal *et al.*, 2010). The interaction between Al and P has been studied and discussed for several crops. Du *et al*. (2009) found that *Stylosanthes* adaptation to low-P acidic soils could be due to superior Al tolerance and efficient absorption of organic P and Al-P fraction by the roots. In wheat plants, Al toxicity is associated with P content in root cell walls (Shao *et al.*, 2015). Al accounts for most of the exchangeable cations and acidity in red soils (Wilson *et al.*, 2004). In south China, an area of 102 million ha is covered with highly weathered acidic red soil where soil P primarily presents as Al-P. Al toxicity and low-P stress negatively affect crop production in this region (Holford, 1997; Hu *et al.*, 2001).

Camellia oleifera Abel. (oiltea) is an important edible and cosmetic oil tree species in China and Southeast Asian countries. The tree has over 2,300 years' history of cultivation and utilization, and is mostly distributed in red acidic soil regions of southern China (He *et al.*, 2011). Consequently, Al toxicity and P deficiency become the major constraints to oiltea production (Zeng *et al.*, 2012). For example, growth, photosynthesis and yield were reduced by low-P and high-Al in soil (He *et al.*, 2011; Huang *et al.*, 2017). Understanding the interaction between Al and P would help to combat these constraints and increase production efficiency. *C. oleifera* is one of the Al hyperaccumulator plants that certain levels of Al are beneficial for its growth (Zeng *et al.*, 2011). In our previous pot study with sand, Al at 0.5–2.0 mmol/L improved oiltea growth, but Al at 4.0 mmol/L inhibits *C. oleifera* growth (Huang *et al.*, 2017). Many researchers found that addition of P alleviated Al toxicity in cereal crops such as wheat (Iqbal, 2013) and rice (Ward *et al.*, 2011; Maejima *et al.*, 2014). However, few Al-P interaction studies were reported on woody species. For *C. oleifera*, it is not clear whether increasing P supply is able to relieve Al toxicity. The objective of this study was to investigate the effectiveness of P addition at different concentrations on alleviation of Al

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toxicity by quantifying plant growth, chlorophyll *a* fluorescence, organic compounds in root exudate, plant Al and P contents, and root tip Al accumulation of oiltea seedlings in a pot experiment cultured by nutrient solutions with a toxic level of Al. The ultimate goal is to improve the production management of oiltea and to understand Al-P interaction and mechanism.

Materials and Methods

Plant Materials and Al and P Treatments

One-year-old grafted seedlings of *C. oleifera* with uniform growth were used. After root pruning and disinfected by 0.15% Formalin, the seedlings were planted into plastic pots (12 cm in diameter and 18 cm in height) containing 3.2 kg sand on September 9, 2015. The potted seedlings were cultured in a greenhouse with normal Hoagland-Arnon solution for three weeks before subjected for treatments. Based on information obtained from our previous studies (Huang *et al*., 2017), plant growth is prohibited by adding 4 mmol/L Al to the culture solution. This Al concentration was used for the current experiment. Four P treatments (concentrations) were created by adding P to Hoagland-Arnon solution containing Al at 4 mmol/L. The P concentrations were 0 mmol/L, 0.5 mmol/L, 1.0 mmol/L, and 1.5 mmol/L P. KH_2PO_4 was the source of P and KCl was used to adjuste the K^+ level in the nutrient solution. Al was provided by $AlCl_3$. The pH of the treatment solutions was adjusted to 5.5 with 0.5% $Ca(OH)_2$ or H_2SO_4. All other elements in the solution were kept the same for all the treatments. Plants were irrigated with 200 mL treatment solutions every three days started on October 1, 2015 and the treatments were terminated in April, 2016. During the experiment, the air temperarutes ranged from 2°C to 31°C with an average of 25°C. Additional 100 mL distilled water was applied in the evenings on hot days (high temperature > 30°C) to insure sufficient moisture. There were 15 replicates (pots) of each treatment and the treated plants (pots) were arranged in a completely randomized design and cultured in the same greenhouse.

Chl a Fluorescence

Using a portable chlorophyll fluorometer (MINI-PAM, Walz, Germany), the Chl *a* fluorescence of each plant was measured on three randomly selected fully expanded leaves on April 3, 2016. The measurements included initial chlorophyll fluorescence (F_o), maximal chlorophyll fluorescence (F_m), light adapted maximum fluorescence (F_m'), minimal fluorescence level in light adapted state (F_o'), steady-state fluorescence yield (F) and the photosynthetically active radiation (PAR). Using these measurements, the following parameters were calculated: Maximal quantum efficiency of PSII is represented as Fv/Fm = (Fm-Fo)/Fm (He *et al.*, 2011); ETR (Relative electron transport rate) = Fv/Fm×PAR×0.5×ETR-factor (0.84) (Motohashi and Myouga, 2015). Quantum yield of PSII electron transport $^{\Phi}$PSII = ΔF/Fm' = (Fm'-F)/Fm' (He *et al.*, 2011) and non-photochemical quenching NPQ = (Fm-Fm')/Fm' = Fm/Fm'-1 (Motohashi and Myouga, 2015).

Antioxidant Enzyme Activity

Roots were collected from three randomly selected plants per treatment on April 5, 2016. About 2 g of fresh roots were grounded with 3 mL 0.05 mol/L phosphate buffer, then centrifuged for 10 min at 12 000 rpm. The supernatant was collected and kept at 4°C until assays of enzyme activity. Superoxide dismutase (SOD) activity was determined using the method reported by Chen and Pan (1996). Enzyme activity that inhibites the photo reduction of nitroblue tetrazolium to blue formazan by 50% represents one unit of SOD activity. Peroxidase (POD) activity was measured by the method provided by Sakharov and Ardila (1999). The amount of enzyme that produces a change of 1.0 absorbance per min at 470 nm measured by an UV spectrophotometer (UV-1200, Shanghai Jingke, China) represents one unit of POD activity. Catalase (CAT) activity was measured as the change of absorbance at 240 nm for 1 min due to H_2O_2 (Gao *et al*., 2008). The amount of enzyme needed to reduce 1 μmol of H_2O_2 per min represents one unit of CAT activity (MacDonald and D'Cunha, 2007). Finally, all enzyme activities were expressed as units per gram fresh weight (U/g/min).

Hematoxylin Staining

To exam Al accumulation at root tips and Al transport, three plants were randomly selected from each treatment and root samples were prepared for hematoxylin staining on April 8, 2016. The roots were washed with deionized water and stained in 0.2% hematoxylin solution containing 0.02% $NaIO_3$ for 15 min (Polle *et al*., 1978). After staining, the roots were rinsed with deionized water for 20 min and photographed under a stereo-microscope.

GC/MS Analysis of Root Exudates

Three randomly selected plants per treatment were removed from the pots carefully (on April 10, 2016) and gently cleaned with slow running tap water. The roots were further washed by submerging in deionized water for 5 min and repeated for three times, and then each plant was placed into a wide-mouth black container containing 250 mL 0.05 g/L $CaSO_4$ to collect root exudates for 24 h. During collection, the container was ventilated constantly with an air pump. After collection, the exudate contained $CaSO_4$ solution was concentrated on a rotary evaporator at 45°C (80–90 r/min) to 50 mL. The concentrated root exudate solutions from the three plants were pooled and preserved in a refrigerator (-20°C) before analysis. Two or

three drops of 0.05% thymol were added to control potential contamination of microorganisms.

Gas chromatography-mass spectrometry (GC-MS) (7890B/5977A A700, Agilent Technologies, New York, USA) was used to identify organic compounds in the root exudates after ethyl acetate extraction (Luo *et al.*, 2017). GC separation was achieved on a capillary column Agilent 122-3832 (30 m × 0.25 mm × 0.25 μm, DB-35 ms, New York, USA). The injector temperature was 280°C with 1:50 split ratio. Helium was the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was 70°C for 2 min, then, increased from 70°C to 280°C at a rate of 10°C/min, then held for 25 min. The transfer-line temperature and ion-source temperature were set at 280°C and 230°C, respectively. Ionization was achieved using a 70 eV electron beam. Mass spectra were recorded from m/z 35 to 500 at a rate of 2 s in full scan mode with the solvent delay time of 3 min. Injection volume was 10 μL. Components were identified by using the standard mass spectrum library NIST02.

Plant Growth Measurement

The remaining six seedlings per treatment were harvested on April 15, 2016 and divided into roots and shoots. The roots were scanned using an Epson Expression 1680 and analyzed by WinRhizo Pro 2013a for total length and volume. Dry weight (DW) of the roots and shoots were obtained after dried at 65°C to a constant weight. The dried shoots and roots were collected and kept separately for P and Al content analysis.

P and Al Content in Plant Tissues

Plant samples (dried shoots and roots) were digested with H_2SO_4+H_2O_2 (EasyDigest 40, AMS, Italy). P concentration was determined using a discrete auto analyser (Smartchem 200, Westco Scientific Instruments, Italy) and the malachite green oxalate method (Murphy and Riley, 1962). The Al concentration was determined colourimetrically using the PCV method (Kerven *et al*., 1989). All measurements were carried out in triplicate.

Statistical Analysis

All collected data were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests for mean comparisons using SPSS for Windows, version 17.0. Graphs were made by Origin 8.5.

Results

Plant Growth

In general, all P treatments increased plant dry weight compared to the control (0 mmol/L P) in a positive linear fashion (Table 1). P treatment of 1.5 mmol/L significantly increased the total length and volume of roots, followed by 1.0 mmol/L P treatment, while there was no significant difference between 0.5 mmol/L P treatment and control. In addtion, the plants in P treatments of 1.5 mmol/L and 1.0 mmol/L had higher root/shoot ratios than that of 0.5 mmol/L P and control.

Chl *a* Fluorescence

The plants in 1.5 and 1.0 mmol/L P treatments had significantly lower F_O values compared to that of 0 and 0.5 mmol/L P treatments. The Fv/Fm and NPQ of the seedlings treated by 1.5 mmol/L P were the highest, followed by 1.0 and 0.5 mmol/L P treatments, while the 0.5 mmol/L P treatment was not significantly different from the 0 mmol/L P treatment. The values of ETR and $^{\Phi}$PSII increased in a linear fashion with rising P concentration (Table 2).

Root SOD, POD and CAT Activity

The SOD, POD and CAT activities were increased by increasing P concentrations (Fig. 1). Compared to the activity level of the control (95.88 U/g/min), the 1.5 mmol/L P treatment increased the SOD activity the most (by 23.0%). Compared to the control, POD activities were increased by 28.8, 57.3 and 65.6% by the 0.5, 1.0 and 1.5 mmol/L P treatments, respectively. The activities of CAT with 0.5, 1.0 and 1.5 mmol/L P treatment were 116, 152 and 163% of the control.

Organic Compounds in Root Exudate

The main constituents in root exudates were esters and alkanes (Table 3). In the root exudates of seedlings in 0, 0.5, 1.0 and 1.5 mmol/L P treatments, 28, 20, 16 and 9 organic compounds were identified, respectively. Triethyl citrate, bis (2-ethylhexyl) phthalate, and 2-ethylhexanol were detected in all four treatments. 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, and undecane were found in 0, 0.5 and 1.0 mmol/L P treatments, while 2,5-furandicarboxylic acid diethyl was found in root exudates of 0.5, 1.0 and 1.5 mmol/L P treatments.

P and Al Concentrations in Plants

The P concentration in both shoots and roots reached a plateau by the 1.0 mmol/L P treatment (Fig. 2a). No statistical difference was found between 0.5 mmol/L P and 1.5 mmol/L P treatments. The lowest concentration of P was found in the control (0 mmol/L P) for both shoots and roots at 1.51 g/kg and 1.03 g/kg, respectively, which were significantly lower than those in all the P treatments. P content in the shoots was generally higher than that in the roots.

Table 1: Dry weight, root to shoot ratio, root length and volume of *Camellia oleifera* seedlings under toxic level of aluminium with addition of phosphorus (P) of different concentrations*

P treatments (mmol/L)	Shoot dry weight (g)	Root dry weight (g)	Root/shoot ratio	Root length (cm)	Root volume (cm^3)
0	1.28 ± 0.09d	1.24 ± 0.11b	0.97 ± 0.15b	374.22 ± 4.94c	1.16 ± 0.09c
0.5	1.32 ± 0.05c	1.31 ± 0.11a	0.99 ± 0.08b	422.86 ± 1.39c	1.54 ± 0.12c
1.0	1.34 ± 0.11b	1.40 ± 0.07a	1.04 ± 0.10a	530.38 ± 1.59b	2.56 ± 0.09b
1.5	1.41 ± 0.20a	1.57 ± 0.08a	1.11 ± 0.20a	1023.49 ± 2.07a	2.78 ± 0.07a

*Mean ± SD. Different letters in the same column indicate significant difference at $p \leq 0.05$ (Duncan's multiple range test)

Table 2: Chl *a* fluorescent parameters of *Camellia oleifera* seedlings under toxic level of aluminium with addition of phosphorus (P) of different concentrations*

P treatments (mmol/L)	Fo	Fv/Fm	ETR	$^{\Phi}$PSII	*NPQ*
0	173.98 ± 0.12a	0.765 ± 0.001c	23.73 ± 1.25d	0.050 ± 0.030d	1.691 ± 0.047c
0.5	172.74 ± 0.05a	0.778 ± 0.035c	26.16 ± 1.17c	0.060 ± 0.087c	1.752 ± 0.034c
1.0	150.08 ± 0.07b	0.782 ± 0.005b	28.31 ± 2.37b	0.065 ± 0.033b	1.917 ± 0.056b
1.5	131.94 ± 0.01c	0.808 ± 0.001a	30.35 ± 1.01a	0.069 ± 0.004a	2.151 ± 0.038a

*Mean ± SD. Different letters in the same column indicate significant difference at $p \leq 0.05$ (Duncan's multiple range test). F_o = Initial chlorophyll fluorescence; Fv/Fm = Maximal quantum efficiency of PSII; ETR=Relative electron transport rate; $^{\Phi}$PSII = Quantum yield of PSII electron transport; and NPQ=Non-photochemical quenching

Table 3: Organic components of *Camellia oleifera* seedling root exudate under toxic level of aluminum and different phosphorus concentration detected by GC/MS

P treatments (mmol/L)	Components of root exudates
0	2-Hydroxy-propanenitrile ($C_6H_{10}O$); 6-Methylheptyl vinyl ($C_{10}H_{20}O$); Carbamic acid, Phenyl ester ($C_7H_7O_2N$); 2-Ethylhexanol ($C_8H_{18}O$); Undecane (C_9H_{20}); 1-Isopropyl-2-methylbenzene ($C_{10}H_{14}$); 5,8-Diethyldodecane ($C_{16}H_{34}$); 4-tert-Amylphenol ($C_{11}H_{16}O$) ; 2-Acetyl-4-methyl-4-pentenoic acid ethyl ester ($C_10H_{16}O_3$); Cyclohexasiloxane,dodecamethyl ($C_{12}H_{36}O_6Si_6$); Propanoic acid, 2-methyl-, 3-hydroxy-2-trimethylpentyl ($C_{12}H_18O_3$); 9-Octadecen-12-ynoic acid methyl ester ($C_10H_10O_4$); 9-keto-2-decenoic acid ($C_{10}H_{16}O_3$); Triethyl citrate ($C_{12}H_{20}O_7$); Tetradecane,2,6,10-trimethyl- ($C_{17}H_{36}$); Hexadecane,2,6,10,14-tetramethyl- ($C_{20}H_{42}$); Phenol,3,5-di-tert-butyl- (7CI,8CI) ($C_{14}H_{22}O$); 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester ($C_{16}H_{22}O_4$); 7-Methyl-Z-tetradecen-1-ol acetate ($C_{17}H_{32}O_2$); Octadecanal, 2-bromo- ($C_{18}H_{35}BrO$); Eicosane ($C_{20}H_{42}$); 4-(Decyloxy)phenol ($C_{16}H_{26}O_2$); 3-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)-Butanoic acid ($C_{15}H_{26}O_2$); 17-Pentatriacontene ($C_{35}H_{70}$); heptacosane ($C_{10}H_{16}O_3$); Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$); tert-Hexadecanethiol ($C_{16}H_{34}S$); n-Dioctyl phthalate ($C_{24}H_{38}O_4$)
0.5	2,4-Hexadien-1-ol ($C_6H_{10}O$); Octamethylcyclotetrasiloxane($C_8H_{24}O_4Si_4$); Phenol ($C_8H_{18}O$); 1-Heptanol,2,4-dimethyl-, (2S,4R)-;1-Heptanol,2,4-dimethyl-, (2S,4R)-(-)- (8CI)($C_9H_{20}O$); 4,6,8-Trimethyl-1-nonene ($C_{12}H_{24}$); 1-Hexanol, 2-ethyl ($C_8H_{18}O$); Undecane(C_9H_{20}); 2,5-furandicarboxylic acid diethyl ($C_{10}H_{14}O$); 2-acetyl-4-methyl-4-pentenoic acid ethyl ester($C_{10}H_{16}O_3$); Dimethyl phthalate ($C_{10}H_{10}O_4$); Methyl-5-(1-methylethyl)phenol($C_{10}H_{14}O$); Triethyl citrate ($C_{12}H_{20}O_7$); Phenol,3,5-bis(1,1-dimethylethyl) ($C_{14}H_{22}O$); 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester ($C_{16}H_{22}O_4$); Nonadecane($C_{19}H_{40}$); Diethyl furan-2,5-dicarboxylate($C_{10}H_{12}O_5$); n-Dioctyl phthalate (C24H38O4); Butyl decyl phthalate($C_{22}H_{34}O_4$); 9-Octadecenamide, (Z)-($C_{18}H_{35}NO$);Bis(2-ethylhexyl) phthalate($C_{24}H_{38}O_4$)
1.0	Propanenitrile, 2-hydroxy(C_3H_5ON); 2,4-Hexadien-1-ol($C_6H_{10}O$); 6-Methylheptyl vinyl($C_{10}H_{20}O$); Cyclopentadecane($C_{15}H_{30}$); Phenol($C_8H_{18}O$); 1-Hexanol, 2-ethyl($C_8H_{18}O$); 4-Methyloctane(C_9H_{20}); Undecane (C_9H_{20}); 2-Butyloctyl alcohol ($C_{12}H_{26}O$); 2,5-furandicarboxylic acid diethyl ($C_{10}H_{14}O$); Dimethyl phthalate ($C_{10}H_{10}O_4$); Docosane ($C_{22}H_{46}$); Triethyl citrate ($C_{12}H_{20}O_7$); 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester ($C_{16}H_{22}O_4$); 9-Octadecenamide, (Z)- ($C_{18}H_{35}NO$); Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$)
1.5	Propanenitrile,2-hydroxy-(C_3H_5ON);6-Methylheptyl vinyl ether($C_{10}H_{20}O$);1-Hexanol,2-ethyl-($C_8H_{18}O$); p-Xylene,2-ethyl- (8CI) ($C_{10}H_{14}$); 2,5-furandicarboxylic acid diethyl ($C_{10}H_{14}O$); Triethyl citrate ($C_{12}H_{20}O_7$); Triethyl acetyl citrate($C_{14}H_{22}O_8$); Octanedioic acid dibutyl ester ($C_{16}H_{30}O_4$); Bis (2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$)

The Al contents in shoots and roots in the control were 4.2 and 11.92 g⁄kg, respectively, which were significantly higher than those in the P treatments (Fig. 2b). Similar levels of Al were found between the 1.0 and 1.5 mmol/L P treatments in both the roots and shoots. In general, roots contained higher Al contents than shoots.

Al Accumulation at Root Tips

Hematoxylin staining showed that the roots of plants receiving no P (control) had lower Al accumulation in their tips than those receiving P of different concentrations (Fig. 3). The amount of Al accumulation at root tips increased as P concentration increased.

Discussion

Aluminium toxicity and P deficiency are often present together in acidic soils, and are considered as two important factors limiting plant growth (Chen *et al.*, 2011). Al stress can significantly inhibit the growth of roots and above ground plant parts. Fortunately this Al negative effect can be partially overcome by P treatment in various plant species (Iqbal *et al.*, 2010; Ward *et al.*, 2011; Iqbal, 2013).

The present study showed that under a toxic level of Al, P treatments increased *C. oleifera* seedling dry weight, root-shoot ratio, total root length and volume when compared to no P treatment (control) with increasing P

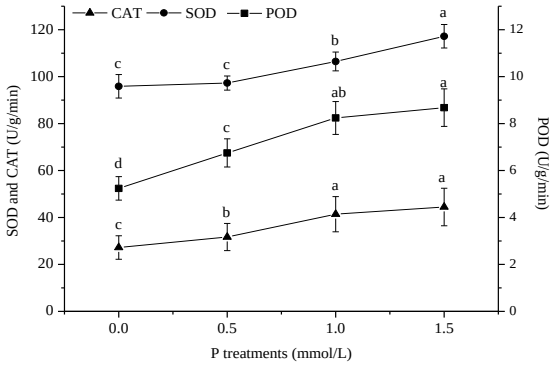

Fig. 1: Antioxidase activities in the roots of *Camellia oleifera* seedlings under a toxic level of aluminum (4 mmol/L Al) and different phosphorus (P) concentrations. Within a data serial, points with different letters indicate significant difference at $p \leq 0.05$ (Duncan's multiple range test). SOD: superoxide dismutase; POD: peroxidase; CAT: catalase

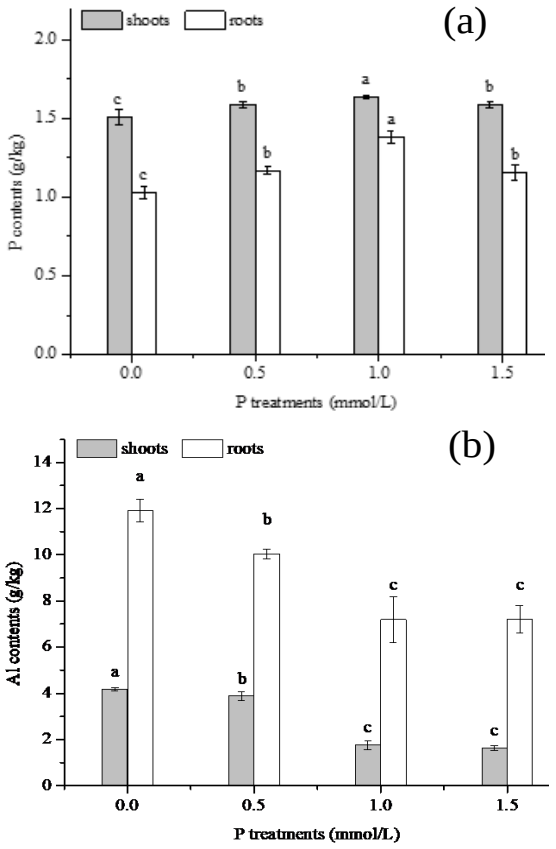

Fig. 2: Phosphorus (P) (a) and aluminum (Al) (b) contents of *Camellia oleifera* seedlings cultured with toxic level of aluminum (4 mmol/L Al) and different P concentrations. Bars with different letters indicate significant difference at $p \leq 0.05$ (Duncan's multiple range test)

concentrations in a linear fashion. Chlorophyll *a* fluorescence measurements also showed that P addition

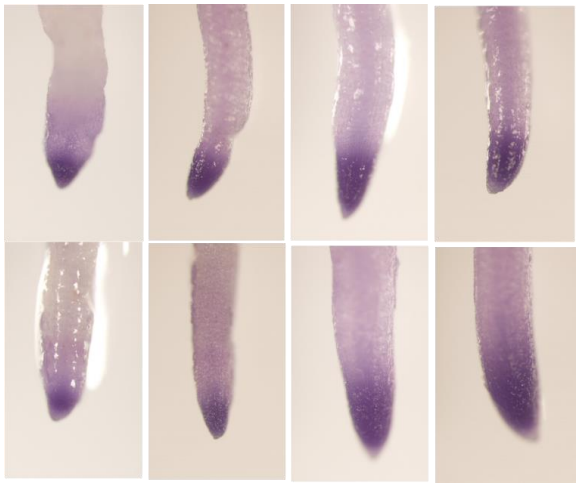

Fig. 3: Root tips of *Camellia oleifera* seedlings visualized by hematoxylin staining. Roots were obtained from seedlings receiving the phosphorus (P) treatments at different concentration: 0 mmol/L, 0.5 mmol/L, 1.0 mmol/L and 1.5 mmol/L. Two different root tips per treatment are showed. The darker area represents Al accumulation. Bar = 200 μma

benefited photosynthetic apparatus. Furthermore, P addition increased P contents in shoots and roots. These results suggest that under Al stress, P supplement can benefit plant growth and increase nutrient absorption capacity of *C. Oleifera*. The Al contents in the shoots and roots of the seedlings decreased with P treatments, even though the hematoxylin staining showed higher Al accumulation at the root tips of P treated plants. This suggests that the overall Al uptake was reduced by P, probably resulted from the precipitation of plant-available Al by soluble P, which is a common chemical reaction occurring in soil (Chen *et al.*, 2011). In addition, the shoots and roots of the seedlings treated with P contained significantly higher P compared to that of the control, and peaked at 1.0 mmol/L P, suggesting that *C. Oleifera* plants do not absorb P continuously with higher P supply.

Chlorophyll *a* fluorescence is a non-invasive quantitative measure of oxygenic photosynthesis. Under our experimental condition (toxic level of Al), Fo decreased as P concentration increased, suggesting that the photoinhibition in the leaves was alleviated by P. Also, Fv/Fm, ETR, ΦPSII and NPQ were significantly increased when 1.0 and 1.5 mmol/L P were applied. These results indicate that only reversible damage occurred to the photosynthetic reaction centers. P additions could prevent Al-induced reduction of PSII efficiency and the components of chlorophyll molecule. In addition, the observed benefit of P addition on chlorophyll fluorescence could be brought by the relief of Al induced magnesium (Mg) deficiency, as P addition can increase Mg uptake (Matsumoto, 2000).

Furthermore, hematoxylin staining indicated that less

Al was accumulated at the root tips in the control (toxic level of Al with no P) compared to that of root tips in treatments receiving P. This P related Al accumulation in root tips may be caused by decreasing amount of Al binding to plasma membranes under P deficiency (Maejima *et al.*, 2014). Another explanation could be that P addition may increase the sequestration or transport of Al^{3+} by the roots tips. This also could be the reason that *C. oleifera* has stronger Al accumulation capacity in leaves (Zeng *et al.*, 2011).

Antioxidant enzyme activities have been shown to contribute to plant resistance to abiotic and biotic stresses. SOD, POD and CAT are the most common antioxidant enzymes detoxifying reactive oxygen species (ROSs) induced by environment stresses (Gao *et al.*, 2008). In the current study, SOD, POD and CAT activity was lower than normal, most likely due to Al toxicity (Chen and Pan, 1996). However, with increasing P concentrations, these enzyme activities were increased significantly compared to the no P treatment, suggesting that P addition increased the enzymes' activity to respond to the toxic level of Al (Meloni *et al.*, 2003). Alternatively, P supply may act as an Al stress defensive mechanism developed by *C. oleifera* seedlings. However, the supplied P was not enough to relief the damage of the Al stress completely since lower than normal enzyme activities were found even with P additions.

In root exudates, organic acids, sugars, phenolics, and amino acids are commonly increased with reduction of environment stresses (Khorassani *et al.*, 2011). Low-P and high-Al stresses frequently induce higher numbers or amounts of organic acids such as citrate, malate and oxalate in root exudates to improve P solubility and bioavailability (Hocking, 2001; Ligaba *et al.*, 2004). Our findings indicate that the number of organic compounds in the *C. oleifera* root exudates declined with increasing P in cultural solutions, which agrees with other study (Chen *et al.*, 2011). This correlation suggests that adjusting organic compound production in root exudates is a mechanism of stress relieve. Triethyl citrate was detected in all four treatments in this study, and can be used as an indicator for evaluation of Al and P stresses in *C. oleifera*.

Conclusion

Under the artificially created Al toxicity conditions, this study showed that P addition alleviates Al toxicity in *C. oleifera* seedlings as evidenced by increased plant growth, SOD, POD and CAT activity, and photosynthetic capacity. However, P alone may not overcome Al toxicity entirely. Other tactics should be applied to fight against Al stress in production of *C. oleifera* in acidic red soils.

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