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Effect of 5-Aminolevulinic Acid (ALA) on antioxidative enzymes, chlorophyll content, and photosynthesis of Pakchoi (*Brassica campestris* ssp. *chinensis*) under salt stress

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Abstract The application of 5-aminolevulinic acid (ALA) solutions significantly increased the net photosynthetic rate (*P*n), stomata conductance (Gs), and intercellular CO_2 concentration (*Ci*), as well as leaf transpiration rate (*E*). Cultivar Qd-1 responded the highest leaf *P*n, being enhanced 1.8 fold and 2 fold, *Gs* was greater by about 3.1 fold and 2.8 fold, *Ci* was increased 1.3 fold and 1.2 fold respectively under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl as compared to control by the effect of ALA, and cultivar Ai-1 (salt tolerant), which responded with the highest *E*, about 67 % higher than that of the control under normal (0 mmol l^{-1}NaCl) conditions. Furthermore, the highest superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities responded in cultivar Ai-1, ALA were enhanced the SOD 1.5 fold and POD 1.2 fold under salt stress(150 mmol l^{-1} NaCl), CAT activity were 1.4 fold greater under salt stress as compared to the control. In the salt sensitive (Li-1) cultivar, the CAT activity increased under salt stress as compared to the control. ALA treatment increased the leaf chlorophyll content of 'Ai-1' and 'Qd-1' leaves.

Keywords: 5-Aminolevulinic Acid; Antioxidative enzymes; Chlorophyll; Photosynthesis; Salt stress.

Introduction

Soil salinity is one of the most important factors limiting plant growth and crop production in many parts of the world, particularly in arid and semi-arid areas (Ashraf and Waheed, 1993). Salinity also decreases photosynthetic

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capacity due to the osmotic stress and partial closure of stomata (Drew *et al.*, 1990). High soil salinity can also cause nutrient imbalances, resulting in the accumulation of elements toxic to plants, and reduced water infiltration if the level of one salt element--sodium--is high (Kotuby *et al.*, 1997). Salt-stressed plants accumulate various molecules found in organic matter such as proline, glucose, glycine betaine, etc. for osmoregulation to occur thereby protecting enzyme activity (Munns and Termaat, 1986). The levels of antioxidative enzyme activity and antioxidant (ascorbic acid and glutathione) concentration are frequently used as indicators of oxidative stress in plants (Mittler, 2002). Bor *et al.* (2003) have demonstrated that the generation of reactive oxygen species (ROS), such as the superoxide radical ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot -}$) and hydrogen peroxide (H_2O_2), alter antioxidant enzyme activity. Antioxidants are induced in plants in response to stressors like salinity. ROS causes oxidative damage to biomolecules such as lipids and proteins, and eventually leads to cell death. To protect against oxidative stress, plant cells produce both non-enzymatic antioxidants such as ascorbate, glutathione and ◦-tocopherol, and antioxidative enzymes such as peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) (Del Rio *et al*., 2003).

Recently, 5-aminolevulinic acid (ALA) has attracted attention as a biodegradable herbicide (Rebeiz *et al* 1984). ALA is a key precursor in the biosynthesis of porphyrins such like chlorophyll and heme. In plants, ALA concentration is strictly controlled to less than 50 nmol/g FW (Stobart and Ameen 1984). Rebeiz *et al* (1984) reported that herbicidal activity increases the accumulation of several chlorophyll intermediates, such as protochlorophyllide, protoporphyrin IX, and Mg-protoporphyrin IX, when plants are treated with 5–40 mmol l^{-1} ALA concentrations. It is supposed that the accumulated chlorophyll intermediates act as a photosensitizer for the formation of singlet oxygen ($^1O_2$), triggering photodynamic damage of ALA-treated plants. Hotta *et al* (1997b) observed that the yields of plants, including kidney beans, barley, potatoes, and garlic, are improved by ALA treatment at low concentrations of 10 to 60%. Hernandez *et al*. (1995), Watanabe *et al*. (2000) and Hotta *et al*. (1998) discussed effects of ALA on the improvement of salt tolerance in cotton seedlings and pea plants, as well as cold resistance in rice. From these studies, it was observed that protection against damage involves a more active ascorbate-glutathione cycle and a high level of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidases (APX), all of which are involved in the development of salt tolerance in cotton and pea plants.

The objective of this study was to investigate the effect of ALA on antioxidant enzyme, chlorophyll and photosynthesis in leaves of pakchoi under different concentrations of salt.

Materials ang methods

Seed materials

The salt tolerant, moderately tolerant and sensitive cvs of Aijiaohuang (Ai-1), Qingdi (Qd-1) and Lichuandasuomian (Li-1) pakchoi (*Brassica campestris* ssp. *chinensis*) were used in this study. Seeds of three cultivars were obtained from Nanjing Agricultural University's experimental farm, located at Jiangpu, Jiangsu, China.

Preparation of plant material

The seeds were soaked in 90mm Petri dishes for 24 hr before sowing. Four seeds were sown in each pot, the pot was filled with soil (soil: vermiculite 2:1)-filled plastic pots (size 12×8 cm containing1000g) (Da Zhongting Lmt, China). Sixteen seeds were sown in three replications (Three pots with 4 seeds in each treatment). The treatments (T1-100mgl^{-1} ALA (EC-1.6ms/cm (1:2-w/v), T2-50 mmol l^{-1} NaCl + 100mg l^{-1} ALA (EC-5.8ms/cm), T3-150 mmol l^{-1} NaCl+ 100mgl^{-1} ALA (EC-8.87ms/cm), T4-50 mmol l^{-1} NaCl (EC-5.3ms/cm), T5-150mgl^{-1} NaCl (EC-8.5ms/cm), Control-distilled water (EC-0.7ms/cm)) were started two weeks after of seed sowing with 40 ml/pot (10ml/plant). ALA (treatment) was purchased from Dikarmun chemical (Industrial Co Ltd China) and grown in a plastic tunnel (10–18 C). Treatments (ALA, ALA+NaCl, NaCl) were applied weekly to soil for the duration of six weeks. Data was recorded after two to three weeks of treatments.

Determination of Chlorophyll

The fully expanded mature leaves were selected randomly. Hundred gram leaf tissues were cut into 1 cm pieces and were placed in 2.5 cm x 20 cm test tubes with 5ml 90% alcohol for 24 hrs, and chlorophyll content was estimated at 470nm, 665nm and 649nm (Arnon, 1949).

Measurement of antioxidative enzymes

One hundred milligrams of leaf tissue sample were ground in 1.6ml phosphate (pH 7.4) buffer solution 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^{o}C. Superoxide dismutase (SOD) was estimated according to Beyer and Fridowich (1987), catalase (CAT) was determined according to Aebi (1983) and Peroxidase (POD) was estimated according to Putter (1974). Each parameter was replicated three times.

SOD activity was measured by monitoring the inhibition of nitro blue tetrazolium (NBT) reduction. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 100 μM Na- EDTA, 750 μM NBT, 130 mM methionine and 20μM Vitamin B_2 by following Beyer and Fridowich (1987). CAT activity was recorded by measuring the decomposition of 45μM H_2O_2 in a 0.05 M phosphate buffer (pH 7.0) at 240 nm (Aebi, 1983). POD activity was observed by measuring the increase in absorbance of the product of reaction among 99% 2-methoxyphenol, 0.05 M phosphate buffer (pH 6.0) and 30% H_2O_2 at 470 nm (Putter, 1974).

Measurement of Photosynthesis Parameters

The photosynthetic rate of mature fully expanded 3rd leaf of pakchoi exposed to different treatments was measured by a LI-6400 portable photosynthesis system (USA), which could simultaneously record the cuvette CO_2 concentration (*Ca*), intercellular CO_2 concentration (*Ci*), leaf transpiration rate (*E*), stomata conductance (*Gs*) and net photo-synthesis rate (*Pn*). All parameters were determined with at least three replications. The photon flux density (PFD) was regulated to 100, 500 and 1000 μmol m^{-2} s^{-1}.

Data collection and statistical analysis

The net photosynthetic rate (*Pn*), stomata conductance (*Gs*), intercellular CO_2 concentration (*Ci*), transpiration rate (*E*), chlorophyll content and antioxidative enzymes were measured. The data were processed by ANOVA from three replicates of all treatments (2002 by SAS Institute Inc., Cary, NC, USA 2002 Version 9.00). Means were separated by Duncan's Multiple Range Test (with significance at $P < 0.05$).

Results and discussions

Antioxidative enzymes activities

The superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities in pakchoi leaf in response to ALA treatment under salt stress (50mmol l^{-1} NaCl and 150mmol l^{-1} NaCl) are shown in Table 1. Superoxide dismutase (SOD) is a major scavenger of superoxide ($O_2^{\cdot -}$), and its enzymatic

action results in the formation of H_2O_2 and $O_2^{\cdot-}$. The reactive oxygen species such as H_2O_2, OH and $O_2^{\cdot-}$ radicals that are produced under salt stress are potentially harmful to plants; and plants have the capacity to cope with these reactive oxygen species by eliminating them with an efficient scavenging system (Breusegem *et al.,* 2001). The SOD activities of each cultivar of pakchoi leaves were decreased under both NaCl conditions. Cultivar 'Li-1' (salt sensitive) and 'Ai-1' (tolerant) of leaves that the activities of SOD over the effect of ALA were 1.6 fold and 1.1 fold greater under 50 mmol l^{-1} NaCl than that of the control, the same tendency was shown under 150 mmol l^{-1} NaCl (Table I). The SOD activities of 'Li-1' and 'Ai-1' leaf were enhanced 1.8 and 1.5 fold compared to control, where as ALA treatment promoted it, regardless of the presence of salt stress (150 mmol l^{-1} NaCl),. The SOD activity cultivar 'Qd-1' (moderately tolerant) at 100 mgl^{-1}ALA rate of application under both salts conditions was lower compared to the control. In this case, the reason for increased SOD activity was assumed to be salt stress. In this experiment the response was very different. SOD activity decreased in all cultivar under salt stress. A similar response was reported by Rahnama and Ebrahimzadeh (2005), that relatively NaCl sensitive potato cultivars the SOD activity showed reduced. Furthermore, ALA (as shown in Table 1) increased the SOD activity of 'Qd-1' leaves, but was less than that of the control under both conditions.

Salt tolerance cultivar (Ai-1), POD activities showed a decrease, 50mmol l^{-1}NaCl and 150mmol l^{-1}NaCl less than that of the control--about 4.4 fold and 5.5 fold. The POD activity under the effect of ALA of under 50mmol l^{-1} NaCl increased about 1.2 fold over that of the control, and under 150mmol l^{-1} NaCl, POD activity decreased 1.3 fold over that of the control under the effect of ALA. Cultivar Qd-1, the leaf POD activity, decreased 2.3 and 2.4 fold under 50mmol l^{-1}NaCl and 150mmol l^{-1}NaCl that of the control. The peroxidase activity increased where as ALA treatment promoted it about 1.1 fold that of the control, regardless of the presence of 150mmol l^{-1}NaCl. Compared with the activity of the control, these activities were under 50 mmol l^{-1} NaCl, a decrease of about 1.3 fold. They maintained a significant difference throughout the experimental period. It is suggested that low or high salt concentration correlated to the pakchoi cultivars increasing or decreasing the POD activity. In potatoes (*Solanum tuberosum*) under higher NaCl levels, POD activity was partially reduced (Rahnama and Ebrahimzadeh *et al*., 2006). Van Huystee (1977) discussed that 5-aminolevulinic acid (ALA) treated pea was incorporated into the peroxidase molecule over a 16-h incubation period, and other porphyrins, such as the prosthetic group of cytochromes and peroxidases increased during the treatment with ALA.

It is well known that CAT (Scandalios, 1994) catalyzes the breakdown of H_2O_2. Catalases (CAT) were also the most efficient antioxidative enzyme, cultivar 'Li-1' (salt sensitive) the CAT activities increased about 5.3 fold and 4.6 fold under 50mmol l^{-1}NaCl and 150mmol l^{-1}NaCl than that of the control. The CAT activities decreased by ALA about 1.1 fold and 1.8 fold in comparison to the control under salt stress (50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl). Increases of the CAT activity under NaCl stress have been reported in peas (Hernandez *et al*., 1995) and cucumbers (Lechno *et al*., 1997). Cultivar 'Qd-1' CAT activities increased about 1.4 fold under 50mmol l^{-1}NaCl than that of the control. The CAT activities decreased about 1.9 fold under high salt stress (150mmol l^{-1}NaCl) as compared to the control. The CAT activities under the effect of ALA decreased about 1.1 fold in comparison to the control under 50mmol l^{-1}NaCl. A similar response was suggested/observed by Ezatollah *et al*. (2007) that in wheat seedlings at higher levels of NaCl, i.e., 100, 150 and 200mM, catalase activity significantly decreased in comparison with 50 mM NaCl. The CAT activities, in the presence of ALA showed no significant difference from that of the control under salt stress (150mmol l^{-1}NaCl) during the experimental period. Furthermore, the result (Table 1) shows that cultivar Ai-1 the CAT activities increased about 1.2 fold under 50mmol l^{-1}NaCl and CAT activities decreased about 1.2 fold under 150mmol l^{-1}NaCl than that of the control. The activities by ALA under 150mmol l^{-1}NaCl and 50mmol l^{-1}NaCl were 1.4 fold that of the control. As we know, ALA is a first precursor of heme biosynthesis in all organisms (Castelfranco and Beal, 1983). In this study, the activity of CAT, which is composed of heme, was increased (Table 1). It is known that isolated chloroplasts can synthesize heme from the exogenously supplied precursor, according to Bhaya and Castelfranco (1985), that administration of ALA results in the increasing accumulation of newly synthesized heme (Yu and Weinstein, 1997). Heme turnover has been demonstrated, directly or indirectly, to occur in several hemoproteins in several types of plant materials, including intact greening barley leaves (Castelfranco and Beal, 1983) and isolated developing pea chloroplasts (Yu and Weinstein, 1997).

Table 1. Effect of ALA on Superoxide dismutase (SOD), Peroxidase (POD), catalase (CAT), of pakchoi leaves (U / g.min)

Cultivars	Treatments	SOD	POD	CAT
Li-1	C	28d	0.077a	0.0026e
	T1	40c	0.020d	0.0024f
	T2	45b	0.033c	0.0048c
	T3	52a	0.070b	0.0028d
	T4	19e	0.019e	0.014a
	T5	17f	0.018e	0.012b
Qd-1	C	52a	0.062c	0.0070d
	T1	49a	0.070a	0.0082b
	T2	32c	0.046d	0.0078c
	T3	38b	0.068b	0.0072d
	T4	20d	0.026e	0.0102a
	T5	14e	0.025e	0.0036e
Ai-1	C	48d	0.066b	0.0056d
	T1	62b	0.027d	0.0032f
	T2	53c	0.080a	0.0080a
	T3	72a	0.048c	0.0078b
	T4	30e	0.015e	0.0066c
	T5	22f	0.012f	0.0046e

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

Chlorophyll Content

The chlorophyll content of pakchoi was examined by application of ALA. It decreased under 50 mmol l^{-1} and 150mmol l^{-1} NaCl (Table 2). Cultivar Li-1 (salt sensitive), in low sodium stress (50 mmol l^{-1}NaCl) only Chl *a* decreased about 5% over that of the control. Chl *b*, total Chl and Chl ratio were not affected under 50mmol l^{-1} NaCl. Cultivar Li-1, Chl *a*, Chl *b*, and total Chl decreased by 18%, 5% and 23% respectively under 150 mmol l^{-1}NaCl over that of control, although Chl ratio almost remained same under both NaCl condition than that of the control. The Chl *a*, Chl *b*, total Chl and Chl ratio increased by 27%, 45%, 45% and 25% respectively, responding to the effect of ALA under 150mmol l^{-1}NaCl. Furthermore, compared with that of the control, ALA treatment increased Chl *a* 5%, Chl *b* 4%, total Chl 9% and Chl ratio 3% under 50 mmol l^{-1}NaCl.

Table 2. Effect of ALA treatment on the chlorophyll (Chl) content of pakchoi leaves (Unit: mg g^{-1} FW). *The unit of ALA solution is mg l^{-1}

Cultivar		Treatments	Chl a	Chl b	Chl a+b	Chl b/a
Lichuandasuomian	(Li-1)	C	0.74c	0.22d	0.96c	0.29c
		T1	0.75c	0.24c	0.99c	0.32b
		T2	0.79b	0.26b	1.05b	0.32b
		T3	1.01a	0.40a	1.41a	0.39a
		T4	0.69d	0.21d	0.90d	0.30c
		T5	0.56e	0.17e	0.73e	0.30c
Qingdi	(Qd-1)	C	0.88b	0.32b	1.20c	0.36b
		T1	1.08a	0.59a	1.67a	0.54a
		T2	0.87b	0.28c	1.15d	0.32c
		T3	1.00a	0.29c	1.29b	0.29d
		T4	0.85c	0.25d	1.10e	0.29d
		T5	0.79d	0.25d	1.04f	0.31c
Aijiaohuang (Ai- 1)		C	0.89c	0.31c	1.20b	0.34c
		T1	0.88c	0.33b	1.21b	0.37c
		T2	1.00a	0.43a	1.43a	0.43a
		T3	0.93b	0.25d	1.18c	0.26d
		T4	0.86d	0.34b	1.20b	0.39b
		T5	0.84d	0.31c	1.15d	0.36c

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

Cultivar Qd-1 (moderate tolerant), 3% to 16% Chl *a*, Chl *b*, total Chl and Chl ratio decreased under both salt conditions compared to the control (Table 2). ALA treatment enhanced the Chl *a*, Chl *b*, total Chl and Chl ratio about 12%, 4%, 5% and 4% respectively under low salt stress (50 mmol l^{-1}NaCl) as compared to the control. ALA treatment increased the Chl *a* 12 % and total Chl 9%, and the Chl *b* and Chl ratio did not increase as compared to the control under high salt stress (150 mmol l^{-1}NaCl). A similar response was reported by Wang *et al*. (2004) ALA treatments increased leaf chlorophyll content, as well as soluble sugar levels, but decreased the rate of respiration under light.

Cultivar Ai-1(salt tolerant), Chl *a* 3 % decreased as compared to control and total Chl remained the same as in the control, Chl *b* 3 % and Chl ratio 5% were higher as compared to the control under low salt stress (50 mmol l^{-1}NaCl). Furthermore, Chl *a* and total Chl decreased about 5%, content of Chl *b* the remained same as in control under 150 mmol l^{-1}NaCl. Chl ratio was a little higher under the salt stress (150 mmol l^{-1}NaCl) as compared-with the control. ALA significantly increased Chl *a* 11%, Chl *b* 12%, total Chl 23% and Chl ratio 9% of 'Ai-1' leaves, respectively, under 50 mmol l^{-1}NaCl over that of the control, which suggests that ALA treatment improved chlorophyll content, and chlorophyll is helpful for photosynthesis. Furthermore, ALA treatment increased Chl *a* 4%, but Chl *b* 6%, total Chl 2% and Chl ratio 8% decreased

under 150mmol l^{-1}NaCl as compared to the control. ALA treated leaves had a significantly the higher chlorophyll content under normal (0mmol l^{-1}NaCl) conditions as compared to the control in cases of all cultivar. As is known, ALA is the first key precursor of chlorophyll biosynthesis, and the biosynthesis of ALA in plants is the boundary during the tetrapyrrol biosynthesis (Wang *et al*., 2003). Chl *a* is the primary electron donor in the reaction center of the two photosystems, and also serves as the light-harvesting (LHC) pigment. In the higher plants, both Chl *a* and Chl *b* are bound to the light-harvesting complex (LHC). In this study, ALA treatment increased the chlorophyll content, which might be beneficial for plants to harvest quantum under salt stress. Chl *b* is the main component of antenna pigment; therefore Chl *b* increase is helpful for quantum harvesting.

Net photo-synthesis rate (P_n), stomata conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (E)

The net photo-synthesis rate (*P*n) of all cultivars of pakchoi increased in response to increased Photon flux density (PFD) up to 1000 µmol m^{-2} s^{-1} (Figs 1-3). The *P*n in ALA treated leaves were generally higher than that of the control regardless of the increase in Photon flux density. However, the higher leaf *P*n of Li-1' leaves with ALA treatment had an increase 1.1 fold and 1.3 fold. The maximum leaf *P*n of cultivar Qd-1 enhanced by 1.8 fold and 2 fold, and cultivar Ai-1' improved 1.3 fold and 1.1 fold under 50 mmol l^{-1} NaCl and 150 mmol l^{-1} NaCl as compared to the control. Wang *et al*. (2005) proposed that 5-aminolevulinic acid (ALA) significantly increased the net photosynthetic rate (*Pn*).

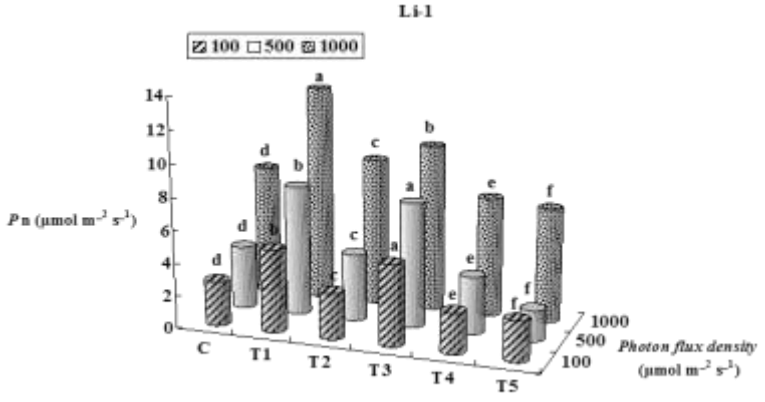

Fig. 1. Net photosynthetic rates (*Pn*) of Li-1 to increase when increase the photon flux density (PED). C-distilled water, T1-100mgl^{-1} ALA, T2-50 mmol l^{-1} NaCl + 100mg l^{-1} ALA, T3-150 mmol l^{-1} NaCl+ 100mgl^{-1} ALA, T4-50 mmol l^{-1} NaCl, T5- 150mgl^{-1} NaCl

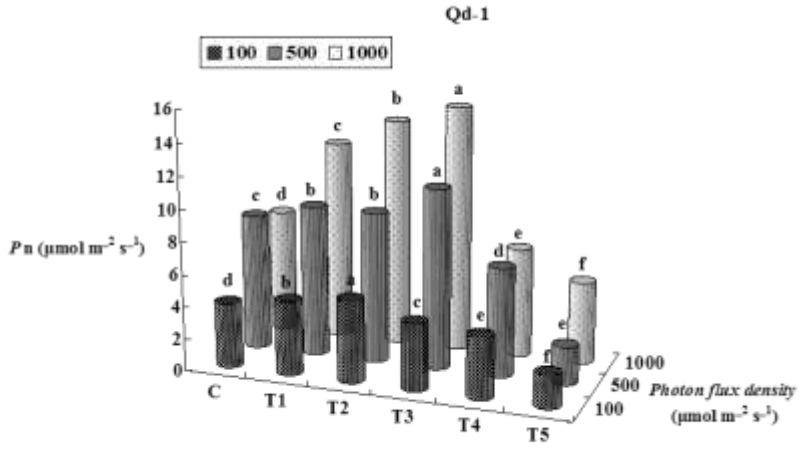

Fig. 2. Net photosynthetic rates (*Pn*) of Qd-1 to increase when increase the photon flux density (PED). C-distilled water, T1-100mgl^{-1} ALA, T2-50 mmol l^{-1} NaCl + 100mg l^{-1} ALA, T3-150 mmol l^{-1} NaCl+ 100mgl^{-1} ALA, T4-50 mmol l^{-1} NaCl, T5- 150mgl^{-1} NaCl

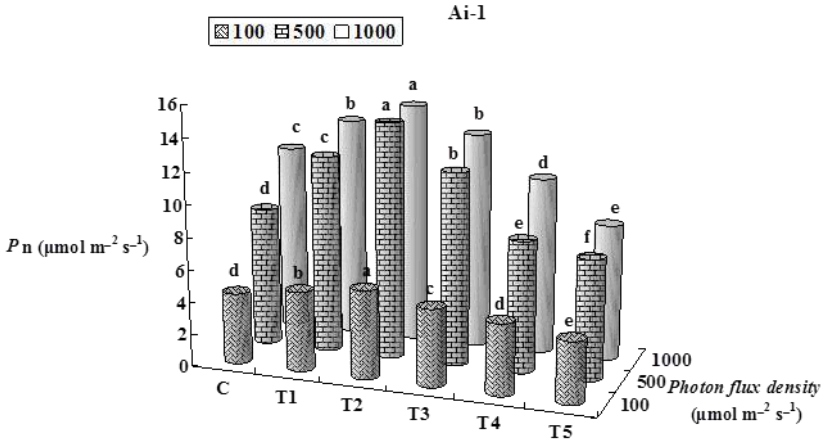

Fig. 3. Net photosynthetic rates (*Pn*) of Ai-1 to increasing when increase the photon flux density (PED). C-distilled water, T1-100mgl^{-1} ALA, T2-50 mmol l^{-1} NaCl + 100mg l^{-1} ALA, T3-150 mmol l^{-1} NaCl+ 100mgl^{-1} ALA, T4-50 mmol l^{-1} NaCl, T5- 150mgl^{-1} NaCl

Data (Table 3) shows that ALA treatments increased the stomata conductance (Gs), intercellular CO_2 concentration (*Ci*) and transpiration rate (*E*) of pakchoi leaves under salt stress of all cultivars, and were significantly higher than salt stress. Cultivar 'Li-1', stomata conductance (*Gs*) 1.4 fold and 1.2 fold decreased under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl than that of control. ALA treated leaves were 2 fold and 1.6 fold as great as that of control under 50

mmol l^{-1} and 150 mmol l^{-1}NaCl. ALA treatment significantly stimulated *Ci* 1.4 fold and 1.6 fold and transpiration rate (*E*) 1.5 fold and 1.9 fold greater than that of the control under 50 mmol l^{-1} and 150 mmol l^{-1}NaCl. Cultivar Qd-1 decreased by 2 fold and 1.4 fold under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl over that of the control. ALA treatment increased *Gs* 3.1 fold and 2.8 fold under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl. *Ci* decreased 1.7 fold under salt stress (50 mmol l^{-1}NaCl) over that of the control. *Ci* was 1.1 fold higher under 150 mmol l^{-1}NaCl as compared to the control. ALA treatment stimulated *Ci* 1.3 fold and 1.2 fold under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl as compared with the control. Transpiration rate (*E*) decreased 1.8 fold and 1.3 fold under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl over that of the control. ALA treatment significantly augmented the *E* of cultivar Qd-1 leaves, and *E* was stimulated 2.3 fold and 2.2 fold under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl as compared with control.

Table 3. Effect of ALA treatment stomata conductance (*Gs*) (mmol m^{-2} s^{-1}), intercellular CO_2 concentration (*Ci*) (µl.l^{-1}) and transpiration rate (*E*) (mmol m^{-2} s^{-1}) of pakchoi leaves. The values were the average when Photon flux density (PFD) 1000µmol m^{-2} s^{-1}

Cultivars	Treatments	*Gs*	*Ci*	*E*
Li-1	C	0.05f	144e	1.04f
	T1	0.13a	209d	2.40a
	T2	0.08c	215c	1.58c
	T3	0.10b	233b	1.95b
	T4	0.07d	239a	1.42d
	T5	0.06d	214c	1.23e
Qd-1	C	0.07d	213e	1.48d
	T1	0.18c	252c	2.90c
	T2	0.22a	274a	3.36a
	T3	0.20b	265b	3.25b
	T4	0.04e	120f	0.82f
	T5	0.05e	237d	1.12e
Ai-1	C	0.15b	247a	2.74b
	T1	0.20a	249a	3.41a
	T2	0.13c	187c	2.50c
	T3	0.13c	196b	2.40d
	T4	0.07d	149e	1.51e
	T5	0.06d	169d	1.36f

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

The effect of ALA treatment on *Gs*, *Ci* and *E* of 'Ai-1' leaves were also evaluated. *Gs* decreased 2.1 fold and 2.5 fold grater under both salt condition (50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl) over that of the control. *Gs* of ALA

treated leaves were 1.8-2.1 folds greater than that of both salt stress conditions, but not higher than that of the control. *Ci* of 'Ai-1' leaves were 1.6 and 1.4 fold lower under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl as compared to the control. *Ci* of ALA treated 'Ai-1'leaves was about 1.2 fold and 1.1 fold enhanced over that of both salt stress conditions (50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl), but not greater than that of the control. *E* of 'Ai-1' leaves were1.8 fold and 2 fold lower under 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl than that of the control. The transpiration rate (*E*) in ALA-treated leaves was generally higher than that of salt stress condition. The *E* of 'Ai-1'leaves, ALA treated leaves were 1.6 fold and 1.7 fold greater as compared with both salt stress conditions (i-e 50 mmol l^{-1}NaCl and 150 mmol l^{-1}NaCl). Wang *et al.* (2005) proposed that 5-aminolevulinic acid (ALA) significantly increased the apparent quantum yield (AQY), carboxylation efficiency (CE) and stomata conductance (Gs). ALA is a first precursor of heme biosynthesis and the heme is essential for other porphyrins such as the prosthetic group of cytochromes and peroxidase, and a more active ascorbate-glutathione cycle related to the increase of the photosynthesis rate is involved in the increase of salt tolerance. This suggests that two different salt concentrations were correlated to cultivars, which were increasing or decreasing the photosynthesis rate over the effect of ALA. Thus highest percentage of *Pn*, *Gs*, *Ci* and *E* over the effect of ALA under high salt stress in pakchoi needs further investigation.

Conclusion

It is concluded that the 100 mgl^{-1}ALA effect on antioxidative enzymes activities increased the chlorophyll content which was followed by an enhancement of photosynthesis activity under salt stress (NaCl 50 and 150 $mmol^{-1}$).

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