ACETYL SALICYLIC ACID AND 24-EPIBRASSINOLIDE ATTENUATE DECLINE IN PHOTOSYNTHESIS, CHLOROPHYLL CONTENTS AND MEMBRANE THERMO- STABILITY IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) UNDER HEAT STRESS

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Abstract

The effect of exogenous application of varying levels of 24-epibrassinolide (0.75, 1.5 and 3 μM) and acetyl salicylic acid (0.25, 0.75 and 1.25 mM) for induction of heat tolerance in terms of their effect on photosynthesis, chlorophyll content, membrane integrity and survival in four weeks old tomato (cultivar: Mei Jie Lo) seedlings under high temperature stress (46°C/4 h daily) for 21 days was investigated. The daily heat stress treatment had deleterious effects on seedlings but chemical treatments significantly reduced the magnitude of losses to different extents. 24-epibrassinolide (3 μM) was over all the best treatment to improve survival (86.11%), photosynthesis (39.4%) and chlorophyll contents (26.12%) accompanied with initiation of flower buds and improved vegetative growth. Whereas acetyl salicylic acid (1.25 mM) best improved photosynthetic activity (40.6%) as compared to the untreated heat stressed control seedlings. Moreover, 3 μM 24-epibrassinolide and 0.75 mM acetyl salicylic acid reduced cell membrane injury to 8.3 and 6.9% respectively as compared with 22.4% in heat stressed control seedlings. However lower doses of acetyl salicylic acid (0.25 and 0.75 mM) had slight (5.6 and 12.8%) inhibition effect on the photosynthesis than the heat stressed controls. Overall both acetyl salicylic acid and 24-epibrassinolide up regulated basal heat tolerance in tomato seedlings and studied concentrations demonstrated signature affect upon different parameters. Thus both chemical agents can be potential candidates for further investigations for exogenous application aiming at extension of tomato growth season in summer.

Key words: Acetyl salicylic acid, 24-epibrassinolide, Photosynthesis, Thermo-stability, *Lycopersicon esculentum*.

Introduction

Extreme temperatures have always been a serious threat to agriculture including tomato production owing to its heat and cold sensitivity. Elevated temperature stress leads to inhibition in plant growth both in vegetative and relatively delicate reproductive developments and yield of several crops (Peet & Willits, 1998; Hussain *et al*., 2006, Singh *et al*., 2007). In general, each 1°C increment in the average temperature during the growth season may reduce the crop yield up to 17% (Lobell & Asner, 2003). The optimum temperature range for growing tomato is 20-26/15-20°C day/night. The prevalence of high ambient temperatures in a significant proportion of the tomato growing areas of the world is one of the most crucial problems in tomato production. Heat stress results in fruit set reduction and declined tomato yields (Peet *et al*., 1997). To devise some adaptable strategies to extend the tomato production spans and improve the yield volumes in rising temperatures is imperative. Besides genetic uplift, induction of thermotolerance in existing high-yielding cultivars by foliar application or pre-sowing seed treatment with low concentrations of growth hormones can be a fruitful effort (Wahid *et al*., 2007). Steroidal hormones are a relatively new class of plant hormones named brassinosteroids. They can act as modulators of plant response to a diversity of abiotic stresses (Clouse & Sasse, 1998). BRs have ability to induce tolerance in plants to salinity, drought, high and low temperatures, heavy metals and others (Bajguz & Hayat, 2009). Acetyl salicylic acid (ASA) has been reported to confer tolerance against several abiotic stresses including heat stress. In potato microplant culture and virus eradication process thermotherapy is a useful practice and depends on the type of virus, present in the plants and the sensitivity of the cultivar to heat. It was demonstrated experimentally that ASA treatment enhanced thermotolerance in potato microplants (Lopez *et al.*, 1998) facilitating production of virus free plantlets through tissue culture. Senaratna *et al*., (2000) observed that plants grown from seeds imbibed in aqueous solutions (0.1-0.5 mM) of salicylic acid or acetyl salicylic acid (ASA) displayed enhanced tolerance and significant (100%) plant survival percentage against heat, chilling and drought stresses. ASA has been reported as inducer of thermotolerance but so far inadequately investigated focusing tomato plants. The present research work was planned for assessment and manipulation of best suitable doses of 24-EBL and ASA for induction of heat tolerance in tomato plants under daily heat stress conditions.

Materials and Methods

Growing seedlings: Uniform sized tomato seeds of heat tolerant tomato cultivar Mei Jie Lo (MJL) were sterilized in 0.1% $HgCl_2$ for two minutes, rinsed in dH_2O and germinated in wet filter paper for 3 days in growth chamber at 26°C and 70% humidity in dark. The peat based plant growth media was procured from Jie Hui Horticulture Co. Yangling and was autoclave-sterilized at 125 Pa/121°C for 21 minutes. The media was filled in rectangular plastic pots (LWD: 8cm x 8cm x 10cm). Germinated seeds were shifted in pots followed by shower irrigation and transferred to the growth chamber with 25/20°C day/night temperature, 12 h light period and 70% humidity. The plants were supplied with commercial nutrition supplement (Rotam Co. Ltd, China), on 2 leaves stage (20 mL per pot in two splits).

Chemical treatments: Acetyl salicylic acid (ASA) and 24-epibrassinolide (EBL) were procured from Sigma-Aldrich, Beijing, China. EBL was dissolved in ethanol to make a stock solution and stored at -20°C for future preparation of selected concentrations. However, ASA doses were directly prepared in distilled water using minimal quantity of ethanol as solvent. Both groups of tomato plants were uniformly foliar sprayed with respective doses of EBL, ASA or distilled water (control) added with equal quantity of ethanol. All the doses including control were added with 0.1% tween-20 as surfactant to enhance chemical absorption and each seedling was sprayed with 8 mL of respective solution. The treated plants were then kept at normal temperature (d/n: 24/20±1°C, humidity: 70%, illumination: 12 h) for 3 days for satisfactory absorption, before heat stress treatment started. The eight treatments were CLHT: control with heat stress treatment, CLNT: control at normal temperature, E1, E2, E3: 0.75, 1.5 and 3 μM and A1, A2, A3: 0.25, 0.75 and 1.25 mM, respectively. Where E and A refer to 24-epibrassinolide and acetyl salicylic acid, respectively.

Heat treatment: In the pre-experiments, LT_{50}: lethal temperature at which about 50% population of four weeks old tomato seedlings (MJL cultivar) would permanently wilt on five hour exposure per day for one week, was found to be 46°C at 70% humidity. Therefore 46°C was selected for daily heat stress treatment for 21 days from 11.00 to 15.00 hours (4 h) keeping in view that it is the peak heat stress time in field conditions. After the heat treatment the GCHT (growth chamber used for heat stress) would return to normal temperature conditions (day/night: 24/20±1°C, 12 h illumination) daily. Ogweno *et al.*, (2008) studied EBL induced thermotolerance in tomato plants and used 40/30°C heat stress for 8 days. However, Singh & Shono (2005) opted a heat stress of 45°C for a brief single exposure of 3 hours for tomato (cultivar: Ailsa Craig) seedlings. In order to avoid sudden heat shock, 46°C was established gradually with increments of 1°C/3 minutes. The first group were kept in GCHT with above described heat stress and chemical treatments along with control (CLHT) and the second group of plants (CLNT) with no chemical treatment but distilled water spray; were placed in the GCNT (growth chamber with normal temperature) operating at (d/n: 24/20±1°C, 12 h illumination). All the seedlings were irrigated with 25mL tap water on alternate days avoiding drought and 70% humidity level was maintained by built in auto-humidifier fed with dH_2O.

Photosynthesis: After 21 days of heat stress period, the heat treatment was halted and seedlings were allowed to recover for three days in the growth chamber at normal temperature conditions. On 25th day the plants were adapted by keeping in open early in the morning and photosynthetic performance was measured using the second fully developed leaf from top of the sample plant between 10:00~12:00 am, using LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). At least five plants per treatment were measured. The net photosynthetic rate (P_N, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) of leaves was measured at a leaf temperature of 25.5±2°C, and relative

humidity (RH) of 80% in the leaf chamber. The ambient CO_2 concentration was ~400 μL of $CO_2 \cdot L^{-1}$ air. Besides, stomatal conductance gs mol·m$^{-2} \cdot$s^{-1}), intercellular CO_2 concentration C_i (mmol·m$^{-2} \cdot$s^{-1}), transpiration rate E (μmol·mol^{-1}) were also measured. Water use efficiency WUE (μmol $CO_2 \cdot$mmol^{-1} was calculated as ratio between the net photosynthesis (P_N) and transpiration rate E.

$$WUE= P_N/E$$

whereas stomatal limitation (Ls) was computed as: Ls=1-(Ci/Ca)

where Ci is intercellular CO_2 and Ca is concentration of CO_2 in the air.

Chlorophyll contents: Following the method of (Zhang, 1985 and Jie *et al.*, 2013) the fresh leaf samples (0.1 g) in triplicate from each treatment were sliced into small uniform pieces avoiding midribs and gently pushed in to glass vials containing 10 mL 95% ethanol, stored for 48 hours in dark, until turned pigment free. The absorbance values of the solution were measured at λ=665, 649 and 470 nm (spectrophotometer: UV-3802, UNIC, Shanghai, China). Pigment concentrations (mg·L^{-1} of extract) of chlorophyll a (C_a), chlorophyll b (C_b), and total chlorophyll content C_t were calculated as follows:

$Ca = (13.95 \times A665) - (6.88 \times A649)$
$Cb = (24.96 \times A649) - (7.32 \times A665)$
$C_t = (C_a + C_b)$

The quantification in terms of (mg·g^{-1} fresh weight) was performed using equation:

$$Q= (Cvn/w) \times 100$$

where C is concentration (mg·L^{-1}), v is volume of solvent (mL), n is dilution factor and W refers to sample fresh weight (g).

Cell membrane thermo-stability: It has been identified as sensitive and rapid method to evaluate heat stress tolerance index in plants (Wu & Wallner, 1993; Saeed *et al.*, 2007) and was determined by electrolyte leakage assessment from leaf tissue by measure of electric conductivity as previously adapted by (Shen & Li, 1982) for measuring heat tolerance in tomato. Briefly, in each treatment at least three uniform plants were selected, third fully expanded leaf was harvested and cut into 1 cm^2 pieces avoiding midribs and were kept in 25 mL deionized H_2O for 15 minutes at 25°C. After machine shaking for fifteen minutes, the initial EC_i values were observed using EC meter, Shanghai Electric Co., China. Then the flasks were kept in boiling water for half hour, allowed to cool to room temperature and then final EC_f was measured. The percent relative membrane injury was computed as:

$$RI (\%) = [1 - \{1 - (T_i / T_f)\} / \{1 - (CLNT_i / CLNT_f)\}] \times 100$$

where, T and CLNT refer to electric conductance values of treatment and control (normal temperature) and subscripts *i*

and *f* correspond to initial and final values, respectively (Yeh & Lin, 2003). Data regarding vegetative growth and flower initiation were also recorded as an index of induced heat tolerance.

Experimental design and data analysis: Completely Randomized Design (CRD) was followed and the experiment was replicated thrice. The data was analyzed with one way ANOVA and the differences among treatment means were determined by LSD at $p < 0.05$ using the analytical software Statistix-8.1. Besides, where ever necessary, the percent change in parameter values was calculated by comparing treatment results to both controls (CLNT, CLHT), using the equation:

$$Change (\%) = [(T-CL)/CL] \times 100$$

where, CL is either control and T refers to treatment value.

Results

Plant survival: The data regarding plant survival was daily recorded in terms of number of live/recovered or dead/irrecoverable plants and was presented on weekly basis (Fig. 1). The wilted but subsequently recovered or re-turgid plants were included among the survivors. Both the chemical agents induced thermal stress tolerance in plants with EBL (3.0 µM) being the best dose with 86.11% plant survival followed by E2: EBL: 1.5 µM (77.77%) and A1: ASA: 0.25 mM (72.22%) contrary to CLHT plants with mere (12.5%) survival.

However, higher dose of ASA and lower dose of EBL had less contribution toward seedling survival, especially during last week.

Effect on membrane thermo-stability: The heat stress caused an enhanced electrolyte leakage in the CLHT seedlings as compared to the CLNT. However, exogenous application of EBL and ASA doses improved membrane integrity to different extents (Fig. 2). It was observed that the best doses were A2: ASA : 0.75 mM, A1: ASA: 0.25 mM and E3: EBL: 3.0 µM with only 6.9, 7.6 and 8.3% relative membrane injuries respectively as compared to CLNT, whereas the untreated heat stressed control seedlings (CLHT) had 22.44% relative membrane injury level. Among chemical treatments, the maximum relative membrane integrity loss (19.01%) was observed in A3: ASA: 1.25 mM treatment (Fig. 2).

Effect on vegetative growth: Daily heat stress for three weeks significantly reduced the vegetative as well as reproductive growth parameters. EBL and ASA treatments up regulated both kinds of growth to some extent under high temperature stress. Treatments E3 and A3 produced highest number of leaves and leaflets per leaf, leaf length and percent dry mass accumulation in stem (Table 1).

Effect on reproductive growth: Pre-treatment with EBL (considerably) and ASA (to a minimum extent) caused initiation of flower buds where as the untreated heat stressed controls showed no signs of flower buds. However, the effect of EBL was more prominent regarding flower bud induction than that of ASA (Table 2).
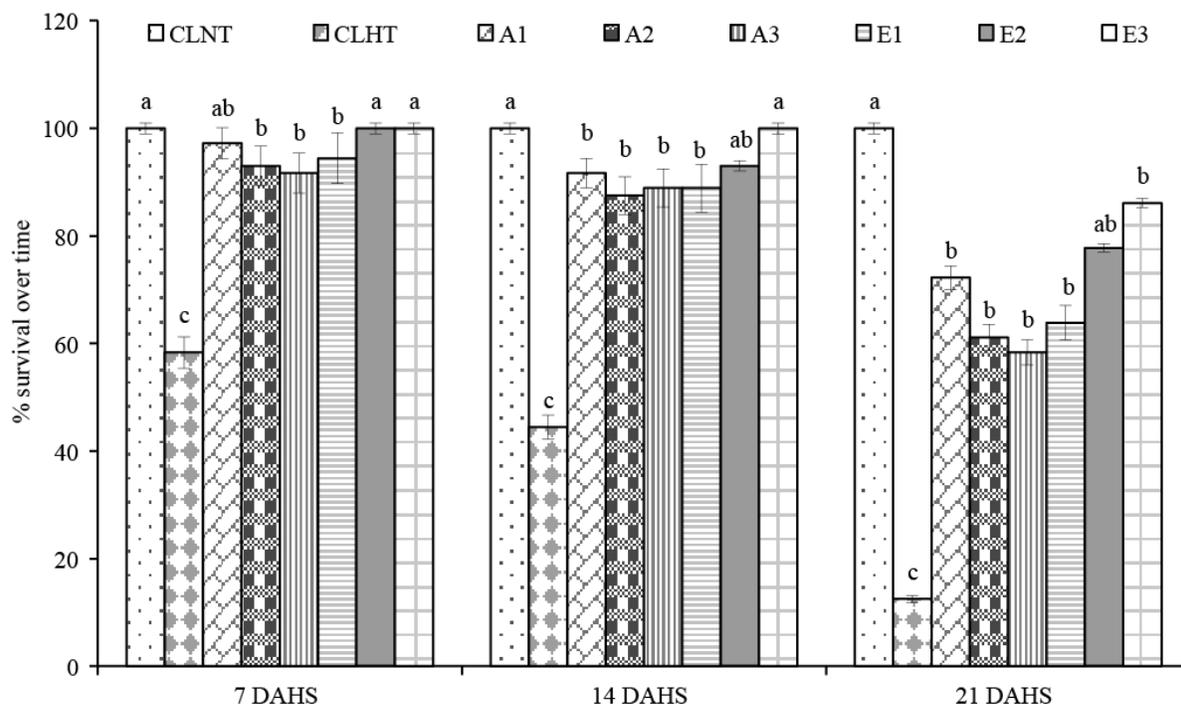
Fig. 1. Effect of varying doses of 24-EBL and ASA on survival of tomato plants (MJL cultivar) on weekly basis against heat stress (46°C/4 h daily). CLNT refers to seedlings grown under normal temperature (d/n: 24/20±1°C) and CLHT is heat stressed control. Different letters on bar heads show significant difference at $p < 0.05$ (ANOVA, LSD) test. DAHS: days after heat shock treatment started.

Table 1. Effect of exogenous EBL and ASA pre-treatment on vegetative growth.

Treatments	Plant height (cm)	Stem thickness (mm)	No. of leaves per seedling	No. of leaflets per leaf	Leaf length (cm)	Stem dry mass (%)
CLNT	29.50 ± 1.0^a	6.36 ± 0.2^{ab}	7.66 ± 0.5^a	9.0 ± 0^a	18.0 ± 0.5^a	4.26 ± 0.7^a
CLHT	14.97 ± 0.5^e	4.68 ± 0.6^f	3.66 ± 0.5^d	5.0 ± 0^e	10.93 ± 0.5^d	1.98 ± 0.2^c
A1	17.17 ± 1.6^d	5.28 ± 0.1^{ef}	5.66 ± 0.5^c	5.33 ± 0.5^e	10.63 ± 1.1^d	2.52 ± 0.5^{bc}
A2	19.30 ± 1.2^c	5.55 ± 0.3^{cd}	5.33 ± 0.5^c	6.33 ± 0.5^d	14.77 ± 1.0^c	3.72 ± 1.3^{ab}
A3	21.25 ± 1.3^b	5.28 ± 0.1^{de}	6.66 ± 0.5^b	7.33 ± 0.5^{bc}	16.20 ± 0.3^b	3.80 ± 0.5^{ab}
E1	16.10 ± 0.5^{de}	5.95 ± 0.1^{bc}	6 ± 0^{bc}	6.66 ± 0.5^{cd}	13.57 ± 0.6^c	2.61 ± 0.6^{bc}
E2	15.33 ± 0.5^e	6.27 ± 0.2^{ab}	6 ± 0^{bc}	7.0 ± 0^{bcd}	14.17 ± 0.3^c	3.03 ± 1.1^{abc}
E3	17.53 ± 0.5^d	6.51 ± 0.1^a	6.66 ± 0.6^b	7.66 ± 0^b	16.300 ± 0.8^b	4.47 ± 1.6^a

Four weeks old tomato seedlings (MJL cultivar) were exposed to daily heat stress (46°C ± 1°C for 4 h) for 21 days. CLNT refers to seedlings grown under normal temperature (d/n: 24/20 ± 1°C) and CLHT is heat stressed control. The data was summarized as means of three replicates. Each value refers to mean ± SD (n = 3). Means followed by different alphabets in a column indicate significant difference among treatments at p<0.05. (ANOVA, LSD Test)

Table 2. Effect of exogenous EBL and ASA on initiation of reproductive indices.

Treatments	No. of seedlings with flower buds	No. of flower buds	No. of opened flowers	Mean size of flower bud (mm)
CLNT	24	32	5	2.1
CLHT	0	0	0	0
A1	4	4	0	1.58
A2	2	2	0	1.49
A3	0	0	0	0
E1	0	0	0	0
E2	6	10	0	1.63
E3	11	14	1	1.92

Four week old tomato seedlings (MJL cultivar) exposed to daily heat stress (46°C±1°C for 4 h) for three weeks. CLNT and CLHT refer to control seedling grown under normal and heat stressed, respectively. The data was summarized as means of three replicates (n = 24)
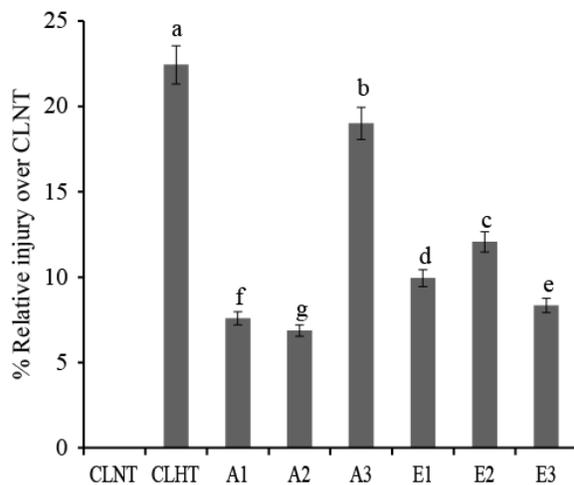
Fig. 2 Effect of different doses of EBL and ASA pre-treatment of tomato seedlings on relative membrane injury after exposure to heat stress (46°C, 4 h daily for 21 days). Each bar represents percent relative injury of cell membranes as compared to seedlings grown at normal temperature (D/N: 24/20 ± 1°C). The data was summarized as means of three replicates. Vertical bars represent mean ± SD (n = 3). Different letters on the bars mention significant difference at 5% level (ANOVA, LSD test).

Effect on chlorophyll contents: Chlorophylls are the core component of pigment-protein complexes which play a major role in the photosynthesis (Ahmad *et al*., 2013). The prolonged high temperature stress (46°C for 4 h per day for 21 days) significantly reduced (~192%) total chlorophyll contents of CLHT treated seedling as compared to CLNT. However, the change of chlorophyll contents was different for different chemical concentrations. The exogenous application of E3 treatment attenuated the deleterious effect (57% over CLHT with reference to CLNT) of heat and significantly enhanced green pigment quantity under heat stress by playing a protective role (Fig. 3C). Rest of all concentration of EBL and ASA treatments A1~3, E1~2 did not offer additive role, instead increased chlorophyll losses were observed. Similar trend was observed in Chl-a and b and maximum losses were also noted in lower doses of EBL and all of ASA. However E3 (3.0 μM) had prominent protective effect and losses were reduced to 69.5 and 45.2% for both Chl-a and Chl-b contrary to CLHT which had 120.1 and 113.4% losses over CLNT, respectively. Conclusively, 3.0 μM EBL significantly improved chlorophyll contents (a, b & total) and reduced a/b ratio under heat stress regime. The data mentions a specific effect of each treatment (Fig. 3).
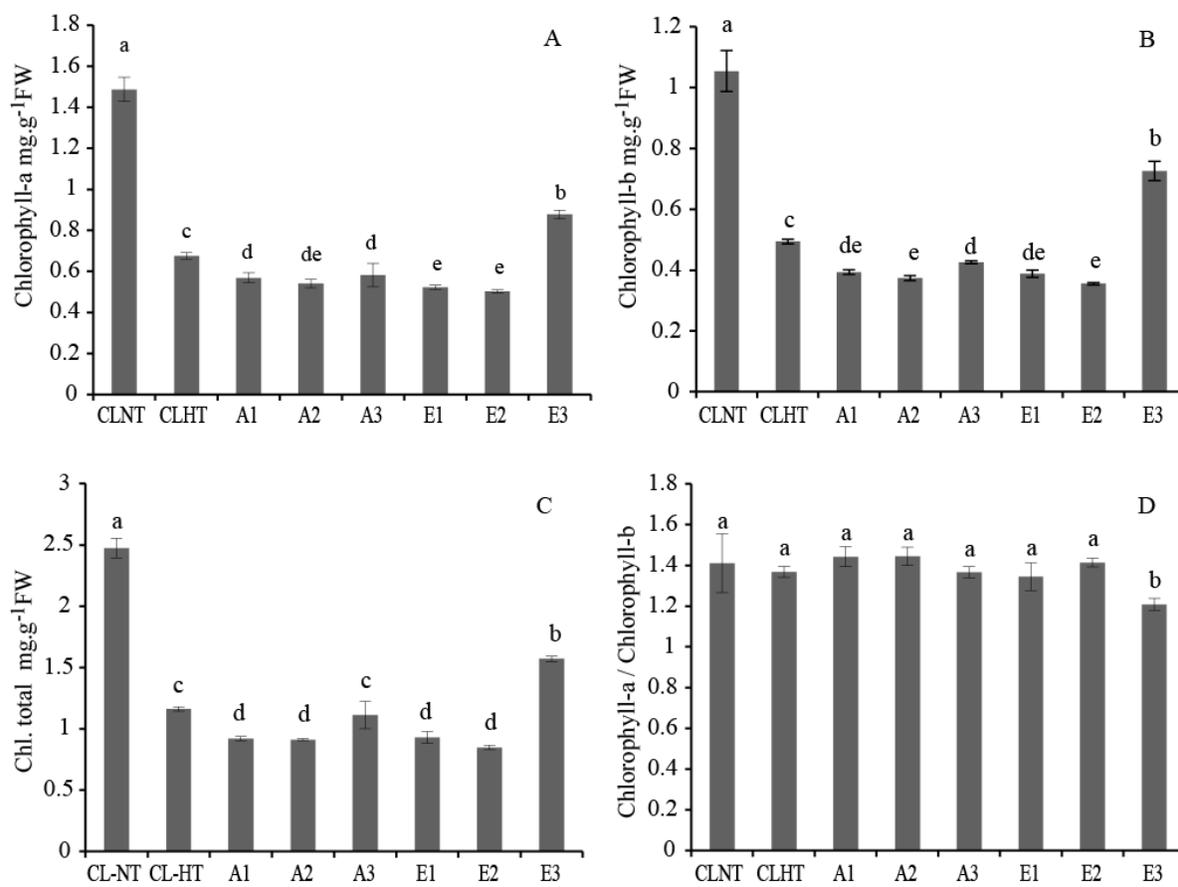
Fig. 3. Effects of exogenously applied 24-epibrassinolide (E₁, E₂, E₃: 0.75, 1.5 and 3 μM) and acetyl salicylic acid (A₁, A₂, A₃: 0.25, 0.75, 1.25 mM) concentrations on Chl-a (A), Chl-b (B), Chl-total (C) and Chlorophyll a/b (D) of tomato seedlings (Mei Jie Lo cultivar) subjected to daily severe heat stress (46°C/ 4 h) for three weeks. CLNT: control at normal temperature and CLHT: heat stressed control. Data are the means of three replicates. Vertical bars refer to mean ± SD (n=3). Bars headed by different alphabets indicate significant difference among treatments at p<0.05. LSD test.

Effect on photosynthesis: Thermal stress had suppressive effect upon net photosynthesis (P_N), stomatal conductance (gs) and transpiration rate (E). Under heat stress conditions, different doses of both chemical treatments significantly (p<0.05) modified photosynthetic parameters. Maximum photosynthetic performance (P_N), was contributed by A3 (9.95) and E3 (9.76) $\mu m \cdot m^{-2} \cdot s^{-1}$ followed by E2 (8.20) (Fig. 4A) while highest (gs) stomatal conductance (Fig. 4B) and (E) transpiration rate (Fig. 4D) were noted in seedlings treated with E3. The heat stress decreased stomatal limitation (Ls) in CLHT but both EBL and ASA had up-regulatory effect on it. However, E3 was closest to that of CLNT with no significant difference (Fig. 4C). Similarly internal CO_2 was increased at heat stress but EBL treatments appear to keep the CO_2 concentrations in normal range (Fig. 4F). However, a downwards trend was observed in water use efficiency (WUE) in the ASA and EBL treatments which was due to increased transpiration rate (Fig. 4E). Higher dose of ASA (A3) and all the doses of EBL had varying level of protective effect and significantly improved the photosynthetic performance. Though the lower doses of ASA (A1 and A2) slightly inhibited P_N but the change was not statistically significant (Fig. 4A).

Discussion

The daily heat stress (46°C/4 h) had sharp declining effect on photosynthesis and a negative regulatory effect on most of allied parameters. The photosynthetic apparatus has been identified as the most sensitive plant component to heat stress (Yordanov et al., 1986; Wise et al., 2004). In the present study, 24-epibrassinolide and ASA treatments demonstrated a protective effect and considerably improved the P_N as compared with untreated heat stressed controls. Our results are in line with findings of Zhang et al., (2013) and Ogweno et al., (2008) who documented that EBR could alleviate the detrimental effects of high temperatures on melon and tomato seedlings growth by increasing plant carboxylation efficiency. Wang et al., (2010) observed that SA pretreatment reduced losses in P_N under heat stress, apparently in part through maintaining a higher Rubisco activation state and greater PSII efficiency. Zhen et al., (2010) found that ASA could alleviate the decrease in P_N caused by low temperature and low light stress. Our results further support the findings of Wang and Li (2007) who reported that SA treatment can maintain a higher P_N in grape leaves under heat stress. In the present study, it was observed that the heat stress caused a decline in stomatal

conductance (gs) and transpiration rate (E) which was subsequently rescued by chemical (EBL & ASA) treatments. The increased gs and E values might possibly have a cooling effect (Jin & Shen, 1999) that would facilitate the plant to continue metabolic activities under heat stress. It is suggested that a reduced gs, might have caused a decreased transpiration (E) and declined CO_2 influx resulting reduction in P_N. Whereas, there was a reduction in the WUE and Ci in the ASA and EBL treatments contrary to untreated controls that might be due to relatively better efficiency of photosynthetic apparatus and CO_2 assimilation at high temperature stress as compared to heat stressed control. The (A3 and E1~3) treatments had a protective effect by improving the stomatal influx and efflux, improving gaseous exchanges

and hence P_N and transpiration. The increased transpiration (E) in EBL treated seedlings, however, might have causes decrease in (WUE) but yet close to that of CLNT (the optimum range at normal temperatures) showing better adaptation. From the chlorophyll data depicted in Fig. 3, it was observed that efficiency of photosynthetic apparatus might depend upon other factors along with the chlorophyll contents since, in A3 there was no prominent increase in chlorophyll contents yet an improved P_N was observed. On the other hand, the increase in Ci in heat stressed seedlings suggests simultaneous involvement of some non-stomatal process as well. Singh & Shono (2005) reported a sharp rise in Ci in the heat stressed control tomato seedlings indicating their less adaptability to high temperature which was reflected by a decline in P_N.
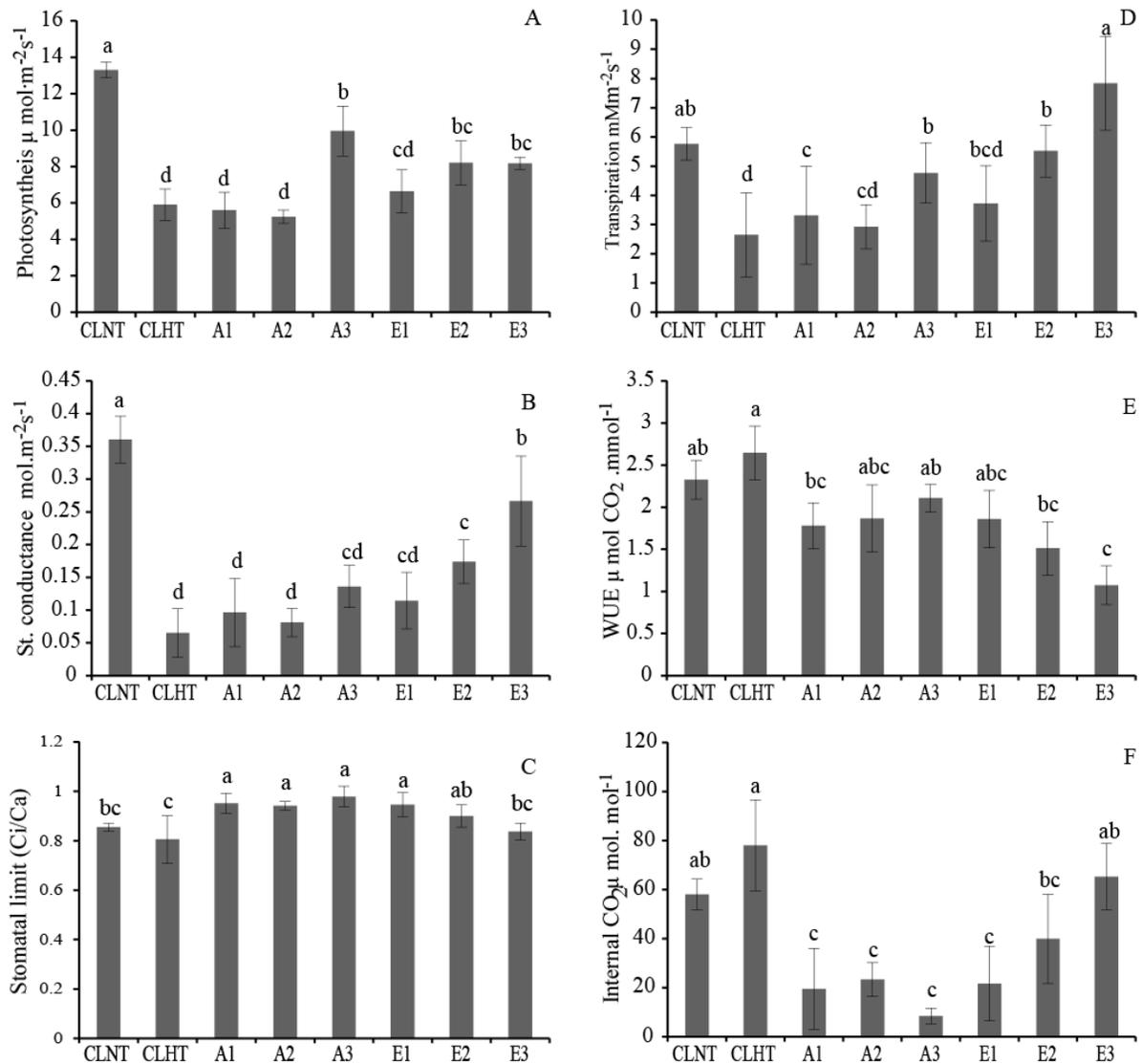
Fig. 4. Effect of exogenously applied 24-epibrassinolide (E₁, E₂, E₃: 0.75, 1.5 and 3 µM) and acetyl salicylic acid (A₁, A₂, A₃: 0.25, 0.75 and 1.25 mM) concentrations on net photosynthetic rate P_N (A), stomatal conductance gs (B), stomatal limitation Ls (C), transpiration rate E (D), water use efficiency WUE (E) and intercellular CO_2 concentration Ci (F) of tomato seedlings (Mei Jie Lo cultivar) subjected to severe heat stress (46°C/4 h daily) for three weeks. CLNT: control at normal temperature and CLHT: heat stressed control. Data are the means of five replicates with ±SD mentioned by vertical bars. Bar heads with different alphabets indicate significant difference among treatments at p<0.05, LSD test.

Based upon the results of the present studies, we suggest that the photosynthesis inhibition is contributed by multiple reasons including heat led damage to the membranes and antioxidant enzyme system, and stomatal and non stomatal limitations (Farquhar & Sharkey, 1982) caused by severe heat stress. However, treatments (A3, E1~3) rescued the plants by improving these aspects in response to specific signal transduction by said concentrations of EBL and ASA. The observed increase in chlorophyll contents by EBL are in agreement with the findings of Fariduddin *et al*., (2003), Hayat *et al*., (2000) and Ghai *et al*., (2002) who reported that the exogenous SA imparts change in the plant pigment contents and it is principally concentration dependent. The plant vegetative and reproductive growth was almost ceased due to acute daily heat stress that is attributed to the steep fall in photosynthates' production as a result of reduced photosynthetic activity. Reduction in plant growth is a major consequence of growing under stress conditions, mainly due to a reduction in net photosynthesis rate (Wahid *et al*., 2007). However, in this study, respective EBL and ASA treatments attenuated the magnitude of loss and produced new leaves as well as initiated flower buds (Tables 1, 2). These results are in line with reports of Li *et al*., (2010) that BR content affect flowering time in arabidopsis and Khurana & Cleland (1992) who demonstrated that lower concentration of SA (3–10 µM) stimulated flowering in various genera of the Lemnaceae family. In the present study, the protective role of EBL and ASA is verified by enhanced plant survival of pretreated seedlings against a frequent high temperature. These results support the findings of Dhaubhadel *et al*., (1999) and Singh & Shono (2005) who observed a significantly improved percent survival in heat stressed brassica and tomato seedlings treated with EBR as compared to untreated controls, suggesting that EBR treatment induces thermotolerance in both plant species. Senaratna *et al*., (2000) observed that ASA treated tomato and bean seedlings showed 100% survival rate against severe heart stress. In the present study, EBL and ASA concentrations were observed to contribute a strengthening effect to the cell membrane integrity. Previously, it was demonstrated that EBL application on tomato plants strengthened the membrane integrity under heat stress and also at normal temperature (Mazorra *et al*., 2002; Singh & Shono, 2005). These results further support findings of Kaur *et al*., (2009) and Qinghua *et al*., (2006) who observed that SA pretreatment in different doses could reduce electrolyte leakage and improve cell membrane stability in brassica and cucumber seedlings, respectively. Though exact mechanism of anti-stress function of EBL and ASA is yet not fully understood, however it is suggested that by virtue of signaling process they initiate a series of biochemical events comprising of antioxidant molecules, several intermediaries and ultimately achieving the goal of increased resistance for a variety of stresses. The findings in the present study may be attributed to the probability that the BRs induced transcription and/or translation of proteins is involved in the synthesis of pigments (Bajguz, 2000) and induction of heat stress tolerance while signal transduction by ASA may play a similar role. Induction of multiple stress tolerance in plants by exogenous application of SA and its derivatives may have a significant practical application in agriculture (Senaratna *et al*., 2000) and in light of present findings further investigations for feasibility of their field application are suggested.

Acknowledgement

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