Promotion of 5-aminolevulinic acid treatment on leaf photosynthesis is related with increase of antioxidant enzyme activity in watermelon seedlings grown under shade condition

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Abstract

Watermelon [*Citrullus lanatus* (Thunb.) Mansfeld] is a photophilic plant, whose net photosynthetic rate was significantly decreased when seedlings were grown under low light condition. However, treatment with 100 mg kg^{-1} 5-aminolevulinic acid (ALA) could significantly restore the photosynthetic ability under the environmental stress. The parameters of leaf gas exchange, chlorophyll modulated fluorescence and fast induction fluorescence of the ALA-treated plants were higher than that of the control. Additionally, ALA treatment increased the activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX). Nevertheless, the treatment of diethyldithiocarbamate (DDC), an inhibitor of SOD activity, dramatically depressed photosynthesis of watermelon leaves, while ALA could reverse the inhibition of DDC. Therefore, it can be deduced that ALA promotion on photosynthesis of watermelon leaves under low light stress is attributed to its promotion on antioxidant enzyme activities, and the increased activities of the enzymes, which are mainly located near the reaction centers of PSI, can scavenge superoxide anions, leading to an increase of apparent electron transport rate and an alleviation of photosynthetic photoinhibition under the stressed environment.

Additional key words: 5-aminolevulinic acid; antioxidant enzymes; chlorophyll fluorescence; JIP test; low light stress; photosynthesis; watermelon.

Introduction

5-aminolevulinic acid (ALA) is the first essential biosynthetic precursor of all porphyrin compounds, such as chlorophyll (Chl), heme, and phytochrome (von Wettstein *et al*. 1995). ALA has been reportedly used as an herbicide or pesticide in higher concentrations (Rebeiz *et al*. 1984) or plant growth regulator in lower concentrations (Bindu and Vivekanandan 1998) to increase crop production (Hotta *et al.* 1997b) and improve stress resistance (Hotta *et al.* 1998, Watanabe *et al.* 2000, Wang *et al*. 2005a). This suggests that ALA may have wide potential applications in agriculture as a natural and environmental friendly substance (Wang *et al.* 2003). However, the mechanisms of ALA regulation on plant growth have not yet been elucidated. Several reports suggested that ALA promotion on plant growth might be related with its stimulation on leaf photosynthesis (Hotta *et al*. 1997b, Wang *et al*. 2005b), and the latter might be related with the increase of activities of antioxidant enzymes (Nishihara *et al.* 2003, Liu *et al*. 2006a, Liu *et al*. 2006b, Memon *et al*. 2008). However, the hypothesis need more evidence in support.

Watermelon is a photophilic plant, often suffering from low light stress in protective cultivation (He *et al.* 1994). We observed that low light intensity severely depressed its leaf photosynthesis whereas exogenous ALA treatment could increase photosynthetic gas exchange

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Abbreviations: ALA – 5-aminolevulinic acid; APX – ascorbate peroxidase; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DDC – diethyldithiocarbamate; *E* – transpiration rate; ETR – electron transport rate; F_m – maximum fluorescence; F_m' – maximum fluorescence of the light-adapted leave; F_o – initial fluorescence; F_v – variable fluorescence; F_v/F_m – maximal photochemical effciency of PSII; FM – fresh mass; M_o – approximate initial slope of the fluorescence transient; g_s –stomata conductance; JIP test – formulate a group of fluorescence parameters, which quantify the stepwise flow of energy through PSII; $O_2^{\cdot-}$ – superoxide anion radical; OEC – oxygen evolving complex; OJIP – chlorophyll *a* fluorescence transients; P_N – net photosynthetic rate; PI_{ABS} – performance index on a basis of absorption; POD – peroxidase; PS – photosystem; Q_A^- – reduced Q_A, one electron acceptor bound in PQ; Q_A^{2-} – completely reduced Q_A; q_P – photochemical quenching; SOD – superoxide dismutase; V_j – relative variable fluorescence intensity at the J-step; W_k – amplitude of the K step; φ_{Eo} – quantum yield of electron transport; Ψ_o – possibility of a trapped exciton moves an electron into the electron transport chain beyond Q_A; Φ_{PSII} – actual photosynthetic efficiency.
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and photochemical efficiency and, furthermore, the evidence obtained from the experiment of antioxidant enzyme inhibitor showed that ALA promotion on photosynthesis was related with the increase of antioxidant enzyme activities.

Materials and methods

Plant growth: The experiment was conducted in September 2007 in a plastic tunnel when the day/night temperatures were about 30/20 °C to mimic the autumn-winter cultivation of watermelon in protective facility system. Seeds of watermelon [*Citrullus lanatus* (Thunb.) Mansfeld cv. Yingpijingxin] were surface-sterilized in 55 °C warm water for 30 min and then sown in Petri dishes filled with two pieces of filter paper, to which 8-10 cm^3 distilled water had been added. The dishes were kept in oven darkly with 28 °C for 2–3 d, and the germinated seeds were transferred into plastic pots with diameter 20 cm, which were filled with about 8 kg garden soil containing 7.5 % mature cattle manure, and placed in a plastic tunnel. All seedlings were divided into two parts. One part of plants were grown in plastic tunnel with full sunny light, where the midday light irradiances were about 1060–1620 μmol m^{-2} s^{-1}. The others were grown in plastic tunnel with a dark mesh shade, where the mid-day light intensities were about 240–360 μmol m^{-2} s^{-1}. Twenty days after seed sowing, when the 4th true leaves of seedlings were expanded, 5 cm^3 of 100 mg kg^{-1} ALA was sprayed to two sides of leaves until liquid dropping. Fifteen days later, 5 cm^3 of 1 % diethyldithiocarbamate (DDC) solution was sprayed on some leaves of plants 2 h before gas exchange parameters and Chl fluorescence were measured. Thus, the experimental designs included unshaded control, shaded control, shaded + ALA, shaded + DDC, and shaded + ALA + DDC. Tween-20 with 0.01 % concentration was added in every treatment, including two controls, which were sprayed with distilled water. The experimental plants were arranged in complete random order, with five repeats for each treatment.

Leaf gas exchange parameters were measured using a portable *LI-6400* system (*LI-Cor Inc.*, Lincoln, NE, USA). Net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i) , stomata conductance (g_s) and transpiration (E) were measured simultaneously with an internal light resource with PAR 1000 μmol m^{-2} s^{-1}. During the measurement, the ambient air CO_2 concentration was about 400 μmol mol^{-1} and the temperature about 25 ºC. Each treatment was measured at least 5 times. The means were used to compare the effect of treatments.

Chl fluorescence of watermelon leaves was measured using a modulated chlorophyll fluorometer (*PAM-2100, Walz*, Effeltrich, Germany) at the day of gas exchange measurement. The initial fluorescence (F_o) and maximal fluorescence (F_m) were measured after a 30-min dark adaptation. Then, variable fluorescence (F_v) and maximal photochemical efficiency of PSII (F_v/F_m) were calculated according to the previous methods (Demmig-Adams *et al*. 1995, 1996, Demmig-Adams and Adams 1996). During actual measurement, dark-adapted leaves were firstly irradiated by a red light emitting diode (LED) with PAR 0.1 μmol m^{-2} s^{-1} (λ = 650 nm) to measure F_o, and then a saturated light with PAR 9 000 μmol m^{-2} s^{-1} was pulsed for 0.8 s to measure F_m. After that, an actinic light with PAR 400 μmol m^{-2} s^{-1} was turned on, and the saturated pulse light was illuminated for the maximum fluorescence of the light-adapted leaves (F_m') measurement. Then, the actinic light was turned off and the far-red light with λ = 730 nm was on for several seconds, and the minimum fluorescence of the light-adapted leaves (F_o') was recorded. After this step, electronic transfer rates (ETR), actual photochemical efficiency of PSII (Φ_{PSII}) and photochemical quench (q_P) were automatically recorded by the instrument. $ETR= [(F_m'–F)/F_m'] \times PFD \times 0.5 \times 0.83$, $\Phi_{PSII}= (F_m'–F)/F_m'$, $q_P = (F_m'–F)/(F_m'–F_o')$. One cycle like this manipulation took 20 s, and a Chl fluorescence was measured for 5 min, thus the time-curves of the parameters were drawn after measurement. All values were measured 3–5 repeats, and means ± SD were presented.

Chl fast induction fluorescence and JIP test: Chl fast induction fluorescence was measured by a *Plant Efficiency Analyzer* (*PEA*, *Hansatech,* King's Lynn, Norfolk, UK) according to Strasser *et al*. (1995). The fluorescence was induced by a red light (λ = 650 nm) of about 3 000 μmol m^{-2} s^{-1} provided by an array of six LED, which focused on the sample surface to give homogenous illumination over the exposed area of the sample (4 mm in diameter). The fluorescence signals were recorded within a time scan from 10 μs to 1 s with a data-acquisition rate of 10^5 points per second for the first 2 ms and of 1 000 points per second after 2 ms. Each treatment was measured at least 5 times.

Chlorophyll fluorescence transients (OJIP) were analyzed according to the JIP test (Appenroth *et al.* 2001, Krüger *et al.* 1997, Strasser *et al.* 2000), by using the following original data: (a) the fluorescence intensity at 50 μs considered as F_o when all reaction centers (RCs) of PSII were open; (b) the maximal fluorescence intensity, F_m, assuming that excitation intensity was high enough to close all the RCs of PSII; (c) the fluorescence intensities at 300 μs (K-step) and 2 ms (J-step).

Antioxidant enzyme activities: One hundred milligrams of leaves were homogenized in 2 cm^3 of 50 mM phosphate buffer (pH 7.8) which contained 0.4 % polyvinyl pyrrolidone (PVP), an inhibitor of phenolic compounds in

a pre-chilled mortar and pestle on ice. The homogenate was centrifuged at 10 000×*g* for 20 min at 4 °C and the supernatant was collected as crude enzyme extraction (Tan *et al.* 2008).

Total superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by measuring the ability of inhibiting the photochemical reduction of nitrobluetetrazolium (NBT). The reaction mixture (3 cm^3) contained 195 mM methionine, 1.125 mM NBT, 3 μM EDTA, and 0.05 cm^3 of enzyme extract in 50 mM PBS (pH 7.8). After addition of 20 μM riboflavin, the cuvettes were exposed to a 15-W circular "white light" tube for photoreaction 10 min. Then the reaction mixture was measured as absorbance of 1 cm cuvette at 560 nm. One unit of SOD activity was defined as the amount of enzyme per fresh mass sample causing 50 % inhibition of the photochemical reduction of NBT (Beauchamp and Fridovich 1971).

Peroxidase (POD, EC 1.11.1.7) activity was measured according to the change in absorption at 460 nm due to guaiacol oxidation. Total 3 cm^3 of reaction solution contained 0.1 M acetic acid buffer (pH 5.4), 0.75 % H_2O_2, and 0.25 % guaiacol. The reaction was started by adding 50 mm^3 of enzyme extraction. Changes of absorbance at 460 nm of 1 cm cuvette were then recorded within 3 min after the start of the reaction at 1-min intervals.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed by measuring the oxidation of ascorbate at 290 nm of 1 cm cuvette. Total 3 cm^3 of reaction solution contained 50 mM PBS (pH 7.0), 1 mM H_2O_2, and 1mM ascorbate. The reaction was started by adding 100 mm^3 of enzyme extraction. Changes of absorbance at 290 nm were then recorded within 3 min after the start of the reaction at 1-min intervals.

Statistical analysis: All data were subjected to *ANOVA* test and means were compared by the Duncan's test. Comparisons with $p<0.05$ were considered significant difference.

Results

Gas exchange: P_N of watermelon leaves under shade condition was significantly lower than that under natural solar light condition (Table 1), suggesting that watermelon is a light-loving plant and low irradiance during growth would degrade the photosynthetic ability. Spraying with 100 mg kg^{-1} ALA could increase P_N by about 40 %, compared with the control under shade condition, suggesting that ALA could improve photosynthesis of plants suffering from low light stress. On the other hand, 1 % DDC treatment decreased P_N, only 67 % of the shaded control, whereas the DDC inhibition was significantly eliminated when the seedlings were pretreated by ALA ($p<0.05$). The P_N of ALA-pretreated leaves were 69 % higher than that without pretreatment.

From Table 1, g_s and E of the watermelon leaves under shade condition are seen to be lower than those of the natural light control, whereas C_i is higher than that of the latter ($p<0.05$). ALA treatment has a tendency to increase g_s and E of watermelon leaves, although the differences are not statistically significant. Compared with the shaded control, DDC treatment also decreased g_s and E, while ALA pretreatment blocked the effect of DDC.

Table 1. Effect of ALA treatment on photosynthetic characteristics of 35 d watermelon seedlings leaves under low light stress. P_N – net photosynthetic rate; g_s – stomata conductance; E – transpiration rate; C_i – intercellular CO_2 concentration. The data in the table were the means ± SD of at least five repeated measurements of separate samples. The different small letters in the same columns represent the significant difference ($p<5$ %). In the table, 'uck' and 'sck' represent the control plants grown without or with shade respectively , 'sala', 'sddc' and 'sa+d' represent the shaded plants treated with 100 mg kg^{-1} ALA, 1 % DDC, or ALA+DDC, respectively.

Treatment	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	C_i [μmol mol^{-1}]
uck	6.03±0.26 a	47.61±1.78 a	1.64±0.07 a	155.75±12.21 b
sck	3.36±0.39 b	39.34±0.32 bc	1.37±0.02 bc	209.44±3.17 a
sala	4.75±0.33 ab	44.89±1.62 ab	1.53±0.01 ab	184.91±1.98 ab
sddc	2.27±0.22 c	32.50±1.12 c	1.22±0.01 c	162.96±5.20 b
sa+d	3.84±0.24 b	38.96±2.12 bc	1.27±0.01 bc	156.38±1.34 b

Chl fluorescence: F_o of dark-adapted leaves of watermelon seedlings grown under shade condition was significantly higher than that of natural light control, while F_v/F_m and the ratio of variable to initial fluorescence (F_v/F_o) were lower than the latter (Table 2). It seems that low light intensity severely affected the development and function of photosynthetic apparatus. ALA and/or DDC treatments did not change the fluorescence parameters of the dark-adapted leaves, implying no obvious modification at the base of photosynthetic structure induced by the chemicals at the short term experiment.

Fig. 1 shows that Φ_{PSII}, ETR and q_P increased gradually when the dark-adapted watermelon leaves were exposed to an actinic light intensity of 400 μmol m^{-2} s^{-1}. However, three photochemical parameters were significantly depressed when seedlings were shaded. Nevertheless, ALA treatment improved Φ_{PSII}, ETR and q_P of the shaded leaves, whereas DDC decreased them, and ALA had an antagonistic effect against DDC. It means that ALA could increase photochemical efficiency of water-

Table 2. Effect of ALA treatment on initial fluorescence (F_o), maximal fluorescence (F_m), the ratios of variable to maximum fluorescence (F_v/F_m) and variable to initial fluorescence (F_v/F_o) of the 35 d watermelon leaves under shade condition. The data in the table were the means ± SD of at least three repeated measurements of separate samples, which were measured by a Hansatech modulated chlorophyll fluorometer after leaves were dark-adapted for 30 min. The different small letters in the same column represent the differences significant (p<5 %).

Treatment	F_o	F_m	F_v/F_m	F_v/F_o
uck	0.3277±0.0050 b	1.7015±0.0707 a	0.8075±0.0051 a	4.196±0.038 a
sck	0.3646±0.0092 a	1.6533±0.0891 a	0.7790±0.0073 b	3.530±0.019 b
sala	0.3712±0.0029 a	1.7090±0.0191 a	0.7825±0.0064 b	3.611±0.074 b
sddc	0.3660±0.0076 a	1.6920±0.0587 a	0.7837±0.0056 b	3.623±0.093 b
sa+d	0.3462±0.0089 a	1.6327±0.0792 a	0.7872±0.0084 b	3.711±0.028 b

Table 3. Effect of ALA treatment on biophysical parameters of 35 d watermelon seedlings leaves when assessed by JIP tests. The data in the table were the means ± SD of at least five repeated measurements of separate samples. The different small letters in the same columns represent the differences significant (p<5 %). Ψ_o – possibility of a trapped exciton moves an electron into the electron transport chain beyond Q_A^-; φ_{Eo} – quantum yield of electron transport; M_o – approximate initial slope of the fluorescence transient; W_k – amplitude of the K step; V_j – relative variable fluorescence intensity at the J-step.

Treatment	Ψ_o	φ_{Eo}	M_o	W_k	V_j
uck	0.6886±0.0166 a	0.5721±0.0148 a	0.3582±0.0237 e	0.2870±0.0119 b	0.3011±0.0166 d
sck	0.5974±0.0292 b	0.4860±0.0276 b	0.5606±0.0353 c	0.3476±0.0197 a	0.4026±0.0292 c
sala	0.6265±0.0167 b	0.5067±0.0143 b	0.4689±0.0371 d	0.3490±0.0217 a	0.3935±0.0168 c
sddc	0.4618±0.0221 d	0.3572±0.0209 d	0.8076±0.0184 a	0.3754±0.0118 a	0.5382±0.0222 a
sa+d	0.5321±0.0292 c	0.4174±0.0257 c	0.6792±0.0413 b	0.3624±0.0202 a	0.4679±0.0292 b

Table 4. Effects of ALA treatment on the antioxidant activities of 35 d watermelon leaves. The data in the table were the means±SD of at least three repeated measurements of separate samples. The different small letters represent the differences significant (p<5 %). The data in the table were the means±SD of at least three repeated measurements of separate samples.

Treatment	SOD activity [unit g^{-1}(FM)]	POD activity [ΔA_{460} g^{-1}(FM) min^{-1}]	APX activity [ΔA_{290} g^{-1}(FM) min^{-1}]
uck	237.8±1.3 b	64.23±0.27 b	8.996±0.380 bc
sck	250.3±0.5 ab	45.16±0.37 b	10.92±0.036 bc
sala	256.3±1.5 a	151.6±1.30 a	16.87±0.145 a
sddc	230.2±1.4 b	23.49±1.02 c	9.433±0.368 c
sa+d	243.8±0.9 ab	63.57±1.55 b	11.56±0.092 b

melon leaves under low light stress, which would be related with DDC-related factors.

JIP test: The data from Chl fast induction fluorescence measurements (Table 3) showed that Ψ_o and φ_{Eo} of the shade-grown seedlings were significantly lower while M_o, W_k and V_j were much higher than that of natural light condition, suggesting that low light depressed the possibility of a trapped exciton moving an electron into the electron transport chain beyond Q_A^- and quantum yield of electron transport, thus, the closure rate of active reaction center Q_A (M_o) was accelerated and the OEC activities were destroyed. In this measurement, we did not observe significant effect of ALA on primary photochemical reaction; however, ALA treatment tended to increase Ψ_o and φ_{Eo}, but decreased M_o. These effects were significant when DDC treatment was conducted (p<0.05). In fact, DDC treatment caused the increase of M_o and V_j by 44 % and 34 %, but the decrease of Ψ_o and φ_{Eo} by 23 % and 27 %, respectively. If the leaves were pretreated with ALA, M_o and V_j were 16 % and 13 % lower, while Ψ_o and φ_{Eo} were 15 % and 17 % higher than that only treated by DDC. Thus, ALA promotion on photochemical efficiency of watermelon leaves under low light stress would tightly be related with DDC-related factors.

Antioxidant enzymes activities: No significant differences of antioxidant enzyme (including SOD, POD and APX) activities of watermelon seedlings were found between seedlings grown under shade and natural light condition (Table 4). However, ALA treatment stimulated all three antioxidant enzymes compared with the controls. Furthermore, DDC treatment depressed the enzyme activities whereas ALA pretreatment could prevent DDC's inhibition. It is worthwhile to notice that the most considerable change induced by ALA and/or DDC treatment was not SOD but POD. The POD activity of

ALA treatment was 3 times as high as that of the shaded control, while DDC treatment induced POD activity decrease to 50 %, but ALA+DDC had almost 3 times of enzyme activity compared with that without ALA treatment. Thus, ALA and/or DDC could induce greater changes of POD activity than the other enzymes.

Discussion

Watermelon is a species originated in the tropic arid deserts of Africa and suitable to be planted in warm and sunshine environments. However, in protective cultivation systems, low light often affects vigorous growth and development of watermelon seedlings (He *et al.* 1994). Previous studies have showed that shade significantly decreased leaf photosynthetic ability of watermelon seedlings (Kang *et al.* 2006, Sun and Wang 2007). The data from this experiment approves the previous observation, and shows that the gas exchange characteristics including P_N, g_s and E were depressed while C_i was increased by shade treatment (Table 1). Additionally, shade treatment increased F_o, but decreased F_v/F_m and F_v/F_o (Table 2), suggesting that low irradiance affects photosynthetic apparatus development and photochemistry of watermelon leaves.

Several reports have demonstrated that ALA treatment can improve the net photosynthetic rate in spinach (Nishihara *et al*. 2003), melon (Wang *et al.* 2004a), pak-choi (Wang *et al.* 2005a, 2004b), radish (Hotta *et al.* 1997a*,* Wang *et al.* 2005b) and strawberry (Liu *et al.* 2006b), either under natural light condition or low irradiance stress. With *PAM-2100* modulated Chl meter, we proved that ALA treatment could increase plant photochemical efficiency (Sun and Wang 2007). It was found that, in most situations, promotion of ALA on photosynthetic electron transport rate was greater than the other parameters. In this study, it was also showed that ALA treatment increased the actual photochemical efficiency, electron transport rate and photochemical quenching (Fig. 1), but had no effects on part of fluorescence parameters of dark-adapted leaves, such as F_v/F_m and F_v/F_o (Table 2). It is similar with previous reports in watermelon (Kang *et al*. 2006, Sun *et al.* 2008), strawberry (Liu *et al*. 2006b) and radish (Wang *et al*. 2005b), suggesting that ALA treatment in the tested dozens did modify the photosynthetic apparatus in short term. However, measurements of transgenic tobacco with ability to overproduce ALA showed that the increase of endogenous ALA led to significantly higher levels of F_v/F_m and F_v/F_o than the wild type, as well as other parameters such as Φ_{PSII}, ETR and q_P, suggesting that promotion of ALA on photochemical efficiency in transgenic plants was greater than exogenous application of one time (Zhang *et al*. 2008b). In a previous report, Kang *et al*. (2006) observed that the promotion of 200 mg kg^{-1} ALA treatment on F_v/F_m and F_v/F_o was significant (p<0.05), while the effect of 50–100 mg kg^{-1} ALA was not (p>0.05). Therefore, it is speculated that response of photosynthetic apparatus may be at higher dozens of ALA application. However, a higher dosage of ALA than 5 mM often induces plant photooxidation, since ALA can be converted into protoporphyrin IX at dark and

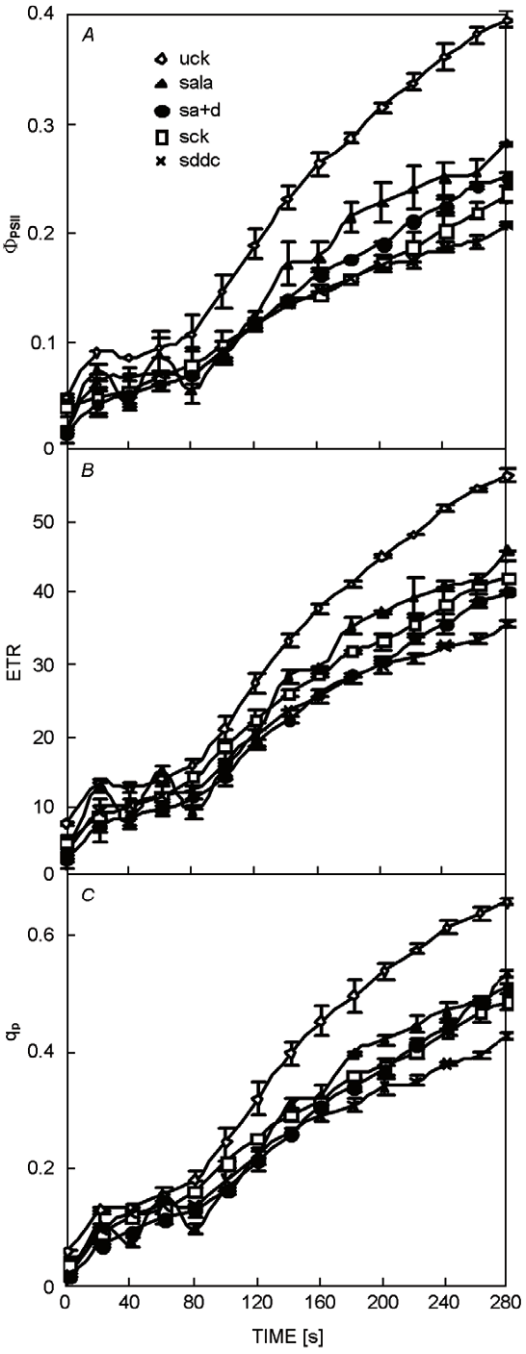

Fig.1. Effect of ALA on the actual photochemical efficiency of PSII (Φ_{PSII}, *A*), the electron transport rate (ETR, *B*) , the photochemical quenching (q_P, *C*) after the dark-adapted leaves of watermelon were transferred to actinic light intensity of 400 μmol^{-1} m^{-2} s^{-1} for 5 min. The data are showed by means of 5 repeats ± SD, which were from the measurements of a *PAM-2100* modulated chlorophyll fluorometer.

induce photosensitive reaction to kill plants (Rebeiz *et al*. 1984, Kumar *et al*. 1999). Thus, it is wise to use ALA with lower concentrations and more times. The concentration of ALA used in our work equaled to 0.6 mM, which was a low concentration. This is the first study to use both *PEA* and *PAM-2100* modulated fluorometer to assess the promotion of ALA on photochemical efficiency. Furthermore, since *PEA* has high time resolution, it can be utilized to record the overall performance on fluxes, yield of trapping and electron transport (Srivastava *et al.* 1997, 1998, 1999). In present study, shade treatment would decrease W_k of watermelon leaves, suggesting that the OEC activity at the donor side of PSII reaction center was affected. However, ALA and/or DDC did not affect W_k, suggesting the compounds had no effect on OEC activity. Thus, ALA and DDC have no direct effect on the activity of the donor side of PSII reaction center. Nevertheless, shade, ALA and DDC induced great changes of M_o, suggesting the closure of active reaction center was accelerated by shade or DDC treatment, but inhibited by ALA treatment. Thus, ALA and DDC mainly affected the acceptor side of PSII reaction center. The decrease of M_o and V_j by ALA treatment represented less Q_A^{2-} accumulation and more electrons could move into the electron transport chain beyond Q_A^-, consequently, Ψ_o and φ_{Eo} in ALA-treated leaves were greater than that of the control, especially at DDC treatment (Table 3).

Because DDC is an inhibitor of SOD, the antagonism ALA and DDC strongly suggests that the promotion of ALA on photosynthesis is related with antioxidant enzymes. In fact, there are several reports suggesting ALA stimulation on antioxidant enzymes. Nishihara *et al.* (2003) firstly found that the increase of enzyme activities induced by ALA was related with the increase of net photosynthetic rate during salt stress. Similarly, Liu *et al*. (2006a), Lukšienė *et al*. (2007) and Zhang *et al*. (2008b) also observed ALA-induced increase of antioxidant enzyme activity during seed germination or seedling growth. Therefore, the increased antioxidant ability induced by ALA may be important for plants to tolerate non-biotic stresses. However, the mechanism of ALA regulating photosynthesis needs to be elucidated. It has been known for a long time that ALA is the key precursor of Chl biosynthesis in plants (von Wettstein *et al.* 1995), thus, the promotion on photosynthesis by exogenous ALA treatment might be a result of higher levels of Chl accumulation (Wang *et al.* 2004a, 2004b, 2005b). However, in strawberry, Liu *et al.* (2006b) observed that 300 mg kg^{-1} ALA treatment did not affect Chl content of leaves but induce higher photosynthetic rate, compared with the control. Thus, ALA promotion on plant photosynthesis cannot merely be attributed to its involvement in Chl biosynthesis. The other more important mechanisms may be working when ALA was treated. Actually, Chl content is not the limiting factor for leaf photosynthesis in most cases. In this study, we do not merely interpret the increase of antioxidant enzyme activities and therefore the ability to detoxify reactive oxygen species as the main mechanism for ALA improvement on photosynthesis of watermelon under low light stress. We consider that ALA treatment increased antioxidant enzyme activity and accelerated reactive oxygen metabolism, which eliminated $O_2^{\cdot -}$ accumulation and photoinhibition near PSI (Mehler 1951), increased photosynthetic electron transport rate, and therefore, photochemical efficiency (Fig. 1). It has been reported that leaf $O_2^{\cdot -}$ accumulation under low light formed $^{\cdot}OH$ through Fenton reaction, the latter would injure PSI and lead to photoinhibition of PSII (Terashima *et al.* 1998). Thus, acceleration of the cycle of production and elimination of reaction oxygen species, *i.e*. water-water cycle (Weng *et al*. 2008), as a by-pass route of photosynthetic electronic transport chain, is beneficial for absorbance and dissipation of light energy in chloroplasts, which can prevent photodamage due to excess light energy. Because the down-stream of electronic transport chain near PSI is open, it is easy for a trapped exciton to move an electron into the electron transport chain beyond Q_A^-, thus, less Q_A^{2-} was accumulated, M_o was decreased, and Ψ_o and φ_{Eo} were increased (Table 3).

ALA has been suggested to regulate plant growth and development as a new hormone (Bindu and Vivekanandan 1998); however, there is no evidence that its physiological functions are direct. Wang *et al.* (2005a) proposed that ALA promotion on plant salt tolerance depended upon its conversion into tetrapyrrole compounds, because levulinic acid, an inhibitor of ALA dehydrase, could completely block the promotion. Germinating seedlings treated by exogenous ALA contained more heme than the control. Therefore, heme was guessed to be an important factor during ALA regulation on plant growth and development. Additionally, heme is known to be a prosthetic group of peroxidases (Jones *et al*. 1998, Tsiftsoglou *et al.* 2006) and it is necessary for the activities of POD and APX. Therefore, in this study, the increased activities of antioxidant enzymes in watermelon seedlings treated by ALA (Table 4) might be a result of ALA conversion into heme. However, it needs further verification.

There are a few reports to demonstrate the relationship between ALA treatment and peroxidase activity (Nishihara *et al*. 2003). Liu *et al*. (2006a) used ascorbic acid to inhibit POD activity, which led ALA-induced germination of watermelon seeds blocked, suggesting that POD activity is an important aspect of ALA physiological function. In this study, the greatest change of antioxidant enzyme activity was found in POD, which is in accordance with previous reports (Liu *et al*. 2006a, Liu *et al*. 2006b, Kang *et al*. 2006, Lukšienė *et al*. 2007, Zhang *et al*. 2008a,b), suggesting that POD activity was closely related with ALA function. Additionally, DDC, as an SOD inhibitor used in the study, was predicted to affect SOD activity more than POD. However, the decline of POD was much greater than that of SOD (Table 4), but the reason has not been elucidated. The decrease of SOD activity induced by DDC would lead to

$O_2^{\cdot -}$ accumulation, which then severely depressed POD activity. Anyway, DDC application clearly demonstrated the role of antioxidant enzymes on ALA-induced increase of photosynthesis of watermelon seedlings under low light stress.

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