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Effects of 5-aminolevulinic acid treatment on photosynthesis of strawberry

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Abstract

Effects of root treatment with 5-aminolevulinic acid (ALA) on leaf photosynthesis in strawberry (*Fragaria ananassa* Duch.) plants were investigated by rapid chlorophyll fluorescence and modulated 820 nm reflection using 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) and methyl viologen (MV). Our results showed that ALA treatments increased the net photosynthetic rate and decreased the intercelluar CO_2 concentration in strawberry leaves. Under DCMU treatment, trapping energy for Q_A reduction per PSII reaction center increased greatly, indicating DCMU inhibited electron transfer from Q_A^-. The maximum photochemical efficiency of PSII (F_v/F_m) decreased under the DCMU treatment, while a higher F_v/F_m remained in the ALA-pretreated plants. Not only the parameters related to a photochemical phase, but also that one related to a heat phase remained lower after the ALA pretreatment, compared to the sole DCMU treatment. The MV treatment decreased PSI photochemical capacity. The results of modulated 820 nm reflection analysis showed that DCMU and MV treatments had low re-reduction of P700 and plastocyanin (PSI). However, the strawberry leaf discs pretreated with ALA exhibited high re-reduction of PSI under DCMU and MV treatments. The results of this study suggest that the improvement of photosynthesis by ALA in strawberry was not only related to PSII, but also to PSI and electron transfer chain.

Additional key words: chlorophyll fluorescence; gas exchange; heat phase; photochemical phase, photosystem I, photosystem II.

Introduction

Strawberry (*Fragaria ananassa* Duch.) is one of the most important fruits in the temperate climatic zone. World production reached more than 4,516,810 t annually (Sowik *et al.* 2015). ALA is the first essential biosynthetic precursor of all porphyrin compounds, such as chlorophyll (Chl), heme, and phytochrome (von Wettstein *et al.* 1995). Results of previous studies suggest that ALA may have wide potential of applications in agriculture, such as increase of photosynthesis and plant growth (Hotta *et al.* 1997, Wang *et al.* 2003), increase in net photosynthetic rate (P_N) under NaCl stress (Zhao *et al.* 2015) and improvement of fruit quality (An *et al.* 2016), as a natural and environmentally friendly substance. However, the mechanisms of ALA effects on plant growth have not yet been elucidated. Several reports suggested that stimulatory effects of ALA on plant growth might be related to its stimulation of leaf photosynthesis (Nishihara *et al.* 2003, Wang *et al.* 2004, Memon *et al.* 2009). The improvement of photosynthesis by ALA may be related to the increase of electron transport rate (Sun *et al.* 2009, Zhao *et al.* 2014). However, it is not known how ALA could improve the electron transport.

DCMU is an inhibitor of electron transfer, which blocks the PSII electron transfer in thylakoid membranes and therefore inhibits photosynthesis (Komenda *et al.* 2000). It is known that DCMU binds to the Q_B site of D1

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Abbreviations: ALA – 5-aminolevulinic acid; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; E – transpiration rate; F_j and F_i – the fluorescence intensity at 2 ms (J-step) and 30 ms (I-step); F_m – the maximum fluorescence intensity; F_o – the minimum fluorescence intensity; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; MR – modulated 820 nm reflection; MR_o – value of modulated 820 nm reflection at the onset of red light illumination; MV – methyl viologen; P680 – PSII reaction center; P700 – PSI reaction center; P_N – net photosynthetic rate; PC – plastocyanin; PQH_2 – plastoquinol; RC/ABS – Q_A reducing reaction centers per PSII antenna chlorophyll; V_J – the relative variable fluorescence intensities at the J-step; V_I – the relative variable fluorescence intensities at the I-step; V_{PSI} – maximum slope of decrease of MR/MR_o; $V_{PSII\text{-}PSI}$ – maximum slope of increase of MR/MR_o.
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protein of PSII and inhibits the reduction of Q_B, blocking the electron transfer. MV is thought to be a very effective electron acceptor that competes strongly with ferredoxins (Fd) for electrons from the FeS-clusters of PSI and as a consequence strongly suppresses also the cyclic electron transfer around PSI (Schansker *et al.* 2005). If electron transfer inhibitors, such as DCMU and MV, are used in study of ALA regulation, the results may be helpful in order to elucidate the mechanisms of ALA on improving photosynthesis of plants.

Photosynthetic electron transport from water to $NADP^+$ is driven by PSII and PSI. There are electron carriers in PSII and PSI, such as primary and secondary quinone electron acceptor of PSII (Q_A and Q_B), plastoquinone (PQ), and plastocyanin (Lazar, 2006). They play an important role in regulating of electron flow between PSII and PSI (Yan *et al.* 2013). When a dark-adapted photosynthetic sample is illuminated by high intensity of excitation light [*ca*. 3,000 μmol(photon) m^{-2} s^{-1} and more], usually two steps appear in chlorophyll (Chl) *a* fluorescence rise between minimal (F_o; denoted as O) and maximal (F_m; denoted as P) levels; the first step at 2 ms and the second step at 30 ms. The steps are frequently denoted as J and I, respectively, and the rise is called the OJIP transient (Lazar, 2009). In recent years, biological roles for kinetic phases of this transient were reported (Schansker *et al.* 2011, Zushi *et al.* 2012). In addition, PSI redox change can be detected by changes in 820 nm reflection, as they significantly correlate with each other (Jiang *et al.* 2006, Gao *et al.* 2014). Recently, simultaneous detection of OJIP and 820 nm reflection transients have been used as a feasible way to explore photosynthetic electron transport process and the interaction between PSII and PSI. By using this technique, the effects of dehydration, drought, and heat stress on the photosynthetic electron transport chain were recently reported in bean, apple, and pea leaves (Li and Ma 2012, Goltsev *et al.* 2012, Oukarroum *et al.*2013). In this paper, we used simultaneous measurement of Chl *a* fluorescence and modulated 820 nm reflection to explore the mechanisms of photosynthetic performance improvement by ALA in strawberry.

Materials and methods

Experimental material and ALA treatment: The experiment was conducted at Horticultural Experimental Station, Institute of Nanjing Agricultural Sciences, Jiangsu Province, China. Plants of strawberry (*Fragaria ananassa* Duch., cv. 'Benihonpe') with four fully expanded leaves were transferred into plastic pots (diameter of 20 cm) which were filled with 8 kg of garden soil containing 7.5% of mature cattle manure at the beginning of September 2013. Then, the potted plants were placed in a plastic greenhouse with usual further management (Han *et al.* 2009). One week later, the plants were treated with 100 ml of water containing 0 (control), 75 μM, or 150 μM ALA per plant through a root irrigation. There were twenty plants in each treatment. Ten days after ALA treatment, the leaf gas-exchange parameters, Chl fluorescence parameters, and modulated 820 nm reflection were measured.

Measurements of leaf gas-exchange parameters: Using a portable *LI-6400* system (*LI-Cor Inc*., Lincoln, NE, USA), net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration *(E*) were measured simultaneously with an internal light source of 1,000 μmol(photon) m^{-2} s^{-1} at 10 d after treatment with ALA. During the measurement, the ambient air CO_2 concentration was about 400 μmol mol^{-1} and the temperature about 20ºC. Each treatment was repeatedly measured 15 times. The means were used to compare the effect of treatments.

DCMU and MV treatments and measurements of Chl *a* fluorescence transient and modulated 820 nm reflection: Ten days after the ALA treatment, the leaves from the 150 μM ALA-treated plants and the untreated ones were detached, leaf discs with a diameter of 2 cm were punched out, then placed on wet filter paper in petri dishes immediately. The leaf discs from the control plants were soaked in 1% ethanol (control, since DCMU was dissolved in ethanol, the final ethanol concentration of solution was diluted to 1%), 0.5 mmol L^{-1} DCMU solution or 0.2 mmol L^{-1} MV solution, respectively. The leaf discs from the ALA-treated plants in 0.5 mmol L^{-1} DCMU solution (ALA + DCMU) or 0.2 mmol L^{-1} MV solution (ALA + MV) were incubated for 2 h in the darkness. Then, all leaf discs were used to measure the Chl fluorescence, with at least 15 leaf discs of each treatment measured.

The measurements were carried out by using a multifunctional plant efficiency analyzer (*M-PEA2*, *Hansatech*, UK). Monitoring modulated reflection change near 820 nm is a very convenient way to follow the redox state of PSI (Strasser *et al.* 2010). The leaf discs incubated in darkness for 2 h with different treatments were illuminated with 1-s pulse of continuous red light [627 nm, 5,000 μmol(photon) m^{-2} s^{-1}] and subsequently, with far-red light [735 nm, 200 μmol(photon) m^{-2} s^{-1}] for 10 s. Chl *a* fluorescence and modulated 820 nm reflection were recorded during the illumination.

Chl fast fluorescence transients were quantified according to the JIP test: (*1*) fluorescence intensity at 20 μs (F_o, when all reaction centers of PSII are open); (*2*) the maximum fluorescence intensity (F_m, when all reaction centers of PSII are closed), and (*3*) fluorescence intensities at 300 μs (K step), 2 ms (J step), and 30 ms (I step). Using these original data, some parameters can be calculated for quantifying PSII behavior (Strasser *et al.*2000, Appenroth *et al.* 2001, Panda *et al.* 2006).

Parameter	Explanation	Calculation
F_v/F_m	Maximum quantum yield for primary photochemistry	$F_v/F_m = 1 - F_o/F_m$
V_t	Relative variable fluorescence intensities at K-step (V_K), J-step (V_J), and I-step (V_I)	$V_t = (F_t - F_o)/(F_m - F_o)$
M_o	Approximate initial slope of the fluorescence transient	$M_o = 4 \times (F_{300\ \mu s} - F_o)/(F_m - F_o)$
W_K	Amplitude of the K step	$W_K = (F_{300\ \mu s} - F_o)/(F_{2ms} - F_o)$
ET_o/TR_o	Probability that an electron moves beyond Q_A	$ET_o/TR_o = 1 - V_J$
RE_o/ET_o	Probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side	$RE_o/ET_o = (1 - V_I)/(1 - V_J)$
RC/CS_o	Density of P680 per excited cross section	$RC/CS_o = F_v/F_m \times (V_J/M_o) \times F_o$
RC/ABS	Q_A reducing reaction centers per PSII antenna chlorophyll	$RC/ABS = M_o \times (1/V_J) \times (ABS/TR_o)$

At the onset of the red light illumination (0.7 ms), PSI (P700 and plastocyanin) was entirely in the reduced state. After the far-red illumination, PSI was completely oxidized. The declined amplitude of the modulated 820 nm reflection intensity due to PSI redox change can reflect PSI photochemical capacity (Li and Ma 2012, Yan *et al.* 2012). In order to exclude interference caused by geometry differences between samples, modulated 820 nm reflections were expressed as MR/MR_o, where MR_o is the modulated reflection value at the onset of actinic illumination (taken at 0.7 ms, the first reliable MR measurement) and MR is the modulated reflection signal during illumination. Maximum decrease in slope (V_{PSI}, in the range of 0.7–3 ms) and maximum increase in slope ($V_{PSII\text{-}PSI}$, in the range of 7–300 ms) of MR/MR_o were calculated (Gao *et al.* 2014).

Statistical analysis: All data were subjected to analysis of variance (*ANOVA*) to assess significant differences at p=0.05 by *Duncan*'s test.

Results

Effect of ALA on gas-exchange parameters of strawberry leaves: The P_N of strawberry leaves was improved dramatically at 10 d after root treatment with 75 μM or 150 μM ALA (Table 1). P_N increased by 17.3% after the 75 μM ALA treatment or by 25.6% after 150 μM ALA treatment. On the other hand, the ALA treatment decreased C_i. C_i decreased by 11.6 or by 21.7% after 75 μM or 150 μM ALA treatment, respectively. The higher concentration of ALA lowered C_i more the lower concentrations. Therefore, the decrease of C_i was induced by the increase of P_N in strawberry leaves. There was no significant influence on g_s and E by ALA treatments (Table 1).

Effects of DCMU and MV on M_o/V_J and V_{PSI}: The DCMU treatment increased trapping energy for Q_A reduction per PSII reaction center (M_o/V_J) by 56.1% (Fig. 1*A*), which reflected that the DCMU treatment inhibited electron transfer afterwards from Q_A^- and increased the trapping energy for Q_A reduction per PSII reaction center. However, MV had no effect on M_o/V_J. On the other hand, the maximum slope decrease of MR/MR_o (V_{PSI}) was not affected by DCMU but significantly decreased by the MV treatment (Fig. 1*B*). These results suggested that DCMU mainly affected PSII, while MV mainly affected PSI in photosynthetic electron transfer.

Effects of different treatments on Chl *a* fluorescence and OJIP transient curves and the related parameters: From dark-adapted state, when all PSII reaction centers are open (Q_A is fully oxidized) and minimal fluorescence is detected (the O-step), the PSII reaction centers are gradually closing upon illumination reaching fully closed state (Q_A is fully reduced) and maximal fluorescence is detected (the P-step). The intermediate waves (the J- and

Table 1. Effects of 5-aminolevulinic acid (ALA) treatment on photosynthetic characteristics in strawberry leaves. P_N – net photosynthetic rate, g_s – stomata conductance, C_i – intercellular CO_2 concentration, E – transpiration rate. The data are the means ± SD of 15 repeated measurements of separate samples. The same *small letters* in the same columns represent no significant difference at p=0.05 level. The strawberry plants were root-treated with 100 ml of water (control), 75 μM ALA, or 150 μM ALA per plant.

Treatment	P_N [μmol m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol(CO_2) mol^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]
Control	13.545 ± 0.046^b	0.200 ± 0.011^a	233.618 ± 11.212^a	8.667 ± 0.042^a
75 μM ALA	15.889 ± 0.037^a	0.171 ± 0.012^a	206.633 ± 5.334^{ab}	7.661 ± 0.017^a
150 μM ALA	17.013 ± 0.021^a	0.186 ± 0.014^a	182.891 ± 4.558^b	8.250 ± 0.033^a

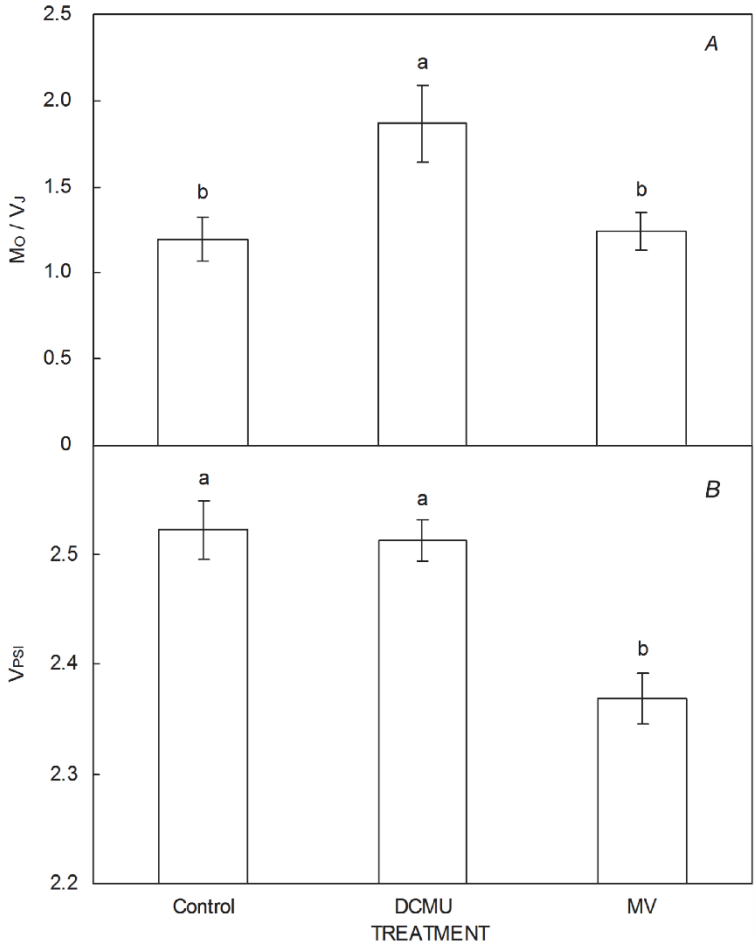

Fig. 1. Effects of DCMU and methyl viologen (MV) treatments on trapping energy for Q_A reduction per PSII reaction center (M_o/V_J) (*A*) and the maximum slope of decrease of MR/MR_o (V_{PSI}) (*B*) in strawberry leaves. The data presented were the means ± SD of 15 repeated measurements of separate samples, The different *small letters* represent the significant differences (p<0.05).

I-steps) in the fluorescence rise curve reflect subsequent kinetic bottlenecks of the electron transport chain. The fluorescence intensity at the K-step and J-step were significantly elevated after the DCMU treatment, while ALA+DCMU showed lower values at those steps than those with only DCMU (Fig. 2). However, the fluorescence intensity increased significantly only at the I-step in the MV treatment, and ALA+MV showed lower fluorescence intensity than that of MV only.

The DCMU treatment increased the minimum fluorescence intensity (F_o) of strawberry leaves greatly (Table 2). The F_o in strawberry leaves with ALA+DCMU was lower than that of the DCMU treatment only without ALA pretreatment. The DCMU treatment decreased F_v/F_m in the dark-adapted state (Table 2). However, the ALA pretreatment followed by DCMU treatment had higher F_v/F_m than that of the DCMU treatment only without ALA pretreatment. Thus, DCMU decreased the photochemical capacity of PSII reaction center of strawberry leaves greatly, while the ALA pretreatment followed by DCMU treatment remained higher photochemical capacity than that of the DCMU treatment only without ALA pretreatment.

The density of P680 per excited cross section (RC/CS_o) after the DCMU treatment decreased significantly, while the relative variable fluorescence intensity at the K-step (V_K), J-step (V_J), and I-step (V_I) after the DCMU treatment increased significantly (Table 2). The leaves pretreated with ALA followed by DCMU treatment had lower V_K, V_J, and V_I than those of DCMU treatment only without ALA pretreatment. The approximate initial slope of the fluorescence transient (M_o) and amplitude of the K- step (W_K) also increased greatly by DCMU, while ALA pretreatment followed by DCMU treatment resulted in lower M_o and W_K values than those of the DCMU treatment only without ALA pretreatment. The DCMU treatment decreased amount of Q_A reducing reaction centers per PSII antenna Chl (RC/ABS) and the probability that an electron moves further than Q_A (ET_o/TR_o), while the ALA pretreatment followed by the DCMU treatment resulted in higher RC/ABS and ET_o/TR_o than those of the DCMU treatment only (Table 2).

The MV treatment increased F_m and relative variable fluorescence intensities at the I-step (V_I). The ALA+MV treatment showed lower F_m and V_I values than those of the MV treatment only without ALA pretreatment (Table 2). The probability that an electron transfer from the intersystem electron carriers enables reduction of the end electron acceptors at the PSI acceptor side (RE_o/ET_o) decreased under the MV treatment, while the ALA pretreatment under MV treatment showed higher RE_o/ET_o (Table 2).

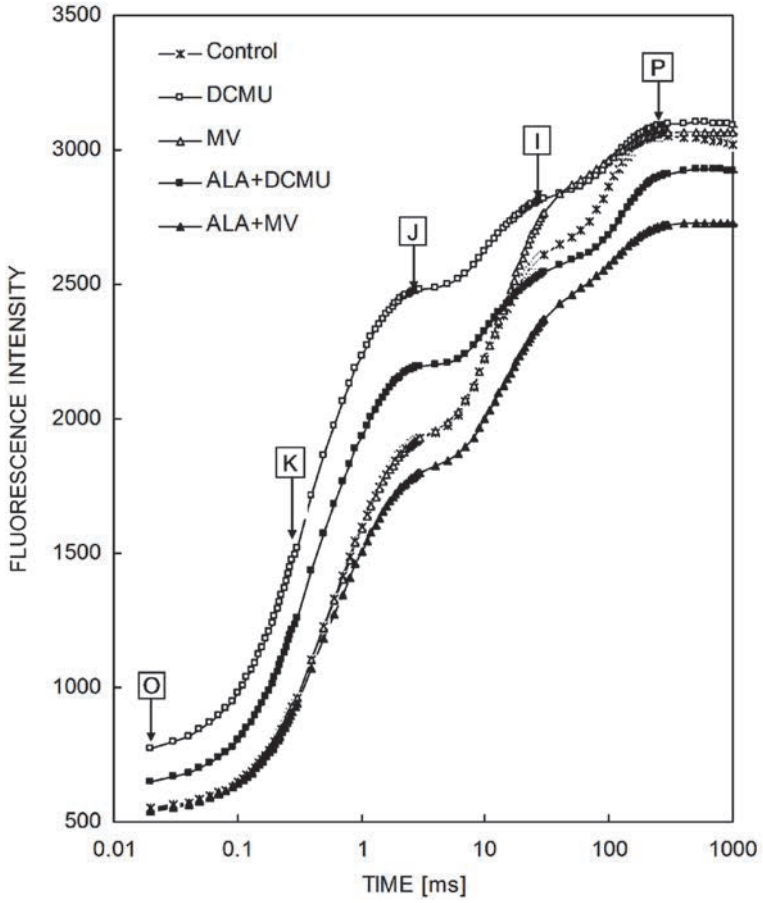

Fig. 2. Effects of 150 μM 5-aminolevulinic acid (ALA) pretreatment on the chlorophyll *a* fluorescence OJIP transient curves under inhibitor DCMU or methyl viologen (MV) treatment. The signals are plotted on a logarithmic time scale from 20 μs to 1 s. The letters O, K, J, I, and P refer to the selected time points used by the JIP-test for the calculation of structural and functional parameters.

Table 2. Effects of different treatments on rapid chlorophyll fluorescence parameters. The different *small letters* in the same row show the significant difference at the level of p=0.05. F_o – fluorescence intensity at 20 μs (when all reaction centers of PSII are open); F_v/F_m – maximal quantum yield of PSII photochemistry; V_K – relative variable fluorescence intensity at 300 μs; V_J – relative variable fluorescence intensity at J step; V_I – relative variable fluorescence intensity at I step; M_o – the approximate initial slope of the fluorescence transient ; W_K – amplitude of the K step; ET_o/TR_o – the probability that an electron moves further than Q_A; RC/CS_o – the density of P680 per excited cross section; RC/ABS – Q_A reducing reaction centers per PSII antenna chlorophyll; RE_o/ET_o – the probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side.

Parameter	Treatment Control	DCMU	ALA+DCMU	MV	ALA+MV
F_o	538.5 ± 19.4^{c}	797.0 ± 25.8^{a}	649.7 ± 27.0^{b}	550.3 ± 19.8^{c}	535.4 ± 12.9^{c}
F_m	2,994.0 ± 115.3^{bc}	3,108.2 ± 121.4^{ab}	2,923.9 ± 127.9^{c}	3,198.5 ± 116.2^{a}	2,869.8 ± 108.4^{c}
F_v/F_m	0.820 ± 0.008^{a}	0.742 ± 0.038^{c}	0.777 ± 0.029^{b}	0.828 ± 0.008^{a}	0.813 ± 0.019^{a}
V_K	0.152 ± 0.024^{c}	0.360 ± 0.071^{a}	0.271 ± 0.081^{b}	0.156 ± 028^{c}	0.153 ± 0.054^{c}
V_J	0.507 ± 0.043^{c}	0.766 ± 0.063^{a}	0.663 ± 0.088^{b}	0.500 ± 0.050^{c}	0.505 ± 0.079^{c}
V_I	0.811 ± 0.027^{b}	0.892 ± 0.033^{a}	0.834 ± 0.031^{b}	0.886 ± 0.020^{a}	0.837 ± 0.014^{b}
M_o	0.608 ± 0.096^{c}	1.440 ± 0.284^{a}	1.084 ± 0.324^{b}	0.623 ± 0.110^{c}	0.611 ± 0.216^{c}
W_K	0.299 ± 0.032^{c}	0.467 ± 0.056^{a}	0.401 ± 0.069^{b}	0.309 ± 0.027^{c}	0.297 ± 0.067^{c}
RC/ABS	0.694 ± 0.080^{a}	0.404 ± 0.065^{c}	0.501 ± 0.099^{b}	0.674 ± 0.066^{a}	0.709 ± 0.128^{a}
ET_o/TR_o	0.493 ± 0.043^{a}	0.234 ± 0.063^{c}	0.337 ± 0.087^{b}	0.500 ± 0.050^{a}	0.495 ± 0.079^{a}
RC/CS_o	371.4 ± 29.9^{a}	317.3 ± 21.74^{b}	318.7 ± 33.28^{b}	369.4 ± 19.74^{a}	382.5 ± 26.86^{a}
RE_o/ET_o	0.382 ± 0.045^{b}	0.466 ± 0.091^{a}	0.509 ± 0.074^{a}	0.229 ± 0.033^{c}	0.315 ± 0.166^{b}

Effects of different treatments on modulated 820 nm reflection (MR/MR_0) and the related parameters: After two hours in the darkness, P700 and PC of the leaf discs were mostly in a completely reduced state. Upon exposure to actinic light, the two photosystems were excited separately. The accumulation of P_{700}^{+} and PC^{+} increased

absorbance at 820 nm, resulting in a decreased MR/MR_o (fast phase). Subsequently, electrons coming from PSII arrived at P_{700}^+ and PC^+ and re-reduced the electron carriers, causing the decrease in MR/MR_o. At the end of the fast phase (minimal MR/MR_o), the oxidation and re-reduction rates equaled, and MR/MR_o fell in a transitory steady state. Once the re-reduction rate was faster than the oxidation rate, MR/MR_o began to increase (slow phase) and stabilized at about the same time as OJIP transient reached maximum. The treatment of DCMU or MV decreased the re-reduction rate of P_{700} and PC (*i.e.*, maximum slope of increase in MR/MR_o), while the pretreatment with ALA (ALA+DCMU or ALA+MV) resulted in a higher re-reduction rate of P_{700} and PC than those with DCMU or MV treatment only (DCMU or MV) (Fig. 3).

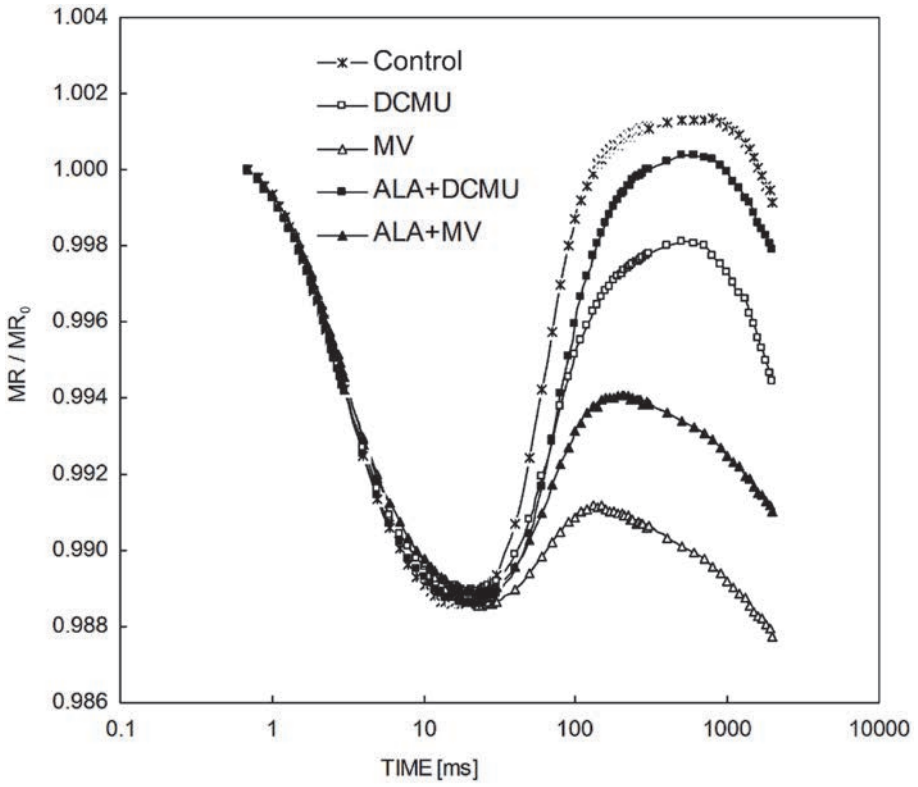

Fig. 3. Effect of pretreatment with 5-aminolevulinic acid (ALA) on modulated 820 nm reflection (MR/MR_o) of strawberry leaf disks under inhibitor DCMU or methyl viologen (MV) treatment. The signals were plotted on a logarithmic time scale. Each data point is the average of 15 replicates.

Table 3. Effects of treatments on parameters derived from modulated 820 nm reflection (MR/MR_o) of strawberry leaf disks. *Different letters* indicate a significant difference at $p<0.05$. Each value is the means ± SD of 15 replicates. V_{PSI} – maximum slope of decrease of MR/MR_o; $V_{PSII-PSI}$ – maximum slope of increase of MR/MR_o.

Parameter	Treatment Control	DCMU	ALA+DCMU	MV	ALA+MV
V_{PSI}	2.523 ± 0.047^a	2.513 ± 0.031^a	2.544 ± 0.066^a	2.369 ± 0.044^b	2.373 ± 0.032^b
$V_{PSII-PSI}$	0.038 ± 0.003^a	0.025 ± 0.002^b	0.033 ± 0.003^a	0.010 ± 0.003^d	0.018 ± 0.002^c

The MV treatment decreased the value of V_{PSI} significantly (Table 3), suggesting the oxidation rates of P700 and PC decreased and the photochemical activity of PSI became lower than that of control. The DCMU and MV treatments decreased the value of $V_{PSII-PSI}$ significantly, indicating the re-reduction of P700 and PC decreased. The strawberry leaves with the ALA pretreatment followed by DCMU or MV treatment had higher $V_{PSII-PSI}$ than those of DCMU or MV treatment only without ALA pretreatment (Table 3). This indicates that the ALA pretreatment caused high re-reduction capacity of P700 and PC when treated with inhibitors DCMU or MV.

Discussion

Results of this study showed that the treatment with ALA can improve photosynthesis of strawberry plants, which is consistent with previous studies. Hotta *et al.* (1997) reported that ALA possesses plant growth-regulating

properties when it was applied at low concentrations and subsequently may enhance agricultural production. Wang *et al.* (2004) showed that application of ALA significantly increased P_N of melon seedlings. Liu *et al.* (2006) also reported that spraying ALA in a concentration of 100 mg L^{-1} increased strawberry P_N. Results of this study also showed that the P_N of strawberry leaves was improved dramatically ten days after the 75 or 150 μM ALA treatment. Recently, Zhao *et al.* (2014) has reported that the enhanced salt tolerance of tomato seedlings by ALA was related to its effect on enhancing the photosynthetic capacity, PSII photochemical efficiency, and apparent electron transfer rate. The improvement of photosynthetic capacity by ALA on strawberry leaves may also be related to its effect on the electron transport chain.

In this work, we found that the DCMU treatment increased significantly trapping energy for Q_A reduction per PSII reaction center (M_o/V_J). Stirbet and Govindjee (2011) showed that M_o/V_J could indicate the maximum specific trapped exciton flux. The increase of M_o/V_J suggests that DCMU inhibited electron transfer afterwards from Q_A^- along the electron transfer chain. The results agreed with the work done by Strasser and Strasser (1995). Results of the MV treatment showed that MV can decrease PSI photochemical capacity of strawberry. The inhibition effects of DCMU and MV on PSII and PSI are useful for investigating improvement of photosynthesis by ALA through the electron transfer chain. The increased relative variable fluorescence intensity at J-step (V_J) and I-step (V_I) under DCMU treatment suggest DCMU affected both the photochemical phase and the thermal phase in strawberry. The results of this study showed that the pretreatment with ALA followed by DCMU (ALA+DCMU) decreased V_J and V_I values compared with the DCMU treatment only. We suggest that ALA can protect both the photochemical phase and the thermal phase in strawberry. The protection of the photochemical phase by ALA was supported by further investigation of the changes in RC/ABS and ET_o/TR_o. These results showed that the amount of Q_A reducing reaction centers per PSII antenna Chl (RC/ABS) and the probability that an electron moves further than Q_A (ET_o/TR_o) decreased after the DCMU treatment without ALA pretreatment but increased after the ALA pretreatment followed by the DCMU treatment. These results provided further evidence that ALA can protect the photochemical phase in strawberry. It has been known that only the first rise phase (O-J) reflected the reduction of Q_A and the second rise phase (J-I-P) represented another process. The fast rise phase has been known as the photochemical phase (strong dependence on the light intensity) and the slower (J-I-P) rise phase has been known as the thermal phase (less light-intensity dependent but more sensitive to high temperature) (Lazar 2006). Therefore, the ALA treatment could protect electron transfer around the PSII reaction center, which is an important reason for the higher photosynthetic rate in strawberry leaves.

The MV treatment increased the maximum fluorescence intensity (F_m) and relative variable fluorescence intensities at I-step (V_I) but had no effects on V_J, indicating that only the parameters related to the thermal phase were influenced by MV. Usually, the time during which MR/MRo is minimal, is the J-I phase of PF (*i.e.*, during the partial reduction of the PQ pool) (Tóth *et al*. 2007), and the slow phase develops mainly during the I-P phase of PF (*i.e.*, during the reduction of the acceptor side of P700) (Strasser *et al*. 2010, Schansker *et al*. 2003). The probability of electron transferring from the intersystem electron carriers to the end electron acceptors at the PSI side (RE_o/ET_o) decreased under the MV treatment. This suggests that MV treatment may decrease the maximum slope increase of MR/MR_o ($V_{PSII\text{-}PSI}$). Gao *et al.* (2014) reported that the increase in the value of $V_{PSII\text{-}PSI}$ implies rapid re-reduction of P700 and PC. It is also reported that the oxidation of P700 and PC can cause an increase in absorbance in the 800−850 nm range (Schansker *et al.* 2003). Therefore, those results showed that the MV treatment can slow down the re-reduction of PSI (P700 and PC). The higher value of RE_o/ET_o in ALA-pretreated strawberry leaf discs followed by the V treatment than MV treatment only suggests that the pretreatment of the strawberry leaves with ALA can increase the re-reduction capacity of PSI.

ALA is the first essential biosynthetic precursor of all porphyrin compounds including phytochrome, so improvement of photosynthesis of strawberry under ALA treatment may be related to the amount of increased phytochrome. The research of Thieli *et al.* (1999) showed that in transgenic potato plants with overexpression of functional phytochrome B not only increased photosynthesis per leaf area, but also reduced the sensitivity of photosynthesis to photoinactivation under prolonged light stress. Further research by Kreslavski *et al.* (2015) showed that the maximal photochemical quantum yield of PSII (F_v/F_m) was suppressed stronger in nontransformed potato plants than in transgenic potato plants with intense expression of phytochrome B when exposed to UV-B irradiation. In this study under the DCMU treatment, the value of F_v/F_m decreased less in strawberry leaf discs pretreated with ALA (ALA+DCMU) than without ALA pretreatment (Table 2). These results suggest that the improvement of photosynthesis under ALA treatment may be related to the increase of phytochrome.

Other mechanisms, such as SOD activity and detoxification, might be also involved in improvement of photosynthesis of strawberry after the ALA treatment. Allen *et al.* (1997) and Kouril *et al.* (2003) showed that increased activity of SOD in chloroplasts of transgenic tobacco plants and overproducing cytosolic glutathione reductase (GR) of transgenic rice plants generally led to increased protection from membrane damage caused by exposure to MV. In this study, the protection effect of ALA on electron transfer chain under MV and DCMU treatments may also be related to the increase of

antioxidants and enzyme activities as reported by Nishihara *et al.* (2003) and Sun *et al.* (2009). Recently, it has been reported that ALA treatment upregulated serine/threonine kinase and chaperone indicating detoxification system may play potential roles in plant protection against stress (Phung and Jung 2015). The detoxification system may also play a role in ALA protection of electron transfer under DCMU and MV treatments as suggested by Phung and Jung (2015). The mechanisms of ALA effects on protection of electron transfer chain in strawberry plants need to be further investigated.

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