Fractionation of Inorganic Phosphorus and Aluminum in Red Acidic Soil and the Growth of *Camellia oleifera*

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Abstract. Aluminum (Al) toxicity and phosphorus (P) deficiency are two crucial factors limiting the production of *Camellia oleifera*, which is grown commercially in red acidic soils in Southern China. The current study characterized the different forms of P and Al in the red acidic soils of *C. oleifera* plantations. Soil and plant tissue samples taken from 32 *Camellia* plantations across Hunan province were analyzed. Furthermore, a pot experiment with nutrient solutions of different Al and P contents was carried out to investigate P and Al uptake and their effect on *C. oleifera* growth. The results showed that the P content extracted by NaOH (Fe-P) was the highest in all types of soil samples (rhizosphere, 0–20 cm, and 20–40 cm zones), followed by P extracted by NH4F (Al-P), H2SO4 (Ca-P), and Na3C6H5O7 (O-P). HCl (In-Al), NH4Ac (Ha-Al), and Na4P2O7 (AP) and Al-P in rhizosphere. P in roots and young leaves also had a positive correlation with Ex-Al, whereas Al in old leaves was positively correlated with In-Al and total Al. Significant correlations between Al-P, Ex-Al, and AP were detected. The pot experiment indicated that adding Al or P alone increased plant growth and Al or P uptake, respectively. When adding both Al and P, significant synergistic effect was found. These results suggest that Al is beneficial to *C. oleifera*, which may be the adaptive mechanism of *C. oleifera* to use insoluble Al-P in red acidic soil.

Acidic soils account for 30% of the world’s total land and as high as 50% of the arable land (Kochian et al., 2004). In these low pH soils, Al is easily fixed by clay minerals and becomes unavailable for root uptake. On the other hand, Al is solubilized into ionic forms, which are easily absorbed by plants, especially when soil pH falls below 5 (Zheng, 2010). Early studies about acidic soils focused on Al toxicity (Kochian, 1995). High Al content in acidic soils inhibits root growth and consequently affects whole plant growth and development (Yi et al., 2010; Zheng, 2010). The lack of essential mineral nutrients has also been attributed to acidic soils (Kochian et al., 2004). P is the most important limiting nutrient in acid soils because of Al-oxyhydroxide fixations that lead to low P solubility and not readily available for root uptake (Zheng, 2010). Thus, Al toxicity and P deficiency are two interrelated factors limiting plant growth and crop production in acid soils (Barcelo and Poschenrieder, 2002; Yu et al., 2016). The concurrence of high Al (Al toxicity) and low P (P deficiency) has been widely investigated for many plant species (Liao et al., 2006; Maejima et al., 2014). Correlation analyses revealed that P in roots was positively associated with available P (AP) and Al-P in rhizosphere. P in roots and young leaves also had a positive correlation with Ex-Al, whereas Al in old leaves was positively correlated with In-Al and total Al. Significant correlations between Al-P, Ex-Al, and AP were detected. The pot experiment indicated that adding Al or P alone increased plant growth and Al or P uptake, respectively. When adding both Al and P, significant synergistic effect was found. These results suggest that Al is beneficial to *C. oleifera*, which may be the adaptive mechanism of *C. oleifera* to use insoluble Al-P in red acidic soil.

Materials and Methods

Soil and plant tissue samples. In China, *C. oleifera* is primarily cultivated in the red acidic soils of Hunan province. Thirty-two plantations with established mature stands of *C. oleifera* forests across the province were identified and sampled (Fig. 1). At each site, five medium-sized trees were selected for sampling. Soil samples of 0–20 cm and 20–40 cm zones were collected from four spots (one each at east, south, west, and north directions) at 1.0 m away from the tree trunks. The collections from each tree were pooled and then quartered for a sample of up to 1 kg. Rhizosphere soil samples were taken by collecting the soil adherent to nonwoody roots by hand or using a spade under the tree canopy.
feeder roots. The pH of the soil samples was 3.6–4.9. At the same time, 5 g of the feeder roots were taken. Forty young (the upper third leaves) and old leaves (the bottom fifth leaves) were collected from the four directions of each tree.

Analysis of P and Al contents in plant tissues. The plant materials (leaves or roots) were rinsed thoroughly in tap water and then in distilled water. After the wash, the samples were dried in an oven at 105 °C for 30 min and then at 60 °C for 24 h. The dried plant materials were ground and digested with H2SO4+H2O2 (EasyDigest 40; AMS, Rome, Italy). Al concentration was determined by means of aluminon (Nieuwenburg and Uitenbroek, 1948). P concentration was determined using a discrete auto analyzer (Smartchem 200; Westco Scientific Instruments, Rome, Italy) after the malachite green oxalate method. All measurements were carried out in triplicate.

Fractionation of soil inorganic P. All soil samples were air-dried and ground manually. Fractionation of soil P was determined by the sequential extraction procedure outlined by Chang and Jackson (1957) as follows: Al-P (0.5 g soil + 10 mL 0.5 mol·L−1 NH4F), Fe-P (the residual + 10 mL 0.1 mol·L−1 NaOH), O-P (the residual + 10 mL 0.3 mol·L−1 Na2C2O4·H2O), and Ca-P (the residual + 10 mL 0.5 mol·L−1 H2SO4). Soil total P was digested with concentrated H2SO4+H2O2, and the AP was extracted by 0.03 mol·L−1 NH4F + 0.03 mol·L−1 HCl (Bray and Kurtz, 1945). Samples were extracted by shaking for 2 h. The P contents of all the extractions were determined by the same method used for plant tissues described previously.

Fractionation of soil Al. Various forms of Al were extracted by sequential extraction procedure with different reagents (Dai et al., 2011; Lu, 2000): For Ex-Al, 1.0 g soil was mixed with 10 mL 1 mol·L−1 KCl and shaken for 30 min; for Ha-Al, the residual was mixed with 10 mL 1 mol·L−1 NH4Ac and shaken for 5 h; for In-Al, the residual with 10 mL 1 mol·L−1 HCl was shaken for 1.5 h; for Hy-Al, the residual with 10 mL 0.5 mol·L−1 NaOH was shaken for 1.5 h; and for Or-Al, 1.0 g soil was agitated for 2 h with 10 mL 0.1 mol·L−1 Na2P2O7·10H2O. Soil total Al (TAl) was digested with concentrated H2SO4+H2O2. Al contents of all the previous samples were determined following the same method used for analysis of plant tissues.

Pot experiment. One-year-old grafted seedlings with uniform growth were transplanted to 2.5 L plastic pots (12 cm diameter with 18 cm height) containing 3.2 kg sand on 27 Apr. 2014. The potted seedlings were

Table 1. Inorganic phosphorus (P) fractions of soil samples taken from *Camellia oleifera* plantation sites.

| Soil P fraction | Soil zone | Content (mg kg−1) | Soil P fraction | Soil zone | Content (mg kg−1) |
|-----------------|-----------|-----------------|-----------------|-----------|-----------------|
| Fe-P            | Rhizosphere | 133.08 ± 22.45 a | O-P            | Rhizosphere | 7.24 ± 1.89 a  |
|                 | 0–20 cm   | 124.50 ± 23.70 ab |                | 0–20 cm   | 6.74 ± 1.42 ab  |
|                 | 20–40 cm  | 111.37 ± 20.00 b |                | 20–40 cm  | 6.01 ± 1.27 b   |
| Total (% of total P) | 368.95 (38.4%) |                | Total (% of total P) | 19.99 (2.1%) |
| Al-P            | Rhizosphere | 108.81 ± 24.03 a | AP             | Rhizosphere | 1.93 ± 0.83 a  |
|                 | 0–20 cm   | 98.65 ± 22.80 b  |                | 0–20 cm   | 1.64 ± 0.70 b   |
|                 | 20–40 cm  | 87.35 ± 16.10 b  |                | 20–40 cm  | 1.39 ± 0.61 c   |
| Total (% of total P) | 294.81 (30.7%) |                | Total (% of total P) | 4.96 (0.5%) |
| Ca-P            | Rhizosphere | 13.43 ± 3.00 a   | Total P        | Rhizosphere | 345.80 ± 52.56 b |
|                 | 0–20 cm   | 12.66 ± 2.60 b   |                | 0–20 cm   | 320.71 ± 63.92 a |
|                 | 20–40 cm  | 11.55 ± 2.17 b   |                | 20–40 cm  | 294.13 ± 52.56 b |
| Total (% of total P) | 37.64 (3.9%) |                | Total (% of total P) | 960.64 (100%) |

*Data presented are mean ± SD. Different letters in the same column within each type of P indicate significant difference at P ≤ 0.05 (Duncan’s multiple range tests). Phosphorus Fractionations were determined by the sequential extraction procedure (Chang and Jackson, 1957): Al-P: 0.5 g soil + 10 mL 0.5 mol·L−1 NH4F; Fe-P: the residual + 10 mL 0.1 mol·L−1 NaOH; O-P: the residual + 10 mL 0.3 mol·L−1 Na2C2O4·H2O; Ca-P: the residual + 10 mL 0.5 mol·L−1 H2SO4. Total P: Soil total P; AP: Soil available P.

Table 2. Aluminum fractions of soil samples taken from *Camellia oleifera* plantation sites.

| Soil Al fraction | Soil zone | Contents (g·kg−1) | Soil Al fraction | Soil zone | Contents (g·kg−1) |
|-----------------|-----------|-----------------|-----------------|-----------|-----------------|
| Ex-Al           | Rhizosphere | 4.78 ± 1.24 b   | Hy-Al           | Rhizosphere | 0.99 ± 0.16 b  |
|                 | 0–20 cm   | 4.86 ± 1.22 a   |                | 0–20 cm   | 1.12 ± 0.20 a  |
|                 | 20–40 cm  | 4.59 ± 1.00 b   |                | 20–40 cm  | 1.00 ± 0.18 b  |
| Total (% of total Al) | 14.23 (11%) |                | Total (% of total Al) | 3.11 (2.3%) |
| Ha-Al           | Rhizosphere | 10.05 ± 2.08 a  | Or-Al           | Rhizosphere | 10.26 ± 4.59 a |
|                 | 0–20 cm   | 9.81 ± 2.16 a   |                | 0–20 cm   | 10.22 ± 5.05 a |
|                 | 20–40 cm  | 10.72 ± 1.96 a  |                | 20–40 cm  | 10.98 ± 4.29 a |
| Total (% of total Al) | 30.58 (23.1%) |                | Total (% of total Al) | 31.46 (23.8%) |
| In-Al           | Rhizosphere | 10.48 ± 2.45 a  | Total Al        | Rhizosphere | 43.64 ± 9.83 a |
|                 | 0–20 cm   | 9.99 ± 2.51 a   |                | 0–20 cm   | 44.33 ± 10.51 a |
|                 | 20–40 cm  | 9.67 ± 2.76 a   |                | 20–40 cm  | 44.40 ± 9.57 a |
| Total (% of total Al) | 30.14 (22.8%) |                | Total (% of total Al) | 323.37 (100%) |

*Data presented are mean ± SD. Different letters in the same column within each type of Al indicate significant difference at P ≤ 0.05. Aluminum forms were extracted by sequential extraction procedure (Dai et al., 2011; Lu, 2000): Ex-Al: 1.0 g soil was mixed with 10 mL 1 mol·L−1 KCl and shaken for 30 min; Ha-Al: the residual mixed with 10 mL 1 mol·L−1 NH4Ac were shaken for 5 h; In-Al: the residual with 10 mL 1 mol·L−1 HCl were shaken for 1.5 h; Hy-Al: the residual with 10 mL 0.5 mol·L−1 NaOH were shaken for 1.5 h; Or-Al: 1.0 g soil were agitated for 2 h with 10 mL 0.1 mol·L−1 Na2P2O7·10H2O.
grown outdoors and protected from rainfall by a plastic canopy. Four treatments of different combinations of P and Al concentrations were created by adding Al or P to Hoagland-Arnon solution: Al-P: no Al or P added; AlP: no Al and 1 mol P L⁻¹ added; AlP⁺: 2 mmol Al L⁻¹ and no P added; and AlP⁺: 2 mmol Al L⁻¹ and 1 mol P L⁻¹ added (all other elements were kept unchanged). Plants were irrigated with 200 mL treatment solutions every 3 d starting from 30 Apr. 2014. The source of P was KH₂PO₄, and the K⁺ in the nutrient solution was adjusted by KCl. Al was provided by AlCl₃. The pH of the treatment solutions was adjusted to 5.5 with 0.5% Ca(OH)₂ or H₂SO₄. Additional 100-mL distilled water was applied in the evenings on hot days to insure sufficient moisture. There were five replicates in each treatment, and the pots were arranged in a completely randomized design. The seedlings were harvested on 27 Aug. 2014.

Total plant dry weight (DW) was obtained after oven dried at 65 °C to a constant weight. The dried plants (whole plants, including stems, leaves, and roots) were analyzed for P and Al contents using the same methods mentioned previously.

**Statistical analysis.** Data from all sample sites were pooled because no site differences were found. One-way analysis of variance (ANOVA) was performed, when significant, Duncan’s multiple range tests were followed to distinguish the differences in P and Al fractionation among the three soil sample layers. Correlation analysis was performed using SPSS for Windows V17.0. All graphs were made by Origin 8.5.

## Results

*Fractionation of soil P.* In all three types of soil samples, Fe-P contents were the highest, followed by Al-P, accounted for 38% and 31% of total P, respectively (Table 1). Ca-P and O-P accounted for 3.9% and 2% of total P, respectively, whereas AP was only 0.5% of total P. Numerically, the rhizosphere soil contained the highest P of all forms. The ANOVA analysis showed that for Fe-P, Ca-P, and O-P, there was no difference between rhizosphere and 0–20 cm and between 0–20 cm and 20–40 cm soils. For Al-P, no significant difference was found between 0–20 cm and 20–40 cm soils. The total P content at 0–20 cm was significantly higher than that at 20–40 cm soils, whereas no significant difference was found between rhizosphere and 0–20 cm soils. The content of AP in rhizosphere soil was significantly higher than that of the nonrhizosphere soils, and the AP in 0–20 cm was higher than that of 20–40 cm soils.

*Fractionation of soil Al.* For Ha-Al, In-Al, Or-Al, and total Al, no differences were observed among rhizosphere and other soil zones (Table 2). The contents of Ha-Al, In-Al, and Or-Al were similar and each accounted for ≈23% of total Al, whereas Ex-Al accounted for 11%, and Hy-Al accounted for 2.3% of total Al. The contents of Ex-Al and Hy-Al in the soil zone of 0–20 cm were greater than that of the rhizosphere and 20–40 cm, and no significant difference was observed between rhizosphere and 20–40 cm soils.

*P and Al contents in plant tissues.* The P contents in roots and young leaves were 0.799 and 0.823 mg kg⁻¹, respectively, and no difference was found between them (Fig. 2). However, they were significantly higher than the P content in the old leaves, which was 0.639 mg kg⁻¹. On the contrary, the highest Al content (11.345 g kg⁻¹) was found in the old leaves.

### Table 3. Correlations (r values) between phosphorus (P) content of *Camellia oleifera* plant tissues and soil P fractions.

| Soil zone | Tissues | Al-P | Fe-P | O-P | Ca-P | AP | TP |
|-----------|---------|------|------|-----|------|----|----|
| Rhizosphere | Roots | 0.3837 | 0.0690 | -0.0889 | -0.1272 | 0.509** | 0.0851 |
| | Young leaves | 0.1322 | 0.2397 | 0.1860 | 0.1275 | -0.3369 | 0.2129 |
| | Old leaves | 0.0924 | 0.1570 | 0.1095 | 0.0028 | -0.2625 | 0.1437 |
| 0–20 cm | Roots | 0.2910 | 0.1940 | 0.1900 | 0.2240 | 0.0160 | 0.2410 |
| | Young leaves | 0.0520 | -0.0210 | 0.1420 | 0.0280 | -0.4190 | 0.0160 |
| | Old leaves | 0.1140 | 0.0410 | 0.1680 | 0.1080 | -0.3080 | 0.0720 |
| 20–40 cm | Roots | 0.0233 | -0.0326 | -0.0274 | 0.0760 | 0.1294 | 0.0605 |
| | Young leaves | 0.0680 | 0.0512 | 0.1738 | 0.0744 | -0.3380 | 0.0602 |
| | Old leaves | 0.0456 | 0.0801 | 0.2120 | 0.1303 | -0.2271 | 0.0996 |

** Indicates significant at P ≤ 0.05. Phosphorus Fractionations were determined by the sequential extraction procedure (Chang and Jackson, 1957): Al-P: no Al or P added; AlP: no Al and 1 mol P L⁻¹ added; AlP⁺: 2 mmol Al L⁻¹ and 1 mol P L⁻¹ added. The P contents and lower case for Al contents.

### Table 4. Correlations (r values) between aluminum (Al) contents of *Camellia oleifera* plant tissues and soil Al fractions.

| Soil zone | Tissues | Ex-Al | Ha-Al | Hy-Al | In-Al | Or-Al | T-Al |
|-----------|---------|-------|-------|-------|-------|-------|------|
| Rhizosphere | Roots | 0.3412** | 0.1832 | -0.3897 | -0.1123 | 0.0851 | 0.2631 |
| | Young leaves | 0.436* | 0.491* | -0.3879 | 0.504* | 0.3321 | 0.618** |
| | Old leaves | 0.3495 | 0.2646 | -0.3127 | 0.552** | 0.2166 | 0.625** |
| 0–20 cm | Roots | 0.3115 | 0.3182 | -0.2656 | -0.1613 | 0.0897 | 0.2285 |
| | Young leaves | 0.537* | 0.447* | -0.1871 | 0.589** | 0.443* | 0.612** |
| | Old leaves | 0.3614 | 0.2571 | -0.1356 | 0.539** | 0.3368 | 0.666** |
| 20–40 cm | Roots | 0.3294 | 0.2664 | -0.2874 | -0.0266 | 0.0105 | 0.2330 |
| | Young leaves | 0.465* | 0.476* | -0.4172 | 0.502* | 0.4194 | 0.621** |
| | Old leaves | 0.3085 | 0.2740 | -0.3008 | 0.558** | 0.2815 | 0.637** |

** Indicates significant at P ≤ 0.05 or P ≤ 0.01. Aluminum forms were extracted by sequential extraction procedure (Dai et al., 2011; Lu, 2000): Ex-Al: 1.0 g soil was mixed with 10 mL 1 mol L⁻¹ KCl and shaken for 30 min; Ha-Al: the residual mixed with 10 mL 0.1 mol L⁻¹ NH₄Ac were shaken for 5 h; In-Al: the residual with 10 mL 0.5 mol L⁻¹ HCl were shaken for 1.5 h; Hy-Al: the residual with 10 mL 0.5 mol L⁻¹ NaOH were shaken for 1.5 h; Or-Al: 1.0 g soil was agitated for 2 h with 10 mL 0.1 mol L⁻¹ Na₃P₂O₅·10H₂O.
leaves, followed by the roots and the young leaves with 8.784 and 6.085 g kg⁻¹, respectively.

Relationship between plant P and Al contents and soil P and Al fractions. Correlation analyses showed that P content in roots was positively correlated with AP in rhizosphere soil ($r = 0.509, P = 0.04$). No other significant correlation between different P forms in the soil and plant tissues was observed (Table 3). More significant correlations were found between Al contents in plant tissues and soil Al fractions (Table 4). In the rhizosphere zone, positive correlations were observed between Al contents in young leaves and soil Ex-Al, Ha-Al, In-Al, and T-Al; between Al content in roots and soil Ex-Al; and between Al contents in old leaves and soil In-Al and T-Al. Similarly, Al contents in young leaves had correlations with soil Ex-Al, Ha-Al, In-Al, and T-Al, but not with Hy-Al, in the 0–20 cm and 20–40 cm soil zones. Also, Al contents in roots did not have any correlation with any Al fraction of soils at 0–20 cm and 20–40 cm.

Relationship between soil P and Al fractions. Significant correlations were found between Al-P and Ex-Al, between O-P and Hy-Al in the rhizosphere, and between AP and Or-Al in the rhizosphere and 0–20 cm soils. All other correlation analyses were not significant (Table 5).

Growth of *C. oleifera* under different P and Al regimes. The pot experiment showed that the supplement of P or Al or both significantly increased the plant biomass (Fig. 3). The DW was the highest when both P and Al were added to the nutrient solution (Al-P⁺) and the lowest when neither P nor Al was added (Al-P⁻). No difference in the DW was found when either P or Al was added to the solution. The significantly higher DW of P-Al treatment indicates a synergistic effect between P and Al.

Adding P or Al alone significantly increased plant P or Al content compared with no addition (Al-P⁻) (Fig. 4). When both P and Al were added (Al-P⁺), the plant P and Al contents were significantly higher than when P (Al-P⁻) or Al (Al-P⁺) were added alone at the same concentrations. This result indicates that the interaction between P and Al promotes plant absorption of both elements.

**Discussion**

At low soil pH (acidic soil), Al becomes more soluble in the soil solution and thus becomes toxic to living organisms (Zheng,
2010). P can strongly react with Al oxide and becomes unavailable to plants causing P deficiency (Kochian et al., 2004; Shen et al., 2011). Therefore, P deficiency and Al toxicity have long been considered as two concurrent limiting factors for plant growth and crop production in acidic soils (Yu et al., 2016). C. oleifera is mainly cultivated in red acidic soils and faces strong challenges of P deficiency and high concentration of Al. However, the growth and fruit yield of C. oleifera seem not suppressed (He et al., 2010; Yuan et al., 2013b; Zeng et al., 2011). Observations of C. oleifera suggest that this plant may have evolved adaptive mechanisms to Al toxicity and P deficiency in red acidic soils (Shen et al., 2011; Zhang et al., 1997). C. oleifera has been proven to be a P-efficient and Al-hyperaccumulator plant (Zeng et al., 2011).

Our study found that all the P fractions in top soils (rhizosphere and 0–20 cm zone) were higher than that of subsoil (20–40 cm zone), which could be due to surface application of P fertilizer, a common practice in the plantations (Caioine et al., 2015). Because Al is abundant in the acidic soil and C. oleifera plants absorbed Al more than the plants needed, which caused higher concentrations in older leaves. This is a similar situation to high soil salinity condition when plants absorbed more sodium and chloride in older leaves than in young leaves (Niu and Cabrera, 2010). On the other hand, the AP content in the acidic soil was very low, which may cause a lower P concentration in the older leaves. The Fe-P and Al-P were the major fractions of P in the red acidic soil and were over 65% of total P in samples of rhizosphere, 0–20 and 20–40 cm soil zones. This suggests that these two fractions (Fe-P, Al-P) were the main components of soil P in the red acidic soils of C. oleifera plantations. Ex-Al and AP contents in the rhizosphere were significantly higher than nonrhizosphere soils. The P content in roots was positively associated with AP, Al-P, and Ex-Al in the rhizosphere. Adding both Al and P significantly increased Al and P contents in plants and plant growth. More importantly, Al is a beneficial element to C. oleifera suggesting that it has an adaptive mechanism to use Al-P in the red acidic soil.

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