Increasing Nutrient Solution pH Alleviated Aluminum-Induced Inhibition of Growth and Impairment of Photosynthetic Electron Transport Chain in *Citrus sinensis* Seedlings

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Although the physiological and molecular responses of *Citrus* to Al-toxicity or low pH have been examined in some details, little information is available on *Citrus* responses to pH and aluminum (Al) interactions. *Citrus sinensis* seedlings were irrigated for 18 weeks with nutrient solution at a concentration of 0 or 1 mM AlCl$_3$·6H$_2$O and a pH of 2.5, 3.0, 3.5, or 4.0. Thereafter, biomass, root, stem, and leaf concentrations of Al and nutrients, leaf gas exchange, chlorophyll a fluorescence (OJIP) transients, and related parameters were investigated to understand the physiological mechanisms underlying the elevated pH-induced alleviation of *Citrus* toxicity. Increasing the nutrient solution pH from 2.5 to 4.0 alleviated the Al-toxic effects on biomass, photosynthesis, OJIP transients and related parameters, and element concentrations, uptake, and distributions. In addition, low pH effects on the above physiological parameters were intensified by Al-toxicity. Evidently, a synergism existed between low pH and Al-toxicity. Increasing pH decreased Al uptake per root dry weight and its concentration in roots, stems, and leaves and increased nitrogen, phosphorus, calcium, magnesium, sulfur, and boron uptake per plant and their concentrations in roots, stems, and leaves. This might be responsible for the elevated pH-induced alleviation of growth inhibition and the impairment of the whole photosynthetic electron transport chain, thus preventing the decrease of CO$_2$ assimilation.

1. Introduction

Aluminum (Al) exists mostly as silicate or oxide precipitates that are biologically inactive in neutral or moderately acidic soils. However, Al solubility increases greatly in acidic soils (pH < 5), resulting in the release of phytotoxic Al$^{3+}$ from clay minerals into soil solution [1]. Micromolar concentration of Al$^{3+}$ can cause a rapid inhibition of root elongation and subsequently impair the uptake of water and nutrients, leading to poor growth and yield loss of crops [2]. Therefore, Al-toxicity in acid soils has been regarded as a major factor limiting crop productivity worldwide, since ~30% of free ice land is acidic [3]. Furthermore, soil acidity is becoming an increasingly serious problem due to the improper farming practices and environmental deterioration [4]. In recent decades, many researchers have investigated Al-toxic effects on plant growth [5, 6], uptake of nutrients [6, 7], leaf CO$_2$ assimilation [8–11], and photosynthetic electron transport [12, 13].

The toxicity of Al to plants depends not only on their Al-tolerance, but also on the soil properties, primarily pH [14]. Hence, it is necessary to investigate the combination effects of Al and pH on plants to better understand the adaption of plants to acidic soils with high active Al. However, such data are very rare, because Al-toxicity and low pH are almost examined separately in different experiments. To our best
knowledge, most of studies regarding pH effects on plant Al-toxicity have focused on the pH-induced alterations in toxic Al species and activities in solution, root growth, and root tissue (cell sap) Al concentration, and the results are not consistent [1, 14, 15]. Because Al-toxicity occurs mainly on acidic soils, it is generally believed that the lower the pH in the culture medium, the greater the toxicity of Al to plants. Degenhardt et al. [16] reported that an Al-induced increase in rhizosphere pH was responsible for the Al-resistance in the Arabidopsis alr-104 mutant and that increasing the solution pH from 4.4 to 4.5 improved root growth rate of both alr-104 and wild type under Al-toxicity. Wang et al. [17] observed that the Al-tolerant wheat cultivar had higher capacity to keep higher rhizosphere pH relative to the Al-sensitive and that increasing the solution pH from 4.5 to 5.0 enhanced the Al-resistance of wheat. Using Eucalyptus trees, Yang et al. [18] found that raising the nutrient solution pH from 4.0 to 4.5 increased net photosynthesis and transpiration under Al-toxicity. However, Kinraide [19] found that the activities of AlIII in soil solutions peaked at ~ pH 4.1 in the range of pH 3.5 to 5.5, implying that Al-toxicity in soils with pH 4.1 might be more severe than that in more acidic or alkaline soils. Al level in rice root cell sap increased as solution pH increased from 4.0 to 6.0 [20].

Citrus often display poor growth and a shortened lifetime in low pH soils with high active Al [21]. In China, Citrus are cultivated mainly on acidic soils. Moreover, soil acidity is becoming an increasing urgent problem in some Citrus orchards [22]. Although the physiological and molecular responses of Citrus to Al-toxicity or low pH have been investigated in some details [9, 23–25], very scarcity is known on Citrus responses to pH and Al interactions. Preliminary study showed that increasing nutrient solution pH prevented Al-toxic effects on Citrus growth and photosynthesis. Here, we used C. sinensis seedlings as materials and investigated the effects of pH and Al interactions on biomass; Al and nutrient elements in roots, stems, and leaves; gas exchange, chlorophyll (Chl) a fluorescence (OJIP) transients and related parameters in leaves. Our objective was to determine the physiological mechanisms underlying the increased pH-induced alleviation of Citrus Al-toxicity.

2. Materials and Methods

2.1. Culture and Treatments of Seedlings. Seeding culture and treatments were conducted according to Guo et al. [26] with some modifications. Five-week-old “Xuegan” (C. sinensis) seedlings were transplanted to 6L pots (two per pot) containing sand thoroughly washed with tap water, and then planted in a greenhouse under a natural photoperiod at Fujian Agriculture and Forestry University, Fuzhou with an annual average relative humidity, temperature, and sunlight of ~ 76%, 20°C and 1600 h, respectively. Six weeks after transplanting, each pot was replenished every day with fresh nutrient solution. The nutrient solution was supplied to the pots until a portion of nutrient solution began to leak out from a hole at the bottom of the pot (~ 500 mL). The pH of the solution was adjusted by NaOH or HCl before irrigation. At the end of the experiment, fully expanded (~ 7-week-old) leaves were used for the assays of all physiological parameters.

2.2. Measurements of Root, Stem, and Leaf Dry Weights. At the end of the experiment, ten seedlings per treatment from different pots were harvested and then divided into roots, stems, and leaves. Their dry weight (DW) was weighted after being dried to a constant weight at 70°C.

2.3. Measurements of Leaf OJIP Transients and Calculation of Related Parameters. Leaf OJIP transients were measured in 3 h dark-adapted seedlings at room temperature using a Handy Plant Efficiency Analyzer (Handy PEA, Hansatech Instruments Limited, Norfolk, UK). All fluorescence parameters were calculated according to Jiang et al. [27] and Banks [28].

2.4. Leaf Gas Exchange. Leaf gas exchange was measured with a CIRAS-2 portable photosynthesis system (PP systems, Herts, UK) at a leaf temperature of ~ 26°C, a controlled light intensity of ~ 1000 μmol m−2 s−1 and a controlled CO2 concentration of ~ 380 μmol mol−1 between 9 and 12 a.m. on a sunny day. The flow rate through the 2.5 cm2 leaf chamber was kept at 200 μmol s−1. Water use efficiency (WUE) was calculated as CO2 assimilation/transpiration rate.

2.5. Assays of Elements, Calculation of Element Uptake, and Distributions in Roots, Stems, and Leaves. The small first- and second-order fibrous roots (< 2.0 mm diameter), the middle sections of stems, and the recent fully expanded mature (~ 7-week-old) leaves were used for the measurements of elements [26]. Al was assayed with a NexION 300X Inductively Coupled Plasma Mass Spectrometer (ICP-MS, PerkinElmer, CT, USA). Phosphorus (P) was measured colorimetrically as blue molybdate-phosphate complexes. Potassium (K) was assayed with a FP640 Flame Photometry (Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China). Calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) were determined with a PinAAcle 900F Atomic Absorption Spectrometer (Perkinelmer Singapore Pte Ltd., Singapore). Sulfur (S) was determined by the simple turbidimetric method. Nitrogen (N) was assayed by the Kjeldahl method with a Kjeltac 8200 Auto Distillation (FOSS Analytical AB, Höganas, Sweden). Boron (B) was determined by the curcumin method [22, 24].

Element uptake and distributions were calculated as described previously [26].

2.6. Data Analysis. There were 12 pots (24 seedlings) per treatment in a completely randomized design. Results represented the mean ± SE of 4-12 replicates (one plant from different pot per treatment) except for the mean OJIP transients and the different expressions derived from the mean transients. Significant differences among the eight treatments
were analysed by four (pH values) × two (Al levels) ANOVA and followed by the least significant difference (LSD) at P<0.05 level.

Principal component analysis (PCA) was performed using a SPSS® statistical software (version 17.0, IBM, NY, USA) [26].

3. Results

3.1. Seedling Growth. Without Al-toxicity, only pH 2.5 decreased stem, leaf, shoot, and whole plant DW and increased root DW/shoot DW ratio relative to control (pH 4.0); with Al-toxicity, all the six parameters kept stable when pH decreased from 4.0 to 3.5, then root, stem, leaf, shoot, and whole plant DW decreased and root DW/shoot DW ratio increased with further decreasing pH. Al decreased or did not alter stem, leaf, shoot, and whole plant DW, but it increased or did not affect root DW and root DW/shoot DW ratio. The interactive effects of pH and Al were significant for root DW, whole plant DW, and root DW/shoot DW ratio. Many rotten and died fibrous roots were observed in the pH 2.5 + 1 mM Al-treated seedlings (Figure 1 and Figure S1).

3.2. Leaf Gas Exchange. Without Al-toxicity, leaf stomatal conductance (g,) and transpiration rate (CO₂ assimilation) did not change as pH decreased from 4.0 to 3.0 (3.5) and then decreased with further decreasing pH, but intercellular CO₂ concentration (Cᵢ), the ratio of intercellular to ambient CO₂

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**Figure 1:** Effects of pH and Al interactions on (a) root, (b) stem, (c) leaf, (d) shoot, (e) whole plant DW, and (f) root DW/shoot DW ratio in C. sinensis seedlings. Bars represent means ± SE (n = 10). Different letters above the bars indicate a significant difference at P < 0.05. NS, * and ** indicate nonsignificant and significant at 5% and 1% level, respectively.
concentration (C_i/C_a), and WUE did not alter in response to pH. With Al-toxicity, leaf CO₂ assimilation, gₛ, transpiration rate, and WUE kept unchanged as pH decreased from 4.0 to 3.5 and then decreased with further decreasing pH, but both Cᵢ and Cᵢ/Cₐ ratio were the highest at pH 2.5. Interactions between pH and Al in leaf gas exchange were significant only for CO₂ assimilation (Figure 2).

Regression analysis showed that CO₂ assimilation increased with increasing gₛ, but it increased with decreasing Cᵢ and Cᵢ/Cₐ ratio in leaves (Figure S2).

3.3. Leaf OJIP Transients and Related Parameters. OJIP transients from the 0 mM Al-treated leaves displayed little alterations in response to pH except for a slight increase in O-step at pH 2.5 compared with OJIP transients from the pH 4.0 + 0 mM Al-treated leaves. Al increased the heterogeneity of samples, especially at pH 2.5. OJIP transients from the 1 mM Al-treated leaves displayed an increased O-step at pH 2.5-3.0 and a suppressed P-step at pH 2.5 compared with OJIP transients from the pH 4.0 + 1 mM Al-treated leaves (Figure S3). OJIP transients from the low pH and/or Al-treated leaves had positive ΔL-, ΔK-, ΔJ-, and ΔI-bands around 150 μs, 300 μs, 2 ms, and 30 ms compared with OJIP transients from the pH 4.0 + 0 mM Al-treated leaves, respectively. The positive ΔL-, ΔK-, ΔJ-, and ΔI-bands were the most pronounced from the leaves treated by pH 2.5 + 1 mM Al (Figure 3).

Without Al-toxicity, minimum fluorescence (F₀), maximum fluorescence (Fₘ), maximum variable fluorescence (Fᵥ), maximum primary yield of photochemistry of photosystem II (PSII; Fᵥ/F₀), fraction of oxygen evolving complex
Figure 3: Effects of pH and Al interactions on the mean OJIP transients expressed as the kinetics of relative variable fluorescence: (a) between $F_o$ and $F_m$: $V_i = (F_i - F_o)/(F_m - F_o)$ and (b) the differences of the eight samples to the reference sample submitted to pH 4.0 + 0 mM Al ($\Delta V_i$); (c) between $F_o$ and $F_{300}$: $W_K = (F_i - F_o)/(F_{300} - F_o)$ and (d) the differences of the six samples to the reference sample submitted to pH 4.0 + 0 mM Al ($\Delta W_K$).
(OEC) relative to control ($F_{\text{OEC}}$), maximum amplitude of IP phase, maximum PSI efficiency of dark-adapted leaves ($F_\text{m}/F_\infty$), quantum yield for energy dissipation ($Q_{\infty}$), quantum yield for electron transport ($Q_{\text{ET}}$), quantum yield for the reduction of end acceptors of PSI per photon absorbed ($Q_{\infty}/Q_{\text{ET}}$), Fv/Fm, RE/RC, ETm/RC, ETo/RC, and DI/CSo, respectively, but it decreased with further decreasing pH. Without Al-toxicity, the uptake of Al, S, Cu, Fe, Mn, and Zn per plant (root DW) increased or did not alter with decreasing pH with the exception that the uptake of Mn and Zn per plant was higher at pH 3.0 than that at pH 2.5, but the uptake of the other elements per plant (root DW) decreased or kept unchanged with decreasing pH. With Al-toxicity, the uptake of Al and Mn per plant (root DW) increased or did not alter with decreasing pH with the exception that the uptake of Mn per plant was slightly higher at pH 3.5 than that at pH 2.5, but the uptake of the other elements per plant (root DW) decreased or kept unchanged with decreasing pH with the exception that the uptake of S per plant (root DW) was higher at pH 3.5 than that at pH 4.0. Al-toxicity increased the uptake of Al per plant (root DW) and increased or did not alter the uptake of B per plant (root DW) with the exception that the uptake of B per plant was decreased by Al at pH 2.5, but it decreased the uptake of N, P, K, Ca, and Mg per plant (root DW) and decreased or did not change the uptake of S, Cu, Fe, and Zn per root DW. The uptake of S, Cu, Fe, Mn, and Zn per plant and of Mn per root DW was decreased by Al at pH 2.5-3.0, but increased or unaltered by Al at pH 3.5-4.0. Interactions between pH and Al on the uptake of elements were significant for the uptake of all elements except for the uptake of Mn and Zn per plant was higher at pH 3.0 than that at pH 2.5, but the uptake of the other elements per plant (root DW) decreased or kept unchanged with decreasing pH with the exception that the uptake of Mn per plant was slightly higher at pH 3.5 than that at pH 2.5, but the uptake of the other elements per plant (root DW) decreased or kept unchanged with decreasing pH with the exception that the uptake of S per plant (root DW) was higher at pH 3.5 than that at pH 4.0. Al-toxicity increased the uptake of Al per plant (root DW) and increased or did not alter the uptake of B per plant (root DW) with the exception that the uptake of B per plant was decreased by Al at pH 2.5, but it decreased the uptake of N, P, K, Ca, and Mg per plant (root DW) and decreased or did not change the uptake of S, Cu, Fe, and Zn per root DW. The uptake of S, Cu, Fe, Mn, and Zn per plant and of Mn per root DW was decreased by Al at pH 2.5-3.0, but increased or unaltered by Al at pH 3.5-4.0. Interactions between pH and Al on the uptake of elements were significant for the uptake of all elements except for the uptake of Al, N, and K per root DW (Figure 7).

Whole plant (root) DW increased with increasing uptake of N, P, K, Ca, Mg, S, B, or Fe (S, B, or Fe) per plant (Figure S5).

Leaf CO$_2$ assimilation decreased with increasing leaf Al or Mn concentration, but it increased with increasing leaf N, K, Ca, Mg, Fe, or Zn concentrations. Leaf CO$_2$ assimilation increased with increasing uptake of N, P, K, Ca, Mg, S, B, or Fe per plant (Figure S6).

Leaf Al increased with increasing F$_o$, M$_o$, DI/ABS, ABS/RC, DI/RC, TR$_o$/RC, or DI/CSo, respectively, but it decreased with increasing F$_m$, F$_v$, F$_v$/F$_m$, F$_o$/F$_m$, F$_o$/OEC, maximum amplitude of IP phase, F$_m$/F$_m$, ET$_o$/ABS, RE$_o$/ABS, ET$_o$/TR$_o$, ET$_o$/RC, RE$_o$/RC, RE$_o$/CSo, or PI$_{abs\,total}$, respectively (Figure S7).

Generally speaking, all element distributions in leaves and stems (roots) decreased (increased) or did not alter
Figure 4: Effects of pH and Al interactions on 21 Chl a fluorescence parameters in dark-adapted C. sinensis leaves. Bars represent means ± SE (n = 12). Different letters above the bars indicate a significant difference at $P < 0.05$. NS, * and ** indicate nonsignificant and significant at 5% and 1% level, respectively.
Figure 5: Effects of pH and Al interactions on concentrations of (a, h, o) Al, (b, i, p) N, (c, j, q) P, (d, k, r) K, (e, l, s) Ca, (f, m, t) Mg, and (g, n, u) S in *C. sinensis* (a-g) leaves, (h-n) stems, and (o-u) roots. Bars represent means ± SE (*n* = 8 except for 4 for K and Mg). Different letters above the bars indicate a significant difference at *P* < 0.05. NS, * and ** indicate nonsignificant and significant at 5% and 1% level, respectively.
with decreasing pH with or without Al-toxicity. Al decreased (increased) or did not affect Al, N, P, K, S, B, Cu, Mn, and Zn distributions in leaves and stems (roots) with the exceptions that P distribution in stems and Cu distribution in leaves were increased by Al at pH 3.5. Al decreased Ca and Mg distributions in leaves and increased or did alter their distributions in stems and roots. At pH 2.5, Al increased (decreased) Fe distribution in leaves and stems (roots), but at pH 3.0-4.0, it decreased (increased) or did not alter Fe distribution in leaves and stems (roots). Interactions between pH and Al on element distributions were significant for all element distributions in leaves, stems, and roots except for the distributions of Ca, Mg, and B in leaves and Al, N, K, and Zn in stems (Figures 8-9).

3.5. PCA Loading Plots. PCA was carried out to examine the physiological patterns of *C. sinensis* seedlings in response to pH with or without Al-toxicity (Figure 10 and Tables S1-S2). The first two components contributed to 60.6% and 68.3% of the total variation in the 0 and 1 mM Al-treated *C. sinensis* seedlings, respectively. These parameters were more highly clustered in the 1 mM Al-treated seedlings than those in the 0 mM Al-treated ones. For the 0 mM Al-treated seedlings, PC1 was heavily loaded on N uptake per root DW (0.985), N uptake per plant (0.982), B uptake per root DW (0.980), B uptake per plant (0.977), Mg uptake per root DW (0.975), leaf N concentration (0.974), Ca uptake per root DW (0.972), Ca uptake per plant (0.963), K uptake per root DW (0.959), and P uptake per root DW (0.952) (Table S1).
mM Al-treated seedlings, PC1 was the mostly affected by the alterations of stem N concentration (0.982), N uptake per root DW (0.981), Mg uptake per root DW (0.981), B uptake per root DW (0.980), Mg uptake per plant (0.978), B uptake per plant (0.977), N uptake per plant (0.977), Ca uptake per plant (0.967), Ca uptake per root DW (0.965), and leaf Mn concentration (-0.962) (Table S2).

Also, we determined the physiological patterns of C. sinensis seedlings in response to Al at pH 2.5, 3.0, 3.5, or 4.0 (Figure 11 and Tables S3–S6). The contribution of PC1 and PC2 to the total variation displayed little change as pH decreased from pH 4.0 to 3.5 and then increased with further decreasing pH. For the pH 4.0-treated seedlings, Ca uptake per root DW (0.981), Ca uptake per plant (0.979), K uptake per root DW (0.974), Al uptake per plant (-0.974), stem Mn concentration (0.973), Mg uptake per root DW (0.972), N uptake per root DW (0.970), Mg distribution in roots (-0.970), P uptake per root DW (0.965), and Al uptake per root DW (-0.958) contributed largely to PC1 (Table S3). For the pH 3.5-treated seedlings, stem Mn concentration (0.985), N uptake per root DW (0.983), Mn distribution in stems (0.982), Mg distribution in leaves (0.982), Mn...
distribution in roots (0.979), P uptake per root DW (0.977), leaf Mg concentration (0.974), root P concentration (0.970), Ca uptake per root DW (0.970), and Mn uptake per plant (-0.969) were the main contributors to PC1 (Table S4). For the pH 3.0-treated seedlings, PC1 was mostly influenced by the alterations of Mg uptake per root DW (0.995), Mg uptake per plant (0.994), P uptake per root DW (0.982), leaf Mg concentration (0.982), P uptake per plant (0.982), N uptake per plant (0.980), N uptake per root DW (0.979), leaf P concentration (0.979), leaf Ca concentration (0.978), and Ca uptake per root DW (0.977), leaf Mg concentration (0.974), root P concentration (0.970), Ca uptake per root DW (0.970), and Mn uptake per plant (-0.969) were the main contributors to PC1 (Table S4). For the pH 3.0-treated seedlings, PC1 was mostly influenced

![Figure 8: Effects of pH and Al interactions on (a, h, o) Al, (b, i, p) N, (c, j, q) P, (d, k, r) K, (e, l, s) Ca, (f, m, t) Mg, and (g, n, u) S distributions in C. sinensis (a-g) leaves, (h-n) stems, and (o-u) roots. Bars represent means ± SE (n = 8 except for 4 for K and Mg). Different letters above the bars indicate a significant difference at P < 0.05. NS, * and ** indicate nonsignificant and significant at 5% and 1% level, respectively.](image-url)
S uptake per plant (0.978) (Table S5). For the pH 2.5-treated seedlings, PC1 was largely accounted for by the modifications of S uptake per plant (0.991), N uptake per plant (0.991), S distribution in roots (-0.991), S uptake per root DW (0.990), N uptake per root DW (0.990), Mg uptake per plant (0.985), leaf S concentration (0.984), Mg uptake per root DW (0.982), and K uptake per plant (0.980) (Table S6).

4. Discussion

4.1. Interactive Effects of Al and Low pH on C. sinensis Seedlings Showed Synergism. The Al-induced alterations of most physiological parameters and OJIP transients became more pronounced with decreasing pH. Many parameters were altered by Al-toxicity only at pH 2.5-3.0, but unaffected at pH 3.5-4.0 (Figures 1–9 and S1 and S3). The exception was that the Al-induced increase in leaf and stem level of B was greater at pH 3.5-4.0 than that at pH 2.5-3.0 or similar between the two (Figure 6). These findings indicated that the Al-induced alterations of these physiological parameters were intensified by low pH. Obviously, increasing the nutrient solution pH from 2.5 to 4.0 alleviated Al-toxicity of C. sinensis seedlings. This agrees with the results obtained on Arabidopsis [16], wheat [17], and Eucalyptus [18]. We observed that both the level of Al in roots, stems, and leaves and Al uptake per plant (root DW) increased with decreasing pH with or without Al-toxicity with the exception that Al uptake per plant was basically unchanged in response to pH in 1 mM Al-treated seedlings (Figures 5 and 7). The increased

Figure 9: Effects of pH and Al interactions on (a, f, k) B, (b, g, l) Cu, (c, h, m) Fe, (d, i, n) Mn, and (e, j, o) Zn distribution in C. sinensis (a-e) leaves, (f-j) stems, and (k-o) roots. Bars represent means ± SE (n = 8 except for 4 for Fe and Mn). Different letters above the bars indicate a significant difference at P < 0.05. NS, * and ** indicate nonsignificant and significant at 5% and 1% level, respectively.
pH-induced decreases in the level of Al in roots, stems, and leaves and Al uptake per root DW might be responsible for the elevated pH-induced alleviation of C. sinensis Al-toxicity. Also, we observed that the low pH-induced alterations of most physiological parameters were greater in the 1 mM Al-treated seedlings than those in the 0 mM Al-treated ones (Figures 1–9 and S1 and S3), demonstrating that the low pH-induced alterations of physiological parameters were enhanced by Al-toxicity. To conclude, there was a synergism between low pH and Al.

4.2. Al-Toxicity Increased Root Al Accumulation, Especially at Low pH. Plant Al-tolerance is associated not only with less uptake of Al by roots, but also with relatively less transport of Al from roots to shoots [29]. Previous studies indicated that the supply of S, B, and P decreased Al level in stems and leaves and increased or did not affect root Al level, thus alleviating Citrus Al-toxicity [6, 7, 23]. Thus, the Al-induced increase in root Al accumulation and decrease in leaf and stem Al accumulation (Figure 8) might be an adaptive strategy of C. sinensis to Al-toxicity. However, the increase in Al-tolerance due to the increased pH could not be explained in this way, because Al distribution in roots of the Al-treated seedlings was higher at pH 2.5-3.0 than that at pH 3.5-4.0. We found that increasing the nutrient solution pH from 2.5 to 4.0 decreased the level of Al in roots, stems, and leaves and the uptake of Al per root DW (Figures 5 and 7), which might play a key role in the increased pH-induced alleviation of Citrus Al-toxicity.

4.3. Increased Uptake and Levels of Nutrients Might Play a Role in the Elevated pH-Induced Alleviation of Al-Toxicity. Micromolar concentration of Al$^{3+}$ can lead to a rapid inhibition of root growth and subsequently interfering with the uptake of nutrients [2, 20]. Al decreased the uptake of N, P, K, Ca, Mg, and Cu per plant at each given pH, especially at low pH with the exception that the uptake of Cu per plant was not altered by Al at pH 4.0. The uptake of S, B, Mn, and Zn per plant was increased and decreased by Al at pH 3.5-4 and pH 2.5-3, respectively (Figure 7). Regression analysis indicated that both whole plant DW and leaf CO$_2$ assimilation decreased with decreasing uptake of N, P, K, Ca, Mg, S, B, or Fe per plant (Figures S5-S6). Generally viewed, Al decreased the levels of N, P, K, Ca, Mg, and S in roots, stems, and leaves, especially at pH 2.5-3.0 with the exceptions of a few, but increased the level of B in leaves, stems, and roots at each given pH. Also, B level in roots, stems, and leaves increased with increasing pH with or without Al-toxicity (Figures 5-6). Regression analysis showed that leaf CO$_2$ assimilation decreased with decreasing leaf concentration of N, K, Ca, Mg, Fe, or Zn (Figure S6). The supply of Ca, Mg, S, B, and P can ameliorate Al-toxicity of plants [6, 7, 23, 30–35]. PCA showed that N, P, K, Ca, Mg, S, and B uptake per plant and/or per root DW was the main contributors to PC1 in the 0 mM Al-, 1 mM Al-, pH 4.0-, pH 3.5-, pH 3.0- and/or pH 2.5-treated seedlings (Tables S1-S6), demonstrating that the uptake of these elements might play a role in Citrus Al-toxicity (tolerance) and/or low pH-toxicity (tolerance). Thus, both the increased uptake per plant and/or root, stem, and leaf levels of N, P, Ca, K, Mg, S, and B might
Figure 11: PCA loading plots of all physiological parameters for (a) pH 4.0-, (b) pH 3.5-, (c) pH 3.0-, and (d) pH 2.5-treated C. sinensis seedlings submitted to 0 and 1 mM Al.

be involved in the increased pH-induced alleviation of Citrus Al-toxicity.

4.4. Causes for the Elevated pH-Induced Alleviation of Photosynthetic Decline in the Al-Treated Leaves. Although $g_s$ in the Al-treated leaves increased with increasing pH (Figure 2(b)) and leaf CO$_2$ assimilation increased with increasing $g_s$ (Figure S2), the ameliorative action of the increased pH against the inhibitory effect of Al on photosynthesis (Figure 2(a)) could not explained by the increased $g_s$ alone, because low pH
increased or did not affect both C\textsubscript{i} and C\textsubscript{i}/C\textsubscript{a} ratio (Figure 2) and leaf CO\textsubscript{2} assimilation decreased with increasing C\textsubscript{i} or C\textsubscript{i}/C\textsubscript{a} ratio (Figure S2).

Previous studies showed that the impaired whole photosynthetic electron transport chain from the donor side of PSII to the reduction of PSI end acceptors was the main cause contributing to the Al-induced inhibition of photosynthesis in Citrus leaves [27] and that the Al-induced impairment of the whole photosynthetic electron transport chain and the subsequent decline in leaf CO\textsubscript{2} assimilation could be alleviated by the supply of B, S, and P [6, 7, 23]. We observed that Al-toxicity lowered F\textsubscript{o}/F\textsubscript{m} (a good indicator of photoinhibitory effects on PSII) and \(\psi_{o/2} \) (ET\textsubscript{o}/TR\textsubscript{o}), increased DL\textsubscript{o}/RC\textsubscript{o}, and altered greatly OJIP transients in low pH treated leaves (Figures 3-4), together demonstrating that photoinhibition occurred in these leaves [36, 37]. Increasing the nutrient solution pH from 2.5 to 4.0 prevented the Al-induced alterations of OJIP transients and all the 21 fluorescence parameters (Figures 3-4 and S3). Regression analysis showed that there was a positive relationships between leaf CO\textsubscript{2} assimilation and F\textsubscript{m}/F\textsubscript{m}. F\textsubscript{o}/F\textsubscript{m}, F\textsubscript{o}/O\textsubscript{ ECB}, maximum amplitude of IP phase, F\textsubscript{o}/F\textsubscript{m}, ET\textsubscript{o}/ABS, RE\textsubscript{o}/ABS, ET\textsubscript{o}/TR\textsubscript{o}, RE\textsubscript{o}/TR\textsubscript{o}, ET\textsubscript{o}/RC, RE\textsubscript{o}/RC, RE\textsubscript{o}/CS\textsubscript{o}, or PI\textsubscript{abs,total}, but a negative relationship between leaf CO\textsubscript{2} assimilation and F\textsubscript{o}, M\textsubscript{o}, DL\textsubscript{o}/ABS, ABS/RC, DL\textsubscript{o}/RC, TR\textsubscript{o}/RC, or DL\textsubscript{o}/CS\textsubscript{o} (Figure S4). These results suggested that the increased pH alleviated the Al-toxic impairment on the whole electron transport chain, thus preventing the Al-induced inhibition of photosynthesis.

Evidence shows that the deficiencies of mineral nutrients (N, P, K, Ca, and Mg) can impair the whole photosynthetic electron transport chain and cause a marked decline in leaf photosynthesis [38–43]. Here, the increased pH-induced alleviation of the Al-induced decreases in leaf levels of N, P, K, Ca, and Mg and their uptake per plant might be one of the causes for preventing the Al-induced decline in leaf CO\textsubscript{2} assimilation, as indicated by the positive correlations between leaf CO\textsubscript{2} assimilation and leaf level of N, K, Ca, or Mg and uptake per plant of N, P, K, Ca, or Mg (Figure S6). Previous study showed that Al-toxicity increased or did not affect B concentration in Citrus grandis roots, stem and leaves, but supply B alleviated the Al-induced impairment occurring in the whole photosynthetic electron transport chain and inhibition of photosynthesis [23]. Our results showed that B concentration in roots, stems, and leaves and its uptake increased with increasing pH (Figures 6-7) and that leaf CO\textsubscript{2} assimilation increased with increasing B uptake per plant and displayed an increased trend with increasing leaf B concentration (Figure S6). These results indicated that the increased pH-induced increase in B uptake per plant might contribute to the alleviation of photosynthesis inhibition in Al-treated leaves. The antagonistic action of the increased pH against the inhibitory effect of Al-toxicity on leaf CO\textsubscript{2} assimilation might also involve the increased pH-induced a decrease in leaf Al concentration (Figure 5(a)), as indicated by the negative and significant relationship between leaf CO\textsubscript{2} assimilation and Al concentration (Figure S6) and the negative or positive relationships between leaf CO\textsubscript{2} assimilation and Chl a fluorescence parameters (Figure S4). Based on these results, we concluded that increasing the solution pH from 2.5 to 4.0 mitigated the Al-induced impairment occurring on the whole photosynthetic electron transport chain, thus preventing the Al-induced decline in CO\textsubscript{2} assimilation via decreasing the level of Al and increasing the uptake per plant of elements (N, P, K, Ca, Mg, and B) and their levels in leaves.

5. Conclusions
Our data clearly demonstrated that a synergism existed between low pH and Al and that increasing the nutrient solution pH from 2.5 to 4.0 alleviated the Al-toxicity of C. sinensis seedlings. Increasing pH decreased Al uptake per root DW and its level in roots, stems, and leaves and increased N, P, K, Ca, Mg, S, and B uptake per plant and their levels in roots, stems, and leaves. This might account for the increased pH-induced alleviation of Citrus Al-toxicity.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure
The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Figure S1: effects of pH and Al interactions on growth of C. sinensis seedlings. Figure S2: CO\textsubscript{2} assimilation in relation to (a) stomatal conductance (g\textsubscript{s}), (b) intercellular CO\textsubscript{2} concentration (C\textsubscript{i}), and (c) ratio of intercellular to ambient CO\textsubscript{2} concentration (C\textsubscript{i}/C\textsubscript{a}) in C. sinensis leaves. Figure S3: effects of pH and Al interactions on the high-light-induced OJIP transients of dark-adapted C. sinensis leaves plotted on a logarithmic time scale (0.01 to 1000 ms). Figure S4: leaf CO\textsubscript{2} assimilation in relation to 21 Chl a fluorescence parameters. Figure S5: element uptake per plant in relation
to (a-l) whole plant DW and (m-x) root DW in C. sinensis seedlings. Figure S6: leaf CO₂ assimilation in relation to (a-l) leaf element concentrations and (m-x) element uptake per plant in C. sinensis seedlings. Figure S7: leaf Al concentration in relation to 21 Chl a fluorescence parameters. Table S1: PCA for physiological parameters of 0 mM Al-treated C. sinensis seedlings. Table S2: PCA for physiological parameters of 1 mM Al-treated C. sinensis seedlings. Table S3: PCA for physiological parameters of pH 4-treated C. sinensis seedlings. Table S4: PCA for physiological parameters of pH 3.5-treated C. sinensis seedlings. Table S5: PCA for physiological parameters of pH 3-treated C. sinensis seedlings. Table S6: PCA for physiological parameters of pH 2.5-treated C. sinensis seedlings. (Supplementary Materials)

References


