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Lin, Ling-Na
Department of Horticulture, National Taiwan University, Taipei, Taiwan, Republic of China

Huang, Kuang Liang
Department of Horticulture, National Chiayi University, Chiayi, Taiwan, Republic of China

Okubo, Hiroshi
九州大学大学院農学研究院環境農学部門農業環境科学園芸学研究室

Chang, Yu-Sen
Department of Applied Biology, College of Agriculture and Life Science, Chungnam National University

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Alleviation of High Temperature Stress in Wax Begonia (*Begonia* × *semperflorens–cultorum* Hort.) by Salicylic Acid

Ling–Na LIN[1], Kuang Liang HUANG[2], Hiroshi OKUBO and Yu–Sen CHANG[1]*

Laboratory of Horticultural Science, Division of Agronomy and Environmental Sciences,
Department of Agro–environmental Sciences, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
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Wax begonia (*Begonia* × *semperflorens–cultorum* Hort.) plants often suffer from high temperature stress during hot seasons in Taiwan. Since salicylic acid (SA) has proved to enhance heat tolerance in many plants, this study evaluates whether exogenous SA applications could alleviate high temperature stress of wax begonias.

Plug seedlings of wax begonia 'Super Olympia' were treated with 25, 100, 400, 800, with 1600 μM SA before 55 °C, for 2 h of high temperature stress. Results indicated that 25–400 μM SA enhanced heat tolerance by reducing the value of Relative Injury (RI) and Malondialdehyde (MDA), whereas 800, 1600 μM SA had adverse effects. Transplanted plants of wax begonia 'Super Olympia' were treated with 25, 100, 400 μM SA before 35 °C, for 16 days of high temperature stress. Results showed that 25–400 μM SA enhanced heat tolerance by maintaining a higher value of Chlorophyll Meter Reading (CMR), reducing leaf thickness and the number of red spotted leaves. Especially, 100 μM SA treatment had most significant improvement among 25–400 μM SA treatments.

The thermo protection period of 100 μM SA maintained significantly for 5 days, based on the lower value of F0, RI, and MDA, and the higher value of Fm and Fv/Fm. Applying times up to 2 or 3 times extended protective effect significantly, based on reducing the value of RI and MDA, and increasing plant height and number of flowers. Either medium drench or foliar spray treatments improved heat tolerance of wax begonias. Medium drench treatments produced better thermo protection based on the results of higher Fm and Fv/Fm value. In conclusion, salicylic acid pretreatments with satisfactory concentrations between 25 to 400 μM proved to enhance heat tolerance in the wax begonia. Results recommend medium drench with 100 μM SA per week, up to 2–3 times as the most optimal application of SA.

INTRODUCTION

Wax begonia (fibrous–rooted begonia, *Begonia* × *semperflorens–cultorum* Hort.) is popular and widely grown for outdoor use in landscapes and gardens. Hamrick (2003) recommended a (18–21/16–18 °C) day/night temperature as optimum for wax begonia growth and development. However, Taipei International Flora Exposition has raised the issue of extending plant vigor easily and economically, especially in the hot season.

Salicylic acid (SA) is a phenolic derivative, distributed in a wide range of plant species. In terms of stress physiology, salicylic acid has demonstrated an important role in response to biotic stress. Many reports have suggested that salicylic acid also plays a role in responses to abiotic stress (such as low or high temperature, UV–B irradiation, ozone, and heavy metals) (Hayat and Ahmad, 2007; Janda *et al*., 2007).

Dat *et al*. (1998a) first revealed that 1–100 μM SA could induce heat tolerance of mustard plants. Many researchers have indicated the protective effect of exogenous salicylic acid against high temperature stress in mustard plants (Dat *et al*., 1998a), potato microplants (Lopez *et al*., 1998), Arabidopsis (Larkindale and Knight, 2002), *Cicer arietinum* L. (Chakraborty and Tongden, 2005), creeping bentgrass (*Agrostis stolonifera*) (Larkindale and Huang, 2004), Kentucky bluegrass (*Poa pratensis*) (He *et al*., 2005), grape and citrus (Wang *et al*., 2002, 2003).

This research evaluates whether exogenous SA treatments enhance heat tolerance of 'Super Olympia' series wax begonias under various high temperature stress conditions. Trials were conducted on different plant growth stages, SA doses, applying methods, and times to provide simple, practical advice for application.

MATERIALS AND METHODS

Plant materials

Plug seedlings and transplants in 12–cm diameter pots of wax begonia (*Begonia* × *semperflorens–cultorum* Hort.) 'Super Olympia' Series, including white, pink, and red cultivars were used in the experiment.

Heat treatments

Plants were subjected to various high temperature stresses (35/30 °C day/night temperature, phytotron of National Taiwan University up to or more than 14 days, or a 45 °C water bath for 20 mins, or 55 °C preheated oven, dark, 2 hrs) to evaluate the SA effect.

Exogenous SA treatments

[1] Department of Horticulture, National Taiwan University, Taipei, Taiwan, Republic of China
[2] Department of Horticulture, National Chiayi University, Chiayi, Taiwan, Republic of China
* Corresponding author (E–mail: yschang@ntu.edu.tw)

Plants were subjected to various concentrations (25–1600 μM, adjusted to pH 6.8 with KOH) by medium drench or foliar spray to evaluate SA effect.

Measurement items

Number of leaves with red spots

Heat injury in leaves was evaluated visually by numbers of leaves with red spots. Data was collected by classifying the red spot area by the following criterion, then calculating the number of leaves at the end of the test.

The degrees of heat tolerance were measured according to the criterion with four grades as follows:

level 0: the ratio of (red spot area/leaf area)=0 or $\leq$ 5%.

level 1: the ratio of (red spot area/leaf area)=5 or $\leq$ 30%

level 2: the ratio of (red spot area/leaf area)=30 or $\leq$ 60%

level 3: the ratio of (red spot area/leaf area)= >60%

Malondialdehyde (MDA)

The extent of lipid peroxidation was estimated by determining MDA reacting to TBA (thiobarbituric acid)–reactive substance. Leaves were sampled and followed the method of Yang *et al*. (2008).

RI (Relative Injury value, %)

Twenty leaf discs (10 mm in diameter) were placed in 30 ml glass vials, rinsed three times with 20 ml of distilled water to remove electrolytes released during leaf disc excision. Vials were then filled with 30 ml of distilled water and allowed to stand in the dark for 24 h at room temperature.

Electrical conductivity (EC1) of the bathing solution was determined at the end of the incubation period. Tubes were heated in a temperature–controlled water bath at 95 °C for 20 min, and then cooled to room temperature for measuring the electrical conductivity (EC2). Electrolyte leakage was calculated as a percentage of EC1/EC2.

RI (Relative Injury value, %) = (EC1/EC2) × 100 Shi *et al*. (2006).

Chlorophyll fluorescence parameters

Leaf photochemical efficiency was determined by measuring chlorophyll fluorescence. Chlorophyll fluorescence parameters were measured using a portable fluorometer (Mini–Pam, Walz Co., Germany). Leaves were dark acclimated for 30 min and the measurements of maximal quantum yield of PSII (Fv/Fm), minimal fluorescence (F0), maximal fluorescence (Fm) were carried out according to the method of Shi *et al*. (2006).

Chlorophyll meter reading (CMR value, SPAD–502 value)

CMR value was measured using the SPAD–502 chlorophyll meter (SPAD–502, Minolta Camera Co., Japan). This measures absorption at 650 and 940 nm wavelengths to estimate chlorophyll levels. Two measurements were immediately taken per leaf and averaged to provide a single value per leaf. The SPAD sensor was placed randomly on leaf mesophyll tissue only, avoiding veins.

Leaf thickness

Leaf thickness was measured using the sensor (SM–112, Dial Thickness Gauges, Dogger Instrument Co., Taiwan). Two measurements were immediately taken per leaf and averaged to provide a single value per leaf. The sensor was placed randomly on leaf mesophyll tissue only, avoiding veins.

Plant width

Two measurements were taken, the biggest and smallest width per plant and averaged to provide a single value per plant.

Statistics

Values presented were means ± one standard deviation (SD) of 5–7 replicates. Statistical analyses were carried out by analysis of variance (ANOVA). Differences between treatments were separated by the least significant difference (LSD) test at a 0.05 probability level.

Application of treatments and evaluations

Identifying optimal concentration of SA to enhance heat tolerance in wax begonia plug seedlings

Plug seedlings were foliar sprayed with salicylic acid solutions (100, 200, 400, 800, and 1600 μM), and with distilled water to act as a control. All seedlings were kept at room temperature for 2 h. Seedlings were then exposed to high temperature (55 °C) for 2 h. Following the high temperature treatment, leaves were sampled for RI and MDA analysis.

Treatments included five replicates, and one seedling per replicate.

Identifying optimal concentration of SA to enhance heat tolerance in wax begonia transplants

Transplants were medium drenched with salicylic acid solutions (25, 100, 400 μM), and in distilled water to act as a control. All plants were kept at room temperature for 2 h and then exposed to high temperature (35/30 °C day/night temperature phytotron) for 16 days. Following high temperature treatment, the measurements of plant height, width, CMR value, leaf thickness, number of leaves with red spots were recorded and analyzed.

Identifying the lasting period of SA for thermo protective effect

Transplants were foliar sprayed with 100 μM salicylic acid, and in distilled water to act as a control. All plants were kept at room temperature for 2 h and then exposed to high temperature (35/30 °C day/night temperature phytotron) for 1, 2, 3, 4, 5, 6, 7 days. Chlorophyll fluorescence parameters were measured every day, then leaves were sampled for RI and MDA analysis.

Identifying optimal applying times of SA for thermo protective effect

Transplants were medium drenched with 100 μM salicylic acid, and in distilled water to act as a control. All plants were kept at room temperature for 2 h and then exposed to high temperature (35/30 °C day/night temperature phytotron). The applying times were increased at 7–day intervals. At the end of the test, plant height, width, and flower numbers were recorded, and leaves were sampled for RI and MDA analysis.

SA thermo protective effect when comparing foliar spray treatment with medium drench treatment

Transplants were medium drenched or foliar sprayed with 100 μM salicylic acid, and drenched in distilled water to act as a control. All plants were kept at room temperature for 2 h and then exposed to high temperature stress (35/30 °C day/night temperature phytotron) 14 days. Chlorophyll fluorescence parameters were measured.

RESULTS

Identifying optimal concentration of SA to enhance heat tolerance in wax begonia plug seedlings

Relative Injury value indicated that the dose of 100–400 μM of SA pretreatments significantly enhanced heat tolerance of plug seedlings by reducing electrolyte leakage and conferring membrane protection, while the higher concentration (800, 1600 μM) had opposite effect. Both RI values were higher than the control values (Fig. 1).

MDA (Malondialdehyde) value showed that the dose of 100–200 μM of SA pretreatments reduced, compared with the control treatment, while the higher concentration (800, 1600 μM) had significant adverse effect (Fig. 2).

Pretreatment of wax begonia seedling leaves with SA solution (100, 200, 400 μM) prior to high temperature stress significantly decreased electrolyte leakage, while the SA solution (100, 200 μM) pretreatment significantly decreased malondialdehyde production in the leaves. The recommended concentration of exogenous SA to enhance heat tolerance of wax begonia plug seedlings was not too high.

Identifying optimal concentration of SA to enhance heat tolerance in wax begonia transplants

The number of leaves with red spots showed that the dose of 25–400 μM of SA pretreatments significantly decreased the appearance of red spot level 3 (Table 1).

100 μM of SA pretreatment by medium drench had the highest significant value of plant width and SPAD–502 value. 100, 400 μM of SA medium drench decreased leaf thickness significantly (Table 1).

Results of the experiment of wax begonia transplants, showed better visual quality, higher width, and CMR (SPAD–502) value than the control, especially the 100 μM pretreatment before high temperature stress (35/30 °C

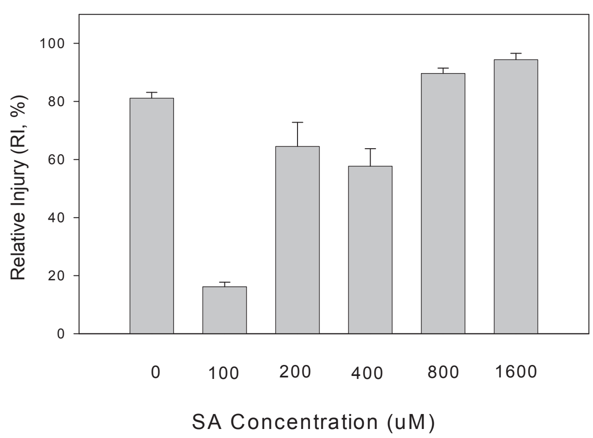

Fig. 1. Effects of various concentrations of SA pretreatment and heat shock (55 °C, dark, 2 hrs) treatment on RI value (%) of wax begonia.

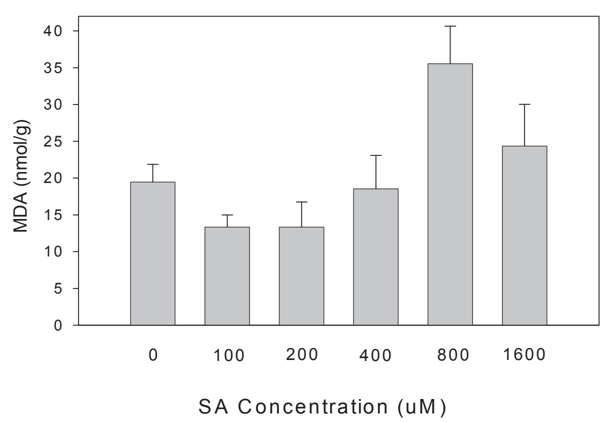

Fig. 2. Effects of various concentrations of SA pretreatment and heat shock (55 °C, dark, 2 hrs) treatment on MDA value (nmol/g) of wax begonia..

Table 1. Effects of various concentrations of salicylic acid pretreatment by medium drench and heat shock (35/30 °C day/night temperature Phytotron, 16 days) on plant growth and development of wax begonia 'Super Olympia' transplants

Salicylic acid (μM)	Plant height (cm)	Plant width (cm)	CMR SPAD–502 value	Leaf thickness (mm)	Number of leaves with red spots			
					Level 0	Level 1	Level 2	Level 3
0	13.3	15.7	20.0	0.55	25.3	18.8	5.2	1.4
25	14.3	12.5	21.0	0.53	21.6	18.8	4.0	0
100	13.7	16.0	25.1	0.51	35.6	19.6	4.4	0
400	16.4	14.6	20.4	0.50	41.8	15.4	3.6	0
LSD.05	2.86	2.55	2.49	0.037	19.64	–	–	0.60
Significance	NS	*	***	*	NS	NS	NS	***

day/night temperature phytotron, 16 days).

Identifying the lasting period of SA for thermo protective effect

Chlorophyll fluorescence parameters were measured after 100 μM of SA foliar spray and high temperature stress in the first week. During 1–4 days, salicylic acid pretreatment plants showed a lower F0 value, and Fm, Fv/Fm were higher than the control treatment plants (Fig. 3).

The RI values of SA 100 μM pretreatment by foliar spray were less than the ones of the control treatment plants during the first five days, though not significant.

The MDA values of SA 100 μM pretreatment by foliar spray were less than the ones of the control treatment plants on the first, third, and seventh day, though not significant (Table 2).

The values of chlorophyll fluorescence, RI, and MDA suggested that the protective effect could maintain about five days after SA 100 μM foliar spray.

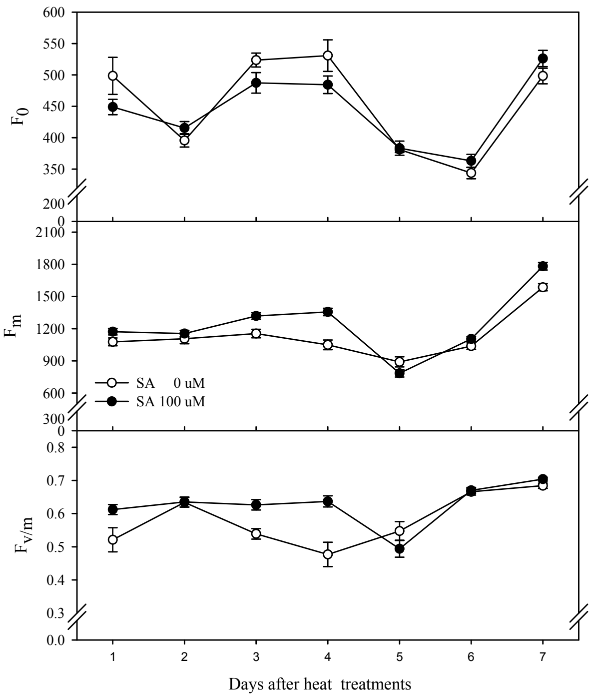

Fig. 3. Effects of salicylic acid treatment by foliar spray on chlorophyll fluorescence parameters of wax begonia on 'Super Olympia' plants under 40–35/30 °C.

Identifying the optimal applying times of SA for thermo protective effect

SA 100 μM pretreatment once by medium drench enhanced heat tolerance of wax begonia plants, based on the lower value of RI and MDA, compared with the control treatment plants.

The applying times of up to two or three times at 7–day intervals significantly decreased the value of RI. The result indicated that plant height and the number of flowers increased while applying times increased (Table 3).

SA thermo protective effect compared to foliar spray treatment with the medium drench treatment

Chlorophyll fluorescence parameters were measured after 100 μM of SA foliar spray and medium drench before high temperature stress (35/30 °C day/night temperature phytotron, 14 days). Compared with the control treat-

Table 2. Effects of salicylic acid pretreatment by foliar spray on RI and MDA value of wax begonia 'Super Olympia' plants under 40–35/30 °C

	Treatment	1st day	3rd day	5th day	7th day
RI (%)	CK	11.11a[z]	8.64a	16.78a	10.30a
	SA 100 μM	8.81a	8.49a	14.84a	12.89a
MDA (nmol/g)	CK	9.00a	11.89a	13.43a	16.07a
	SA 100 μM	8.28a	10.71a	14.69a	14.85a

[z]Mean separation within columns by LSD test, P<0.05

Table 3. Effects of various applying times of salicylic acid pretreatment by medium drench on growth and injury of wax begonia 'Super Olympia' plants under high temperature stress (40–35/30 °C day/night temperature Phytotron, 28days)

Treatment	RI (%)	MDA (nmol/g)	Plant height (cm)	Plant width (cm)	Number of flowers
Control	9.38a[z]	0.99a	15.1b	18.6a	13.5b
SA, once	7.98b	0.86b	15.2b	18.2a	12.6b
SA, 2 times	7.21bc	0.82bc	16.4ab	17.1a	21.4a
SA, 3 times	6.56c	0.71c	17.0a	18.3a	24.6a

Each value is the mean of five replications.
[z]Mean separation within columns by LSD test, P<0.05

ment plants; both foliar spray and medium drench significantly lowered the value of F0, and raised the value of Fv/Fm. The result indicated that either foliar spray or medium drench could enhance the tolerance of wax begonia plants under high temperature stress (Fig. 4).

Compared with various applying methods, medium drench showed higher value of Fm and Fv/Fm. At the end of the test, medium drench showed no leave necrosis and grew more vigorously than foliar spray treatment plants (Figs. 4 and 5).

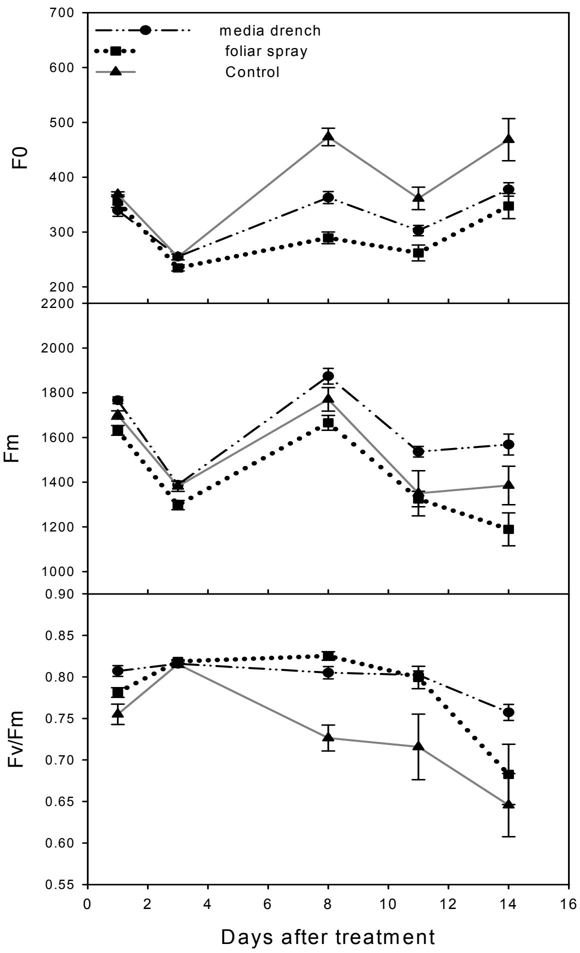

Fig. 4. Effects of 100 μM salicylic acid treatment by medium drench or foliar spray on chlorophyll fluorescence of wax begonia plants.

medium drench foliar spray control

Fig. 5. Effects of 100 μM salicylic acid treatment by medium drench or foliar spray on growth status of wax begonia plants after high temperature stress (35/30 °C day/night temperature Phytotron, 14 days).

DISCUSSION

This study demonstrates that applying satisfactory concentrations (25–400 μM) of salicylic acid enhances heat tolerance of wax begonia plug seedlings and transplants, while 800, 1600 μM SA treatments had adverse effects (Figs. 1 and 2, Table 1). The results agree with previous research findings in alleviating high temperature stress of mustard plants (Dat *et al.*, 1998a), potato microplants (Lopez *et al.*, 1998), Arabidopsis (Larkindale and Knight, 2002), *Cicer arietinum* L. (Chakraborty and Tongden, 2005), creeping bentgrass (Larkindale and Huang, 2004), Kentucky bluegrass (He *et al.*, 2005), grape, and *Citrus* (Wang *et al.*, 2002; 2003).

Plants treated with higher concentrations of SA were often susceptible to stress injury. This is because lower SA doses might induce moderate stress, affecting the oxidative status of the plant similar to stress–acclimation effect (Horváth *et al.*, 2007). However, high exogenous concentrations of SA might cause overly strong oxidative stress for plants to overcome and suffer to death (Kováčik *et al.*, 2009).

Janda (2007) described the primary effect of high temperature is to alter protein conformations and membrane status, indicating that one of the first signs of high temperature stress is reduced a reduction in photosynthetic activity. SA pretreatments helped stabilize the membrane and reduced heat injury, evidenced by the lower value of RI, MDA (Figs. 1 and 2), and the number of leaves with red spots (Table 1). Higher chlorophyll CMR (SPAD–502) value and photochemical efficiency (Fv/Fm) helped plants to maintain photosynthetic activity (Figs. 3 and 4, Table 4).

Results show that both foliar spray and medium drench alleviate heat stress if the doses were lower than 400 μM (Table 4). In general, SA treatments were applied by foliar spray (Amutha *et al.*, 2007; Dat *et al.*, 1998a, b; He *et al.*, 2005; Shi *et al.*, 2006; Wang and Li, 2006), and a few studies conducted the medium drench treatment in beans and tomatoes (Senaratna *et al.*, 2000). Results

Table4. Effects of 100 μM salicylic acid treatment by medium drench or foliar spray on chlorophyll fluorescence of wax begonia plants at the end of experiment (35/30 °C day/night temperature Phytotron, 14 days after application)

SA treatment	F0	Fm	Fv/Fm
Control	468.4a[z]	1385ab	0.646b
100 μM drench	377.7b	1568a	0.757a
100 μM spray	347.3b	1189b	0.683ab

Each value is the mean of six replications.
[z]Mean separation within columns by LSD test, $P<0.05$

of this study emphasize that medium drench treatments enhance heat tolerance more than foliar spray treatments do, based on chlorophyll fluorescence parameters during the high temperature period and plant growth performance at the end of the trial (Table 4). The wax texture on the leaf surface of the wax begonia might limit the foliar absorption of salicylic acid. Another possibility is that SA transportation via phloem in the wax begonia might be more efficient than that via xylem. Future study is still needed for advanced research in the future. This study recommends lower concentrations (25–400 μM) as the medium drench for desired and stable protective effects, or combining medium drench and foliar spray. The concentrations of 100 μM of SA proved to be the best protective effect.

This study claims that SA protective effect maintained about five days (Fig. 3, Table 2), and one or two more SA application every 7 days extended protective effect (Table 3). The chlorophyll fluorescence parameters (lower F0 or higher Fv, Fv/Fm) demonstrated temporary significant protective effect. Eraslan (2007) revealed that the long–term effects of SA under stress conditions were not as effective in alleviating abiotic stress as reported in the literature conducted with short–term studies. Results proved that two or three SA applications are more effective than one or no treatment.

In conclusion, salicylic acid pretreatments with satisfactory concentrations between 25 to 400 μM enhanced heat tolerance in the wax begonia, whereas treatments applied with 800, 1600 μM concentrations had adverse effect. Results recommend the most optimal application of SA treatment as medium drench with 100 μM SA per week up to 2–3 times.

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