INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–1222/2021/25–3–623–631
DOI: 10.17957/IJAB/15.1709
http://www.fspublishers.org

Full Length Article

Phosphorus Reduces Aluminum Toxicity in Oil Tea Roots by Regulating the Cell Wall Components and Antioxidant Defense System

Xinjing Qu, Jiao Liao, Chenhui Zhang and Jun Yuan*
Key Laboratory of Cultivation and Protection for Non-Wood Forest Trees, Ministry of Education, Central South University of Forestry and Technology, Changsha, Hunan, China
For correspondence: yuanjun@csuft.edu.cn; Xinjingqu@126.com
Received 26 August 2020; Accepted 12 November 2020; Published 25 November 2021

Abstract

Aluminum (Al) toxicity is one of the most important impeding factors for plant growth and productivity in acidic soils. Phosphorus (P) application may alleviate Al stress in many plants. In this study we investigated the effect of P on Al toxicity in cell wall components and oxidative stress and to explore the underlying mechanisms in oil tea (*Camellia oleifera* Abel.) roots. Results indicated that Al toxicity severely inhibited root elongation, changed cell wall components, and caused oxidative damage to the roots of oil tea. However, P supply reduced the adsorption of Al in the cell wall by decreasing the demethylesterfied pectin content and hemicellulose 1 content that decreased the Al binding sites. Moreover, the addition of P alleviated the inhibition of xyloglucan endotransglucosylase and endo-β-1,4-glucanases activities under Al stress, which enhanced the loosening of the cell wall. P addition reduced the activities of polyphenol oxidase and phenylalanine ammonia lyase and enhanced the activities of reactive oxygen species scavenging enzymes, which reduced the oxidative damage caused by Al toxicity. The results reveal important mechanisms of P-induced mitigation of Al stress in oil tea roots that might be useful in the cultivation of plants on acidic soils.

Keywords: Al stress; P deficiency; Pectin; Hemicellulose; Oxidative stress

Introduction

Aluminum (Al) toxicity is one of the most important factors inhibiting plant growth in acidic soils, and about 60% of acidic soils are located in the subtropics and tropics (Kochian *et al.* 2004). An important symptom of Al stress is the inhibition of root elongation, which can occur within a short period after Al supply (Kopittke *et al.* 2015). In addition, long-term Al stress can cause disorder of the ROS scavenging system, leading to the accumulation of the superoxide anion and H_2O_2, membrane lipid peroxidation, protein degradation, and cell death (Yu *et al.* 2018; Riaz *et al.* 2018a). Root tips are the primary target of Al stress, which accumulate more Al than any other part (Kopittke *et al.* 2015). Although Al stress induces various Al-tolerance mechanisms in plants, such as release of secret organic acids to chelate Al in the rhizosphere, compartmentalize Al in vacuoles, and increase the rhizosphere pH to decrease Al adsorption to roots in most plants (Kochian *et al.* 2004), Al in roots mainly accumulates in the cell wall because pectin and hemicellulose provide negatively charged sites to bind it (Yang *et al.* 2011). Additionally, Al accumulation in the cell wall changes the properties of cell wall components, resulting in rigid cell walls and limited cell elongation, which ultimately inhibit root elongation (Safari *et al.* 2018). However, the addition of other substances can reduce the accumulation of Al in cell walls, such as boron application, which can increase the degree of pectin methyl-esterification in roots of trifoliate orange to decrease Al accumulation (Riaz *et al.* 2018b). Similarly, the addition of putrescine decreases the pectin content, hemicellulose content, and pectin methyl esterase (PME) activity, which reduces the Al content in roots and promotes root growth (Zhu *et al.* 2019).

Phosphorus (P) is a vital nutrient element involved in the synthesis of macromolecules, such as phospholipids, nucleic acids, NADP, and ATP; however, the bioavailable P content of plants is very low in acidic soils. P deficiency limits plant growth and influences the cumulative patterns of amino acids, organic acids, and carbohydrates in roots (Mo *et al.* 2019). Reportedly, the P deficiency may enhance Al toxicity on the inhibition of plant growth by affecting wall properties. For instance, Nagarajah *et al.* (1970) reported that pectin in the cell walls of roots can bind Fe and Al through ligand exchange of phosphate to solubilize P from in acidic soils. Zhao *et al.* (2018) and Zhu *et al.* (2015) reported that short term P-deficiency caused an increase in the pectin content and a decrease in the degree of pectin methyl-esterification, which increased the binding ability of

To cite this paper: Qu X, J Liao, C Zhang, J Yuan, (2021). Phosphorus reduces aluminum toxicity in oil tea roots by regulating the cell wall components and antioxidant defense system. *Intl J Agric Biol* 25:623–631

cell wall to Fe and Al. The adsorption of Al by cell walls caused by P deficiency undoubtedly aggravates Al stress in the root system. P addition has long been considered an important factor to alleviate Al toxicity because it may enhance the utilization of nutrient elements and reduce the Al content in plants (Yu *et al.* 2018).

Oil tea (*Camellia oleifera* Abel.) is an important edible oil tree that has been cultivated in southern China for more than 2300 years (Yang *et al.* 2016). At present, the planting area of oil tea in China is greater than four million hectares, mostly distributed in acidic soils in southern China. In these areas, Al toxicity and P deficiency are two crucial factors limiting the production of oil tea as a result of strong mineral leaching and acid deposition. Zhou *et al.* (2019) reported that the supply of P can decrease the accumulation of Al in oil tea and inhibition of plant growth by Al toxicity. Root tip is the most sensitive root part to P deficiency and Al stress, and the mechanism through which P relieves root Al stress needs further study. We hypothesized that P application may mitigating Al toxicity by changing physiological and biochemical characteristic of oil tea roots. The aim of this study was to investigate the mitigation effect of P on Al induced oxidative damage and modification in the cell wall components of the roots of oil tea.

Materials and Methods

Experiment treatments

The experimental treatments were the same as previously described in Qu e*t al.* (2020). Specifically, four-month-old healthy and uniform seedlings of *C. oleifera* 'Huajin' with 14-16 cm height were selected and planted in plastic pots filled with a mixture of sand and perlite. The seedlings were transformed into a greenhouse and grown at 28/22°C day/night temperature. The clear nutrient solution with pH 4.2 containing one of two Al (0 and 4 mM $AlCl_3{\cdot}6H_2O$) concentrations and one of two P (0.025 and 0.5 mM KH_2PO_4) concentrations were used to irrigate the seedlings. The components and concentrations of nutrient solution were chose according to Ghanati *et al.* (2005). The seedlings were randomly divided into four groups *viz.* +P-Al (0 mM Al and 0.5 mM P), +P+Al (4 mM Al and 0.5 mM P), -P-Al (0 mM Al and 0.025 mM P), and -P+Al (4 mM Al and 0.025 mM P). Each group consisted of three independent replications, and +P-Al treatment was considered a control. A 1 mM KCl was used to compensate for K^+ concentration between -P and +P treatments. Seedlings were subjected to the above treatments for 8 weeks before termination of the experiment.

Determination of relative root elongation and root activity

Before and after eight-weeks of P and Al treatment, root length was assessed by WinRhizo Pro 2013 image analysis software after scanning root images by root scanner. Relative root elongation was assessed based on the root elongation under different P and Al treatments/root elongation in control treatment ×100%. Root activity of oil tea root segments (0-10 mm) was assayed using the triphenyl tetrazolium chloride (TTC) method (Gai *et al.* 2017).

Histochemical analysis

After P and Al treatment, the distribution of Al in roots was detected by staining with hematoxylin solution. Then, the roots were photographed under light stereomicroscopic microscope (Olympus SZX16) as described by Polle *et al.* 1978. Plasma membrane integrity of the roots was detected by staining with Evan's blue solution. Then, the roots were photographed under a light stereomicroscope (Yamamoto *et al.* 2001).

Cell wall preparation, fractionation, and Al content determination

The root cell wall (CW) was prepared according to Li *et al.* (2016). Root segments (1.0 g per replication) were homogenized and centrifuged at 4500×g. The precipitate was washed with 80% ethanol and methanol: chloroform [1:1 (v/v)] mixture followed by acetone. Then, the precipitate was dried at 60°C for further use. The dried powder was the crude cell wall.

Cell wall components were extracted sequentially from the crude cell wall according to the method of Yang *et al.* (2011). Pectin was first extracted with hot water; then hemicellulose 1 (HC1) was extracted with 4% KOH; hemicellulose 2 (HC2) was extracted with 24% KOH; and the residue consisted mainly of cellulose.

The Al content in roots, cell walls, and pectin-free, HC1-free, and HC2-free fractions of cell walls was extracted by 2 M HCl. The Al content was determined using the aluminum colorimetric method (Nieuwenburg and Uitenbroek 1948).

Al adsorption in root cell wall fractions

Roots in the +P-Al and -P-Al treatments were used for the Al adsorption experiment. Cell walls and the pectin-free, HC1-free, and HC2-free fractions of cell walls were placed in 15 mL centrifuge tubes, and then 5 mL 4 mM $AlCl_3$ containing 0.5 mM $CaCl_2$ at pH 4.2 was added. The centrifuge tubes were shaken occasionally for 24 h. After adsorption, the Al mixtures were subsequently centrifuged for 10 min at 4500×g. The Al content in the supernatant was determined using the aluminum colorimetric method. The concentrations of Al in pectin and HC1and HC2 fractions were calculated as above.

Analysis of cell wall pectin and hemicellulose

The pectin content was determined by M-hydroxybiphenyl colorimetry according to Li *et al.* (2016). The

demethylesterfied pectin content was determined according to Louvet *et al.* (2011) at 620 nm using a spectrophotometer, and formaldehyde was used as the standard solution. The degree of pectin methyl esterification (DME) was calculated using the following equation.

$$DME\ (\%) = (C_{Methyl\ pectin}/C_{Total\ pectin}) \times 100.$$

The total sugar content in the HC1 and HC2 fractions was determined by anthrone colorimetry using glucose as a standard.

PME, XET, and EGase activity assay

For extraction of PME, roots (0.5 g per replicate) were homogenized using liquid N_2 and were suspended in 5 mL extraction buffer (1 M NaCl and 0.1 M Tris at pH 7.5) at 4°C for 1 h. Extracts were centrifuged and the supernatants were used to determine PME activity using the method of Anthon and Barrett (2004) based on the condensation of aldehyde with MBTH under neutral conditions.

The xyloglucan endotransglucosylase (XET) and endo-β-1,4-glucanases (EGase) activity were assayed by a plant xyloglucan endotransglucosylase ELISA testing kit and plant endo-β-1,4-glucanase ELISA testing kit (Shanghai Jianglai Biotechnology Co. Ltd.), respectively, according to the operating instructions.

Assay of PAL, PPO activity, and soluble phenolic content

Phenylalanine ammonia lyase (PAL) activity was assayed after extraction in 0.1 M $Na_2B_4O_7$ buffer containing 5 mM 2-mercaptoethanol and 2% (w/v) polyvinylpyrrolidone (pH 8.8). The reaction mixture comprised 1 mL enzyme extract, 2 mL 0.1 M $Na_2B_4O_7$ buffer (pH 8.8), and 1 mL 0.2 M L-phenylalanine. After incubation, the reaction was stopped with 0.1 mL of 5 M HCl. The absorbance was assayed at 290 nm (Wang and Huang 2015). Polyphenol oxidase (PPO) activity was determined by calculating the oxidation of catechol according to Singh *et al.* (1999). Soluble phenolics were extracted with aqueous methanol and determined with Folin-Ciocalteu reagent using gallic acid as a standard (Morrison 1972).

Antioxidant enzyme activity, free proline and H_2O_2 content assays

Superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity, and free proline content were determined using the method of Wang and Huang (2015). Ascorbate peroxidase (APX) activity was determined using the method of Nakano and Asada (1981). The H_2O_2 content was determined using the xylenol orange method according to Zhang *et al.* (2016).

Statistical analysis

Experiments were carried out using a complete randomized block design with three replications. Data were analyzed using the statistical program SPSS software (version 22.0).

Results

Effect of P and Al on relative root elongation, root activity and plasma membrane integrity

Compared to the control (+P-Al), the relative root elongation decreased significantly in the -P+Al treatment (Fig. 1a). The +P+Al treatment significantly increased the relative root elongation by 58.97% compared to the -P+Al treatment. Combined P deficiency and Al stresses decreased root activity significantly, while P addition (+P+Al) significantly increased the root activity by 52.09% (Fig. 1b). To visualize the loss of plasma membrane integrity, we used the Evans blue dye to staining the roots under different treatments. Evan's blue staining showed that -P+Al treatment was stronger and showed greater damage to the plasma membrane than the +P-Al treatment (Fig. 1c). The +P+Al treatment showed weaker staining than the -P+Al treatment, which indicated the decrease in plasma membrane damage.

Effect of P on the Al content in the roots

Hematoxylin staining results qualitatively demonstrated the Al distribution by a purple color (Fig. 2a). The color of roots in the -P+Al treatment was darker than that in the +P+Al treatment, indicating that roots in the -P+Al treatment had higher Al accumulation than those in the +P+Al treatment. To confirm the distribution of Al in roots, we assayed the Al content in roots and root cell walls. Al content in roots and cell walls significantly increased in +Al treatments than in -Al treatments (Fig. 2b). Interestingly, P application under Al stress significantly decreased Al content in roots and root cell walls compared to -P+Al treatment. More than 55% of Al in roots was located in the cell wall (Fig. 2c). The distribution ratio of Al in the cell wall was nearly 80% higher in +Al treatments than in -Al treatments.

Effect of P on Al allocation and adsorption in root cell wall fractions

To further confirm the effect of P on the distribution of Al in cell wall components, we measured the Al accumulation in different cell wall components under -P+Al and +P+Al treatments. The results showed that most of the Al (about 80%) in cell walls accumulated in pectin and HC1 (Table 1). The Al content in pectin and HC1 decreased under P addition. The proportion of Al in pectin decreased to 40.51% from 45.48% under P addition.

The effect of P on Al adsorption in different cell wall components showed that pectin and HC1 adsorbed more Al compared with HC2 and cellulose (Table 2). The adsorption of Al to pectin and HC1 fraction with P supply was lower than that under the P deficiency.

Table 1: Effect of P on the Al content in different cell wall components of +Al treatments of oil tea roots

Treatment	Al content in different cell wall components (mg/kg)			
	Pectin	HC1	HC2	Cellulose
-P	243.43±12.90a	188.46±0.98a	50.48±2.74a	51.06±7.79a
	(45.48±0.32%)	(35.27±1.47%)	(9.43±0.10%)	(9.82±1.10%)
+P	189.37±26.10b	175.08±2.42b	48.47±4.35a	53.19±10.01a
	(40.51±1.99%)	(37.74±2.97%)	(10.41±0.46%)	(11.34±1.18%)

Values (mean ± SD, n=3) with different letters indicate significant differences between -P and +P treatments within the same cell wall component according to an Independent sample *t* test ($P<0.05$)

Table 2: The effect of P on the adsorption of Al in different cell wall components of oil tea roots

Treatment	Adsorption of Al in different cell wall components (mg/kg)			
	Pectin	HC1	HC2	Cellulose
-P	186.35±5.17a	196.81±0.95a	26.14±5.19a	31.64±14.05a
+P	162.24±12.60b	177.31±2.81b	21.07±2.19a	23.43±2.52a

Values (mean ± SD, n=3) with different letters indicate significant differences between -P and +P treatments within the same cell wall component according to an Independent sample *t* test ($P<0.05$)

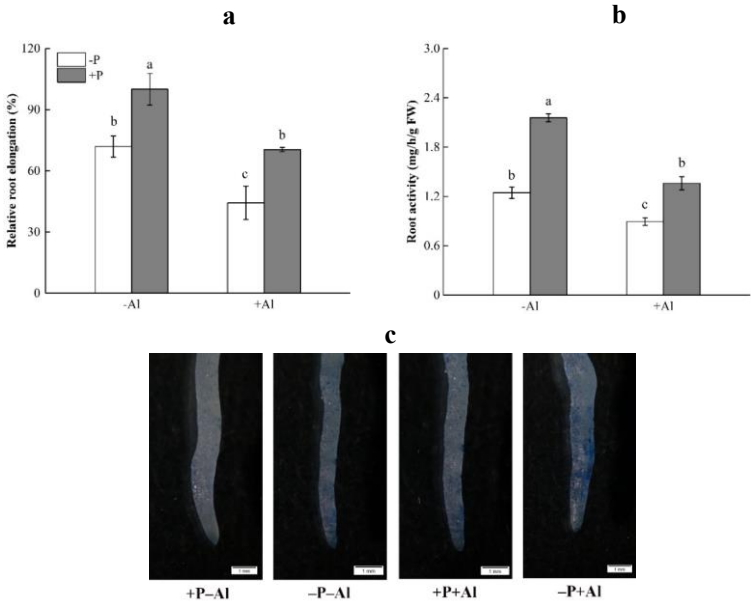

Fig. 1: Effect of P and Al on relative root elongation (**a**), root activity (**b**), and plasma membrane integrity (**c**) in oil tea roots. Error bars indicate the SD; different letters above error bars indicate significant differences at $P < 0.05$ according to Duncan's test

Effect of P and Al on pectin properties

Exposure to Al resulted in a significant increase in pectin, of 94.37% and 58.27% under P supply and P deficiency, respectively (Fig. 3a). P deficiency also induced an increase in the pectin content regardless of Al presence. The degree of pectin methyl esterification (DME) was at a high level without Al supply, whereas the Al stress significantly decreased DME to 32.23% and 20.26% under -P and +P treatments, respectively (Fig. 3b). However, application of P significantly decreased the DME under Al toxicity. Al toxicity significantly increased the demethylesterified pectin content by 4.78- and 4.27-folds under P deficiency and P supply, respectively (Fig. 3c). P application under Al toxicity decreased the demethylesterified pectin content significantly. P application under Al toxicity significantly decreased the PME activity, though Al stress slightly increased the PME activity under P deficiency (Fig. 3d).

Variations in hemicellulose content in response to P and Al treatment

The content of HC1 increased in oil tea after Al supply (Fig. 4a). After P application, the HC1 content in roots decreased significantly under Al stress. The content of HC2 was not significantly affected by P and Al treatments (Fig. 4b). The -P+Al treatment significantly decreased the XET and EGase activities by 24.67% and 30.57%, respectively, compared to the +P-Al treatment (Fig. 4c-d). However, the application of P under Al toxicity reduced the inhibition of XET and EGase activities.

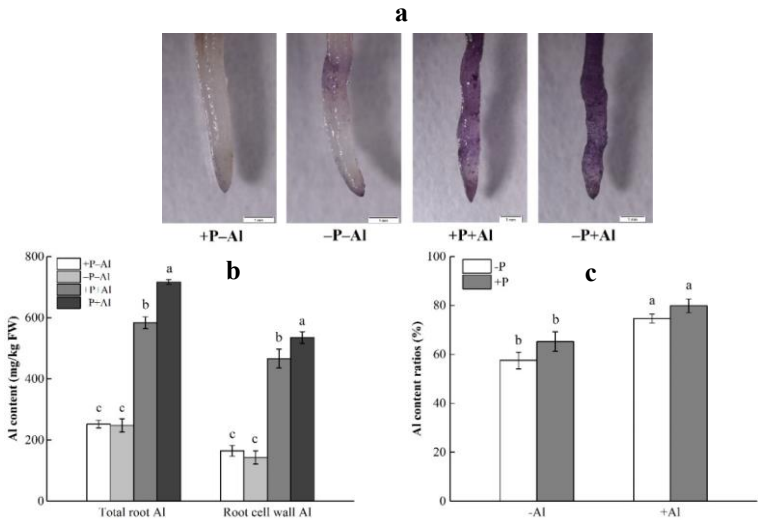

Fig. 2: The distribution of Al in roots of oil tea under different P and Al treatments. (**a**) The distribution of Al in roots was determined by hematoxylin staining. (**b**) The content of Al in roots and their cell walls. (**c**) The Al content ratios were calculated using the ratio of Al content in root cell walls and roots. Error bars indicate the SD; different letters above error bars indicate significant differences at $P < 0.05$ according to Duncan's test

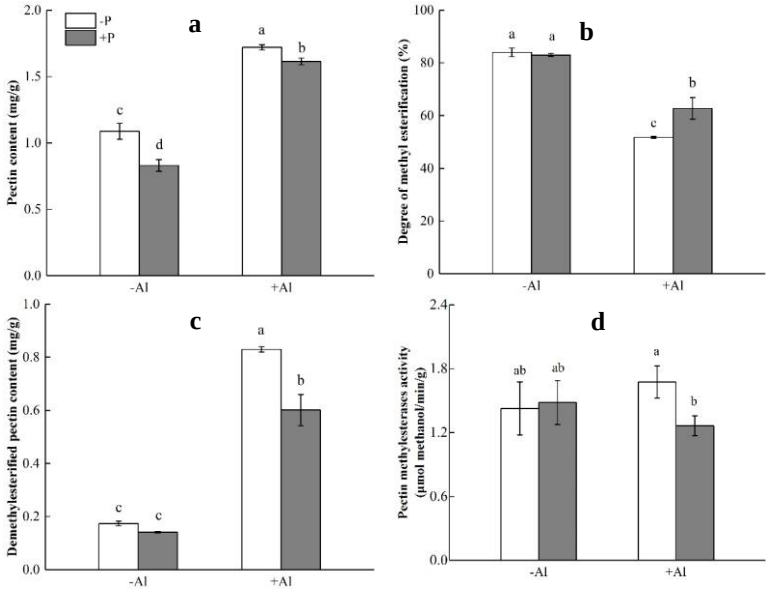

Fig. 3: Effect of P and Al on the pectin content (**a**), degree of pectin methyl esterification (**b**), demethylesterified pectin content (**c**), and PME activity (**d**). Error bars indicate the SD; different letters above error bars indicate significant differences at $P < 0.05$ according to Duncan's test

Effect of P and Al on the PAL and PPO activities and soluble phenolic content

On the condition of P deficiency, the PAL and PPO activities increased significantly under Al stress compared to the control (+P-Al) plants (Fig. 5a-b). However, P application significantly decreased the PAL and PPO activities under Al stress. Soluble phenolic content was increased under Al stress, and the supply of P did not significantly change the soluble phenolic content under Al stress (Fig. 5c).

Effect of P and Al on free proline and H_2O_2 contents and antioxidant enzyme activities

Phosphorus deficiency significantly decreased the activity of SOD in oil tea roots regardless of Al stress (Fig. 6a). The -P+Al treatment significantly decreased the activities of APX and CAT (Fig. 6b-c). However, the +P+Al treatment increased APX and CAT activities by 11.37% and 37.04%, respectively, compared to the -P+Al treatment. Al stress significantly decreased the activity of POD regardless of P

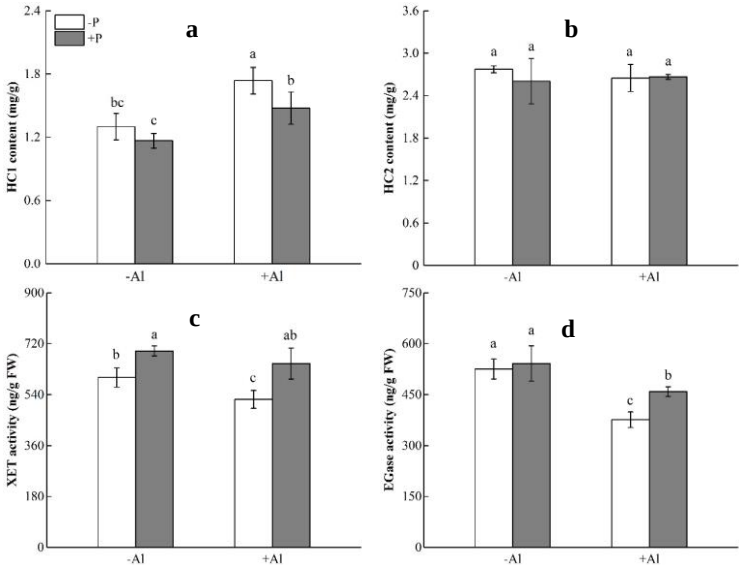

Fig. 4: Effect of P and Al on hemicellulose (HC1 and HC2) content and XET and EGase activities. Error bars indicate the SD; different letters above error bars indicate significant differences at $P < 0.05$ according to Duncan's test

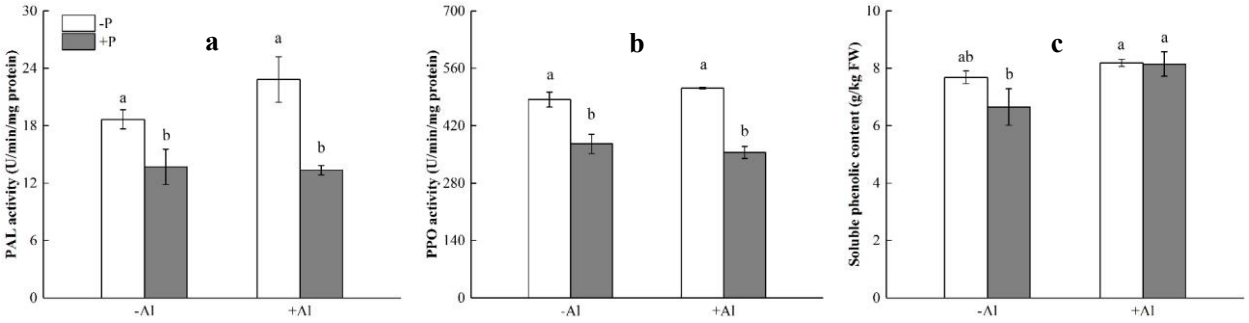

Fig. 5: Effect of P and Al on the activities of PAL and PPO and the content of soluble phenolic in oil tea roots. Error bars indicate the SD; different letters above error bars indicate significant differences at $P < 0.05$ according to Duncan's test

addition compared to the +P-Al treatment (Fig. 6d). Al stress significantly increased H_2O_2 content regardless of P application, while the +P+Al treatment decreased the H_2O_2 content compared to the -P+Al treatment (Fig. 6e). The -P+Al treatment significantly increased the free proline content, while P application under Al stress decreased the free proline content (Fig. 6f).

Discussion

In this study, Al stress under P deficiency significantly inhibited the relative root elongation rate, decreased root activity, and destroyed the plasma membrane integrity in oil tea roots. However, the appreciable improvement in root elongation, root activity, and plasma membrane integrity by P application under Al toxicity, indicating that P is involved in alleviating the Al stress. Roots are the first site of metal absorption and accumulation in many plants. Many studies revealed that Al stress inhibited relative root elongation mainly due to the accumulation of Al in the roots (Riaz *et al.* 2018b; Zhu *et al.* 2019). Reducing the accumulation of Al in roots will relieve the disruption and severe lesions in the root elongation zone caused by the accumulation of a large amount of Al in root tips (Mukhopadyay *et al.* 2012; Kopittke *et al.* 2015). Increasing evidence indicates that the cell wall is a major target of Al accumulation in plant roots, and reducing the Al content in the cell wall could alleviate root elongation inhibition by Al stress (Li *et al.* 2017b; Safari *et al.* 2018; Zhu *et al.* 2019). Our results revealed that the cell wall was the main site of Al accumulation in roots and nearly 80% of Al distributed in cell wall when exposed to Al, consistent with observations reported by Li *et al.* (2017a) for tea. Roots in the -P+Al treatment were more susceptible to Al than those in the +P+Al treatment, and the application of P decreased the accumulation of Al in roots and cell walls (Fig. 2a-b).

Current increasing physiological and biochemical evidence shows that the adsorption of Al to the cell wall is associated with polysaccharides in cell walls (Li *et al.* 2017b; Safari *et al.* 2018). The ability of cell wall fractions

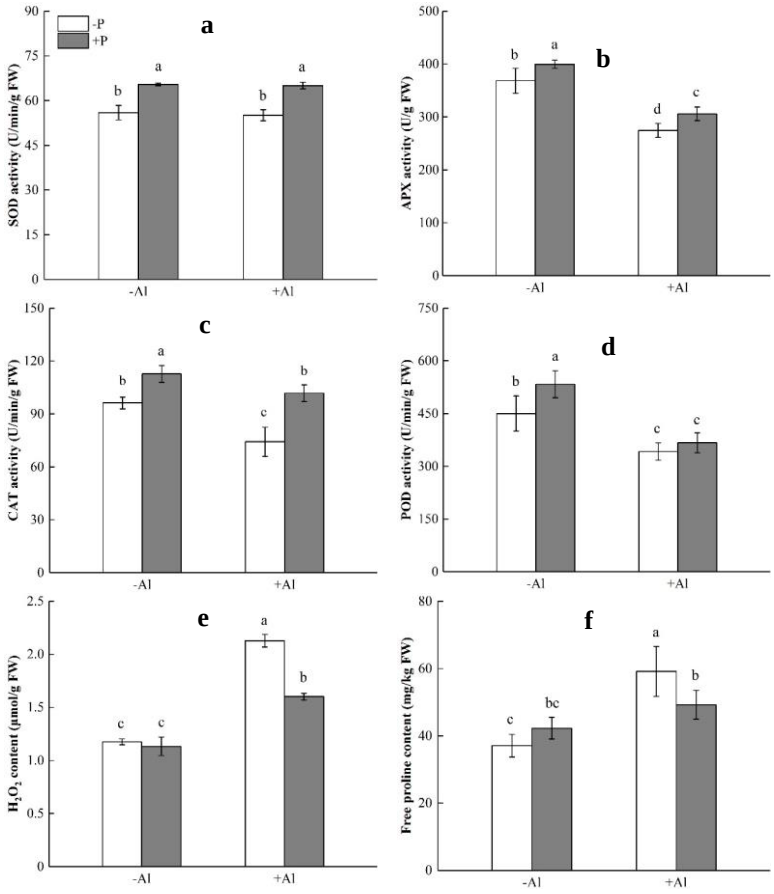

Fig. 6: Effect of P and Al on the activities of SOD, APX, CAT, and POD and the content of H_2O_2 and free proline in oil tea roots. Error bars indicate the SD; different letters above error bars indicate significant differences at $P < 0.05$ according to Duncan's test

to bind with Al was determined in our study (Table 1). The pectin fraction has a large number of negatively charged carboxylic groups that can easily chelate Al, and plants with higher pectin content in roots have a greater potential to accumulate Al (Horst *et al.* 2010). However, the actual Al accumulation capacity of pectin depends on both the pectin content and the degree of pectin methylesterification (Li *et al.* 2016). Al stress increased the pectin content in oil tea roots (Fig. 3), while the -P+Al treatment resulted in a higher cell wall demethylesterified pectin content and a lower degree of pectin methylesterification in oil tea roots (Fig. 3). Interestingly, P application significantly decreased the pectin content, especially the demethylesterified pectin content, and increased the degree of pectin methylesterification, which significantly reduced the binding sites of Al. It has been reported that plant resistance to Al is negatively related to PME activity (Zhu *et al.* 2012). Therefore, the decreased Al content in the pectin fraction may further be due to the lower PME activity under the +P+Al treatment (Fig. 3).

For a long time, the investigations of root cell wall responses to Al stress were focused on the properties of pectin, while recent studies pointed out that the hemicellulose as the second wall component was very susceptible to Al stress (Safari *et al.* 2018). It is shown that hemicellulose content was enhanced significantly under Al stress (Zhu *et al.* 2019), and Yang *et al.* (2011) showed that hemicellulose was the principal binding site of Al in Arabidopsis. Our result showed that the -P+Al treatment significantly increased the HC1 content, while the HC1 content and Al content in HC1 decreased with P application. A decrease in the HC1 content may decrease the binding sites of Al. The modification of hemicellulose polysaccharides, such as xyloglucan, affects wall loosening and expansion (Safari *et al.* 2018). Our results showed that P application reduced the inhibition of XET and EGase activities by Al stress (Fig. 4). The increase in XET activity catalyzed the splitting of xyloglucan chains, and an increase in EGase activity hydrolyzed β-1,4 glucan bonds to loosen the xyloglucan-cellulose network, thereby loosening the cell wall and promoting the elongation of roots (Yang *et al.* 2011; Safari *et al.* 2018).

PAL is a key enzyme involved in the biosynthesis of phenolic compounds and shows a positive response to various stress conditions (Hajiboland *et al.* 2015). However, the increase in PPO activity is able to promote the oxidation of phenolic to quinones, which in turn produce ROS and are

toxic to cells (Yoruk and Marshall 2003). It has been reported that Al stress can increase the activities of PAL and PPO and enhance the oxidative stress of cells, thus disrupting the intracellular environmental stability (Riaz *et al.* 2018a). Our results show that the addition of P effectively reduced the activities of PAL and PPO, thereby reducing the production of harmful substances. Studies have shown that Al stress can induce the production of soluble phenolic and the application of ^{27}Al-nuclear magnetic resonance identified the Al-catechin complex in cell sap, which reduced the damage caused by Al^{3+} to organelles inside the protoplast (Morita *et al.* 2008; Hajiboland *et al.* 2013). Our results indicated that the addition of Al significantly increased the content of soluble phenolic regardless of P application, which may be used for chelating Al in cell sap.

It has been reported that ionic stress such as P deficiency and Al toxicity breaks down the balance between antioxidant enzymes and ROS, resulting in oxidantive damage and growth inhibition of plants (Yu *et al.* 2018; Zhou *et al.* 2019). The working mechanism of antioxidant enzymes is that SOD converts the excess superoxide radical into H_2O_2, and POD, CAT, and APX decompose H_2O_2 into H_2O and O_2 (Wang *et al.* 2009). The results showed that -P+Al treatment inhibited the activities of antioxidant enzymes, which resulted in the overproduction of H_2O_2 and ultimately led to oxidative damage, and the plasma membrane integrity of cells was destroyed. The P addition decreased the H_2O_2 content and increased the activities of antioxidant enzymes. Proline is an important osmoregulator that can be induced by abiotic stress such as heavy metal toxicity and water deficit (Hou *et al.* 2016; Qu *et al.* 2019). P application significantly decreased the free proline content under Al stress, which further confirmed that P application decreased the toxicity of Al to roots.

Conclusion

The present study demonstrated that Al stress severely inhibited root elongation by inducing variations in the cell wall components as well as oxidative damage in roots of oil tea. However, P supply promoted root elongation. The mechanism could be summarized as follows: (1) P application reduced the accumulation of Al in cell walls by decreasing de-methyl esterified pectin content and HC1 content to decrease Al binding sites; (2) P application under Al stress enhanced cell wall loosening by alleviating the inhibition of XET and EGase activities; (3) P application eliminated oxidative damage by inhibiting the activities of PAL and PPO and by enhancing the activities of ROS scavenging enzymes.

Acknowledgement

This research was funded by Provincial Science and Technology Major Project of Hunan, China under Grant, grant number 2018NK1030.

Author Contributions

Xinjing Qu, carried out the experiment, collected and organized data, and wrote the manuscript. Jiao Liao and Chenhui Zhang carried out the experiment, collected and organized data. Jun Yuan raised the hypothesis underlying this work, designed the experiment, and reviewed the manuscript. All authors have read and approved the final manuscript.

References

Anthon GE, DM Barrett (2004). Comparison of three colorimetric reagents in the determination of methanol with alcohol oxidase. Application to the assay of pectin methylesterase. *J Agric Food Chem* 52:3749–3753

Gai Z, J Zhang, C Li (2017). Effects of starter nitrogen fertilizer on soybean root activity, leaf photosynthesis and grain yield. *PLoS One* 12; Article 0174841

Ghanati F, A Morita, H Yokota (2005). Effects of aluminum on the growth of tea plant and activation of antioxidant system. *Plant Soil* 276:133–141

Hajiboland R, S Bahrami Rad, J Barceló, C Poschenrieder (2013). Mechanisms of aluminum-induced growth stimulation in tea (*Camellia sinensis*). *J Plant Nutr Soil Sci* 176:616–625

Hajiboland R, S Bahrami Rad, S Bastani (2015). Phenolics metabolism in boron-deficient tea [*Camellia sinensis* (L.) O. Kuntze] plants. *Acta Biol Hung* 64:196–206

Horst WJ, Y Wang, D Eticha (2010). The role of the root apoplast in aluminum-induced inhibition of root elongation and in aluminum resistance of plants: A review. *Ann Bot* 106:185–197

Hou W, H Hu, Y Lu, T Li, W Teng, L Wang (2016). Relieve effect of exogenous P on the eucalyptus growth and physiological feature under Al stress. *J Northeast For Univ* 44:5–9

Kochian LV, OA Hoekenga, MA Piñeros (2004). How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu Rev Plant Biol* 55:459–493

Kopittke PM, KL Moore, E Lombi, A Gianoncelli, BJ Ferguson, FP Blamey, NW Menzies, TM Nicholson, BA McKenna, P Wang, PM Gresshoff, G Kourousias, RI Webb, K Green, A Tollenaere (2015). Identification of the primary lesion of toxic aluminum in plant roots. *Plant Physiol* 167:1402–1411

Li D, Z Shu, X Ye, J Zhu, J Pan, W Wang, P Chang, C Cui, J Shen, W Fang, X Zhu, Y Wang (2017a). Cell wall pectin methyl-esterification and organic acids of root tips involve in aluminum tolerance in *Camellia sinensis*. *Plant Physiol Biochem* 119:265–274

Li X, Y Li, M Qu, H Xiao, Y Feng, J Liu, L Wu, M Yu (2016). Cell wall pectin and its methyl-esterification in transition zone determine Al resistance in cultivars of Pea (*Pisum sativum*). *Front Plant Sci* 2016; Article 00039

Li X, J Liu, J Fang, L Tao, R Shen, Y Li, H Xiao, Y Feng, H Wen, J Guan, L Wu, Y He, HE Goldbach, M Yu (2017b). Boron supply enhances aluminum tolerance in root border cells of Pea (*Pisum sativum*) by interacting with cell wall pectins. *Front Plant Sci* 2017; Article 00742

Louvet R, C Rayon, JM Domon, C Rusterucci, F Fournet, A Leaustic, MJ Crépeau, MC Ralet, C Rihouey, M Bardor, P Lerouge, F Gillet, J Pelloux (2011). Major changes in the cell wall during silique development in Arabidopsis thaliana. *Phytochemistry* 72:59–67

Mo X, M Zhang, C Liang, L Cai, J Tan (2019). Integration of metabolome and transcriptome analyses highlights soybean roots responding to phosphorus deficiency by modulating phosphorylated metabolite processes. *Plant Physiol Biochem* 139:697–706

Morita A, O Yanagisawa, S Takatsu, S Maeda, S Hiradate (2008). Mechanism for the detoxification of aluminum in roots of tea plant (*Camellia sinensis* (L.) Kuntze). *Phytochemistry* 69:147–153

Morrison IM (1972). A semi-micro method for the determination of lignin and its use in prediction the digestibility of forage crops. *J Sci Food Agric* 23:455–463

Mukhopadyay M, P Bantawa, A Das, B Sarkar, B Bera, P Ghosh, T Kumar Mondal (2012). Changes of growth, photosynthesis and alteration of leaf antioxidative defence system of tea [*Camellia sinensis* (L.) O. Kuntze] seedlings under aluminum stress. *Biometals* 25:1141–1154

Nagarajah S, AM Posner, JP Quirk (1970). Competitive adsorption of phosphate with polygalacturonate and other organic anions on kaolinate and oxide surface. *Nature* 228:83–84

Nakano Y, K Asada (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880

Nieuwenburg CJV, G Uitenbroek (1948). On the detection of aluminium by means of aluminon. *Anal Chim Acta* 2:88–91

Polle E, C Konzak, J Kittrick (1978). Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. *Crop Sci* 18:823–827

Qu X, H Wang, M Chen, J Liao, J Yuan, G Niu (2019). Drought stress-induced physiological and metabolic changes in leaves of two oil tea cultivars. *J Amer Soc Hortic Sci* 144:439–447

Qu X, J Zhou, J Masabni, J Yuan (2020). Phosphorus relieves aluminum toxicity in oil tea seedlings by regulating the metabolic profiling in the roots. *Plant Physiol Biochem* 152:12–22

Riaz M, L Yan, X Wu, S Hussain, O Aziz, Y Wang, M Imran, C Jiang (2018a). Boron alleviates the aluminum toxicity in trifoliate orange by regulating antioxidant defense system and reducing root cell injury. *J Environ Manage* 208:149–158

Riaz M, L Yan, X Wu, S Hussain, O Aziz, M Imran, MS Rana, C Jiang (2018b). Boron reduces aluminum-induced growth inhibition, oxidative damage and alterations in the cell wall components in the roots of trifoliate orange. *Ecotox Environ Safe* 153:107–115

Safari M, F Ghanati, MR Safarnejad, NA Chashmi (2018). The contribution of cell wall composition in the expansion of *Camellia sinensis* seedlings roots in response to aluminum. *Planta* 247:381–392

Singh N, R Singh, K Kaur, H Singh (1999). Studies of the physico-chemical properties and polyphenoloxidase activity in seeds from hybrid sunflower (*Helianthus annuus*) varieties grown in India. *Food Chem* 66:241–247

Wang X, J Huang (2015). *Principle and Technology of Plant Physiological and Biochemical Experiments*, 3rd edn. Higher Education Press, Beijing, China

Wang X, P Liu, H Luo, Z Xie, G Xu, J Yao, K Chen (2009). Effect of Al and F interaction on physiological characteristics of tea plants. *Acta Hort Sin* 36:1359–1364

Yamamoto Y, Y Kobayashi, H Matsumoto (2001). Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant Physiol* 125:199–208

Yang C, X Liu, Z Chen, Y Lin, S Wang (2016). Comparison of oil content and fatty acid profile of ten new *Camellia oleifera* cultivars. *J Lipids* 2016; Article 3982486

Yang L, X Zhu, Y Peng (2011). Cell wall hemicellulose contributes significantly to aluminum adsorption and root growth in Arabidopsis. *Plant Physiol* 155:1885–1892

Yu J, L Xia, D Yin, C Zhou (2018). Effects of phosphorus on aluminum tolerance of Chinese fir seedlings. *Sci Silvae Sin* 54:36–47

Yoruk R, MR Marshall (2003). Physicochemical properties and function of plant polyphenol oxidase: A review. *J Food Biochem* 27:361–422

Zhang Z, W Qu, X Li (2016). Experimental Guidance on Plant Physiology, 4th edn. Higher Education Press, Beijing, China

Zhao X, X Zhu, Q Wu, R Shen (2018). Study on mechanism of demethylation of pectin promoting reutilization of cell wall phosphorus in Rice (*Oryza sativa*) root. *Acta Pedol Sin* 55:1190–1198

Zhou J, Z Ai, H Wang, G Niu, J Yuan (2019). Phosphorus alleviates aluminum toxicity in *Camellia oleifera* seedlings. *Intl J Agric Biol* 21:237–243

Zhu C, X Cao, Z Bai, L Zhu, W Hu, A Hu, B Abliz, C Zhong, Q Liang, J Huang, J Zhang, Q Jin (2019). Putrescine alleviates aluminum toxicity in rice (*Oryza sativa*) by reducing cell wall Al contents in an ethylene-dependent manner. *Physiol Plantarum* 167:471–487

Zhu X, G Lei, T Jiang, Y Liu, X Li, S Zheng (2012). Cell wall polysaccharides are involved in P-deficiency-induced Cd exclusion in *Arabidopsis thaliana*. *Planta* 236:989–997

Zhu X, Z Wang, J Wan, Y Sun, Y Wu, G Li, R Shen, S Zheng (2015). Pectin enhances rice (*Oryza sativa*) root phosphorus remobilization. *J Exp Bot* 66:1017–1024