BRIEF COMMUNICATION

Pretreatment with 5-aminolevulinic acid mitigates heat stress of cucumber leaves

J. ZHANG[1], D.-M. LI[1], Y. GAO[1], B. YU[1], C.-X. XIA[2] and J.-G. BAI[1]*

State Key Laboratory of Crop Biology/College of Life Sciences[1] and College of Food Science and Engineering[2], Shandong Agricultural University, Tai'an, Shandong 271018, P.R. China

Abstract

Cucumber seedlings were pretreated with 3 μM 5-aminolevulinic acid (ALA) followed by cultivation at normal (25/18 °C) or high (42/38 °C) day/night temperature to investigate the protective effects of ALA on heat stress in plants. Heat elevated the content of malondiadehyde (MDA), superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) in leaves of all plants but less in ALA-pretreated plants. Heat treatment resulted in higher antioxidant enzyme activities and proline and soluble sugar content and weaker growth inhibition in ALA-pretreated plants than in those treated with heat alone. These results indicate that ALA pretreatment increased the tolerance of seedlings to heat stress.

Additional key words: 5-aminolevulinic acid, antioxidant enzyme, *Cucumis sativus,* heat, proline, soluble sugar.

Heat stress inhibits plant growth (Huang and Xu 2008) and generates reactive oxygen species (ROS) including superoxide radical and hydrogen peroxide (Dat *et al.* 1998), which cause damage to plant cells (Fath *et al.* 2001). Therefore, plants have evolved antioxidant enzymes to alleviate this damage (Foyer *et al.* 1994).

An important precursor in the biosynthesis of porphyrin compounds is 5-aminolevulinic acid (ALA; Von Wettstein *et al.* 1995). Exogenous application of ALA regulates antioxidant enzyme activities and thereby increases the resistance of plants to different stresses such as cold (Balestrasse *et al.* 2010), chilling (Korkmaz *et al.* 2010), low irradiance (Sun *et al.* 2009) and salinity (Naeem *et al.* 2010). Although ALA pretreatment has not been reported to alleviate the damage of heat stress, it is hypothesized that pretreatment with ALA can enhance the ability of plants to resist heat stress through regulation of antioxidant enzyme activities. Therefore, cucumber seedlings were pretreated with ALA and then subjected to heat stress to investigate the protective effects of ALA application against heat stress. The roles of antioxidant enzyme activities, proline and soluble sugar were also investigated.

Cucumber (*Cucumis sativus* L. cv. Chunguang No. 2) seedlings were cultivated at 25 °C and watered twice daily with Hoagland nutrient solution (Gao *et al.* 2010). At the two-leaf stage, seedlings were selected and divided into four groups. In preliminary studies, the pretreatment with 3 μM ALA for 2 d resulted in lower content of malondialdehyde (MDA), $O_2^{\bullet-}$ and H_2O_2 in heat-stressed cucumber leaves. Therefore, two groups of cucumber seedlings were sprayed with 3 μM ALA, and two groups of cucumbers were sprayed with distilled H_2O. After 2 d, the ALA-pretreated groups were separately exposed to normal (25/18 °C) and high (42/38 °C) day/night temperatures together with 75 % relative humidity and a 12-h photoperiod (irradiance of 300 μmol m^{-2} s^{-1}). The ALA-untreated groups were separately subjected to identical conditions. After 2 d of heat treatment, the second leaves from all four groups were harvested.

MDA was extracted from leaves with 10 % trichloroacetic acid and was determined spectrophotometrically at

Received 18 July 2011, *accepted* 1 December 2011.
Abbreviations: ALA - 5-aminolevulinic acid; APX - ascorbate peroxidase; CAT - catalase, DHAR - dehydroascorbate reductase; GPX - guaiacol peroxidase; GR - glutathione reductase; GSH-Px - glutathione peroxidase; MDA - malondiadehyde, MDHAR - monodehydroascorbate reductase; SOD - superoxide dismutase.
* Corresponding author; fax: (+86) 538 8242217, e-mail: baijg@sdau.edu.cn

450, 532 and 600 nm (Dhindsa *et al.* 1981, Xu *et al.* 2008). H_2O_2 content was determined according to Bernt and Bergmeyer (1974) and the rate of $O_2^{\cdot -}$ formation according to Elstner and Heupel (1976). Activity was measured for the enzymes: superoxide dismutase (SOD; Hwang *et al.* 1999), catalase (CAT; Pereira *et al.* 2002), guaiacol peroxidase (GPX; Ramiro *et al.* 2006), glutathione peroxidase (GSH-Px; Xue *et al.* 2001), ascorbate peroxidase (APX; Zhu *et al.* 2004), mono-dehydroascorbate reductase (MDHAR; Hoque *et al.* 2007), dehydroascorbate reductase (DHAR; Doulis *et al.* 1997) and glutathione reductase (GR; Foyer and Halliwell 1976). The content of protein in each enzyme extract (Bradford 1976) and the content of proline (Bates *et al.* 1973) were also assayed. The soluble sugar content was estimated with anthrone reagent (Yemm and Willis 1954). Data were collected from three independent experiments and expressed as the mean ± standard errors. Differences were analyzed using the one-way *ANOVA* and least significant difference (LSD) at $P < 0.05$.

Heat stress adversely affected growth of many plant species (Huang and Xu 2008). Similarly, in this study, heat treatment significantly decreased cucumber height in comparison to the control and obviously reduced the fresh masses of the second leaves, shoots and roots (Fig. 1). In contrast, suitable concentrations of ALA promote plant

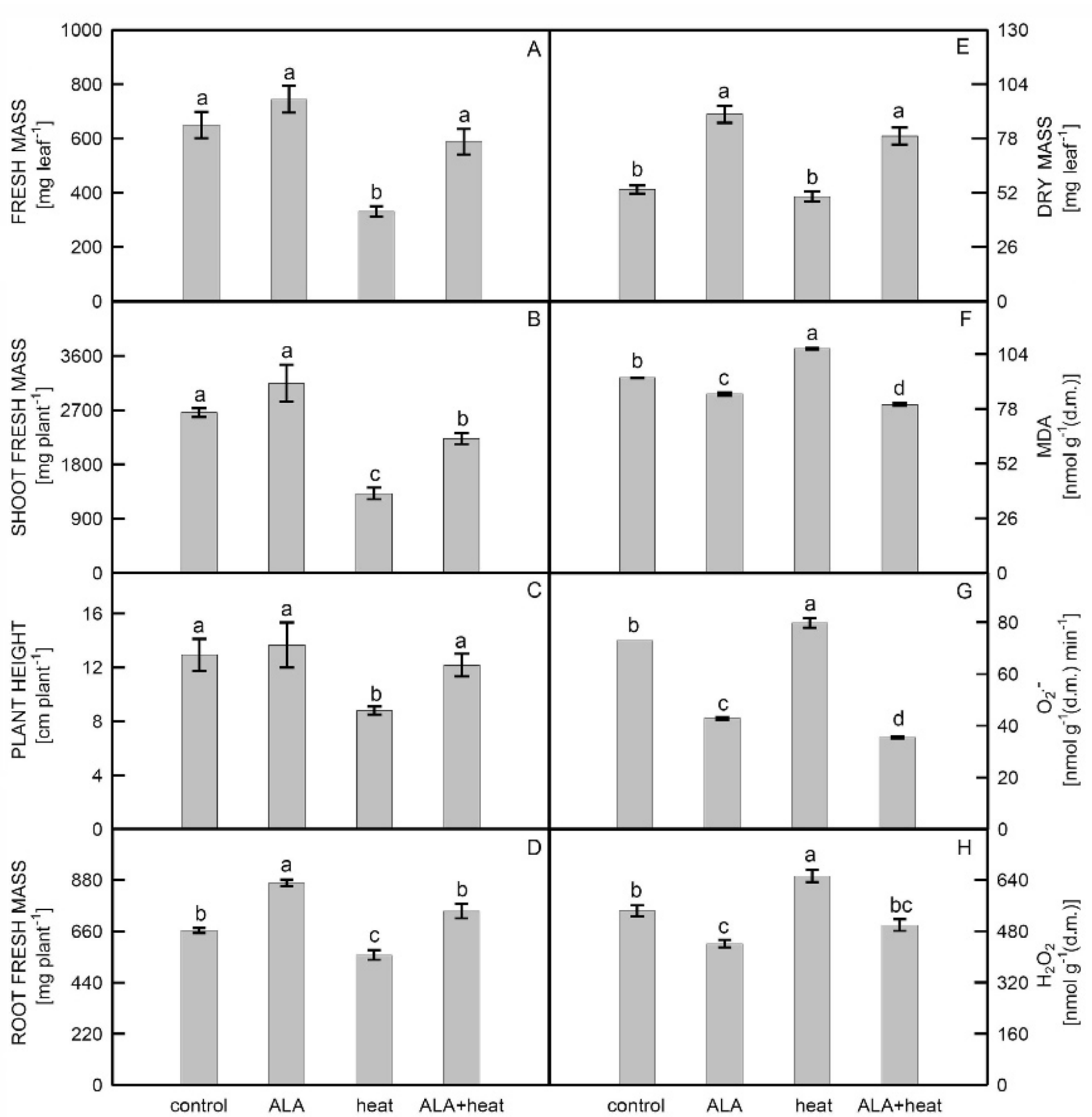

Fig. 1. Changes in fresh mass of the second leaf (*A*), shoot fresh mass (*B*), plant height (*C*), root fresh mass (*D*), dry mass of the second leaf (*E*), MDA content (*F*), $O_2^{\cdot -}$ production (*G*) and H_2O_2 content (*H*) in the second leaves induced by ALA pretreatment and/or heat stress. Control - 25/18 °C; ALA - ALA pretreatment followed by 25/18°C; Heat - 42/38 °C; ALA + heat - ALA pretreatment followed by 42/38 °C for 2 d. Each value represents the mean of at least three replicates ± SE. Different letters indicate statistically significant differences between treatments at $P < 0.05$.

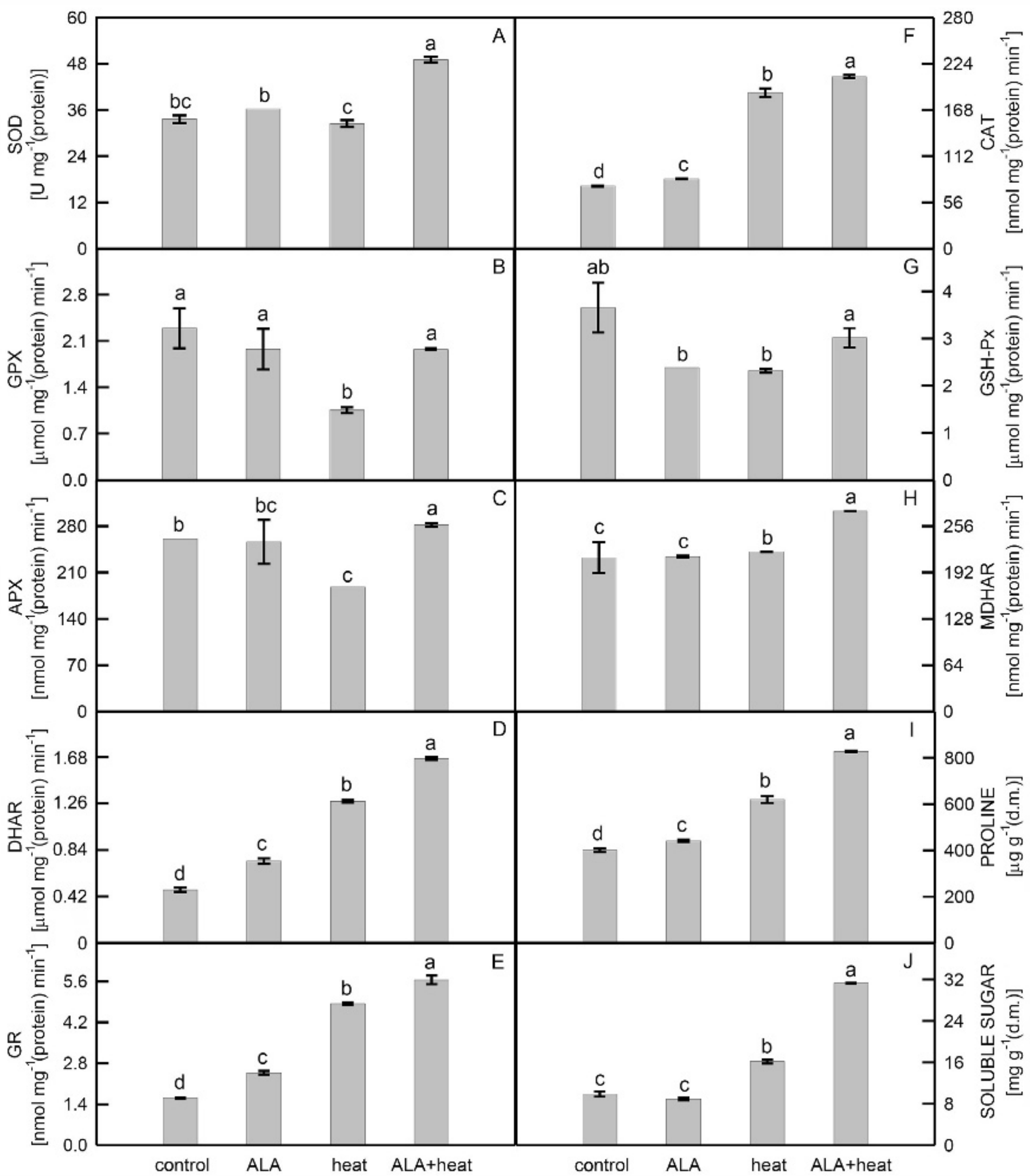

Fig. 2. Changes in activities of SOD (*A*), GPX (*B*), APX (*C*), DHAR (*D*), GR (*E*), CAT (*F*), GSH-Px (*G*) and MDHAR (*H*) and content of proline (*I*) and soluble sugars (*J*) in the second leaves induced by ALA pretreatment and/or heat stress. Control - 25/18 °C; ALA - ALA pretreatment followed by 25/18°C; Heat - 42/38 °C; ALA + heat - ALA pretreatment followed by 42/38 °C for 2 d. Each value represents the mean of at least three replicates ± SE. Different letters indicate statistically significant differences between treatments at $P < 0.05$.

growth of several crops (Hotta *et al.* 1997). Similarly, dry mass of the second leaves and root fresh mass were significantly enhanced in the ALA pretreatment group compared with the control group. ALA application protected soybean plants from cold stress without adverse effects on plant growth (Balestrasse *et al.* 2010). The results of this study were consistent with this observation. Growth parameters in ALA + heat treatment were significantly higher compared with the heat treatment alone, suggesting that 3 μM ALA protects cucumbers from heat stress.

Heat increased MDA content in *Anabaena doliolum* (Mishra *et al.* 2005), but heat acclimation results in gradual decrease in MDA content in turfgrass species (Xu *et al.* 2006). In this study, the MDA content in cucumber increased under heat stress. However, MDA content in the both ALA pretreatment groups was lower than in the respective untreated groups (Fig. 1). It can be due to

ALA-triggered increased CAT, DHAR and GR activities.

Compared to the control, the $O_2^{\bullet-}$ production and H_2O_2 content were significantly increased in leaves of the heat treatment group, which is in accordance with the reported $O_2^{\bullet-}$ and H_2O_2 accumulation in wheat under increased temperature (Luo *et al.* 2008). Pretreatment with ALA significantly decreased the content of $O_2^{\bullet-}$ and H_2O_2 in heat-stressed cucumber leaves, which was consistent with the altered MDA content. ROS content in cucumber leaves was also increased by chilling and this increase was reduced by application of cinnamic acid (Li *et al.* 2011a).

The activities of CAT in mulberry (Chaitanya *et al.* 2002) and MDHAR, DHAR and GR in cucumber (Song *et al.* 2005) were increased by heat stress. Comparable results were obtained in this study with increased CAT, MDHAR, DHAR and GR activities in the heat treatment group compared with the control group (Fig. 2). ALA + heat treatment increased the activities of SOD, CAT, GPX, GSH-Px, APX, MDHAR, DHAR and GR to a greater extent than heat treatment alone, and these coincided with the change in MDA, $O_2^{\bullet-}$ and H_2O_2 as well as plant growth indexes. Similarly, cinnamic acid pretreatment mitigated chilling stress of cucumber leaves through elevating the activities of antioxidant enzymes such as SOD, CAT, GPX, GSH-Px and APX (Li *et al.* 2011a). Pretreatment with salicylic acid increased the activities of MDHAR, DHAR and GR in heat-stressed mustard seedlings (Dat *et al.* 1998).

The content of proline and soluble sugars was increased under heat stress of *Lonicera japonica* (Li *et al.* 2011b). Analogous results were obtained in this study with significantly enhanced content of both compounds in response to heat treatment compared with the control group. Further, the content of proline and soluble sugars were significantly enhanced in the ALA + heat treatment group compared with the heat treatment alone. Application of glycinebetaine increased the content of proline and soluble sugars and improves the heat tolerance of sprouting sugarcane buds (Rasheed *et al.* 2011). It was reported that proline regulated antioxidant enzymes under heat stress (Rasheed *et al.* 2011). As concern soluble sugars, glucose increased the activities of MnSOD and Cu/ZnSOD (Ślesak *et al.* 2006), while sucrose balances the negative effect of atrazine on SOD activity (Ramel *et al.* 2009). Moreover, it was observed that soluble sugar quenched ROS, thus contributing to the stress tolerance (Bolouri-Moghaddam *et al.* 2010). In the ALA-pretreated heat-stressed cucumber plants, the increased proline and soluble sugar content correlated with weaker growth inhibition and increased antioxidant enzyme activities. This indicated that ALA pretreatment might modulate antioxidant enzymes indirectly *via* increased proline and soluble sugar content.

In conclusion, heat stress increased the content of $O_2^{\bullet-}$, H_2O_2 and MDA and inhibited plant growth. The combination of ALA pretreatment and heat resulted in higher increase in activities of antioxidant enzymes (SOD, CAT, GPX, GSH-Px, APX, DHAR, MDHAR and GR) compared with heat treatment alone. Furthermore, the increased proline and soluble sugar content, decreased $O_2^{\bullet-}$, H_2O_2 and MDA content and diminished growth inhibition were observed in the ALA + heat treatment compared with heat treatment alone. It is hypothesized that ALA pretreatment increases antioxidant enzyme activities directly or indirectly and thereby induced tolerance of cucumber seedlings to heat stress.

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