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The photosynthetic performance of two Citrus species under long-term aluminum treatment

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Abstract

The effect of Al stress on leaf pigment contents, chlorophyll (Chl) a fluorescence transient, and leaf gas exchange of Citrus sinensis and Citrus grandis were investigated in sandy culture with nutrient solution (control) or supplemented by 0.5 mM AlCl₃·6H₂O (Al toxic) for 54 weeks. We found a significant decline of Chl a, carotenoids (Car), Ch a/b, and Car/Chl (a+b) in C. grandis caused by Al stress. Except the significant increase of Car/Chl (a+b), no remarkable difference was found in C. sinensis. The Al-induced downregulation of CO₂ assimilation was related to the imbalance of reduction and oxidation of primary quinone acceptor of PSII in two Citrus species. Compared to C. grandis, increasing ratio of Car/Chl (a+b) for photooxidation protection and decreasing inhibition of electron transfer contributed to electron conversion efficiency maintenance of PSII under Al stress in C. sinensis, a relatively Al-tolerant species.

Additional key words: Al resistance; excessive Al; OJIP curve; photoinhibition.

Introduction

The soil degradation, such as soil salinization, soil acidification, and heavy metal pollution, represents major obstacles to crop production (Mbarki et al. 2018). In subtropical and tropical regions, the acid soil is widely distributed, which accounts for 60% of acid soil globally (Kochian et al. 2015). Soil acidification activates aluminum (Al⁵⁺) from insoluble deposits typically when the soil pH is below 5.0, which depresses the plant development and crop yield (Liang et al. 2013). For instance, Lin and Myhre (1991) reported the Citrus shoot growth was retarded and root tips were thickened under Al stress. Kopittke et al. (2015) observed that 75 μM Al⁵⁺ downregulated the soybean root elongation within 5 min. Thung et al. (1987) reported a negative relationship between common bean yield and soil Al content under field conditions. The Al-toxic soil was also found to decrease the maize grain yield by 46–73% (Welcker et al. 2005) and wheat grain yield by 23–100% (Valle et al. 2009). Therefore, the Al toxicity was considered as one of the major abiotic stresses for crops in subtropical and tropical areas.

In south China, a number of Citrus orchards encounter soil acidification. Our previous investigation on the soil chemical properties of pummelo orchard in Fujian province indicated that over 90% of tested soil samples had the pH lesser than 5.0, and the soil pH of Citrus orchard decreased continuously during last decade due to improper fertilization (Li et al. 2015). Hence, the potential Al toxicity as well as Al tolerance strategies of Citrus species should be well illustrated for a better orchard management.

The Al resistance of Citrus species was related to the photosynthetic performance. For instance, Guo et al. (2018) observed that Al-tolerant Citrus seedlings tended to maintain a better photosynthetic homeostasis than the sensitive ones. By a cultivar comparison, Pereira et al.

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Abbreviations: Car – carotenoids; CE – carboxylation efficiency of Rubisco (= Pn/C); Chl – chlorophyll; Ci – intercellular CO₂ concentration; E – transpiration rate; ET₉₀ – energy flux beyond Qₑ; F₀ – minimal fluorescence of dark-adapted state; Fₘₐₓ – maximal fluorescence; Fv – variable fluorescence (= Fₘₐₓ – F₀); Fv/F₀ – the ratio of variable fluorescence to initial fluorescence; Fv/Fm – maximum photochemical efficiency of PSII; gₛ – stomatal conductance; Pₘᵢₑᵢ – performance index of absorption; Pₙ – net photosynthetic rate; RC – reaction center; TR – energy trapping flux by PSII center; WUE – water-use efficiency (= Pn/E); Ψₑ – efficiency that an electron moves further than Qₑ.

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reported that Citrus species suffering from Al toxicity resulted in photosynthetic inhibition which was related to thylakoid destruction and impaired electron transport. Moreover, the Citrus species with a higher photosynthetic inhibition by Al stress were prone to show a higher decrease of $F_a/F_o$ compared with control.

So far, in most of the studies on Citrus photosynthesis, plants were treated for 20 weeks or less under Al stress (Chen et al. 2005a,b; Jiang et al. 2008). Nevertheless, such Al-stress duration might not to be enough to disclose the photosynthetic mechanisms under Al stress. One reason is that the Citrus species is native to the tropical and subtropical areas (Nicolosi et al. 2000), with the superb intrinsic adaptation to high Al$^+$, therefore a short-term Al duration might not result in adequate physiological damage for Citrus species. Secondly, Citrus species is perennial with a longer growth regime and a relatively higher biomass than that of herbaceous plants; it would also offer itself many flexible strategies for Al toxicity combating. In view of this hypothesis, the potential Al stress and related responses of Citrus should be reconsidered at a much broader time scale; therefore, a prolonged duration of Al stress was applied to Citrus seedlings.

The objectives of present study were to characterize the effects of Al stress on leaf pigment contents, chlorophyll a fluorescence (OJIP) transient, and leaf gas exchange of two Citrus species under long-term stress.

Materials and methods

Plant materials, growth conditions, and stress treatments: For germination, the plump seeds of Citrus sinensis (L.) Osbeck and Citrus grandis (L.) Osbeck were covered with moist-washed river sand in a tray after surface sterilization in August 2017. Six weeks after sowing, the uniformed size seedlings (about 10 cm) were transplanted to a 6-L pot filled with clean river sand and supplied with 1/4 modified Hoagland nutrient solution containing 1 mM KNO$_3$, 1 mM Ca(NO$_3$)$_2$, 0.1 mM K$_2$HPO$_4$, 0.5 mM MgSO$_4$, 10 μM H$_2$SO$_4$, 2 μM MnCl$_2$, 2 μM ZnSO$_4$, 0.5 μM CuSO$_4$, 0.065 μM (NH$_4$)$_2$MoO$_4$$_3$, and 20 μM Fe-EDTA. The Al treatments started 11 weeks after transplanting, including 1/4 nutrient solution plus 0 (Control) and 0.5 mM AlCl$_3$·6 H$_2$O (Al toxic) with pH adjusted to 4.1–4.2 by 1 M NaOH or HCl. The treatments lasted for 54 weeks. All plants were grown in a greenhouse in Fujian Agriculture and Forestry University, Jinshan Campus (26°5’N, 119°14’E) throughout the experiment with an annual temperature of 20–25°C, air humidity of 76%, light intensity of 1,000–1,980 h. All plants were grown in a greenhouse in Fujian Agriculture and Forestry University, Jinshan Campus (26°5’N, 119°14’E) throughout the experiment with an annual temperature of 20–25°C, air humidity of 76%, light intensity of 1,000–1,980 h. Annual sunshine duration of 1,700–1,980 h.

Leaf pigment contents: The Citrus leaf pigments were extracted with 80% acetone under dark. Briefly, five leaf discs (0.2826 cm$^2$ each) were soaked in 6 ml of 80% acetone overnight and shaken every 2 h. The supernatant extracted for 24 h was used for quantification of leaf pigment by measuring the absorbance at 470, 646, and 663 nm with a Libra S22 UV/Vis spectrophotometer (Biochrom Ltd., Cambridge, UK). The contents of Chl a, Chl b, and Car were calculated according to Lichtenthaler (1987).

Chl a fluorescence (OJIP) transient: The leaf Chl a fluorescence transient was measured using a Handy PEA (Hansatech Instruments Ltd., Norfolk, UK) after dark-adaptation for 3 h at room temperature according to Jiang et al. (2008). The leaf fluorescence signals were read from 10 μs to 1,000 ms under continuous light [maximum light intensity of 3,000 μmol(photon) m$^{-2}$ s$^{-1}$]. The stepwise energy flow through PSI was analyzed based on JIP-test (Strasser et al. 2000) using original fluorescence records. The relative variable fluorescence transient curves were normalized as $V_i = (F_i – F_0)/(F_m – F_0)$, and the $\Delta V_i$ was achieved by subtracting $V_{\text{control}}$ from $V_{\text{Al stress}}$ according to Tsimilli-Michael and Strasser (2013a). $V_i$ at 0–300 μs (O–K), 0–2 ms (O–J), 2–30 ms (J–I), and 30–1,000 ms (I–P) were normalized as $W_{\text{OK}}$ [$W_{\text{OK}} = (F_i – F_0)/(F_m – F_0)$], $W_{\text{OJ}}$ [$W_{\text{OJ}} = (F_i – F_0)/(F_m – F_0)$], $W_{\text{OJ}}$ [$W_{\text{OJ}} = (F_i – F_0)/(F_m – F_0)$], and $W_{\text{IP}}$ [$W_{\text{IP}} = (F_i – F_0)/(F_m – F_0)$], respectively. Then $W_{\text{OK}}$, $W_{\text{OJ}}$, $W_{\text{OJ}}$, and $W_{\text{IP}}$ were renormalized by subtracting $V_{\text{control}}$ from $V_{\text{Al stress}}$, and expressed as $\Delta W_{\text{OK}}$, $\Delta W_{\text{OJ}}$, $\Delta W_{\text{OJ}}$, and $\Delta W_{\text{IP}}$ respectively.

Leaf gas exchange: The net photosynthetic rate ($P_n$), intercellular CO$_2$ concentration (C$i$), stomatal conductance (g$s$), and transpiration rate (E) of Citrus leaves were measured by a CIRAS-2 portable photosynthesis system (PP System, Herts, UK). The measurements were conducted 3–5 min after closing the leaf to leaf chamber with PPFD of 1,000 μmol(photon) m$^{-2}$ s$^{-1}$, ambient CO$_2$ concentration of 380 μmol(CO$_2$) mol$^{-1}$, leaf chamber temperature of 30.7 ± 0.5°C, and ambient vapor pressure of 1,010 ± 5 Pa on a sunny day from 09:00 to 11:00 h.

Statistical analysis: The experiments were carried out in a completely randomized design with ten pots (two plants per pot) per treatment for each Citrus species, five seedlings of each treatment with the most similar size were selected for the test. All data were analyzed by SPSS v. 16.0 (SPSS Corp., Chicago, IL, USA). The results were presented as means ± SE, n = 5. Two-way analysis of variance (ANOVA) was used to determine the significant interaction between Citrus genotypes and Al treatment. The significant differences between means were tested using Duncan’s multiple range test at a significant level of P≤0.05.

Results

Leaf pigment contents: The Al stress significantly decreased the contents of Chl a and Car without a remarkable difference in Chl b and Chl (a+b) in C. grandis leaves (Table 1). Accordingly, the ratios of Chl a/b and Car/Chl (a+b) were depressed dramatically in C. grandis leaves by Al stress compared with control. In contrast, the ratio of Car/Chl (a+b) was upregulated by Al stress compared with control in C. sinensis leaves. However, the results indicated insignificant difference in Chl a, Chl b, Chl (a+b), Car, and Chl a/b in C. sinensis leaves under Al stress.
OJIP transient and fluorescence parameters: The OJIP transient of Citrus leaves for control and Al toxicity were shown in Fig. 1A. We found that O–J phase in the OJIP transient curve of C. sinensis leaves dropped slightly by Al toxicity. Nevertheless, compared with control, a decrease of the OJIP transient curve was found in C. grandis leaves when exposed to Al toxicity. The results of Fig. 1B revealed that the curve decreased from 0 to 0.5 ms, while increased from 0.6 to 4.0 ms. Noticeably, the positive △Vt was found in C. grandis from 2.6 to 6.0 ms. As shown in Fig. 1C,D; the negative L-band (0.13 ms) and K-band (0.3 ms) were found in two Citrus species with a higher amplitude in C. grandis leaves. The positive bands were found in △WJ and △WIP of C. sinensis. However, a positive band was

Table 1. The effects of Al stress on leaf pigment contents of Citrus sinensis and Citrus grandis seedlings. Values represent means ± SE (n = 5). Significant differences (P≤0.05) between treatments are indicated by different letters. Chl – chlorophyll, Car – carotenoids.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatments</th>
<th>Chl a [mg m⁻²]</th>
<th>Chl b [mg m⁻²]</th>
<th>Chl (a+b) [mg m⁻²]</th>
<th>Car [mg m⁻²]</th>
<th>Chl a/b</th>
<th>Car/Chl (a+b)</th>
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<tr>
<td>C. sinensis</td>
<td>Control</td>
<td>484.52 ± 5.42ab</td>
<td>154.44 ± 5.75ab</td>
<td>638.96 ± 9.09a</td>
<td>125.89 ± 1.76a</td>
<td>3.15 ± 0.11a</td>
<td>0.20 ± 0.01ab</td>
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<tr>
<td></td>
<td>Al toxicity</td>
<td>483.75 ± 3.44ab</td>
<td>153.55 ± 3.77a</td>
<td>637.31 ± 6.64a</td>
<td>141.93 ± 1.10b</td>
<td>3.16 ± 0.06a</td>
<td>0.22 ± 0.01a</td>
</tr>
<tr>
<td>C. grandis</td>
<td>Control</td>
<td>529.86 ± 21.05a</td>
<td>169.07 ± 5.71ab</td>
<td>698.94 ± 25.88a</td>
<td>152.99 ± 5.49a</td>
<td>3.13 ± 0.08a</td>
<td>0.22 ± 0.01ab</td>
</tr>
<tr>
<td></td>
<td>Al toxicity</td>
<td>455.13 ± 37.35b</td>
<td>170.13 ± 4.85ab</td>
<td>625.26 ± 40.73a</td>
<td>122.42 ± 15.29b</td>
<td>2.67 ± 0.18b</td>
<td>0.19 ± 0.01c</td>
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Fig. 1. The effects of Al stress on the leaf Chl a fluorescence transient of Citrus sinensis and Citrus grandis seedlings. (A) Chl a fluorescence; (B) △Vt, Vt = (F – F0)/(Fm – F0), △Vt = Vt (Al toxicity) – Vt (Control); (C) △WJ, WJ = (F – F0)/(FJ – F0), △WJ = WJ (Al toxicity) – WJ (Control); (D) △WOK, WOK = (F – F0)/(FK – F0), △WOK = WOK (Al toxicity) – WOK (Control); (E) △WIP, WIP = (F – F0)/(FI – F0), △WIP = WIP (Al toxicity) – WIP (Control); (F) △WII, WII = (F – F0)/(FII – F0), △WII = WII (Al toxicity) – WII (Control). Values represent means of five replicates.
PHOTOSYNTHESIS OF CITRUS SPECIES UNDER ALUMINIUM STRESS

only presented in $\Delta W_3$ of C. grandis.

Based on the data of Chl a fluorescence transients, we found a significant increase of $F_0$ (Fig. 2A), significant decrease of $F_m$ (Fig. 2B), $F_{\pi}$ (Fig. 2C), $F_v/F_m$ (Fig. 2D), $F_v/F_0$ (Fig. 2E) in Al-treated C. grandis leaves compared with control. However, the above mentioned parameters were not affected remarkably in C. sinensis leaves by Al stress compared with control (Fig. 2A–E). The Al stress had no obvious effect on $ET_0/ TR_0$ (Fig. 2F) in two Citrus species. Also, the Al stress did not reduce absorption flux per RC ($\text{ABS}/\text{RC}$, Fig. 2G), whereas the trapped energy per RC ($\text{TR}_0/\text{RC}$, Fig. 2H) was significantly reduced in two Citrus species. Besides, the electron transport per RC ($ET_0/ \text{RC}$, Fig. 2I) was inhibited remarkably by Al stress in C. grandis, while no obvious decline was found in C. sinensis. Under Al stress, C. sinensis kept a remarkably higher electron flux reducing end electron acceptors at the acceptor side per RC ($\text{RE}_0/\text{RC}$, Fig. 2J) and performance index on absorption basis ($\text{PI}_{\text{abs}}$, Fig. 2L) than that of C. grandis.

Fig. 2. The effects of Al stress on Chl a fluorescence parameters in leaves of Citrus sinensis and Citrus grandis seedlings. Values represent means ± SE ($n = 5$). Significant differences ($P < 0.05$) between treatments are indicated by different letters. Minimal fluorescence of dark-adapted state ($F_0$) (A), maximal fluorescence ($F_m$) (B), variable fluorescence ($F_v$) (C), maximum photochemical efficiency of PSII ($F_v/F_m$) (D), the ratio of variable fluorescence to initial fluorescence ($F_v/F_0$) (E), electron transport probability ($ET_0/ \text{TR}_0$) (F), the absorption flux per reaction center ($\text{ABS}/\text{RC}$) (G), the trapped energy per reaction center ($\text{TR}_0/\text{RC}$) (H), the electron transport per reaction center ($ET_0/ \text{RC}$) (I), reduction of end acceptors at PSI electron acceptor side per reaction center ($\text{RE}_0/\text{RC}$) (J), the density of active PSII reaction centers per absorption ($\text{RC}/\text{ABS}$) (K), performance index on the absorption basis ($\text{PI}_{\text{abs}}$) (L).
**Leaf gas exchange:** The Al stress lowered the $P_N$ and CE significantly in two *Citrus* species compared with control. It was also noteworthy that the *C. sinensis* leaves maintained a higher $P_N$ and CE than *C. grandis* leaves under Al stress (Fig. 3A,F). The decreased $P_N$ by Al stress coincided with a remarkable increment of $C_i$, while no significant difference of $g_s$ was found in *C. grandis* leaves (Fig. 3B,C). However, a significant decrease of $g_s$ with no obvious difference in $C_i$ was observed in *C. sinensis* leaves under Al stress. Compared with control, the Al stress downregulated the $E$ of two *Citrus* species without significant difference (Fig. 3D). Moreover, the Al stress significantly inhibited WUE in *C. grandis* but not in *C. sinensis* compared with control (Fig. 3F).

**Discussion**

The plant suffering from Al toxicity is an integrative result of stress duration and stress concentration (Costa de Macedo et al. 1997). For instance, it was evidenced that a time-dependent adjustment mechanism played roles in Al stress recovery in sorghum cultivars (Peixoto et al. 2002), which emphasized the influence of stress duration on plant Al resistance. As a perennial fruit trees, the *Citrus* species are native to acid soil with a relatively higher Al content. Hence, the Al adaptation of *Citrus* species should be monitored at a longer duration.

Previously, we have identified that *C. sinensis* was more tolerant to Al stress than *C. grandis* (Yang et al. 2021).

![Fig. 3.](image-url) The effects of Al stress on the leaf gas exchange of *Citrus sinensis* and *Citrus grandis* seedlings. Values represent means ± SE ($n = 5$). Significant differences ($P \leq 0.05$) between treatments are indicated by different letters. Net photosynthetic rate ($P_N$) (A), intercellular CO₂ concentration ($C_i$) (B), stomatal conductance ($g_s$) (C), transpiration rate ($E$) (D), water-use efficiency (WUE) (E), carboxylation efficiency of Rubisco (CE) (F).
PHOTOSYNTHESIS OF CITRUS SPECIES UNDER ALUMINIUM STRESS

2011). Different from our previous treatments with a higher Al concentration under relative short stress duration (Chen et al. 2005b, Guo et al. 2018), the present study mainly focused on Citrus photosynthetic performance with 0.5 mM Al under a longer duration. Herein, we found that the contents of Chl (a+b) and Chl b were not affected remarkably by 0.5 mM Al after 54 weeks in two Citrus species, which indicated a good adaptation of Citrus species to Al stress. The Chl a of C. sinensis leaves was not affected obviously by Al stress. However, the Al stress significantly decreased the Chl a of C. grandis leaves. Similarly, Al toxicity downregulated the ratio of Chl a/b in C. grandis leaves but not in C. sinensis leaves. Dale and Causton (1992) proposed that the ratio of Chl a/b is an indicator of plant light availability. Reyes-Díaz et al. (2009) identified that Al-sensitive blueberry showed a higher decline of Chl a/b ratio than Al-tolerant one in response to Al stress. The decline of Chl a, concomitantly with a lower Chl a/b, in C. grandis leaves was also in agreement with the results in soybean leaves (Milićević and Stojačević 2003), suggesting a higher photosynthetic efficiency of C. sinensis than that of C. grandis. In addition to Chl a and Chl b, Car are also included in PSII antennae complexes in thylakoid membrane, which plays a role in PSII structure maintenance and excess energy dissipation (Niyogi 1999). Decline of Chl a would result in structural damage of antennae complex and inhibition of electron flow in C. grandis. On the other hand, a remarkable increment of the ratio of Car/Chl (a+b) in C. sinensis leaves would result in a protection from photooxidation (Havaux and Klopfstech 2001) by increasing heat dissipation (Niyogi et al. 1998).

The JIP-test is a valid and rapid technique to investigate the plant photosynthetic functions regarding the structure and behavior of PSII based on the theory of energy flux in biomembrane (Strasser et al. 2004). Abiotic stresses, such as temperature (Chen and Cheng 2009, Mathur et al. 2011, Snider et al. 2018), heavy metal (Appenroth et al. 2001, Li et al. 2010), salt (Lu et al. 2003), and drought (Shao et al. 2010, Rapacz et al. 2019), altered the shape of the OJIP test. In our study, the more pronounced decrease of fluorescence yield by Al stress was found in C. grandis leaves than that of C. sinensis (Fig. 1A), which might be attributed to the structural and/or functional imbalance of PSII under Al stress. The original OJIP curve was then double-normalized at specific phases to further reveal the fluorescence changes at O–J, O–K, J–I, and I–P steps (Fig. 1C–F). Tsirilis-Michael and Strasser (2013 a,b) reported \( \Delta W_0 \) as an indicator of energy conservation in photochemical phase. \( \Delta W_0 \) implied the energy connectivity between antennae complexes and reaction center. \( \Delta W_{\text{et}} \) characterized the electron transferred from \( Q_{s} \) to plastoquinone pool, and \( \Delta W_p \) demonstrated the electron flowed from reduced plastoquinone (PQH\(_{2}\)) to PSI end electron acceptors. Based on the theory, we found negative L-bands at 0.13 ms and K-bands at 0.3 ms in \( \Delta W_{\text{OK}} \) (Fig. 1C) and \( \Delta W_{\text{O}} \) (Fig. 1D) of two Citrus species, respectively. A similar finding was reported in drought-tolerant barley leaves under drought stress (Oukarroum et al. 2007). Mlinarić et al. (2017) reported that negative L-band in mature leaf compared to positive L-band in young leaf of common fig was associated with a higher utilization efficiency of excitation energy. As a probe of PSII donor side, the positive K-step was proposed to be related with the inactivation of oxygen-evolving system (Strasser et al. 2004). The present results of negative L-band and K-band indicated lesser destruction on energy connectivity among PSII units and oxygen-evolving system by 0.5 mM Al in two Citrus species, especially for C. grandis. With increasing Al concentrations, positive shifts of the L-band and K-band were found in two Citrus species (Fig. 1S, supplement). However, positive \( \Delta W_{\text{et}} \) reflected the impaired electron flow to plastoquinone in two Citrus species by Al stress. The impairment was more pronounced in C. grandis than that in C. sinensis under Al stress. Ceppi et al. (2012) proposed \( \Delta W_{\text{et}} \) as an indicator of leaf PSI content. Kalaji et al. (2018) reported negative IP-bands in strong Fe-deficiency treatment compared to positive IP-bands in less Fe-deficiency treatments of rapeseed leaf, which was ascribed to the greater accumulation of NADP\(^+\) under heavier stress. Similarly, in the present study, the C. grandis had a negative band, while C. sinensis had a partial positive band in \( \Delta W_{\text{et}} \) phase, which implied that the C. grandis might experience more severe inhibition of electron flow from PQH\(_{2}\) to electron acceptors at PSI end under Al stress compared to C. sinensis. In conclusion, the 0.5 mM Al-induced photosynthetic electron transport chain impairment was mainly attributed to the imbalance of reduction and oxidation of \( Q_{s} \) in two Citrus species. Overall, C. grandis suffered more from photoinhibition resulting from Al stress compared to C. sinensis. The inference was supported by a significant increase of \( F_{o} \), a characteristic of PSII RCs destruction (Moustakas et al. 1996), and also by a significant decrease of \( F_{v}/F_{m} \), associated with higher photoinhibition (Pereira et al. 2000). Besides, downregulation of energy fluxes for electron transport (ET\(_{o}\)/RC) and upregulation of \( F_{o} \), manifested less energy flowed to RCs (Kalaji et al. 2018) in C. grandis under Al stress.

The evidence that C. sinensis is much tolerant to Al stress than C. grandis was also supported by the regulation of \( F_{v}/F_{m} \) and Pl\(_{\text{abs}}\) under Al stress. \( F_{v}/F_{m} \) and Pl\(_{\text{abs}}\) are two most crucial parameters deduced from Chl a fluorescence test that indicated the plant PSII efficiency conferring environmental stresses (Force et al. 2003, Shapcott et al. 2007, Baker 2008). The value of \( F_{v}/F_{m} \) showed the energy conversion efficiency of PSII RCs and the Pl\(_{\text{abs}}\) served as an indicator of plant vitality (Clark et al. 2000, Strasser et al. 2000). In our study, Al stress decreased \( F_{v}/F_{m} \) in C. grandis compared with control; a similar finding was also reported in Al-treated tobacco leaves regardless of stress duration and stressor concentration (Li et al. 2012). Pl\(_{\text{abs}}\) was deduced from three parameters including \( \Psi_{m}, \Psi_{q_{0}} \) (\( F_{v}/F_{m} \)), and RC/ABS, of which the parameter \( \Psi_{m} \) calculated as \( ET_{o}/TR_{o} \), reflected the probability of electron transport beyond \( Q_{s} \) at the acceptor side of PSII (Lu and Vonshtak 1999), while RC/ABS provided information on the density of active PSII RCs per absorption. It was observed that the C. sinensis achieved significantly higher \( \Psi_{m} \), RC/ABS, and \( \Psi_{q_{0}} \) than C. grandis under Al stress. In contrast, despite
previous studies indicated that Al stress downregulated the \( P_{\text{ABS}} \) in \( C. \text{grandis} \) leaves (Jiang et al. 2008) and maize leaves (Zhao et al. 2017), our results showed that \( P_{\text{ABS}} \) was upregulated in \( C. \text{sinensis} \) leaves and significantly higher than that of \( C. \text{grandis} \) under long-term Al stress.

The Al stress downregulated the \( P_{\text{N}} \) without affecting the \( E \) significantly in two \( C. \text{citrus} \) species. Based on the stomatal limiting theory (Bethke and Drew 1992), the decreased \( P_{\text{N}} \) along with an increased \( C_{\text{i}} \) indicated that a nonstomatal factor, such as photodamage, resulted in decreased CO\(_2\) assimilation in \( C. \text{grandis} \) leaves by Al stress. However, the declined \( P_{\text{N}} \) and \( g_{\text{s}} \) without affecting \( C_{\text{i}} \) implied a significant nonstomatal limitation of photosynthesis in \( C. \text{sinensis} \), and the contribution of stomatal effect in \( C. \text{sinensis} \) could be also significant. Interestingly, the \( g_{\text{s}} \) was depressed in \( C. \text{citrus} \) leaves but not in \( C. \text{grandis} \) leaves. According to Ramani et al. (2006), a decreased \( g_{\text{s}} \) represented protective mechanism by lowering the toxic ions transported from roots to shoots under stress. Diaz-López et al. (2012) also evidenced that decreasing leaf \( g_{\text{s}} \) was effective for plants to avoid water loss under environmental stress. Besides, the CE was inhibited by Al stress in two \( C. \text{citrus} \) species, while the significant downregulation of WUE was only found in \( C. \text{grandis} \). The significant depression of CE was also observed in Al-treated longan seedlings (Xiao et al. 2005) and apparent inhibition of WUE was similar to the finding of Al-treated \( C. \text{citrus} \) species (Pereira et al. 2000), \( C. \text{Cacao} \) genotypes (Ribeiro et al. 2013) and \( Eucalyptus \) clones (Yang et al. 2015). Overall, it appeared that the \( C. \text{sinensis} \) was superbly adapted to long-term Al stress compared to \( C. \text{grandis} \) according to their photosynthetic performance.

In conclusion, the photosynthetic performance reflected by leaf pigment contents, JIP-test, and gas exchange comparisons verified that \( C. \text{citrus} \) species differed in Al tolerance. The Al stress-induced downregulation of CO\(_2\) assimilation was related to the imbalance of reduction and oxidation of Q\(_{a}\) in two \( C. \text{citrus} \) species. Compared to \( C. \text{grandis} \), \( C. \text{sinensis} \) had a higher ratio of Car/Chl \((a+b)\) and less inhibition of electron transfer which contributed to the photooxidation protection and electron conversion efficiency maintenance of PSII under Al stress.

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PHOTOSYNTHESIS OF CITRUS SPECIES UNDER ALUMINIUM STRESS


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