

## Uptake of Insoluble Phosphorus by Sugarcane Seedlings and Root System Responses to Low Phosphorus Stress: Postprint

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### Abstract

Southern acidic soils are characterized by high total phosphorus content but generally low available phosphorus. Insufficient soil phosphorus is a crucial factor limiting sugarcane growth. Elucidating the physiological and biochemical mechanisms underlying sugarcane adaptation to low-phosphorus stress and exploring the phosphorus utilization potential of sugarcane hold significant theoretical and practical implications for guiding sugarcane breeding and cultivation management. This study utilized two sugarcane varieties, ROC22 and ROC10, as experimental materials, employing hydroponic and soil culture experimental methods to investigate the absorption of insoluble phosphorus by sugarcane seedlings, as well as root architecture and physiological responses of the root system under low-phosphorus stress, in order to elucidate the potential mechanisms of sugarcane adaptation to low-phosphorus stress. The results demonstrated: C1) Sugarcane cultured in nutrient solutions with insoluble phosphorus sources CCa-P and A1-P exhibited significantly increased leaf number, shoot dry weight, and biomass compared to the phosphorus-deficient (-P) treatment, comparable to the control (+P) treatment. Total phosphorus accumulation in sugarcane was also significantly enhanced, reaching 30%~77% of the phosphorus accumulation in the control (+P) treatment. C2) Under low-phosphorus conditions, the root system of sugarcane seedlings exhibited a trend toward deeper soil distribution, with increased total root volume, longer maximum root length, and enhanced shallow root distribution. C3) Under low-phosphorus environments, the rhizosphere of sugarcane seedlings was significantly acidified, and root secretions could dissolve insoluble aluminum phosphate, while acid phosphatase activity within plant tissues was also significantly enhanced. These findings indicate that sugarcane seedlings possess a strong capacity for absorbing and utilizing insoluble phosphorus. Increased root number under low-phosphorus conditions, root gravitropism, enhanced shallow root distribution, rhizosphere acidification,

and elevated acid phosphatase activity within plant tissues may constitute important mechanisms for sugarcane seedlings to adapt to phosphorus-deficient environments.

## Full Text

### Preamble

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**Title:** Absorption of Poorly Soluble Phosphorus by Sugarcane Seedlings and Root Response to Low Phosphorus Stress

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### Abstract

Acidic soils in southern China contain high total phosphorus but generally low available phosphorus, and soil phosphorus deficiency represents a critical constraint on sugarcane growth. Elucidating the physiological and biochemical mechanisms underlying sugarcane adaptation to low phosphorus stress and tapping the phosphorus utilization potential of sugarcane hold important theoretical and practical significance for guiding sugarcane breeding and cultivation management. This study investigated the absorption of poorly soluble phosphorus by sugarcane seedlings and the physiological responses of root architecture and root systems under low phosphorus stress using hydroponic and pot culture experiments with two sugarcane varieties, ROC22 and ROC10, to reveal potential mechanisms of sugarcane adaptation to low phosphorus stress. The results showed that: (1) Sugarcane cultivated in nutrient solution with poorly soluble phosphorus (Ca-P and Al-P) as the phosphorus source exhibited significantly increased leaf number, shoot dry weight, and biomass compared to phosphorus-deficient (-P) treatment, reaching levels comparable to the control (+P). Total phosphorus accumulation also increased significantly, achieving 30%-77% of the phosphorus accumulation in the control (+P) treatment. (2) Under low phosphorus conditions, sugarcane seedling roots tended to distribute deeper into the soil, with increased total root volume, longer maximum root length, and greater shallow root distribution. (3) In low phosphorus environments, the rhizosphere of sugarcane seedlings became significantly acidified, root exudates could dissolve poorly soluble aluminum phosphorus, and acid phosphatase activity in plant tissues was significantly enhanced. These results indicate that sugarcane seedlings possess a strong capacity to absorb and utilize poorly soluble phosphorus. The increased root number, enhanced gravitropism of primary roots, greater shallow root distribution, rhizosphere acidification, and enhanced acid phosphatase activity under low phosphorus conditions may represent important

mechanisms enabling sugarcane seedlings to adapt to phosphorus-deficient environments.

**Keywords:** sugarcane, low phosphorus stress, poorly soluble phosphorus, root architecture, adaptive mechanism

## Introduction

Phosphorus is not only an essential component of nucleic acids, proteins, phospholipids, and other organic compounds, but also participates in numerous physiological processes including photosynthesis, respiration, enzyme activation, and signal transduction, exerting significant influence on plant growth, development, yield, and quality (Olday, 1972; 刘辉, 2003). Due to strong chemical fixation of phosphorus in acidic southern soils, soil phosphorus exists primarily in poorly soluble forms such as Fe-P, Al-P, and O-P, resulting in high total phosphorus content (于姣娣等, 2017) but low phosphorus fertilizer use efficiency of only 5%-20% during the current season (闫金珪, 2018). Consequently, soil phosphorus deficiency has become one of the main limiting factors in crop production. To adapt to phosphorus scarcity in soils, plant roots often exhibit pronounced morphological and physiological responses. Studies by 孙淼 (2018) and 韦如萍 (2018) have shown that crops undergo morphological changes under low phosphorus stress, with the most obvious change being increased root-to-shoot ratio, longer roots, and expanded root coverage area to enhance phosphorus absorption and adapt to low phosphorus environments. Meanwhile, root exudates play a crucial role in improving soil phosphorus bioavailability (Zou et al., 2018) by activating and dissolving poorly soluble inorganic phosphorus and participating in the decomposition of organic phosphorus to increase rhizosphere available phosphorus concentration for crop uptake and utilization (梁翠月等, 2015). Numerous studies have demonstrated that root exudates from alfalfa (杨利宁等, 2015), maize (陶佩琳等, 2013), and rice (Hu et al., 2016) can enhance soil phosphorus availability and promote plant absorption and utilization of poorly soluble phosphates. Currently, no studies have reported on the effects of low phosphorus stress on sugarcane root architecture or sugarcane activation and utilization of poorly soluble phosphorus. Therefore, this study investigated sugarcane adaptation mechanisms to low phosphorus stress from the perspectives of root morphological characteristics, root exudates, and acid phosphatase activity. This research holds significant importance for further exploring the molecular mechanisms of sugarcane tolerance to low phosphorus stress and breeding low phosphorus-tolerant sugarcane varieties, and also contributes to resource conservation, environmental protection, and reduced sugarcane production costs.

## Materials and Methods

### 1.1 Experimental Materials

The sugarcane varieties used were ROC22 and ROC10, obtained from the College of Agriculture, Guangxi University.

## 1.2 Experimental Treatments

**1.2.1 Hydroponic Experiment** Stem sections with single buds approximately 5 cm long were cut from the middle and upper portions of sugarcane stalks. The cut stem sections were soaked in saturated lime water for germination and sterilization for 12 h, then removed and grown in nursery substrate until three leaves had unfolded. Seedlings were then transferred to hydroponic plastic buckets. At transplanting, half of the seed roots were removed to minimize their influence on the experiment. Initially, seedlings were cultured for one week in  $0.1 \text{ mmol} \cdot \text{L}^{-1}$  CaCl solution to facilitate adaptation to the hydroponic environment and root development. They were then grown for four days in 1/5 Hoagland nutrient solution (pH = 6.0) before being used as experimental materials.

To understand the absorption capacity of sugarcane seedlings for poorly soluble phosphorus, four treatments were designed: 1/5 Hoagland nutrient solution (+P, CK,  $0.25 \text{ mmol} \cdot \text{L}^{-1}$  NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>), phosphorus-free nutrient solution (-P), phosphorus-free nutrient solution supplemented with  $0.25 \text{ mmol} \cdot \text{L}^{-1}$  aluminum phosphate (-P+Al-P), and phosphorus-free nutrient solution supplemented with  $0.25 \text{ mmol} \cdot \text{L}^{-1}$  calcium phosphate (-P+Ca-P). Sugarcane seedlings were cultivated in these solutions with aeration for 15 min per hour using an electromagnetic air pump, and the nutrient solution was changed every 2 days. Plants were harvested after 38 days. Before harvest, root exudates were collected for complexation dissolution tests and in situ coloration assays. At harvest, phenotypic traits including plant height, leaf length, leaf width, and leaf number were measured. The first fully expanded leaf was collected for acid phosphatase determination, while the remaining shoot and root tissues were chopped, oven-dried, and used for biomass and phosphorus content measurements.

**1.2.2 Pot Experiment** To investigate the effects of different phosphorus distributions on sugarcane seedling root architecture, four treatments were established: no phosphorus fertilizer in either soil layer (-P/-P), phosphorus fertilizer in the upper layer only (+P/-P), phosphorus fertilizer in the lower layer only (-P/+P), and phosphorus fertilizer in both upper and lower layers (+P/+P). Both upper and lower soil layers were 15 cm thick, separated by non-woven fabric with two PVC water pipes inserted in the middle. Phosphorus application (as P<sub>2</sub>O<sub>5</sub>) was  $0.3 \text{ g} \cdot \text{kg}^{-1}$  for phosphorus treatments and  $0 \text{ g} \cdot \text{kg}^{-1}$  for non-phosphorus treatments. Nitrogen (N) application was  $0.4 \text{ g} \cdot \text{kg}^{-1}$  and potassium (K<sub>2</sub>O) application was  $0.44 \text{ g} \cdot \text{kg}^{-1}$  for all treatments.

Each treatment had three replicates with two plants per pot. Plants were harvested after 80 days. At harvest, root systems were carefully cleaned, and a root scanning-WinRHIZO image analysis system was used to obtain two-dimensional root architecture parameters including maximum root length and total root volume. Roots were then cut at the non-woven fabric separator and harvested separately from upper and lower layers before being chopped and oven-dried for root dry weight determination. The experimental soil was sandy soil developed

from Quaternary red earth parent material, with total phosphorus  $0.13 \text{ g} \cdot \text{kg}^{-1}$ , available phosphorus  $0.79 \text{ mg} \cdot \text{kg}^{-1}$ , and pH 4.62.

### 1.3 Measurement Methods

**Root Tip In Situ Coloration:** Following the method of 苏宝玲等 (2000), 6 mg of bromocresol purple and 0.5 g of agar were added to 100 mL of  $1 \text{ mmol} \cdot \text{L}^{-1}$   $\text{CaSO}_4$  solution, heated to boiling with continuous stirring on an electric furnace. After cooling to room temperature with stirring, the pH was adjusted to dark red using  $0.01 \text{ mol} \cdot \text{L}^{-1}$  HCl. Roots of uniform thickness from +P and -P treatments were placed in petri dishes (two roots per plate), the mixture was poured in, and after cooling and solidifying to form plates of uniform thickness, color changes in the rhizosphere were observed and photographed after 2 h.

**Root Exudate Collection:** One day before hydroponic harvest, -P and +P treatments were washed several times with deionized water and cultured overnight in  $0.5 \text{ mmol} \cdot \text{L}^{-1}$   $\text{CaCl}_2$ . The next morning, fresh  $\text{CaCl}_2$  solution of the same concentration was added and cultured for another 24 h before collection in a cold storage room. During collection, the culture solution was passed sequentially through H-type cation resin and formic acid-type anion resin. After collection, the anion resin was removed and root exudates adsorbed on the anion resin were eluted three times with  $0.2 \text{ mol} \cdot \text{L}^{-1}$  HCl prepared with ultrapure water (3 mL each time) into a 150 mL concentration flask, then evaporated to dryness under reduced pressure using a rotary evaporator. Concentration conditions were vacuum with  $40 \text{ }^\circ\text{C}$  water bath. The residue was dissolved three times with 1.5 mL ultrapure water by shaking, filtered through a water-based microporous membrane into a 2 mL centrifuge tube, and stored at  $-20 \text{ }^\circ\text{C}$  for later use.

**Root Exudate Aluminum Complexation:** The mixed solution used for aluminum complexation by root exudates consisted of 25 mL  $5 \text{ mmol} \cdot \text{L}^{-1}$   $\text{AlCl}_3$ , 4 mL  $2 \text{ mol} \cdot \text{L}^{-1}$  HCl, 67 mL ultrapure water, and 120 mL acetone. Chromatography filter paper was immersed in the mixed solution for 15 min, then submerged in pH 6.8 phosphate buffer for 15 min, removed, washed with distilled water, and dried for later use. Exudates were spotted on the dried filter paper in four applications of 5  $\mu\text{L}$  each, then dried and immersed in pyrocatechol violet solution for staining for 5–10 min. The filter paper was washed multiple times with deionized water to remove excess dye, dried, and photographed.

**Acid Phosphatase Assay:** Following the method of McLachlan et al. (1987) with slight modifications, 0.2 g of chopped first fully expanded leaf was ground with a small amount of quartz sand and liquid nitrogen into a homogenate. Five milliliters of  $0.2 \text{ mol} \cdot \text{L}^{-1}$  sodium acetate-acetic acid buffer (pH 5.6) was added, transferred to a 10 mL centrifuge tube, and centrifuged at 10,000 rpm for 10 min to obtain the supernatant. The reaction mixture contained 0.5 mL enzyme solution, 0.45 mL acetate buffer, and 4.5 mL  $5 \text{ mmol} \cdot \text{L}^{-1}$  p-nitrophenyl phosphate disodium. After mixing, the reaction proceeded in darkness at  $30 \text{ }^\circ\text{C}$

for 30 min, then 2 mL of  $2 \text{ mol} \cdot \text{L}^{-1}$  NaOH was added to terminate the reaction, and OD was measured at 405 nm. A standard curve was prepared using  $1 \text{ mmol} \cdot \text{L}^{-1}$  p-nitrophenol as the solute and  $2 \text{ mol} \cdot \text{L}^{-1}$  NaOH as the solvent. Enzyme activity was expressed as the amount of p-nitrophenol produced by hydrolysis of p-nitrophenyl phosphate disodium per unit fresh weight per unit time, i.e.,  $\text{mol NP} \cdot \text{mg Pro}^{-1} \cdot \text{min}^{-1}$ .

#### 1.4 Data Processing

Data were processed and graphed using Excel 2007. Significance testing and multiple comparisons (Duncan's new multiple range test) were performed using SPSS 17.0 software.

### Results

#### 2.1 Effects of Poorly Soluble Phosphorus on Sugarcane Seedling Growth

Table 1 shows that after phosphorus deficiency (-P) treatment, plant height, leaf length, leaf width, and leaf number of both sugarcane varieties decreased compared to the control (+P). Except for leaf length in ROC22 and plant height and leaf length in ROC10, which showed no significant differences, all other parameters reached significant levels, indicating that phosphorus deficiency significantly inhibited sugarcane seedling growth. However, after adding poorly soluble phosphorus, plant height, leaf length, leaf width, and leaf number all increased compared to the phosphorus deficiency (-P) treatment, with leaf width and leaf number in ROC22 reaching significant levels. Under Ca-P treatment, ROC22 showed plant height, leaf length, and leaf number comparable to the CK (+P) treatment with no significant differences. These results indicate that poorly soluble phosphorus alleviated phosphorus deficiency in sugarcane seedlings.

Table 2 also shows that after phosphorus deficiency (-P) treatment, shoot dry weight and biomass of both varieties decreased significantly compared to the control (+P), while root-to-shoot ratio increased significantly ( $p < 0.05$ ). For ROC22, shoot dry weight and biomass decreased by 47% and 38%, respectively, while root-to-shoot ratio increased by 1.3-fold. For ROC10, shoot dry weight and biomass decreased by 37% and 30%, respectively, while root-to-shoot ratio increased by 0.9-fold. After adding poorly soluble phosphorus (Ca-P and Al-P), shoot dry weight and biomass increased significantly compared to the phosphorus deficiency (-P) treatment, with root-to-shoot ratio decreasing to levels comparable to the control (+P) with no significant differences.

These results demonstrate that sugarcane seedlings can absorb and utilize poorly soluble phosphorus to promote growth, with ROC22 showing stronger capacity to absorb and utilize poorly soluble phosphorus than ROC10.

#### Table 1 Effect of poorly soluble P on agronomic traits of sugarcane

| Cultivar | Treatment | Plant height (cm) | Leaf length (cm) | Leaf width (cm) | Leaf number |
|----------|-----------|-------------------|------------------|-----------------|-------------|
| ROC22    | CK (+P)   | 191.8±4.29 a      | 130.1±4.01 a     | 3.3±0.21 a      | 3.5±0.12 a  |
|          | -P        | 178.3±6.77 ab     | 123.6±1.62 ab    | 2.8±0.07 b      | 3.2±0.26 a  |
|          | Ca-P      | 174.0±2.02 b      | 118.2±2.98 b     | 2.6±0.10 b      | 3.3±0.03 a  |
|          | Al-P      | 166.9±3.25 b      | 120.5±3.40 ab    | 1.6±0.03 c      | 2.5±0.03 b  |
| ROC10    | CK (+P)   | 163.7±7.26 a      | 114.6±5.48 a     | 3.3±0.12 a      | 3.2±0.15 a  |
|          | -P        | 163.8±4.37 a      | 116.8±2.90 a     | 3.0±0.06 ab     | 3.4±0.10 a  |
|          | Ca-P      | 149.8±5.43 a      | 104.5±4.78 a     | 3.0±0.09 ab     | 3.0±0.10 ab |
|          | Al-P      | 147.3±6.21 a      | 106.6±4.92 a     | 2.7±0.21 b      | 2.7±0.12 b  |

Note: Different lowercase letters after values in the same column indicate significant difference ( $P < 0.05$ ). The same below.

**Table 2 Effect of poorly soluble P on biomass and root-to-shoot ratio of sugarcane**

| Cultivar | Treatment | Shoot dry weight (g · plant <sup>-1</sup> ) | Root dry weight (g · plant <sup>-1</sup> ) | Biomass (g · plant <sup>-1</sup> ) | Root-to-shoot ratio |
|----------|-----------|---|--|------------------------------------|---------------------|
| ROC22    | CK (+P)   | 26.37±2.31 a                                | 4.06±0.56 a                                | 30.43±2.87 a                       | 0.15±0.01 b         |
|          | Ca-P      | 22.49±3.55 a                                | 3.60±1.01 a                                | 26.09±4.54 ab                      | 0.15±0.02 b         |
|          | Al-P      | 21.14±0.53 a                                | 3.51±0.26 a                                | 24.65±0.75 ab                      | 0.17±0.01 b         |
|          | -P        | 13.88±0.40 b                                | 4.87±0.34 a                                | 18.75±0.42 b                       | 0.35±0.03 a         |
| ROC10    | CK (+P)   | 22.87±0.76 a                                | 3.26±0.13 a                                | 26.13±0.84 a                       | 0.14±0.00 b         |
|          | Ca-P      | 20.90±0.07 a                                | 2.79±0.23 a                                | 23.69±0.20 a                       | 0.13±0.01 b         |
|          | Al-P      | 19.65±1.16 a                                | 2.86±0.24 a                                | 22.50±1.56 a                       | 0.15±0.02 b         |
|          | -P        | 14.31±1.33 b                                | 3.96±0.71 a                                | 18.28±1.91 b                       | 0.27±0.04 a         |

## 2.2 Absorption and Utilization of Poorly Soluble Phosphorus by Sugarcane

Figure 1 [Figure 1: see original paper] shows that total phosphorus accumulation differed significantly among all treatments ( $P < 0.05$ ). Compared to CK (+P), Ca-P, Al-P, and -P treatments in ROC22 decreased by 32%, 70%, and 92%, respectively, while in ROC10 they decreased by 23%, 63%, and 91%, respectively, indicating differences in utilization between poorly soluble and soluble phosphorus sources. Total phosphorus accumulation in Ca-P and Al-P treatments of both ROC22 and ROC10 increased significantly compared to -P treatment, being 8.5-fold and 3.8-fold higher in ROC22, and 8.9-fold and 4.2-fold higher in ROC10, respectively. These results demonstrate that sugarcane possesses a strong capacity to utilize poorly soluble phosphates.

In terms of phosphorus use efficiency (Figure 2 [Figure 2: see original paper]), Ca-P treatment was comparable to the control, while Al-P treatment showed significant improvement compared to both Ca-P and control treatments ( $p < 0.05$ ). In ROC22, Al-P treatment increased phosphorus use efficiency by 1.7-fold and 1.2-fold compared to Ca-P and control treatments, respectively, while in ROC10 it increased by 1.4-fold and 1.0-fold, respectively. These findings indicate that sugarcane can utilize Al-P more effectively than Ca-P.

### 2.3.1 Effects of Different Treatments on Sugarcane Root Systems

Table 3 shows that low phosphorus stress significantly affected root distribution and growth. Compared to other treatments, the no-phosphorus treatment (-P/-P) significantly increased total root volume and maximum root length, with significant differences observed between (+P/-P) and (-P/-P) treatments for total root volume. For both ROC22 and ROC10, the upper-layer phosphorus treatment (+P/-P) also showed significantly greater total root volume and maximum root length compared to the lower-layer phosphorus treatment (-P/+P), with increases of 28.9% and 8.4% in total root volume and 4.5% and 21.0% in maximum root length, respectively. Additionally, the upper-to-lower root dry weight ratio in (+P/-P) treatment was significantly higher than in (-P/+P) treatment for both varieties. These results indicate that under low phosphorus conditions, sugarcane seedlings exhibited increased total root volume, deeper primary root growth, and predominantly shallow root distribution, forming a root architecture adapted for phosphorus absorption from shallow soil layers.

**Table 3** Root characteristics of sugarcane under different soil phosphorus supply

| Cultivar | Treatment | Upper-to-lower root dry weight ratio | Root volume (cm <sup>3</sup> ) | Maximum root length (cm) |
|----------|-----------|--------------------------------------|--------------------------------|--------------------------|
| ROC22    | +P/+P     | 1.08±0.04 a                          | 92.7±5.70 b                    | 58.3±4.41 b              |
|          | +P/-P     | 1.12±0.04 a                          | 82.7±4.37 b                    | 63.7±3.18 b              |

| Cultivar | Treatment | Upper-to-lower root dry weight ratio | Root volume (cm <sup>3</sup> ) | Maximum root length (cm) |
|----------|-----------|--------------------------------------|--------------------------------|--------------------------|
|          | -P/+P     | 1.01±0.07 a                          | 123.3±1.67 a                   | 80.0±2.89 a              |
|          | -P/-P     | 0.79±0.09 b                          | 100.2±3.35 ab                  | 78.5±1.04 ab             |
| ROC10    | +P/+P     | 1.52±0.05 a                          | 87.7±8.09 b                    | 73.3±4.41 ab             |
|          | +P/-P     | 1.31±0.06 a                          | 95.7±10.17 ab                  | 62.0±1.00 b              |
|          | -P/+P     | 1.17±0.09 b                          | 140.0±2.89 a                   | 78.3±6.57 a              |
|          | -P/-P     | 1.14±0.02 b                          | 122.3±15.62 a                  | 87.7±12.35 a             |

### 2.3.2 Phosphorus Deficiency-Induced Rhizosphere Acidification and Dissolution of Poorly Soluble Phosphorus by Root Exudates

As shown in Figure 3 [Figure 3: see original paper], the bromocresol purple agar plates around sugarcane roots in +P treatment showed no obvious color change, while distinct yellow zones appeared around root tips in -P treatment, indicating secretion of acidic substances from root tips under low phosphorus stress and significant rhizosphere acidification.

Results from the pyrocatechol violet staining-filter paper method (Figure 4 [Figure 4: see original paper]) showed that root exudates collected from sugarcane seedlings after -P treatment could dissolve AlPO<sub>4</sub> on filter paper, appearing as bright spots, while exudates from +P treatment could not dissolve AlPO<sub>4</sub>. This demonstrates that root exudates under low phosphorus stress can dissolve poorly soluble aluminum phosphorus, thereby promoting phosphorus absorption.

### 2.3.3 Effects of Low Phosphorus Stress on Acid Phosphatase Activity in Sugarcane

As shown in Figure 5 [Figure 5: see original paper], under -P treatment, acid phosphatase activity in leaves of ROC22 and ROC10 reached 29.54 mol NP · mg Pro<sup>-1</sup> · min<sup>-1</sup> and 36.01 mol NP · mg Pro<sup>-1</sup> · min<sup>-1</sup>, respectively, representing significant increases of 4.3-fold and 2.5-fold compared to +P treatment. These results indicate that acid phosphatase activity in sugarcane seedlings increases significantly under phosphorus deficiency.

## Discussion

### 3.1 Absorption and Utilization of Poorly Soluble Phosphorus by Sugarcane Seedlings

In acidic red soils of southern China, phosphorus readily combines with aluminum and iron to form poorly soluble phosphorus. Generally, plants cannot directly absorb and utilize this phosphorus fraction, making activation and dissolution of poorly soluble phosphorus a prerequisite for plant phosphorus uptake and improved phosphorus use efficiency. This study found that different sugarcane varieties exhibit varying efficiencies in utilizing poorly soluble phosphorus forms, consistent with previous findings in eucalyptus and rice (翁彩凤等, 2014; 李锋等, 2003). When comparing physiological characteristics among different phosphorus treatments, sugarcane varieties supplied with poorly soluble phosphorus (Ca-P and Al-P) as phosphorus sources showed significantly increased leaf number, shoot dry weight, and biomass compared to phosphorus-deficient (-P) treatment, reaching levels comparable to the control (+P) treatment (Tables 1 and 2). Total phosphorus accumulation also increased significantly, achieving 30%-77% of the phosphorus accumulation in the control (+P) treatment (Figure 1). These results demonstrate that sugarcane seedlings possess a strong capacity to activate and utilize poorly soluble phosphates.

### 3.2 Adaptation of Sugarcane Seedlings to Low Phosphorus Environments and Potential Mechanisms

Roots serve as the link between crops and soil and are important organs for nutrient and water absorption. During perception of environmental nutrient changes, roots can produce plastic morphological and physiological changes to enhance crop self-adaptability in response to nutrient stress (罗佳, 2016). Previous studies have shown that under low phosphorus stress, some plant roots become shallower, lateral root length and density increase, total root length increases, and primary roots deepen, forming “umbrella-shaped” or “fibrous” root architectures with shallow roots for phosphorus absorption and deep primary roots for water and nutrient uptake, thereby improving phosphorus acquisition (翁彩凤等, 2014; George et al., 2011; Rouached et al., 2010; 高家合, 2010; 刘灵, 2008). When facing low phosphorus stress, sugarcane also adapts to the adverse environment by altering its root architecture and various root traits, such as increasing total root length or maintaining stability, primarily due to decreased root diameter and thinner roots. This represents an adaptive response where plants increase root length to shorten the diffusion distance of nutrient ions to the root and expand the root absorption area (郑超等, 2015; 曾巧英等, 2015). This study also found that sugarcane seedling root architecture changed accordingly under low phosphorus conditions, with significantly increased total root volume and maximum root length, and significantly increased upper-to-lower root dry weight ratio after phosphorus supply to the upper soil layer. These results indicate that under low phosphorus stress, sugarcane exhibits increased total root volume, more roots in the surface soil, and a tendency for primary roots to dis-

tribute deeper into the soil. These changes in root characteristics are beneficial for absorbing more phosphorus in low phosphorus environments and facilitate absorption and utilization of deep soil phosphorus by young roots. Therefore, regulating root absorption and utilization of phosphorus from different soil layers may represent an important mechanism for sugarcane adaptation to acidic low phosphorus soil environments.

Activation and utilization of poorly soluble phosphorus includes plant root synthesis and secretion of organic acids to activate inorganic phosphorus and plant secretion of acid phosphatase to participate in organic phosphorus activation (梁翠月等, 2015). This study found that low phosphorus stress induced sugarcane seedling roots to secrete acidic substances that acidified the rhizosphere (Figure 3), and these acidic root exudates could dissolve poorly soluble aluminum phosphorus (Figure 4), transforming it into soluble phosphorus for sugarcane absorption and utilization. This demonstrates that rhizosphere acidification is an important mechanism for plant adaptation to phosphorus stress environments, consistent with findings in Chinese milk vetch (兰忠明等, 2012) and maize (陶佩琳等, 2013). Acid phosphatase plays an important role when plants face low phosphorus stress (Cai et al., 2018). This study found that acid phosphatase activity in sugarcane seedlings increased significantly under low phosphorus stress, primarily because acid phosphatase can hydrolyze organic phosphorus to release inorganic phosphorus that can be absorbed and utilized by plants.

In summary, sugarcane seedlings possess a strong capacity to absorb and utilize poorly soluble phosphates. Under low phosphorus conditions, increased total root volume, deeper primary root growth, greater shallow root distribution, secretion of acidic substances to acidify the rhizosphere soil environment for activation and utilization of poorly soluble phosphorus, and enhanced acid phosphatase activity may represent the adaptive mechanisms of sugarcane to low phosphorus stress.

## References

- CAI Z, CHENG Y, XIAN P, et al., 2018. Acid phosphatase gene GmHAD1 linked to low phosphorus tolerance through fine mapping in soybean[J]. *Theor Appl Genet*, 131:1715-1728.
- GAO JH, DENG BE, ZENG XC, et al., 2010. Genotypic differences of phosphorus efficiency in tobacco and their relationship with root morphology and architecture[J]. *J NW Bot*, 30(8): 1606-1613. [高家合, 邓碧儿, 曾秀成, 等, 2010. 烟草磷效率的基因型差异及其与根系形态构型的关系 [J]. *西北植物学报*, 30(8):1606-1613.]
- GEORGE TS, FRANSSON AM, HAMMOND JP, et al., 2011. Phosphorus nutrition: rhizosphere response and adaptations[M]// BUNEMANN EK, OBERSON A, FOSSARD E. *Phosphorus In Action: Biological processes in soil phosphorus cycling*. New York: Springer: 245-271.
- HU X, SHI C, DING Y, et al., 2016. Response of gene expression related to

efficient phosphorus absorption and utilization to low-P stress in rice roots[J]. *Chin J Rice Sci*: 1001-7216.

LAN ZP, LIN XJ, ZHANG WG, et al., 2012. Effects of phosphorus deficiency on root exudates production and insoluble phosphorus activation of Chinese milk vetch[J]. *Chinese Agricultural Sciences*, 45(8): 1521-1531. [兰忠明, 林新坚, 张伟光, 等, 2012. 缺磷对紫云英根系分泌物产生及难溶性磷活化的影响 [J]. *中国农业科学*, 45(8):1521-1531.]

LI F, QU XY, PAN XH, et al., 2003. Preliminary study on the utilization of insoluble phosphorus by different rice varieties[J]. *Acta Plant Nutr Fert*, (4): 420-424. [李锋, 曲雪艳, 潘晓华, 等, 2003. 不同水稻品种对难溶性磷利用能力的初步研究 [J]. *植物营养与肥料学报*, (4):420-424.]

LIANG CY, LIAO H, 2015. Mechanism of plant roots responding to low phosphorus stress[J]. *Life Sciences*, 27(3): 389-397. [梁翠月, 廖红, 2015. 植物根系响应低磷胁迫的机理研究 [J]. *生命科学*, 27(3):389-397.]

LIU H, 2003. Studies on the growth response and physiological and biochemical mechanisms of barley and wheat to low phosphorus stress[D]. Chongqing: Southwest Agricultural University: 6-16. [刘辉, 2003. 大麦和小麦对低磷胁迫的生长反应及其生理生化机制研究 [D]. 重庆: 西南农业大学: 6-16.]

LIU L, LIAO H, WANG XR, et al., 2008. Adaptive changes of soybean root architectures to low phosphorus and their relationship with phosphorus efficiency[J]. *Chin Agric Sci*, 41(4): 1089-1099. [刘灵, 廖红, 王秀荣, 等, 2008. 不同根构型大豆对低磷的适应性变化及其与磷效率的关系 [J]. *中国农业科学*, 41(4):1089-1099.]

LUO J, HOU YY, CHENG JH, et al., 2016. Root morphological characteristics of different phosphorus efficiency genotypes of cotton under low phosphorus stress[J]. *Chin Agric Sci*, 49(12): 2280-2289. [罗佳, 侯银莹, 程军回, 等, 2016. 低磷胁迫下不同磷效率基因型棉花的根系形态特征 [J]. *中国农业科学*, 49(12):2280-2289.]

Olday FC, 1972. Mineral nutrition of plants: principles and perspectives[J]. *Bioscience*, 22(12):739-739.

Rouached H, Arpat AB, Poirier Y, 2010. Regulation of phosphate starvation responses in plants: signaling players and cross talks[J]. *Molecular Plant*, 3(2):288-299.

SHEN H, YANG CY, FAN XW, et al., 2004. Activation of insoluble phosphorus by root exudates and root cell walls of soybean[J]. *Eco-environment*, (4): 633-635. [沈宏, 杨存义, 范小威, 等, 2004. 大豆根系分泌物和根细胞壁对难溶性磷的活化 [J]. *生态环境*, (4):633-635.]

SUN M, LI PC, ZHENG CS, et al., 2018. Effects of low phosphorus stress on root morphology and physiological characteristics of different genotypes of cotton seedlings[J]. *Acta Cott*, 30(1): 45-52. [孙淼, 李鹏程, 郑苍松, 等, 2018. 低磷胁迫对不同基因型棉花苗期根系形态及生理特性的影响 [J]. *棉花学报*, 30(1):45-52.]

TAO PL, LIU HH, DENG H, et al., 2013. Absorption and utilization of insoluble

phosphate in different genotypes of maize under low phosphorus stress[J]. China Agricultural Bulletin, 29(24): 28-35. [陶佩琳, 刘寒寒, 邓红, 等, 2013. 低磷胁迫下不同基因型玉米对难溶性磷酸盐的吸收和利用 [J]. 中国农学通报, 29(24):28-35.]

WEI RP, HU DH, CHEN JH, et al., 2018. Response of root morphology and nutrient utilization of Chinese fir clones to low phosphorus stress[J]. J Nanjing For Univ (Nat Sci Ed), 42(2): 1-8. [韦如萍, 胡德活, 陈金慧, 等, 2018. 低磷胁迫下杉木无性系根系形态及养分利用响应研究 [J]. 南京林业大学学报 (自然科学版), 42(2):1-8.]

WENG CF, TANG J, WU LJ, et al., 2014. The uptake of insoluble phosphorus by eucalyptus seedlings and the response of their roots to low phosphorus stress[J]. Journal of Northwest Botany, 34(5): 970-975. [翁彩凤, 唐健, 吴柳杰, 等, 2014. 桉树幼苗对难溶性磷的吸收及其根系对低磷胁迫的响应 [J]. 西北植物学报, 34(5):970-975.]

YAN JB, LU JM, HOU WF, et al., 2018. Effects of phosphorus fertilizer consumption on yield and phosphorus fertilizer utilization rate of different rice varieties[J]. Chin Agric Sci Technol Rep, 20(8): 74-81. [闫金奎, 鲁君明, 侯文峰, 等, 2018. 磷肥用量对不同水稻品种产量和磷肥利用率的影响 [J]. 中国农业科技导报, 20(8):74-81.]

YANG LN, AOTEGEN BY, LI QF, et al., 2015. Effects of alfalfa root exudates on insoluble phosphorus in soil[J]. Grass Sci, 32(8): 1216-1221. [杨利宁, 敖特根·白银, 李秋凤, 等, 2015. 苜蓿根系分泌物对土壤中难溶性磷的影响 [J]. 草业科学, 32(8):1216-1221.]

YU D, LI Y, YIN DY, et al., 2017. Response and physiological adaptation mechanism of Chinese fir to low phosphorus stress[J]. Forestry Science, 30(4): 566-575. [于姣姣, 李莹, 殷丹阳, 等, 2017. 杉木对低磷胁迫的响应和生理适应机制 [J]. 林业科学研究, 30(4):566-575.]

ZENG QY, JIANG Y, HUANG Y, et al., 2015. Differences in response of different sugarcane strains to nutrient stress[J]. Guangdong Agricultural Science, 42(12): 38-43. [曾巧英, 江永, 黄莹, 等, 2015. 不同甘蔗品系对养分胁迫响应的差异研究 [J]. 广东农业科学, 42(12):38-43.]

ZHENG C, LI QW, HUANG ZR, et al., 2015. Effects of phosphorus levels on root traits of different genotypes of sugarcane at seedling stage[J]. Trop Agric Sci, 35(2): 1-7. [郑超, 李奇伟, 黄振瑞, 等, 2015. 磷水平对不同基因型甘蔗苗期根系性状的影响 [J]. 热带农业科学, 35(2):1-7.]

ZOU X, WEI D, WU P, et al., 2018. Strategies of organic acid production and exudation in response to low-phosphorus stress in Chinese fir genotypes differing in phosphorus-use efficiencies[J]. Trees, 32: 897-912.

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