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ORIGINAL PAPER

Promotive effect of 5-aminolevulinic acid on chlorophyll, antioxidative enzymes and photosynthesis of Pakchoi (*Brassica campestris* ssp. *chinensis* var. communis Tsen et Lee)

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Abstract The objective of this article is to study the effect of 5-aminolevulinic acid (ALA) and enhanced chlorophyll content, antioxidative enzymes and photosynthesis rate by foliar application of ALA. We evaluated three concentrations (control-distilled water, T1-50 mg l^{-1}, T2-150 mg l^{-1}, T3-250 mg l^{-1}) of ALA and seven cultivars, "Sanchidaye" (Sa-1), "Lichuandasuomian" (Li-1), "Aijiaohuang" (Ai-1), "Qingyou" No. 4 (Qi-1), "Aikang" No. 5 (Ak-1), "Hanxiao" (Ha-1) and "Shulv" (Sl-1). "Ak-1" showed strongest response of POD (peroxidase) enzyme activity (0.4 U g^{-1} min^{-1}) in 250 mg l^{-1} ALA solution. The highest CAT (catalase) activity (0.8 U g^{-1} min^{-1}) after administration of 250 mg l^{-1} ALA was observed in "Li-1". Meanwhile, highest (1.42 mg l^{-1}) total chlorophyll content was also observed in "Ak-1", when leaves were treated in 50 mg l^{-1} ALA, "Li-1" and "Ai-1" showed strongest response of specific activity of superoxide dismutase (SOD) in 50 mg l^{-1} and 50 mg l^{-1} ALA. Two hundred and fifty milligram per milliliter of ALA-treatment significantly improved the net photosynthetic rate.

Keywords Pakchoi · Promotion · 5-Aminolevulinic acid · Chlorophyll · Antioxidative enzymes · Photosynthesis

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Introduction

Pakchoi (*Brassica campestris* ssp. *chinensis*) originated from China, and it has especially large area of production in winter season. The ideal temperature during growth is between 15 and 20°C, and while it is best grown in spring and autumn (Larkcom 1991). There are a considerable number of varieties in China. Usually, different varieties are used in different seasons of a year for the local production (Li 1985). Many growers have reported that hot weather causes seed stalk formation (bolting); however, studies have shown that bolting is due to the longer day usually associated with the warm weather (http://edis.ifas.ufl.edu). Under high-temperature conditions, the plant grows slowly, but it can tolerate temperatures above the optimum if there is enough soil moisture (http://hort-devel-nwrec.hort.oregonstate.edu/chincabb.html).

Many physiology processes in plants are impaired by hot temperature, including photosynthesis, enzyme activity, membrane stability and ultimately growth (Nguyen and Joshi 1992). Chlorophyll biosynthesis is affected, when plants are exposed to cold or hot season (van Hasselt and Strikwerda 1976; Feierabend 1977). It is well established that, whatever its nature, stress causes the production of large amount of highly reactive oxygen species (ROS), including free radicals ($O_2^{-\cdot}$: superoxide radicals; H_2O_2: hydrogen peroxide; OH′: hydroxyl radical), in living cells (Wardman and Candeias 1996). Living organisms,

particularly photosynthetic organisms, are continuously exposed to ROS, but their exposure is significantly enhanced in oxidative conditions. For this reason, they have evolved as efficient enzymatic and nonenzymatic detoxifying systems to overcome damage due to ROS (Larson 1988).

It is known that 5-aminolevulinic acid (ALA) is an essential biosynthetic precursor of all porphyrin compounds, including chlorophyll and heme (von Wettstein et al. 1995). ALA is an essential precursor of tetrapyrrole compounds, such as vitamin B12 and heme which are found in plants (Rebeiz et al. 1984), animals (Shemin and Russell 1953) and bacteria (Jacobs 1977); ALA serve as prosthetic groups of respiratory enzymes, and chlorophyll in bacteria (Brunham and Lascelles 1963) and plants (Granick 1961); ALA are the major photosynthetic light-harvesting pigments (Senge 1993). Suitable concentration of ALA had promotive effects on the growth rates and photosynthesis, and also crop yields were enhanced by the application of ALA at the leaf-stage in the life cycle of rice, barley, potato and garlic (Tanaka et al. 1992). ALA presumably promoted chilling tolerance of the plants under low light (Wang et al. 2004), in relation to chlorophyll biosynthesis, photosynthesis activity and suppression of respiration (Hotta et al. 1997). Hotta et al. (2004) proposed that, at low concentrations, foliar application of ALA increased the growth and yield of barley, garlic, potato and kidney bean. Hundred milligrams of ALA per milliliter enhanced the growth and photosynthesis activity of grapevines (Watanabe et al. 2006).

The effects of ALA increased the rate of photosynthesis with Pakchoi cultivars in hot season, implying that ALA has potential for use under these environments. This is the first evidence foliar spray had promotive effect on antioxidative enzymes activities of Pakchoi.

Materials and methods

Seven cultivars of *Brassica campestris* ssp. *chinensis* var. communis Tsen et Lee were used in this study (Table 1). Seeds were obtained from the experimental farm of Nanjing Agricultural University located at Jiangpu, Jiangsu, China. The experiment was done during the month of June–August.

Preparation of plant material

The seeds were soaked into 90 mm Petri dishes before sowing for 24 h. Twelve seeds were sowed in sterilized soil (soil:vermiculite:manure 1:1:0.2) in plastic trays (Da Zhongting Ltd, China) in each replication and was replicated three times. Plastic trays contained eight cells in width and twelve cells in length. The spraying treatments (Control-distilled water, T1-50 mg l^{-1} ALA, T2-150 mg l^{-1} ALA, T3-250 mg l^{-1} ALA) were started after 5–6 weeks of seed-sowing with approximately 10 ml/plant. ALA (spraying treatment) was purchased from Dikarmun Chemical Company (Chemical Industrial Co. Ltd, China). ALA spraying treatments were applied biweekly in the morning for during 3 weeks. Data was recorded after 3 weeks of ALA treatment.

Table 1 Effect of ALA treatment on the chlorophyll (Chl) content of Pakchoi leaves (Unit: mg g^{-1} FW)

Cultivar		Treatments	Chl *a*	Chl *b*	Chl *a* + *b*	Chl *b/a*
"Sanchidaye"	(Sa-1)	Control	0.63c	0.2b	0.83b	0.31b
		T1	0.70a	0.23a	0.93a	0.32b
		T2	0.68b	0.24a	0.92a	0.35a
		T3	0.68b	0.15c	0.83b	0.22c
"Lichuand-asuomian"	(Li-1)	Control	0.71b	0.2c	0.91b	0.28d
		T1	0.73a	0.26b	0.99a	0.35c
		T2	0.64c	0.26b	0.90b	0.40a
		T3	0.71b	0.27a	0.98a	0.38b
"Aijiaohuang"	(Ai-1)	Control	0.60c	0.17c	0.77c	0.28b
		T1	0.65b	0.10d	0.75d	0.15c
		T2	0.58d	0.65a	1.23a	1.12a
		T3	0.94a	0.25b	1.19b	0.26b
"Qingyou" No. 4	(Qi-1)	Control	0.62c	0.15c	0.77d	0.24c
		T1	0.72b	0.19b	0.91c	0.26b
		T2	0.75b	0.22a	0.97b	0.29a
		T3	0.79a	0.23a	1.02a	0.29a
"Akang" No. 5	(Ak-1)	Control	0.59c	0.25b	0.84d	0.42b
		T1	0.96b	0.46a	1.42a	0.47a
		T2	1.00a	0.25b	1.25b	0.25c
		T3	1.09a	0.09c	1.18c	0.08d
"Hanxiao"	(Ha-1)	Control	0.75b	0.14c	0.89c	0.18b
		T1	0.76b	0.17b	0.93b	0.22a
		T2	0.66c	0.11d	0.77d	0.16c
		T3	0.87a	0.20a	1.07a	0.22a
"Shulv"	(Sl-1)	Control	0.60d	0.17c	0.77c	0.28b
		T1	0.79b	0.23a	1.02a	0.29b
		T2	0.64c	0.20b	0.84b	0.31a
		T3	0.87a	0.20b	1.07a	0.22c

The unit of ALA solution is mg l^{-1}

Means followed by the same letter in the column do not differ statistically at $P = 0.05$

Determination of chlorophyll

Chlorophyll content was determined by randomly selecting fully expanded mature leaves. Hundred-gram leaf tissues were cut into 1 cm pieces for the measurement of total

chlorophyll content. The leaf pieces were placed in 2.5 cm × 20 cm test tubes with 5 ml 90% alcohol for 24 h and chlorophyll content was estimated at 470, 665 and 649 nm (Arnon 1949).

Measurement of antioxidative enzymes and protein content

Hundred milligram-leaf tissue samples were homogenized in 1.6 ml phosphate buffer solution (pH 7.4) with a chilled pestle and mortar. The homogenate was centrifuged at 12,000*g* for 20 min and the resulting supernatant was used for determination of the enzyme activity. All operations were carried out at a temperature of 4°C.

For protein content, the Bradford reagent was prepared. Hundred milligrams of Comassie Brilliant Blue G-250 was dissolved in 50 ml 95% ethanol, and then 100 ml 85% (w/v) phosphoric acid was added. The reagent was filtered through Whatman #1 paper. For protein assay, 20 µl supernatant was pipetted into 1.5 cm × 6.5 cm test tube. Three milliliters of protein reagent was added to the test tube and the contents mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min (Bradford 1976).

Superoxide dismutase (SOD) activity was measured by monitoring the inhibition of nitro blue tetrazolium (NBT) reduction at 560 nm. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 100 µM Na-EDTA, 750 µM NBT, 130 mM methionine and 20 µM Vitamin B_2. Reaction was carried out in 2.5 cm × 20 cm test tubes at 25°C under the illumination of a fluorescent lamp (60 µmol m^{-2} s^{-1}). The reaction was allowed to run for 15 min. Blanks and controls were run in the same manner but without illumination and enzyme, respectively. During the experimental condition, the initial rate of reaction, as measured by the difference in increase of absorbance at 560 nm in the presence and absence of extract, was proportional to the amount of enzyme (Beyer and Fridowich 1987). Catalase (CAT) activity was obtained spectrophotometrically by measuring the decomposition of 45 µM H_2O_2 in 0.05 M phosphate buffer (pH 7.0) at 240 nm (Aebi 1983). Peroxidase (POD) activity was estimated spectrophotometrically by measuring the increase in absorbance of the product of reaction among 30% H_2O_2, 99% 2-methoxyphenol and 0.05 M phosphate buffer (pH 6.0) at 470 nm (Putter 1974).

Measurement of photosynthesis parameter

The photosynthetic rate of the third leaf undergoing treatment was measured by a photosynthetic and transpiratory rate analyzer (LI-6400 portable photosynthesis system USA), which could at the same time record the cuvette CO_2 concentration (*Ca*), intercellular CO_2 concentration (*Ci*), leaf transpiration rate (*E*), stomata conductance (*Gs*) and net photosynthesis rate (*Pn*). All parameters were determined with at least three replications. The photon flux density (PFD) was 1,000 µmol m^{-2} s^{-1}. The leaf was placed in the cuvette of LI-6400 portable photosynthesis system, by which photosynthesis was measured.

Data collection and statistical analysis

All data obtained were subjected to ANOVA (2002 by SAS Institute Inc., Cary, NC, USA 2002 Version 9.00). Means were separated by Duncan's multiple range test.

Results

Effect of ALA on chlorophyll content

5-Aminolevulinic acid (ALA) treatment significantly increased the chlorophyll content of Pakchoi leaves (Table 1). The total chlorophyll and chl *b*/*a* ratio in ALA-treated leaves were higher than control, of all cultivars. The general increment of Chl *a* was 18% by 50 mg l^{-1} ALA, 10% by 150 mg l^{-1} ALA, 33% by 250 mg l^{-1} ALA, meanwhile general increment of Chl *b* was 28% by 50 mg l^{-1} ALA, 20% by 150 mg l^{-1} ALA, 8% by 250 mg l^{-1} ALA. Ak-1 showed strongest response of Chl *a* (1.09 mg g^{-1} FW) in T3 (250 mg l^{-1}), while Ai-1 showed strongest response of Chl *b* content (0.65, 1.12 mg g^{-1} FW) and Chl *b*/*a* ratio by 150 mg l^{-1} ALA. Meanwhile, highest (1.42 mg g^{-1} FW) total chlorophyll content was observed in Ak-1, when leaves were treated by 50 mg l^{-1} (T1) ALA solution.

Effect of ALA on activity of antioxidative enzymes

The effect of ALA addition (50–250 mg l^{-1}) on the activity of antioxidative enzymes of Pakchoi were examined.

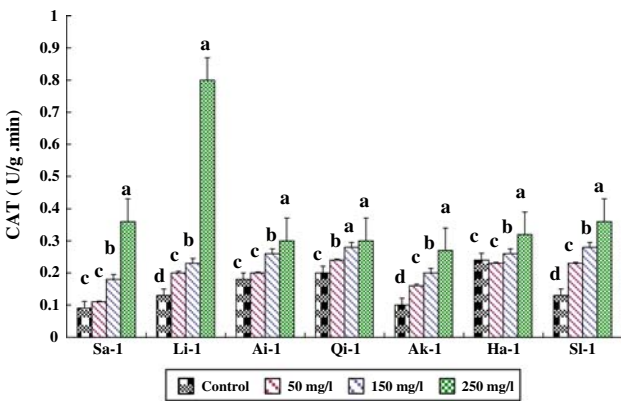

Fig. 1 Effect of 5-aminolevulinc acid (ALA) on activity of CAT in Pakchoi leave. Means with the *same letters* are not significantly different at $P < 0.05$

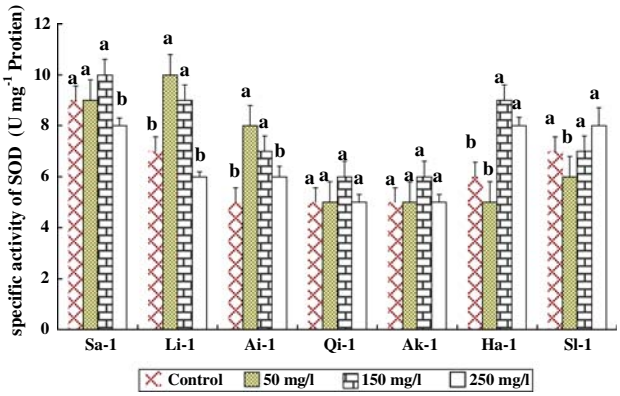

Fig. 2 Effect of 5-aminolevulinc acid (ALA) on specific activity of SOD in Pakchoi leave. Means with the *same letters* are not significantly different at $P < 0.05$

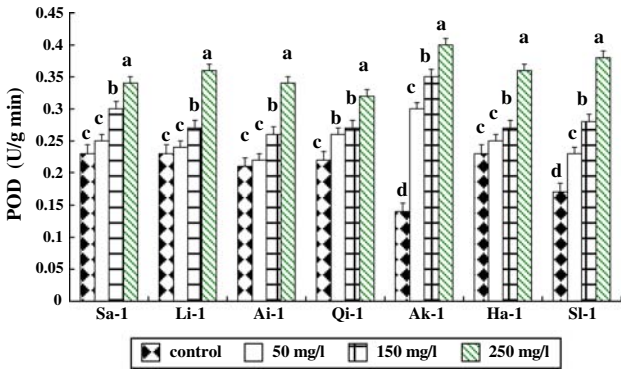

Fig. 3 Effect of 5-aminolevulinc acid (ALA) on activity of POD in Pakchoi leave. Means with the *same letters* are not significantly different at $P < 0.05$

Table 2 Linear correlation between SOD+CAT, SOD+POD, POD+CAT by foliar application of ALA treatments on Pakchoi leaves

Cultivars		SOD+CAT	SOD+POD	POD+CAT
"Sanchidaye"	(Sa-1)	0.35	0.18	0.90
"Lchuandasuomian"	(Li-1)	0.42	0.38	0.96
"Aijiaohuang"	(Ai-1)	0.002	0.01	0.97
"Qingyou" No. 4	(Qi-1)	0.14	0.001	0.88
"Akang" No. 5	(Ak-1)	0.02	0.09	0.89
"Hanxiao"	(Ha-1)	0.42	0.29	0.93
"Shulv"	(Sl-1)	0.30	0.47	0.96

The unit of specific activity of SOD is U g protein^{-1}, and the unit of CAT and POD is U g^{-1} min^{-1}

Figures 1 and 2 show that the activities of POD and CAT were significantly affected and the effect is in correlation with the applied dose of ALA, and significantly increased in response to increased ALA concentration. The highest CAT activity (0.8 U g^{-1} min^{-1}) after administration of 250 mg l^{-1} ALA (Fig. 2) was observed in Li-1, while highest POD enzyme activity (0.4 U g^{-1} min^{-1}) was observed in Ak-1 by 250 mg l^{-1} ALA solution. Ai-1 showed strongest response of linear correlation between POD and CAT activities among all cultivar (Table 2).

From the data illustrated in Fig. 3, the specific activity of superoxide dismutase (SOD) of Pakchoi leaves was significantly higher than control. By foliar application of 50 mg l^{-1} ALA and 150 mg l^{-1} ALA, Li-1 and Ai-1 showed strongest response of specific activity of SOD, and by the highest concentration (250 mg l^{-1}) of ALA, specific activity of SOD was not increased. Furthermore, only "Sl-1" specific activity of SOD significantly increased in response to increased ALA concentration among cultivars. On the other hand, in "Sa-1", "Qi-1" and "Ak-1" treated with 150 mg l^{-1} specific activity of SOD higher than control, and in "Ha-1" specific activity of SOD significantly higher to control (Fig. 3). It is suggested that different cultivars had increased specific activity of SOD in different levels of (ALA) concentrations. POD and CAT were not correlated with specific activity of SOD (Table 2).

Effect of ALA on photosynthesis

The net photosynthesis rate (*Pn*) of Pakchoi increased in response to increased ALA concentration (Table 3). Two hundred and fifty milligrams per milliliter of ALA-treatment significantly improved the net photosynthetic rate. Ak-1 showed strongest response of net photosynthesis rate (*Pn*) among all cultivars. The general increment of net photosynthesis rate (*Pn*) was 13% by 50 mg l^{-1} ALA, 32% by 150 mg l^{-1} ALA and 60% by 250 mg l^{-1} ALA.

In Table 3, data shows that ALA treatment increased the stomata conductance (*Gs*) of Pakchoi leaves; thus, *Ci* and transpiration rate (*E*) of ALA-treated leaves of all cultivars were significantly higher than control. Sa-1 showed strongest response (0.50 μmol m^{-2} s^{-1}) of *Gs* in T3 (250 mg l^{-1}), meanwhile highest intercellular CO_2 concentration was obtained in Qi-1 by same concentration (250 mg l^{-1}). Furthermore, highest transpiration rate (9.83 μmol m^{-2} s^{-1}) was recorded in Ha-1 by 150 mg l^{-1} ALA. In general, G_s was 42% by 50 mg l^{-1} ALA, 30 % by 150 mg l^{-1} ALA and 71% by 250 mg l^{-1} ALA were higher than control, C_i was 4% by 50 mg l^{-1} ALA, 4% by 150 mg l^{-1} ALA and 9% by 250 mg l^{-1} ALA were greater than control, meanwhile, *E* was 50 by 50 mg l^{-1} ALA, 72 % by 150 mg l^{-1} ALA and 65% by 250 mg l^{-1} ALA were more than control.

Discussion

Pakchoi is a cool-season crop preferring moist and uniform conditions in full sunlight. Chlorophyll biosynthesis of Pakchoi might be affected by high-temperature stress. ALA

Table 3 Effect of ALA treatment on the net photosynthesis rate (*Pn*) (μmol m^{-2} s^{-1}), stomata conductance (*Gs*) (mmol m^{-2} s^{-1}), intercellular CO_2 concentration (*Ci*) (mmol m^{-2} s^{-1}) and transpiration rate (*E*) (Ci μl l^{-1}) of Pakchoi leaves

Cultivar		Treatments	*Pn*	*Gs*	*Ci*	*E*
"Sanchidaye"	(Sa-1)	C	7.39c	0.343c	340b	4.67c
		T1	8.40b	0.480b	353a	10.0a
		T2	9.40a	0.301d	314c	4.70c
		T3	9.56a	0.50a	344b	7.18b
"Lichuanda-suomian"	(Li-1)	C	4.62b	0.147d	364b	2.87c
		T1	2.97d	0.201b	380a	4.59b
		T2	3.98c	0.174c	377a	4.36b
		T3	8.50a	0.372a	367b	7.71a
"Aijiaohuang"	(Ai-1)	C	4.88d	0.100d	271c	1.99d
		T1	6.378c	0.212c	326b	4.03c
		T2	7.21b	0.276b	328b	5.16b
		T3	8.39a	0.402a	331a	5.28a
"Qingyou" No. 4	(Qi-1)	C	4.44c	0.153b	333b	3.59b
		T1	4.27d	0.127c	330b	2.98c
		T2	4.78b	0.375a	325c	7.25a
		T3	6.77a	0.079d	405a	1.1d
"Aikang" No. 5	(Ak-1)	C	8.28d	0.381b	324b	4.91d
		T1	9.10c	0.490a	323b	5.24c
		T2	10.24b	0.360c	324b	5.47b
		T3	11.0a	0.497a	341a	6.95a
"Hanxiao"	(Ha-1)	C	4.87d	0.212d	354b	4.47d
		T1	6.65c	0.355c	350c	6.26c
		T2	9.37b	0.610a	355b	9.83a
		T3	10.17a	0.430b	376a	8.57b
"Shulv"	(Sl-1)	C	3.74c	0.045c	295d	1.39c
		T1	5.46b	0.099b	317c	2.67b
		T2	5.65b	0.188a	351a	4.25a
		T3	6.68a	0.09b	329b	2.60b

The values were the average when Photon flux density (PFD) 1,000 μmol m^{-2} s^{-1}

Means followed by the same letter in the column do not differ statistically at $P = 0.05$

is the first key precursor of chlorophyll biosynthesis, and in plants is the limiting step during the tetrapyrrol biosynthesis (Ilag et al. 1994; von Wettstein et al. 1995; Wang et al. 2003), in which the exogenous ALA treatment may increase the chlorophyll content of Pakchoi leaves. The results show that the ALA treatment induces biosynthesis of chloroplasts, which is reflected by the raised chlorophyll content (Table 1). On the other hand, Tanaka et al. (1992, 1993) suggested that 5-aminolevulinic acid (ALA) increased Chl *a* mass and suggested that the apoproteins of light-harvesting complex of photosystem II were first stabilized by binding with Chl *a* and that the mass of the apoproteins was necessary for the formation of Chl *b*. ALA treatment increased the Chl *b* in Pakchoi leaves and Chl *b* is the main component of antenna pigment; therefore, Chl *b* increase is helpful for quantum-harvesting. ALA might be beneficial for plants to harvest quantum under hot season. Our unpublished data also shows that ALA greatly promoted Chl *a* and Chl *b* under salt stress conditions (Saba et al., unpublished), as well as promote stress tolerance of plants in low concentrations (Hotta et al. 1998; Watanabe et al. 2000); Hotta et al. (1997) also proposed the ALA effect in plants in relation to chlorophyll biosynthesis and photosynthesis activity.

In fact, plant cells usually keep the ROS level under tight control by production or activation of scavenging enzymes as well as nonenzymatic components (Bailly 2004).

The enzyme superoxide dismutase (SOD), catalase (CAT) (Scandalios et al. 1984) and peroxidases (POD) play an important role in the antioxidative response of plant cells to damaging conditions, as an effective quencher of reactive intermediary forms of oxygen and peroxide radicals, and consequently in the related H_2O_2 increase (Scandalios 1993).

5-ALA induces either marginal or significant activities of antioxidant enzymes of wheat, which can be associated with enhanced cellular capacity to detoxify reactive oxygen species (Zivile et al. 2006). Moreover, 5-ALA induces the antioxidative enzyme activities of spinach seedlings (Nishihara et al. 2003) and can greatly promote dark respiration (Wang et al. 2003) and germination of Pakchoi seeds (Wang et al. 2005).

H_2O_2 could play an important role in the mechanism of action of hot temperature. Sairam and Srivastava (2000) found that tolerant genotypes had the highest CAT activity and the lowest H_2O_2 content at elevated temperature. They proposed that heat tolerance in wheat was associated with higher levels of antioxidants responsible for detoxification of H_2O_2. Anderson (2002) reported that hydrogen peroxide levels were unchanged in heat stress pepper leaves. Other researchers reported that in pea (*Pisum sativum* L.), mung bean (*Vigna radiata* (L.) Wilczek) and cucumber CAT activity was significantly decreased under chilling stress, however, H_2O_2 content was also significantly decreased (MacRae and Ferguson 1985). Futhermore Boo and Jung (1999) observed that in rice (*Oryza sativa* L.) plants exposed to water stress, CAT activity decreased but levels of O_2^- did not change and H_2O_2 content decreased. Jeffrey and Sonali (2004) suggested that H_2O_2 did not play direct role in heat stress injury in vinca or sweet pea leaves. In this study, we did not investigate the effects of ALA on H_2O_2 and under stress environment, so that was not sure H_2O_2 decreased either increased, it is possible that ALA, influences on the H_2O_2 in Pakchoi plants under hot

temperature. Thus effect of ALA on H_2O_2 in pakchoi needs further investigation.

It is known that hemes are biosynthesized from the precursor 5-aminolevulinic acid (ALA), and that heme is the cofactor for cytochromes in various cell compartments, including mitochondria, chloroplasts and the cytoplasm. ALA treated was incorporated into the peroxidase molecule over a 16-h incubation period, and other porphyrins, such as the prosthetic group of cytochromes and peroxidase, increased during the treatment with ALA, as discussed by Van Huystee (1976, 1977). Therefore, ALA-stimulated heme and the activity of CAT was increased (Nishihara et al. 2003). The increasing activities catalyzed might be scavenging of H_2O_2 and an increase in its activities was related with increase in stress tolerance. On the other hand, superoxide ($O_2^{\cdot-}$) anion production rate was lower in ALA over_ production transgenic tobacco than wild type tobacco (Wang et al., unpublished data).

Photosynthesis activity is further reduced under hot season because hot temperatures will increase the water loss from the leaf, which causes the stomata to close (http://croptechnology.unl.edu). Net photosynthesis rate of Pakchoi leaves during hot season was considerably decreased in Nanjing region China. The synthesis of two precursor molecules of chlorophyll, 5-aminolevulinic acid and protochlorophyllide, is affected during hot season, due to the inhibition of several enzymes involved in the biosynthesis pathway (Feierabend 1977). Wang et al. (2004) observed that the reduction of *Pn* was associated with lower levels of AQY, CE, *Gs*, *Ci* and chlorophyll. The result showed that the net photosynthetic rate increased in response to increased ALA concentration (Table 2). The net photosynthesis was increased due to the increase of available CO_2 by stomatal opening. ALA enhanced the photosynthesis activity of grapevines (Watanabe et al. 2006), rice, barley, potato and garlic (Tanaka et al. 1992) and increased the fruit flesh percentage, total and reducing sugars, fruit volume and weight of date palm (Al-Khateeb et al. 2001).

Conclusion

Foliar application of ALA effect on antioxidative enzyme activities increased the chlorophyll content, which was followed by an enhancement of photosynthetic activity in hot season. Different cultivars had increased chlorophyll content, antioxidative enzymes and photosynthesis rate in different levels of ALA concentrations.

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