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Physiological responses of *Scaevola aemula* seedlings under high temperature stress

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ABSTRACT

Global climate change is expected to result in a relative high frequency of a short period of extreme high temperature (HT) on plant ecosystems and can have an adverse impact on plant growth and development, yet the response of plants to such damage is not fully understood. In this study, physiological responses of *Scaevola aemula* seedlings to a short-term (a 3-day period) HT stress were investigated in order to examine the adaptation of *S. aemula* to the thermal environment. The *S. aemula* seedlings were cultivated under four temperature treatments of 25/20, 35/27, 40/30, 46/35 °C (day/night). The HT stress-induced injure symptoms in leaves were recorded and several selected important physiological variables were measured. The results showed that the leave injuries were not apparent under HT (35/27 °C), but serious damages were observed at days two and three post-treatment under severe HT (40/30 and 46/35 °C). For adapting the thermic environments, *S. aemula* seedlings exhibited a rapid increase of photosynthetic pigments, soluble sugar contents, and superoxide dismutase activity, and simultaneously a decrease of soluble protein contents, proline contents and catalase activity. The HT tolerance of *S. aemula* species depends upon both the elevated temperature and the period of time under the increased temperature. Our study suggests that *S. aemula* could grow well under 35/27 °C. The results provide evidence for the introduction and resource assessment of *S. aemula* species.

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1. Introduction

Plant growth, metabolism, and development are remarkably affected by a variety of abiotic stress, such as drought, high salinity, extreme temperature, flooding, and heavy metals (Mittler, 2006; Ashraf and Foolad, 2007; Cramer et al., 2011; Atkinson and Urwin, 2012). Out of these abiotic stresses, high temperature (HT) stress is considered one of the most disadvantageous environmental stresses and is recognized as the second most important stress (Levitt, 1980; Wang et al., 2003). The HT stress can have a significant harmful effect on almost all aspects of plant growth, development, and reproduction through primarily denaturing the structure of cellular cytoskeletons, disturbing the stability of various proteins, affecting the fluidity of plasma membranes, and altering the efficiency of enzymatic reactions (Mittler et al., 2012; Bita and Gerats, 2013; Hasanuzzaman et al., 2013). Consequently, the effect of HT stress on plant growth and productivity has been a central theme of plant scientists. As the average surface temperature on the earth is predicted to be increased by 2–5 °C at the end of this century due to climate change (IPCC, 2012), a major concern is raised about how the enhancing threat of HT might result in potential damages on the whole life cycle of plants. Particularly, the global environmental change is likely to result in a high frequency of extreme meteorological events, such as a short period of abnormally high temperature. Therefore, more studies are needed for better understanding of plant responses and adaptation to the elevated global temperatures (King et al., 2006).

Plant responses to HT stress are very complex (Ashraf and Foolad, 2007; Cramer et al., 2011; Hasanuzzaman et al., 2013). This might be due to structural complexity of a plant at genetic, organellular and cellular levels, functional complexity of plants at molecular, biochemical and physiological processes, and interactional complexity of plant ecosystems with their surrounding environments in terms of energy flow and matter cycling (Zhu, 2002; Cramer et al., 2011; Hasanuzzaman et al., 2013). In order to maintain growth, production, and development for completing the life cycle, plants have to adapt HT stress by reorganizing their cellular structure and altering their metabolic processes (Weis and Berry, 1988; Valliyodan and Nguyen, 2006). It was reported that HT stress significantly influences the photosynthetic capacity of plants by reducing the amount of photosynthetic pigments (Marchand et al., 2005), declined antioxidant enzyme activities (Hurkman et al., 2009), and increasing malondialdehyde (MDA) content (Mohammed and Tarpley, 2010). Ou et al. (2008) reported that high temperature affected the morphological characteristics and physio-biochemical indexes in *Ginkgo biloba*. Under HT stress, proline (Pro) accumulation

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was observed in Tobacco cells (Kuznetsov and Shevyakova, 1997) and the acclimatized response of plants to temperature stress has also been investigated (Iba, 2002). Moreover, membrane thermostability was successfully used as suitable measure for the evaluation of heat resistance in cotton (Ashraf et al., 2010). Previous studies demonstrated that plant responses and adaptation to HT stress varied across plant species (Hasanuzzaman et al., 2013).

Scaevola aemula is a perennial ornamental plant species native to Australia with the characteristics of fast-growing, good adaptability, long blooming period and profusion of flowers (Elliot et al., 1990). Because of its advanced scenic view, *S. aemula* has become a common and valuable plant species in urban landscaping, gardening decoration and commercial trade and has been successfully introduced from Australia to other countries in North America and Europe (Seaton et al., 2014). The species of *S. aemula* has been introduced and cultivated in China since 2006. Because of the great difference in geography and climate between the original place and the introduced region for *S. aemula*, it is required to investigate how this species adapts to the new habitats. It is a particular case for *S. aemula* which is introduced and cultivated at Chongqing city in Southwestern China because this area is an extremely hot region, where the maximum daily temperature often exceeds 40 °C during summer. However, few studies have been reported to the response of *S. aemula* under a short period of extreme HT stress.

The overall objective of the research project was to investigate the response of *S. aemula* to the new environment under different HT regimes for a short period. The specific objectives of the study were: (1) to determine the HT stress-induced injure symptoms of *S. aemula* due to a 3-day period of increasing extreme temperatures, (2) to quantify the changes in the photosynthetic pigments with different HT treatments, (3) to examine the variations of selected sugar, protein, enzyme contents and activities under HT extremity and duration, and (4) to explore the relationships among the selected physiological valuables under normal temperature conditions. The research may provide a better understanding of physiological adaptive capacity of *S. aemula* in a new habitat and under a short period of extreme HT stress.

2. Materials and methods

2.1. Study site and temperature sets

The experiment was carried out at the Southwest University, Chongqing city, China (29° 33′N 106° 34′E). The study area contained a monsoon humid subtropical climate. Annual mean temperature was 18.2 °C, with 7.8 and 28.5 °C in January and August, respectively. Annual mean precipitation was approximately 1104 mm. In the present study, four temperature sets were set up as the treatment levels and they were 25/22 °C (temperature at day for 10 h/temperature at night for 14 h), 35/27 °C, 40/30 °C, and 46/35 °C. The four temperature sets were determined based on 46-year weather records in Chongqing city. The long-term weather records showed that the highest air and soil surface temperatures were 43 °C and 50 °C in the city. The difference of monthly average temperature was about 8 and 9 °C between daytime and nighttime during June to August. Particularly, there was often a short-time period of extreme HT (lasting two to three days) occurring in this region during summer. Additionally, this short-time event of extreme HT occurred more frequently in the past years. Therefore, the temperature set of 25/22 °C represented a normal weather condition in the study area during the summer times and was treated as a control. The other temperature sets were considered as HT stresses.

2.2. Plant material and experimental procedure

The two-year-old *S. aemula* seedlings were obtained from a local nursery and were cultivated under open-air conditions for five months, starting in December 2010. Then, the seedlings were transplanted into plastic pots (30 cm top diameter × 20 cm bottom diameter × 30 cm

height) with a mixed substrate (25% purple soil, 25% perlite and 50% humus) in the greenhouse under the control condition (25/22 °C) for another two months before the HT treatment started. No fertilizer or plant hormones were utilized for the seedlings in the greenhouse, but watering was regularly performed. Thirty *S. aemula* seedlings were selected with the similar size, over hundred leaves and health statues for each of the four temperature sets. These seedlings were cultivated in an artificial climate chamber (RDN-1000D-4, Ningbo Southeast Equipment CO. Ltd., China) for each temperature treatment. Air humidity and light intensity were set up at the similar values of $50 \pm 10\%$ and $120 \ \mu mol \cdot m^{-2} \cdot s^{-1}$ in the chamber, respectively.

The HT experiments were repeatedly performed three times in summer of 2011, and each experiment lasted for three days. The 3-day period was recognized as a short-term in the present study but might be long enough to severely damage physiological processes in plants. The HT stress-induced injure symptoms of *S. aemula* seedlings were observed and recorded on a daily basis. Five injure classes were used to describe the injury degree of each seedling under HT treatments: (class 0) no injure symptoms of the seedling; (class 1) visible chloroses in one or two leaves; (class 2) visible chloroses in all leaves of the seedling; (class 3) one or two leaves were wilting and abscission; and (class 4) all leaves were wilting and the whole seedling would be dead. The injury index (D_{JI}) was calculated based on the below equation at days 1, 2 and 3 after HT treatment, respectively:

$$D_{JI}(\%) = \frac{\sum (i \times N_i)}{(H \times M)} \times 100\%$$

where, i was the injured class (i = 0, 1, 2, 3, and 4); N_i was the number of injured seedlings at the injured class i; H was the highest injured class in the experiment; and M was the total number of seedlings in the experiment. The higher the D_{JI} value, the more serious the injure degree of the seedlings. The D_{JI} should be zero when no injure symptoms were found in the seedlings.

Leaf samples were taken from the seedlings for each temperature set for chemical analysis at days 1, 2 and 3 after treatment, respectively. The leaves were picked up at the similar position on the seedling (bottom, middle and top) for each sampling time. The selected physiological variables included chlorophyll (Chl), chlorophyll *a* (Chla), chlorophyll *b* (Chlb), carotenoid (Car), MDA, Pro, soluble sugar and soluble protein contents, as well as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities.

A leaf sample (0.4 g f. wt) was mashed in a mortar and pestle with 80% of acetone (v/v) for 12 h, and the extract was filtered through two layers of nylon and centrifuged in sealed tubes at 15,000g for 5 min. Absorbance of the clear supernatant at 663, 647 and 750 nm was recorded and concentrations of Chla, Chlb, and Car were calculated by the equations of Lichtenthaler and Buschmann (2001):

$$Chla = 12.25 A_{663} - 2.79 A_{647}$$

$$Chlb = 21.50 A_{647} - 5.10 A_{663}$$

$$Car = (1000 A_{470} - 1.82 Chla - 95.15 Chlb)/225$$

Soluble sugar and MDA contents were determined by the thiobarbituric acid method (Wang, 2006; Li et al., 2008). A 0.5 g leaf sample (f. wt) was homogenized in 10 ml of 10% trichloroacetic acid. The homogenate was centrifuged at 4000 rpm for 10 min. To a 2 ml aliquot of the supernatant, 2 ml 0.6% thiobarbituric acid (TBA) was added. The mixture was incubated at 100 °C for 15 min, for cooling. After the tube was centrifuged at 4000 rpm for 10 min, absorbance values of the supernatant were recorded at 532, 600 and 450 nm and the soluble sugar and MDA contents were calculated by the formulae of Li et al. (2008).

Total soluble protein content was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. 1.0 g fresh leaf samples were homogenized with 4 ml sodium phosphate

buffer (pH 7.2) and then centrifuged at 10,000g for 10 min at 4 °C. Supernatants and dye were pipetted in spectrophotometer cuvettes and absorbance was measured at 595 nm.

Proline content was determined by the method of Wang (2006) and Li et al. (2008). A 0.2 g fresh leaf tissue was homogenized with 5 ml 3% sulfosalicylic acid and then the solution was placed in a boiling water bath for 10 min. A 2 ml aliquot of the supernatant was filtered with filter paper, and 2 ml acetic acid and 3 ml acidic ninhydrin solution was added. The mixture was incubated for 40 min at 100 °C and then cooled. The reaction mixture was extracted with 5 ml toluene and absorbance was determined at 520 nm. Pure Pro was used as a standard.

For assays of CAT, POD and SOD, 0.5 g fresh leaf samples were homogenized with 2 ml phosphate buffer in an ice bath and centrifuged at 10,000g for 15 min at 4 °C (Wang, 2006; Li et al., 2008). SOD activity was assayed based on the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture contained 1.5 ml 0.05 mol/l phosphate buffer (pH 7.8), 0.3 ml 750 μmol/l NBT, 0.3 ml 130 mmol/l L-methionine, 0.3 ml 100 μmol/l EDTA-Na2, 0.3 ml 20 μmol/l riboflavin and 0.2 ml enzyme extract. The reaction was carried out at 25 °C under a light intensity of 4000 lm/m^2 for 20 min, then spectrophotometric absorbance was measured at 560 nm. One unit of SOD activity was defined as the quantity of enzyme required to inhibit NBT reduction by 50%. POD activity was determined by measuring the ability of the enzyme extract to increase absorbance at 470 nm due to the guaiacol oxidation. A reaction mixture that contained 3 ml 0.1 mol/l phosphate buffer (pH 7.8), 15 μl guaiacol, and 30 μl 30% H_2O_2 was prepared and incubated at 32 °C. A 20 μl enzyme extract was added to the mixture and the increase in absorbance at 470 nm each minute for a total of 4 min was then measured. The average change per min in the absorbance of guaiacol was used to calculate POD activity. CAT activity was determined by measuring the ability of the enzyme extract to decompose H_2O_2. The reaction mixture contained 2 ml 0.1 mol/l phosphate buffer (pH 7.8), 1 ml 0.08% H_2O_2, and 0.2 ml enzyme extract. Absorbance was measured at 240 nm. One unit of catalase was the amount of enzyme which decomposed 1 mmol H_2O_2/min at 25 °C.

2.3. Data analysis

All data represented means of three experiments. A two-factor ANOVA was performed with factors high temperate treatment (three levels) and lasting time (three levels). The Duncan and Dunnett tests were used for multiple comparisons and to determine differences among treatments means at significance levels of P ≤ 0.05 and P ≤ 0.01, respectively. Pearson's correlation coefficient was used for the correlation analysis among the different parameters. All statistical analyses were performed with SPSS 18.0.

3. Results

Compared to the control, 35/27 °C treatment did not resulted in injuries on *S. aemula* seedlings during the time of HT treatment, and the IJI value was zero at days 1 and 2, and 10% at day 3 (Table 1). However, the injury indexes of the seedlings were significantly higher under the 40/30 °C treatment for the last 2 days and under the 46/35 °C treatment for all 3 days than that under the control treatment (P < 0.01). Particularly, more than 50% and 80% of the seedlings were severely damaged in 40/30 °C and 46/35 °C sets at day 3 post-treatment, respectively.

HT extremity and duration, as well as their interaction had significant influence on photosynthetic pigment levels (P < 0.001). Under 35/27 °C, the contents of Chla, Chlb, Chl and Car in *S. aemula*'s leaves significantly increased for all three days when compared to the control (P < 0.01). Particularly, the contents of the photosynthetic pigments increased by 132.8, 135.2, 133.5 and 91.8% at day 1 post-treatment, respectively compared to the control (Fig. 1). The photosynthetic pigment contents significantly increased under 40/30 °C at both day 1 and day 2 (P < 0.01) after HT treatment when compared to the control, but no significant difference was found between 40/30 °C and the control at day 3. The Chla, Chlb, Chl and Car contents were significantly higher at 46/35 °C than at control at day 1 (P < 0.01), but these contents decreased with HT duration and the corresponding values were reduced by 41.3, 31.5, 39.0 and 35.2% at day 3, respectively (Fig. 1).

The MDA contents were significantly reduced by approximately 31, 44 and 49% in 35/22, 40/30 and 46/35 °C sets at day 1 post-treatment when compared to the control (P < 0.05), but there was no significant difference of MDA among all four temperature sets at days 2 and 3 (P > 0.05), except under the 46/35 °C treatment in which the MDA content significantly increased by about 94% (Fig. 2, A). The soluble sugar contents significantly increased under the HT treatments at day 1, except under the 46/35 °C set (Fig. 2, B). At day 2, no significant difference of the soluble sugar contents was found between the 35/22 °C treatment and the control, but the soluble sugar contents were found to significantly increase under the 40/30 °C set and significantly decrease under the 46/35 °C set. At day 3, the soluble sugar contents were as 1.01, 1.84 and 1.50 times higher at 35/27, 40/30 and 46/35 °C treatments as in the control, respectively (Fig. 2, B). When compared to the control, the soluble protein contents increased under the 35/27 °C set, but reduced under the 40/30 and 46/35 °C sets after all three days of the treatments (Fig. 2, C). The Pro contents significantly decreased under HI treatments compared to the control during the three days (P < 0.05) (Fig. 2, D).

HT stress likely enhanced SOD activity, especially in day 1 for all HT treatments and in days 2 and 3 for the 35/27 and 46/35 °C treatment sets (Fig. 2, E). On the contrary, the CAT activity was significantly reduced under HT stresses (P < 0.05), except in 35/27 °C at day 2 (Fig. 2, F). The POD activity did not significantly differ between 25/22 °C and 35/27 °C sets for all 3 days of treatments. But the POD activity decreased by about 26% in 40/30 °C for the three days of treatments when compared to the control. In contrast, the POD activity increased by about 20% at day 3 after treatment under the 46/35 °C treatment (Fig. 2, G).

Under control condition (25/22 °C), the Chl content was positively related to the CAT content, but negatively related to the POD activity (Table 2). The CAT content was negatively in relation with the MDA and Pro contents, and CAT and POD activities. The MDA content was positively related to the Pro content and POD activity. The Pro content was positively related to the soluble protein content, and CAT and SOD activities. The CAT activity was negatively related to soluble sugar content, but positively to soluble protein contents. The POD activity is positively related in both CAT and SOP activities.

4. Discussion

In the present study, we found that HT stress-induced injury symptoms in *S. aemula* seedlings did not occur under the treatment of 35/27 °C, but the proportion of morphological injury in the seedlings increased with the rising HT stress and duration (Table 1). Correspondingly, the selected physiological variables were altered in order to cope with the elevated HT stresses. The contents of Chl and Car increased initially but were reduced with increases of the HT stresses and lasting time (Fig. 1). The MDA content decreased at day 1 for all temperature treatments, but it significantly increased under the treatment of 46/35 °C at day 3. With the increases of HT stress and duration, soluble sugar

Table 1
Changes in injury indexes of *S. aemula* seedlings under different temperature treatments.

Temperature treatments (°C)	Post-treatment time (day)		
	1	2	3
25/22	0.0 ± 0.0a	5.0 ± 0.0a	10.0 ± 0.0a
35/27	0.0 ± 0.0a	0.0 ± 0.0a	10.0 ± 0.0a
40/30	0.0 ± 0.0a	25.0 ± 0.5b	50.2 ± 0.6b
46/35	10.0 ± 0.7b	43.3 ± 1.1c	80.0 ± 0.3c

Injury index: mean ± S.D. Temperature treatments as day/night for 10/14 h. Different letters within each column meant significant different among treatments (P < 0.01).
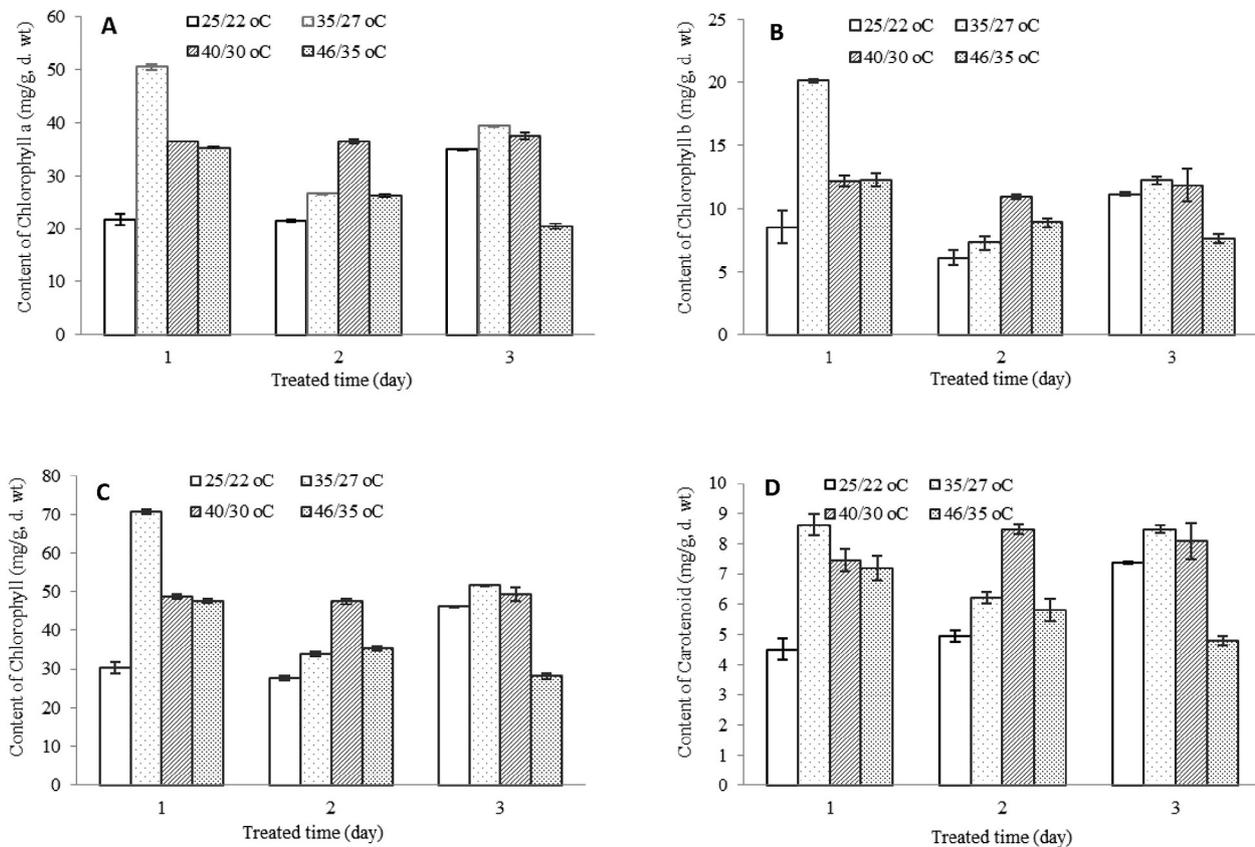
Fig. 1. Temporal changes in the contents of photosynthetic pigments under different temperature treatments. A: chlorophyll a; B: chlorophyll b; C: chlorophyll; and D: carotenoid. Data are means ± S.D.

content and SOD activity increased, while soluble protein content, Pro content and CAT activity decreased (Fig. 2, from A to F). These results were consistent with other previous findings. For instance, between 20 and 35% leaf damages were observed at 1 day under an experiment of 46 °C for 6 h (Fischer, 1980). Omae et al. (2012) reported that HT stress caused rolling, drying and necrosis of leaves in the common bean species (*Phaseolus vulgaris*). The leaf morphological damages were due to HI stress, which are consistent with the changes in the amount of photosynthetic pigments (Marchand et al., 2005), such as the contents of Chl and Car in leaves which reflect the photosynthetic ability of the plants in some ways. The initial increase and then decrease of Chl and Car contents in *S. aemula* foliates are likely a normal response pattern of plants to HT stress. The rising temperature might be favorable to the biosynthesis of Chl and Car in *S. aemula* leaves for a short time. With HT stress intensity and duration increased, the amount of photosynthetic pigments decreased. Particularly at day three following the HT experiment, the contents of Chl and Car were significantly lower in 46/35 °C treatments than the control, meaning the severe HT stress resulted in damage of the biosynthesis process of Chl and Car in *S. aemula* leaves. The photosynthetic pigments significantly declined by 7% for Chla and 18% for total Chl in soybean under moderate HT stress (Hasanuzzaman et al., 2013). A reduction of both Chl and Chla was also observed in sorghum (Mohammed and Tarpley, 2010).

The reduction of photosynthetic pigments was due to HT stress which caused the accumulation of unwanted and harmful oxygen radicals within foliates (Willekens et al., 1994). The increase of MDA content in leaves was a common phenomenon under HT stress (Hurkman et al., 2009). The MDA content increased by 75% in sorghum under HT experiment of 40/30 °C (Mohammed and Tarpley, 2010), and 27 to 58% in another experiment (Miller et al., 2009). Our results that showed that the MDA content increased under 46/35 °C treatment at day 3 supported

these findings. With the increase of the temperature, the soluble sugar increased after day two or day three treatments. This suggests that *S. aemula* could induce the leaves to produce more soluble sugar to adapt to the unfavorable condition when it encounters a certain stress (at 40/30 and 46/35 °C). Under the very HT condition (at 46/35 °C), however, the osmotic adjustment substance like the soluble protein in the leaves of *S. aemula* decreased (Nakamoto et al., 2000; Haslbeck and Buchner, 2002). This phenomenon might be due to two reasons. One is that the decreasing of the photosynthetic rate under excessive HT stress influences the production of soluble protein, and the other is the plant synthesizes more soluble sugar to cope with unfavorable environmental conditions, such as HT.

Proline is the main accumulated osmotic adjustment substance in plants under HT stress. Many studies have demonstrated that there is a positive correlation between the Pro accumulation and the stress tolerance of plants (Kuznetsov and Shevyakova, 1997; Claussen, 2005). In this research, the Pro content was dramatically reduced at different time points and stress temperatures. This suggests that HT stress affects the tolerance of *S. aemula*. Interestingly, although the SOD activity showed a slight fluctuation, this change was not dramatic at different time points and HT stress. Thus, *S. aemula* might have sufficient capacity to balance the external adversity stress.

The CAT activity significantly decreased at day 1 post-treatment, but its activity was slightly restored at day 2 and day 3, which indicated that high temperature induced a stress reaction in *S. aemula*. There was no significant difference in the POD activity between the 35/27 °C treatment and the control, but the POD activity obviously decreased at 40/30 °C. Interestingly, the POD activity at 46/35 °C gradually increased with the prolongation of the stress duration. These data suggest that temperature (35/27 °C) has no effect on the POD activity, but high temperature (40/30 °C) impairs the POD activity. However, with a
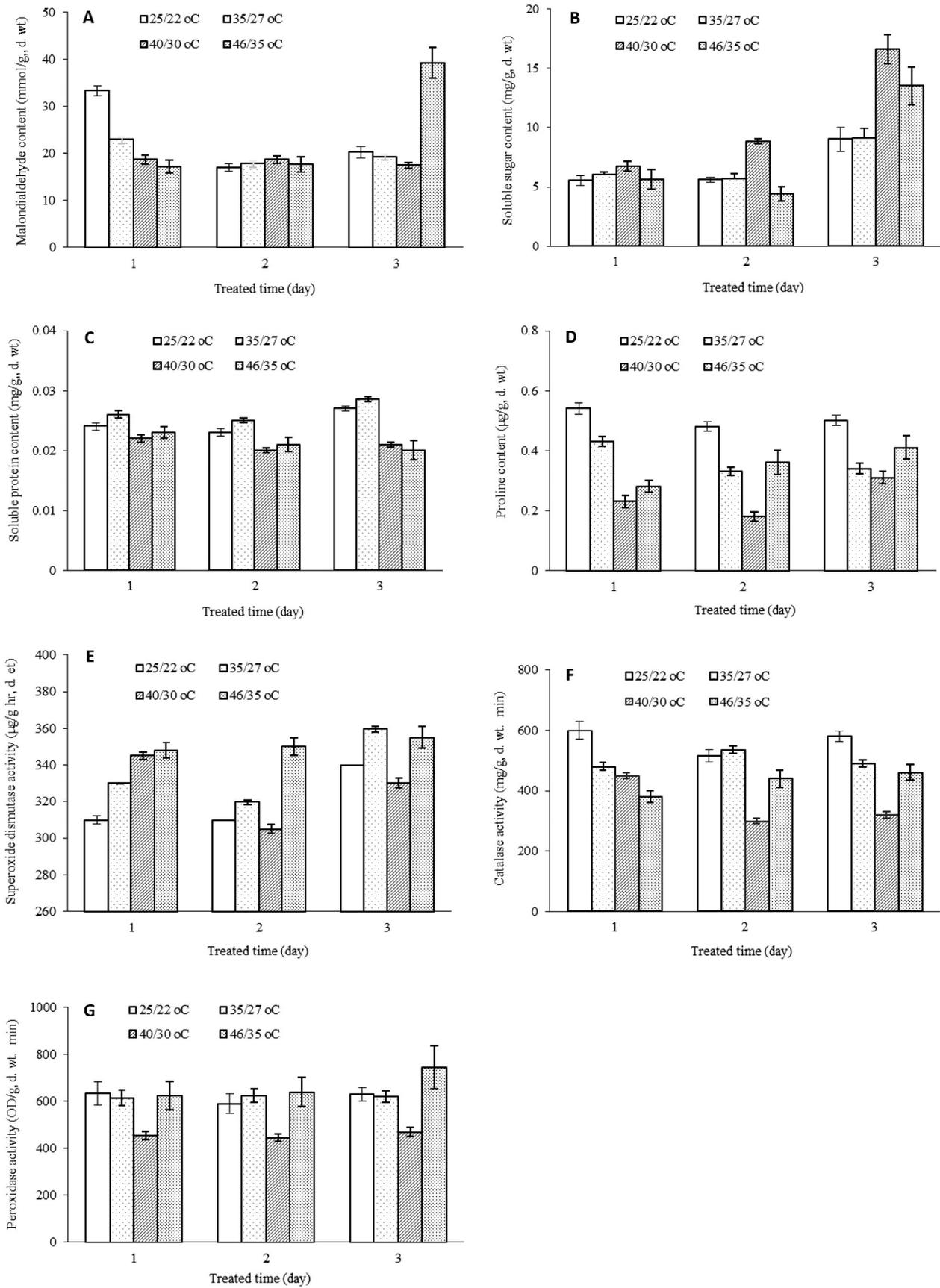
Fig. 2. Temporal changes in several important physiological variables under different temperature treatments. A: Malondialdehyde content; B: soluble sugar content; C: soluble protein content; D: proline content; E: super oxide dismutase activity; F: catalase activity; and G: peroxidase activity. Data are means ± S.D.

Table 2
Correlation analysis of physiological parameters in S. aemula leaves.

	Chl content	CAT content	MDA content	Pro content	Soluble sugar content	Soluble protein content	CAT activity	POD activity	SOD activity
Chl content	1	0.880**	−0.279	−0.279	0.052	0.305	−0.270	−0.384*	0.252
CAT content		1	−0.467**	−.525**	0.204	0.252	−0.444**	−0.559**	0.197
MDA content			1	0.443**	0.252	−0.030	0.318	0.538**	0.146
Pro content				1	−0.145	0.443**	0.783**	0.621**	−0.046
Soluble sugar content					1	−.198	−0.397*	−0.143	0.223
Soluble protein content						1	0.658**	0.264	0.223
CAT activity							1	0.577**	0.030
POD activity								1	0.395*
SOD activity									1

Chl: chlorophyll; CAT: catalase; MDA: malondialdehyde; Pro: proline; POD: peroxidase; SOD: superoxide dismutase.
** Correlation is significant at P ≤ 0.01 (2-tailed).
* Correlation is significant at P ≤ 0.05 (2-tailed).

continuous increase of temperature, the high temperature had an obvious effect on the POD activity and the POD activity increased gradually at 46/35 °C. This suggests that S. aemula adapts to the environment gradually.

According to the correlation analysis, there were significant correlations between the physiological parameters in S. aemula, and there was a synergism between them under HT stress. Temperature stress controlled the production of Chl and CAT, induced the production of permeation control matters like Pro, soluble sugar, and soluble protein, and facilitated cutting seedlings producing CAT, POD and SOD to adapt to the environmental stress.

5. Conclusions

Plant responses to HT stress have been a hot topic in plant science worldwide. The climate change and global warming indicate that the knowledge of the growth, development, and productivity of plants under the influence of elevated temperature can only become increasingly important. Particularly, the global environmental changes likely result in a relatively high frequency of short-term periods of extreme events such as HT on plant ecosystems. Our experiments support the concept that HT stress depends on not only the elevated temperature, but also the time-duration of the increased temperature. The results from our study suggest that S. aemula is able to tolerate the elevated temperature at the 35/27 °C set. The mechanisms underlying the HT toleration of S. aemula are in relation to a short-time increase of photosynthetic pigments, soluble sugar contents and SOD activity; and decrease of soluble protein contents, Pro contents and CAT activity. Many studies indicated that plant response, adaptation and tolerance to HT stress are very complex processes and may differ at cellular, organelle and whole plant levels. Therefore, further studies are needed to investigate plant responses to HT stress under field conditions, long-term bases, and involvement of multiple factors in order to better understand the mechanisms of HT stress tolerance in S. aemula.

Acknowledgements

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