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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 $AlCl_3 \cdot 6H_2O$ (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 CO_2 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 (Al^{3+}) 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 Al^{3+} 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 (= P_N/C_i); Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; ET_0 – energy flux beyond Q_A; F_0 – minimal fluorescence of dark-adapted state; F_m – maximal fluorescence; F_v – variable fluorescence (= $F_m - F_0$); F_v/F_0 – the ratio of variable fluorescence to initial fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; PI_{abs} – performance index of absorption; P_N – net photosynthetic rate; RC – reaction center; TR_0 – energy trapping flux by PSII center; WUE – water-use efficiency (= P_N/E); Ψ_0 – efficiency that an electron moves further than Q_A^-.

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(2000) 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_v/F_0 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^{3+}, 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 spouting, 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 KH_2PO_4, 0.5 mM $MgSO_4$, 10 µM H_3BO_3, 2 µM $MnCl_2$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.065 µM $(NH_4)_6Mo_7O_{24}$, 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 $AlCl_3 \cdot 6\ H_2O$ (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 1,000–1,200 µmol(photon) m^{-2} s^{-1}, and 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 PSII was analyzed based on JIP-test (Strasser *et al*. 2000) using original fluorescence records. The relative variable fluorescence transient curves were normalized as $V_t = (F - F_0)/(F_m - F_0)$, and the $\triangle V_t$ was achieved by subtracting $V_{Control}$ from $V_{Al\ stress}$ according to Tsimilli-Michael and Strasser (2013a). V_t 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_{OK} [$W_{OK} = (F - F_0)/(F_K - F_0)$], W_{OJ} [$W_{OJ} = (F - F_0)/(F_J - F_0)$], W_{JI} [$W_{JI} = (F - F_J)/(F_I - F_J)$], and W_{IP} [$W_{IP} = (F - F_I)/(F_m - F_I)$], respectively. Then W_{OK}, W_{OJ}, W_{JI}, and W_{IP} were renormalized by subtracting $W_{Control}$ from $W_{Al\ stress}$, and expressed as $\triangle W_{OK}$, $\triangle W_{OJ}$, $\triangle W_{JI}$, and $\triangle W_{IP}$, 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 $P \leq 0.05$.

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. 1*A*. 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. 1*B* revealed that the curve decreased from 0 to 0.5 ms, while increased from 0.6 to 4.0 ms. Noticeably, the positive $\triangle V_t$ was found in *C. grandis* from 2.6 to 6.0 ms. As shown in Fig. 1*C*,*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 $\triangle W_{JI}$ and $\triangle W_{IP}$ 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 represents means ± SE ($n = 5$). Significant differences ($P \le 0.05$) between treatments are indicated by *different letters*. Chl – chlorophyll, Car – carotenoids.

Cultivar	Treatments	Chl *a* [mg m^{-2}]	Chl *b* [mg m^{-2}]	Chl (*a*+*b*) [mg m^{-2}]	Car [mg m^{-2}]	Chl *a*/*b*	Car/Chl (*a*+*b*)
C. sinensis	Control	484.52 ± 5.42^{ab}	154.44 ± 5.75^{ab}	638.96 ± 9.09^{a}	125.89 ± 1.76^{b}	3.15 ± 0.11^{a}	0.20 ± 0.01^{bc}
	Al toxicity	483.75 ± 3.44^{ab}	153.55 ± 3.77^{b}	637.31 ± 6.64^{a}	141.93 ± 1.10^{ab}	3.16 ± 0.06^{a}	0.22 ± 0.01^{a}
C. grandis	Control	529.86 ± 21.05^{a}	169.07 ± 5.71^{ab}	698.94 ± 25.88^{a}	152.99 ± 5.49^{a}	3.13 ± 0.08^{a}	0.22 ± 0.01^{ab}
	Al toxicity	455.13 ± 37.35^{b}	170.13 ± 4.85^{a}	625.26 ± 40.73^{a}	122.42 ± 15.29^{b}	2.67 ± 0.18^{b}	0.19 ± 0.01^{c}

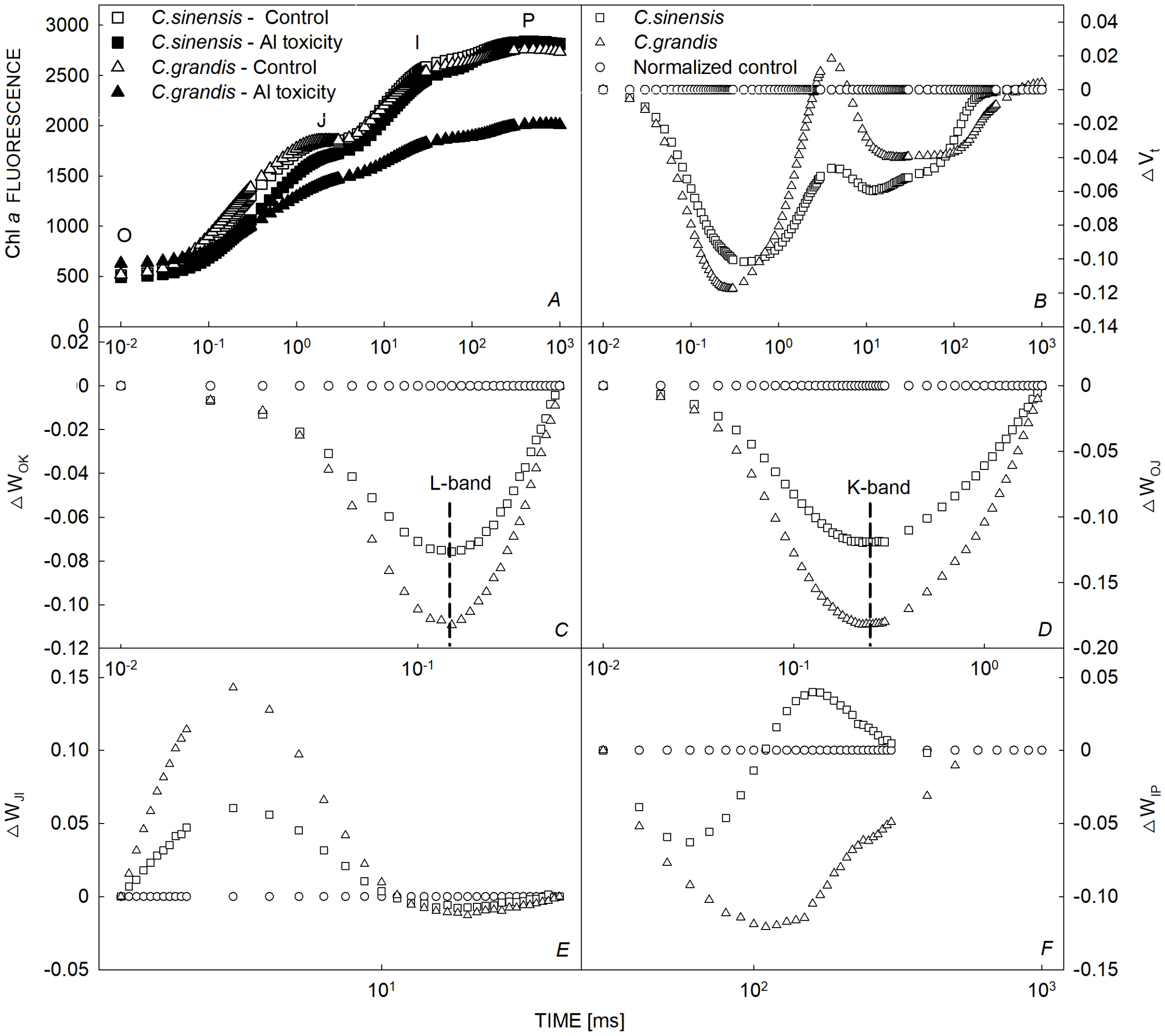

Fig. 1. The effects of Al stress on the leaf Chl *a* fluorescence transient of *Citrus sinensis* and *Citrus grandis* seedlings. (*A*) Chl *a* fluorescence; (*B*) $\triangle V_t$, $V_t = (F - F_0)/(F_m - F_0)$, $\triangle V_t = V_t$ (Al toxicity) – V_t (Control); (*C*) $\triangle W_{OJ}$, $W_{OJ} = (F - F_0)/(F_J - F_0)$, $\triangle W_{OJ} = W_{OJ}$ (Al toxicity) – W_{OJ} (Control); (*D*) $\triangle W_{OK}$, $W_{OK} = (F - F_0)/(F_K - F_0)$, $\triangle W_{OK} = W_{OK}$ (Al toxicity) – W_{OK} (Control); (*E*) $\triangle W_{JI}$, $W_{JI} = (F - F_J)/(F_I - F_J)$, $\triangle W_{JI} = W_{JI}$ (Al toxicity) – W_{JI} (Control); (*F*) $\triangle W_{IP}$, $W_{IP} = (F - F_I)/(F_m - F_I)$, $\triangle W_{IP} = W_{IP}$ (Al toxicity) – W_{IP} (Control). Values represent means of five replicates.

only presented in △W_{JI} of *C. grandis*.

Based on the data of Chl *a* fluorescence transients, we found a significant increase of F_0 (Fig. 2*A*), significant decrease of F_m (Fig. 2*B*), F_v (Fig. 2*C*), F_v/F_m (Fig. 2*D*), F_v/F_0 (Fig. 2*E*) 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. 2*A–E*). The Al stress had no obvious effect on ET_0/TR_0 (Fig. 2*F*) in two *Citrus* species. Also, the Al stress did not reduce absorption flux per RC (ABS/RC, Fig. 2*G*), whereas the trapped energy per RC (TR_0/RC, Fig. 2*H*) was significantly reduced in two *Citrus* species. Besides, the electron transport per RC (ET_0/RC, Fig. 2*I*) 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 (RE_0/RC, Fig. 2*J*) and performance index on absorption basis (PI_{abs}, Fig. 2*L*) than that of *C. grandis*.

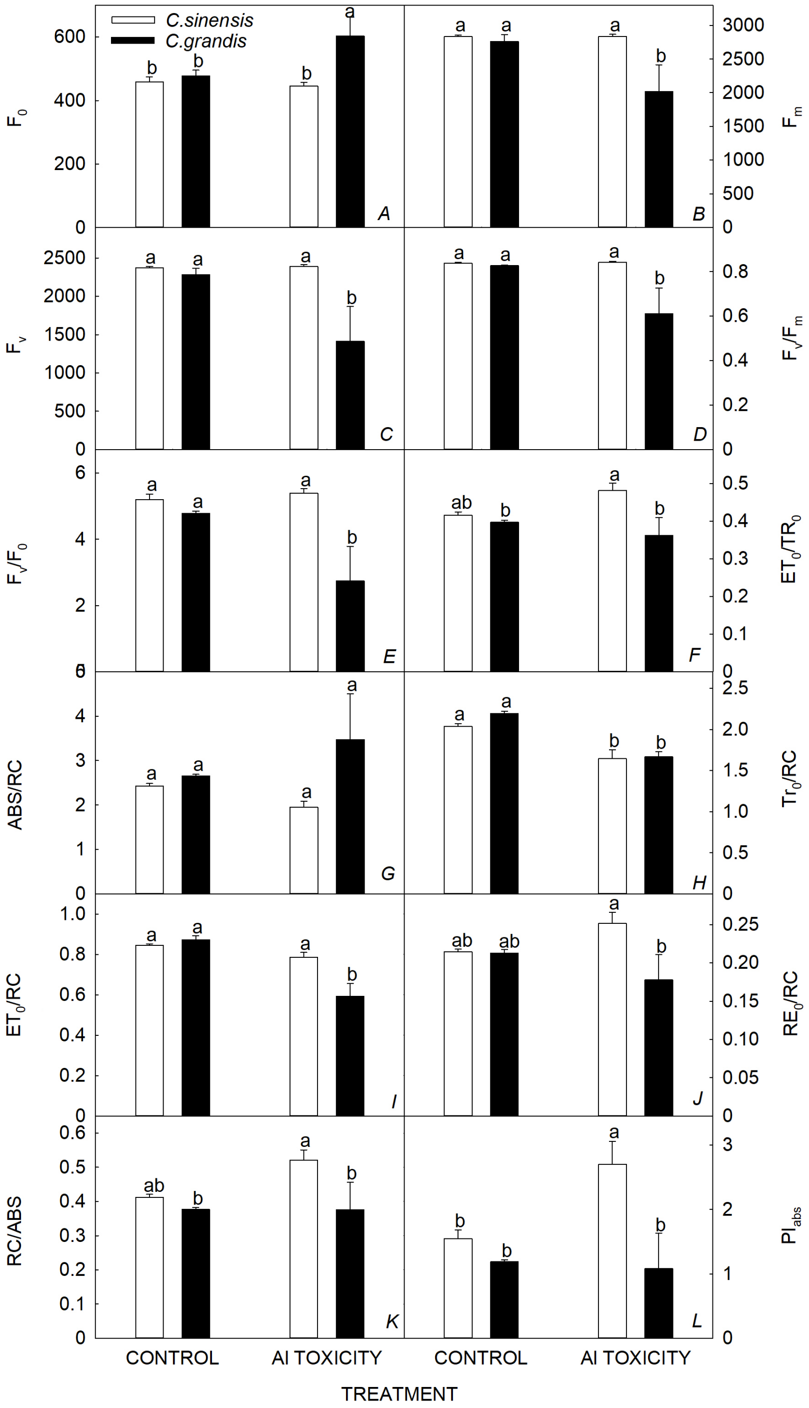

Fig. 2. The effects of Al stress on Chl *a* fluorescence parameters in leaves of *Citrus sinensis* and *Citrus grandis* seedlings. Values represent means ± SE (*n* = 5). Significant differences ($P \leq 0.05$) between treatments are indicated by *different letters*. Minimal fluorescence of dark-adapted 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/TR_0) (*F*), the absorption flux per reaction center (ABS/RC) (*G*), the trapped energy per reaction center (TR_0/RC) (*H*), the electron transport per reaction center (ET_0/RC) (*I*), reduction of end acceptors at PSI electron acceptor side per reaction center (RE_0/RC) (*J*), the density of active PSII reaction centers per absorption (RC/ABS) (*K*), performance index on the absorption basis (PI_{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. 3*A,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. 3*B,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. 3*D*). Moreover, the Al stress significantly inhibited WUE in *C. grandis* but not in *C. sinensis* compared with control (Fig. 3*F*).

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*.

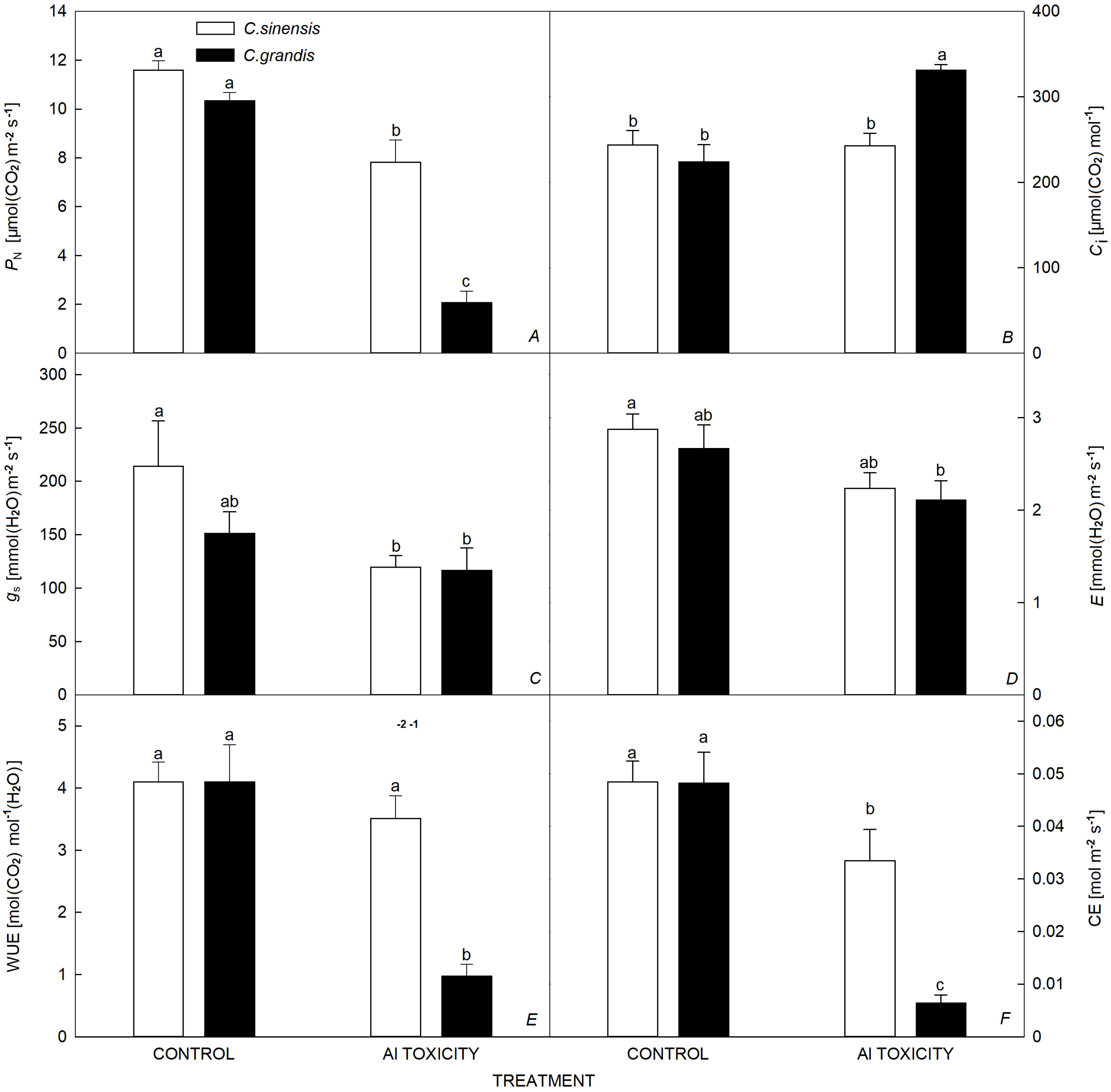

Fig. 3. The effects of Al stress on the leaf gas exchange of *Citrus sinensis* and *Citrus grandis* seedlings. Values represent means ± SE (*n* = 5). Significant differences ($P \leq 0.05$) between treatments are indicated by *different letters*. Net photosynthetic rate (P_N) (*A*), intercellular CO_2 concentration (C_i) (*B*), stomatal conductance (g_s) (*C*), transpiration rate (*E*) (*D*), water-use efficiency (WUE) (*E*), carboxylation efficiency of Rubisco (CE) (*F*).

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-Diaz *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 (Milivojević and Stojanović 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 Kloppstech 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 transient. 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. 1*A*), 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. 1*C*–*F*). Tsimilli-Michael and Strasser (2013 a,b) reported $\triangle W_{OJ}$ as an indicator of energy conservation in photochemical phase. $\triangle W_{OK}$ implied the energy connectivity between antennae complexes and reaction center, $\triangle W_{JI}$ characterized the electron transferred from Q_A^- to plastoquinone pool, and $\triangle W_{IP}$ 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 $\triangle W_{OK}$ (Fig.1*C*) and $\triangle W_{OJ}$ (Fig.1*D*) 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 $\triangle W_{JI}$ 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 $\triangle W_{IP}$ 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 $\triangle W_{IP}$ 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_A 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_0, a characteristic of PSII RCs destruction (Moustakas *et al*. 1996), and also by a significant decrease of F_v/F_0, associated with higher photoinhibition (Pereira *et al*. 2000). Besides, downregulation of energy fluxes for electron transport (ET_0/RC) and upregulation of F_0, 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 PI_{abs} under Al stress. F_v/F_m and PI_{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 PI_{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). PI_{abs} was deduced from three parameters including Ψ_0, φ_{P0} (F_v/F_m), and RC/ABS, of which the parameter Ψ_0, calculated as ET_0/TR_0, reflected the probability of electron transport beyond Q_A at the acceptor side of PSII (Lu and Vonshak 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 Ψ_0, RC/ABS, and φ_{P0} than *C. grandis* under Al stress. In contrast, despite previous studies indicated that Al stress downregulated the PI_{abs} in *C. grandis* leaves (Jiang *et al*. 2008) and maize leaves (Zhao *et al*. 2017), our results showed that PI_{abs} was upregulated in *C. sinensis* leaves and significantly higher than that of *C. grandis* under long-term Al stress.

The Al stress downregulated the P_N without affecting the E significantly in two *Citrus* species. Based on the stomatal limiting theory (Bethke and Drew 1992), the decreased P_N along with an increased C_i indicated that a nonstomatal factor, such as photodamage, resulted in depression of CO_2 assimilation in *C. grandis* leaves by Al stress. However, the declined P_N and g_s without affecting C_i implied a significant nonstomatal limitation of photosynthesis in *C. sinensis*, and the contribution of stomatal effect in *C. sinensis* could be also significant. Interestingly, the g_s was depressed in *C. sinensis* leaves but not in *C. grandis* leaves. According to Ramani *et al*. (2006), a decreased g_s represented protective mechanism by lowering the toxic ions transported from roots to shoots under stress. Díaz-López *et al*. (2012) also evidenced that decreasing leaf g_s was effective for plants to avoid water loss under environmental stress. Besides, the CE was inhibited by Al stress in two *Citrus* species, while the significant downregulation of WUE was only found in *C. 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 *Citrus* species (Pereira *et al*. 2000), *Cacao* genotypes (Ribeiro *et al*. 2013) and *Eucalyptus* clones (Yang *et al*. 2015). Overall, it appeared that the *C. sinensis* was superbly adapted to long-term Al stress compared to *C. grandis* according to their photosynthetic performance.

In conclusion, the photosynthetic performance reflected by leaf pigment contents, JIP-test, and gas exchange comparisons verified that *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 *Citrus* species. Compared to *C. grandis*, *C. 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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