Effects of calcium and phosphorus on aluminium phytotoxicity in soil-Chinese fir system

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Abstract. The potential toxicity of soil active aluminium (Al) on Chinese fir was an urgent problem due to the global soil acidification. The aim of this paper was to study the effects of calcium and phosphorus on Al phytotoxicity in soil-Chinese fir system by using soil culture. One year old seedlings of Chinese fir were used as plant materials. The changes and relationship of pH and active form of Al in rhizosphere soil, leaf Al and physiological indexes in seedlings of Chinese fir were analysed. The results showed that the soil pH of all treatments rose at different degree, while the content of exchangeable Al in soil was decreased under different treatments of calcium and phosphorus as compared with the control. The results also showed that the content of Al and MDA in leave was decreased, while an increase in soluble protein content in leave was observed. It was concluded that the alteration in soil rhizosphere environment may be the main factor improving physiological indexes, consequently alleviating Al phytotoxicity in soil-Chinese fir system.

1. Introduction
Like heavy metals, excess aluminium (Al) in soil has also been confirmed to be toxic to plants. Al phytotoxicity becomes more severely in acid soil because the insoluble Al compounds can be transformed into soluble and active forms, such as Al³⁺, Al(OH)₂⁺ and Al(OH)₃⁻. Al in plant would attack cell membrane, proteins, photosynthetic system and nucleic acids, and then influence the growth, cell membrane integrity, photosynthesis, nitrogen metabolism, nutrient uptake and genetic behavior of plants. The potential toxicity of active Al on terrestrial ecosystem was a major factor leading to the forest degradation overspreading along Middle Europe in the 1970s. Chinese fir (Cunninghamia lanceolata) is one of the most important timber tree species in Southern China. The acidic soil here and the continuous planting of Chinese fir had increased the risk of Al toxicity in Chinese fir.

Therefore, the relief of Al toxicity to Chinese fir and other forest plants are very important and many methods have been reported. Controlling soil acidity was one of the most common methods to alleviate Al toxicity because Al forms were found to be soil pH dependent. Under acidic condition, Al would be solubilized into more toxic forms. It was reported that the addition of alkali materials could increase soil pH and alleviate Al toxicity to plants. Moreover, the alleviating effects of some mineral elements were also reported.

Calcium is one of such elements which can help plant to reduce negative influence of Al on
plants. As a secondary messenger, calcium in plants can directly or indirectly modulate various biological processes \[9\]. It has been reported that calcium was involved in plant resistance to multiple environmental stresses, such as salinity, temperature, drought and heavy metals \[^{10-12}\]. Calcium also played an important role in alleviating Al phytotoxicity through several mechanisms, such as displacement of Al from the cell membrane surface and restoration of Ca\(^{2+}\) on the cell membrane surface, or ionic interaction between Ca\(^{2+}\) and Al\(^{3+}\) \[^{13}\]. Besides, phosphorus (P) was another mineral element focused because it could reduce Al accumulation and enhance aluminum tolerance in a number of plants \[^{8,14}\].

Generally, Ca is added as calcium carbonate, and P is added as phosphate. All of these compounds are alkali, which may change the activity of Al in soil. However, there are few detail reports on the changes of Al activity in soil and its relationship with Al toxicity to Chinese fir after the addition of Ca and P. In this paper, we investigated the combination influence of Ca and P on Al exchangeable form in soil and its relationship with some physiology indexes of Chinese fir. One-year-old seedlings of Chinese fir were used as plant material.

2. Materials and Methods

2.1. Research area

The experiment was performed at the greenhouse located at the campus of Fujian Agriculture and Forestry University, Fujian Province, China with geographic coordinates: East Latitude 26° 05' and Northern Longitude 119°14', characterized as subtropical monsoon climate.

2.2. Pot culture

One year old seedlings of Chinese fir were used as plant materials for soil pot culture. Three seedlings were planted in each plastic pot (29 cm in diameter and 31 cm in height) with 16 kg yellow soil sieved. The soil contained 10.98% total Al, 0.015% total N, 0.022% total P, 2.61% total K, 13.2 mg/kg available N and 0.3% organic matters, respectively. Nine treatments were designed, including Control (CK), Low P (LP), High P (HP), Low Ca (LC), Low Ca+Low P (LCLP), Low Ca+High P (LCHP), High Ca (HC), High Ca+Low P (HCLP) and High Ca+High P (HCHP). The treatment was followed by table 1. Each treatment had four replicates. All pots were watered regularly during the experimental period. Rhizosphere soils and leaf were sampled after 6 months. The soil was air-dried at room temperature and then filtered through 2-mm sieves. Leaf samples were dried in and grounded for the determination of Al. Sample were stored frozen at -80°C for further experiments before MDA and soluble protein measurement.

| No. | Treatment        | Al (mg/kg) | CaCO\(_3\) (g/kg) | KH\(_2\)PO\(_4\) (mg/kg) |
|-----|------------------|-----------|-------------------|-------------------------|
| 1   | Control (CK)     | 150       | 0                 | 0                       |
| 2   | Low P (LP)       | 150       | 0                 | 200                     |
| 3   | High P (HP)      | 150       | 0                 | 400                     |
| 4   | Low Ca (LC)      | 150       | 1                 | 0                       |
| 5   | Low Ca+Low P(LCLP) | 150     | 1                 | 200                     |
| 6   | Low Ca+High P (LCHP) | 150    | 1                 | 400                     |
| 7   | High Ca (HC)     | 150       | 3                 | 0                       |
| 8   | High Ca+Low P(HCLP) | 150    | 3                 | 200                     |
| 9   | High Ca+High P(HCHP) | 150    | 3                 | 400                     |
2.3. Determination of soil pH and exchangeable Al in soil

2.3.1. Soil pH
Soil pH was measured in 1M KCl (1:2.5, w:v) using a PHS—3C pH meter (Shanghai INESA Scientific Instrument Co., Ltd, China).

2.3.2. Exchangeable Al in soil
Content of exchangeable Al in soils were extracted by using 1mol/L KCl. Al in the extract was determinate by an Auto Discrete Analyzer (SmartChem 200, WestCo Scientific Instruments Inc., Italy).

2.4. Determination of MDA and soluble protein content in leave

2.4.1. MDA content
The MDA content in leave was determined according to the modified method of Hodges et al.\cite{16}. One gram of frozen material for each sample was ground in 8 mL of 0.05 mol/L ice-cold phosphate buffer solution (PBS, pH 7.8). The homogenate was centrifuged at 15,000 × g for 30 min at 4°C. A reaction was carried out by mixing 2 mL of sample supernatant with 2 mL of 0.6% thiobarbituric acid containing 10% trichloroacetic acid (TCA) and placing it on a temperature controlled bath at 100°C for 15 min. The reaction solution was then quickly cooled on ice. After centrifugation at 15,000 × g for 15 min at 4°C, the supernatant was collected and its absorbance was measured at 450 nm, 532 nm and 600 nm respectively on a Libra S22 UV-visible spectrophotometer (Biochrom, UK). The concentration of MDA in reaction solution was expressed as µmol/g and calculated as described in the method.

2.4.2. Soluble protein content
The concentration of total soluble protein in leave of Chinese fir was measured based on the method described by Brandford\cite{16}.

2.5. Statistical analysis
Data were analyzed using Microsoft Excel 2010. Values were represented as the means of four replicates (mean ± SD) for each treatment. The correlation between different indexes was statistically calculated using SPSS 13.0.

3. Results and Discussion

3.1. Effect of calcium and phosphorus on soil pH
As showed in Figure 1, the rhizosphere pH increased in various degrees under Ca and P treatment as compared with the control (150 mg/kg Al without Ca or P treatment). The greatest changes were observed in HC, HCLP and HCHP, rising 46.0%, 45.8% and 48.4% respectively. However, HP showed the least increase in rhizosphere pH, rising only 3.0%.
Figure 1. Effect of calcium and phosphorus on pH in rhizosphere soil of Chinese fir (n = 4 for all the treatments).

3.2. Effect of calcium and phosphorus on the content of exchangeable Al in rhizosphere soil
Content of exchangeable Al (Figure 2) in rhizosphere soil were much different among different treatments. It changed the most greatly in HCHP, with a decrease of 96.4%. HC and HCLP take the second place, with a decrease of 85.1% and 87.7%, respectively. LP showed the least effect on content of exchangeable Al in rhizosphere soil, with a decrease of 20.7%.

Figure 2. Effect of calcium and phosphorus on the content of exchangeable Al in rhizosphere soil of Chinese fir. Error bars were standard errors of the Mean (n = 4 for all the treatments).

3.3. Effect of calcium and phosphorus on the content of Al, MDA and soluble protein in leave of Chinese fir
3.3.1. Al content in leave
Figure 3 showed the effect of Ca and P treatment on Al content in Chinese fir leave. Compared to the control, Al content in Chinese fir leave decreased in some extent after calcium and phosphorus
treatment. The change varied slightly among different Ca and P treatments, with a range from 23.4% to 33.4%.

Figure 3. Effect of calcium and phosphorus on Al content in leave of Chinese fir. Error bars were standard errors of the mean (n = 4 for all the treatments).

3.3.2. MDA content in leave
Just like Al content in leave, a decrease in MDA content in Chinese fir leave was also observed after calcium and phosphorus treatment (Figure 4). MDA content in the leave showed the most significant decrease (40.5%) in HC, while there was a slight change in LP. For the treatments of HP, LC, LCLP, LCHP, HCLP and HCHP, the decrease was in the range of 23-38%. MDA is an important index for membrane lipid peroxidation. Its decrease indicated that the Al toxicity on Chinese fir was alleviated after the addition of Ca and P.

Figure 4. Effect of calcium and phosphorus on MDA content in leave of Chinese fir. Error bars were standard errors of the mean (n = 4 for all the treatments).
3.3.3. Soluble protein content in leaf
As showed in Figure 5, the soluble protein content in Chinese fir leaf increased in various degrees under Ca and P treatment as compared with the control. The increment followed the order of HCHP>HCLP>LC>LCHP> HC>LP>HP >LCLP, with a range from 81.8% to 193.2%. The increase of protein content in Chinese fir may meant that Al stress on plant was improved.

![Figure 5. Effect of calcium and phosphorus on soluble protein content in leave of Chinese fir. Error bars were standard errors of the mean (n = 4 for all the treatments).](image)

3.4. Relationship of pH and exchangeable Al with other indexes
Table 2 showed that the soil pH in rhizosphere area was very significantly negatively correlated with content of exchangeable Al in soil, and the exchangeable Al content had a significantly positive correlation with MDA in leave of Chinese fir, and a significantly negative correlation with leaf soluble protein. These results indicated that the changes in physiological indexes of Chinese fir may be due to the changes of pH and exchangeable Al in soil.

| Index          | pH         | Exchangeable Al |
|----------------|------------|-----------------|
| Exchangeable Al | -0.878**   | 1               |
| Al in leave    | -0.354     | 0.621           |
| MDA            | -0.454     | 0.675*          |
| Soluble protein| 0.662      | -0.763*         |

Note:*indicates a significant correlation at 0.05 level, **indicates a significant correlation at 0.01 level.

4. Conclusion
The results showed that the soil pH of all treatment rose at different degree under treatment of calcium and phosphorus, whereas the exchangeable Al content in soil, leaf Al and MDA content were decreased, and the soluble protein in leaf of Chinese fir was increased accordingly, indicating that the alteration in soil rhizosphere environment may be one of the main factor improving physiological indexes, consequently alleviating Al phytotoxicity in soil-Chinese fir system.
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References
[1] Xu X L, Zhang J B 2017 Research progress on aluminum toxicity and its control in forest soil-plant system Chinese J. Ecol. 36:1106-1116
[2] Yan L, Riaz M, Wu X, Wang Y, Du C, Jiang C 2018 Interaction of boron and aluminum on the physiological characteristics of rape (Brassica napus L.) seedlings. Acta Physiol. Plantarum 40:33
[3] Arunakumara K K I U, Walpola B C, Yoon M H 2013 Aluminum toxicity and tolerance mechanism in cereals and legumes - a review J. Korean Soc. Appl. Biol. Chem. 56:1-9
[4] Vicentini T M, Cavalheiro A H, Dechandt C R P, Alberici L C, Vargas-Rechia C G 2019 Aluminum directly inhibits alternative oxidase pathway and changes metabolic and redox parameters on Jatropha curcas cell culture Plant. Physiol. Bioch. 136: 92-97
[5] Chakraborty S, Mishra A, Verma E, Tiwari B, Mishra A K, Sing S S 2019 Physiological mechanisms of aluminum (Al) toxicity tolerance in nitrogen-fixing aquatic macrophyte Azolla microphylla Kaulf: phytoremediation, metabolic rearrangements, and antioxidative enzyme responses. Environ. Sci. Pollut. R. 26: 9041-9054
[6] Ulrich B, Mayer R, Khanna P K 1980 Chemical changes due to acid precipitation in a loess derived soil in central Europe. Soil Sci. 130:193-199
[7] Liu B, Luo C D, Li X W, Gray L, Zhang F, Liu M, Ju J L, Lei B 2014 Research on the threshold of aluminum toxicity and the alleviation effects of exogenous calcium, phosphorus, and nitrogen on the growth of Chinese fir seedlings under aluminum stress. Commun Soil Sci. Plant Anal. 45:126-139
[8] Iqbal M T 2013 Phosphorus enhances aluminium tolerance in both aluminium-tolerant and aluminium-sensitive wheat seedlings S. Afr. J. Plant Soil. 30:13–21
[9] Lan T, You J, Kong L, Yu M, Liu M, Yang Z 2016 The interaction of salicylic acid and Ca2+ alleviates aluminum toxicity in soybean (Glycine max L.) Plant. Physiol. Bioch. 98: 146-154
[10] Talukdar D 2012 Exogenous calcium alleviates the impact of cadmium-induced oxidative stress in Lens culinaris medic. Seedlings through modulation of antioxidant enzyme activities J. Crop Sci. Biotechnol. 15: 325–334
[11] Khushboo, Bhardwaj K, Singh P, Raina M, Sharma V, Kuma, D 2018 Exogenous application of calcium chloride in wheat genotypes alleviates negative effect of drought stress by modulating antioxidant machinery and enhanced osmolyte accumulation In Vitro Cell.Dev.Biol.-Plant 54: 495–507
[12] Roy P R, Tahjib-Ul-Arif M, Polash M A S, Hossen M Z, Hossain M A 2019 Physiological mechanisms of exogenous calcium on alleviating salinity-induced stress in rice (Oryza sativa L.) Physiol. Mol. Biol. Plants 25: 611–624
[13] Rahman M A, Lee S H, Ji H C, Kabir A H, Jones C S , Lee K W 2018 Importance of mineral nutrition for mitigating aluminum toxicity in plants on acidic soils: current status and opportunities Int. J. Mol. Sci. 19:3073
[14] Qu X J, Zhou J Q, Masabni J, Yuan J 2020 Phosphorus relieves aluminum toxicity in oil tea seedlings by regulating the metabolic profiling in the roots Plant. Physiol. Bioch. 152:12-22
[15] Hodges D M, Delong J M, Prange F R K 1999 Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds Planta 207:604–611
[16] Brandford M 1976 A rapid and sensitive method for the quantitation of microgramquantities of protein utilizing the principle of protein-dye binding Anal. Biochem. 72:248–254