Exogenous Application of Absciscic Acid, Putrescine, or 2,4-Epibrassinolide at Appropriate Concentrations Effectively Alleviate Damage to Tomato Seedlings from Suboptimal Temperature Stress

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ADDITIONAL INDEX WORDS. plant growth regulators, abiotic stresses, vegetable production, *Solanum lycopersicum*

SUMMARY. The application of plant growth regulators (PGRs), such as abscisic acid (ABA), putrescine (Put), and 2,4-epibrassinolide (EBR), has been shown to enhance a plant's resistance to various abiotic stresses. However, the protective effects of these PGRs on tomato (*Solanum lycopersicum*) seedlings under suboptimal temperature stress have not yet been evaluated. We also do not know the most effective method of application of PGRs for various tomato cultivars. We studied the effects of three rates of exogenous ABA, Put, or EBR in limiting damage from suboptimal temperature stress on two tomato cultivars, Zhongshu6 (considered sensitive to suboptimal temperatures) and SANTIAM (considered tolerant to suboptimal temperatures). Results showed that application of these PGRs at appropriate concentrations could effectively reduce the decline in the net photosynthetic rate (P_n) and the chlorophyll (Chl) content in leaves caused by suboptimal temperature stress in both 'Zhongshu6' and 'SANTIAM' and could promote an increase in organic osmolyte (proline and soluble sugar) contents and root 2,3,5-triphenyltetrazolium chloride (TTC)-reducing activity for 'Zhongshu6'. However, these effects were inferior on 'SANTIAM'. For both cultivars, the best treatment concentrations are 1 mM ABA, 0.1 mM Put, or 0.02 μ M EBR. Results indicate that in tomato production, exogenous application of ABA, Put, or EBR at appropriate concentrations can effectively limit damage from suboptimal temperature stress.

Thermophilic fruit vegetables, such as tomato, often suffer from suboptimal temperature stress, which is one of the limiting factors in winter vegetable production in China, leading to a decline in production and quality (Wang et al., 2001). Suboptimal temperatures, which refer to day temperatures less than 20 °C and night temperatures less than 10 °C, are categorized as a chilling stress, one of the major environmental stresses affecting plant development and performance. Many tropical and subtropical plant species

are considerably affected when exposed to low temperatures during their life cycles. Many plants can increase their tolerance to freezing temperatures after exposure to a period of non-freezing temperatures, a phenomenon known as cold acclimation. In the process, plants cope with the low temperatures by employing a number of physiological and cellular changes, including inhibition of plant growth, decrease of photosynthesis and increase of leaf thickness, and increasing osmolytes with cryoprotective properties (such as proline and soluble sugars) and root TTC-reducing activity (Ristic and Ashworth, 1993;

Uemura et al., 1995; Xin and Browse, 2000).

A number of genetic and molecular studies on plant response to low temperatures have resulted in the cloning and characterization of many components of the cold signaling pathway, in which the phytohormone ABA plays an important role (Xin and Browse, 2000). Moreover, Put and another class of phytohormones known as brassinosteroids (BRs) were also shown to be involved in low-temperature response (Cuevas et al., 2008; Kagale et al., 2007).

Absciscic acid is the central regulator of abiotic stress resistance in plants and is involved in many stress responses. A plant's ABA content rapidly accumulates under cold or drought stresses, which helps to stimulate stomatal closure, change gene expression, and increase osmocompatible solutes to improve the plant's capacity to cope with the stress conditions (Cutler et al., 2010; Kim et al., 2010; Seki et al., 2007). In plants, Put is a type of polyamine, a low-molecular-mass polycation ubiquitous in all living organisms, and was considered to act as a free radical scavenger to protect the plant from oxidative and chilling stress (Drolet et al., 1986; Galston et al., 1997; Nayyar and Chander, 2004; Shen et al., 2000; Velikova et al., 1998). BRs are a recently classified group of plant hormones that were first isolated from the pollen of rape (*Brassica napus*) in 1979 (Grove et al., 1979). Research has shown that BRs might be a plastic-promoting hormone that allows a plant to adapt to multiple environmental stresses, such as heavy metals, salt, biotic stress, hypoxia, osmotic stress, and temperature stress (Dhaubhadel et al., 1999; Houimli et al., 2008; Kagale et al., 2007; Kang et al., 2006; Masidur et al., 2007; Nakashita et al., 2003; Vidya and Seeta, 2003).

Studies on the effects of growth regulators on suboptimal temperature stress, which is common in vegetable

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This work was funded by 973 Program (2009CB19001), Ministry of Science and Technology, and supported by the earmarked fund for China Agricultural Research System (CARS-25-C-09), Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture, People's Republic of China, and National Key Project (2011BAD12B01).

We gratefully acknowledge assistance from Xiaoxuan Wang in supplying tomato seeds.

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Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	μ L	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
28.3495	oz	g	0.0353
0.001	ppm	$\text{mg}\cdot\text{g}^{-1}$	1000
1	ppm	$\mu\text{g}\cdot\text{g}^{-1}$	1
$(^{\circ}\text{F} - 32) \div 1.8$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$(1.8 \times ^{\circ}\text{C}) + 32$

production, were relatively rare. This study, therefore, investigated what is the induction effect of these exogenous PGRs on tomato seedlings, and whether various chilling-tolerant genotypes of tomato respond differently to these exogenous PGRs. The elucidation of these problems will provide the theoretical basis for understanding the physiological mechanism by which PGRs alleviate the damage in a horticultural crop under chilling stress. It is also important to explore effective ways to apply ABA, Put, or EBR in vegetable production.

Materials and methods

TOMATO SEEDLING CULTIVATION.

Two genotypes of tomato were used in this experiment: chilling-sensitive cultivar Zhongshu6 [Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China] and chilling-tolerant cultivar SANTIAM (IVF, CAAS). Tomato seeds were sown in plugs with a mixture of peatmoss and vermiculite (v/v = 1:1) as culture substrate. When plants reached three fully expanded leaves, ABA (0.2, 0.6, 1 mM), Put (0.1, 0.5, 1.0 mM), EBR (0.01, 0.02, 0.1 μ M) (Sigma, St. Louis, MO), and water were applied to the foliage of selected uniform seedlings. A second application of the PGR with same concentration was made 5 d later. Three replications were used for each treatment, and the experimental unit had 15 plants.

SUBOPTIMAL TEMPERATURE TREATMENT. As soon as the fluid sprayed at the second time was no longer visible, and there were no water drops on the leaf surface, the seedlings were divided into two groups, and one group was moved to climatic chambers (PRX-450B; SaiFu, Ningbo, China) at 16/8 °C (day/night) with a light period of 10 h at a photosynthetic photon flux of 250 μ mol·m⁻²·s⁻¹; the relative humidity was 75%, including the plants sprayed with ABA, Put, EBR, and distilled water. The other group, control samples (CK) including the plants sprayed with distilled water, was then grown in identical climatic chamber kept at 28/22 °C (day/night) under a 10-h photoperiod with the same conditions described above. Sample collection was carried out on days 0, 2, 4, 6, and 8 of suboptimal temperature stress, including the plants sprayed

with distilled water. Three plants were harvested for each sample period.

PHOTOSYNTHESIS MEASUREMENT.

Plant photosynthesis was tested with the second fully expanded leaf under the growing point, on days 0, 2, 4, 6, and 8 of suboptimal temperature stress, using a portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE) according to the manufacturer's instructions. Three plants were used on each sample period.

CHLOROPHYLL CONTENT MEASUREMENT. The second fully expanded leaf under the growing point was sampled on days 0, 2, 4, 6, and 8 of suboptimal temperature stress to measure Chl content according to the previous report using an ultraviolet spectrophotometer (ultraviolet-2102PC/

PS; UNICO, Shanghai, China) (Li, 2000); three plants were used for each treatment.

PROLINE AND SOLUBLE SUGAR CONTENTS MEASUREMENT. Soluble sugar measurement was carried out according to previous report (Li et al., 2004). The second fully expanded leaf were harvested, weighed, and ground to powder in liquid nitrogen and then incubated in 75% ethanol overnight with gentle shaking. After centrifugation at 20,000 g_n , 20 μ L of each extract was incubated with 1000 μ L of anthrone reagents [0.15% (w/v) anthrone, 72% (v/v) H₂SO₄, and 28% (v/v) water] at 100 °C for 1 h. The soluble sugar value was measured at 630 nm. Proline measurement was carried out according to a previously

Fig. 1. The effects of exogenous (A) abscisic acid (ABA), (B) putrescine (Put), and (C) 2,4-epibrassinolide (EBR) on leaf net photosynthetic rate (P_n) of the chilling-sensitive tomato cultivar Zhongshu6 and the chilling-tolerant cultivar SANTIAM under suboptimal temperature stress. Water = control samples sprayed with distilled water and grown at 16/8 °C (60.8/46.4 °F) (day/night) and CK = control samples sprayed with distilled water and grown at 28/22 °C (82.4/71.6 °F) (day/night). Each value is the mean of three replications, and the vertical bars indicate SD ($n = 3$).

described method (Bates et al., 1973). The second fully expanded leaf were weighed and subjected to extraction using 3% sulfosalicylic acid. After filtration, 2 mL of each filtrate was incubated with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin reagent [2.5% (w/v) ninhydrin, 60% (v/v) glacial acetic acid, and 40% (v/v) 6 M phosphoric acid] at 100 °C for 1 h. The reaction was ended in an ice bath. Toluene (4 mL) was added into the extractions followed by vigorous shaking for 20 s. After incubation at 23 °C for 24 h, the proline values were measured at 520 nm.

ROOT ACTIVITY MEASUREMENT. Measurement of root activity was performed according to the TTC method (Wang et al., 2010a). The substrate was removed from the root using tweezers, and then the roots were washed with sterile water. The surface liquid of roots was blotted with tissue paper and their fresh weights were measured. Roots with weights 0.5 g were placed in tubes and filled with 5 mL of 0.4% TTC and 5 mL phosphate buffer (0.06 mol·L⁻¹, pH 7.0). Control treatment (blank runs) was always carried out using the same procedure, but adding 2 mL of 1

mol·L⁻¹ sulfuric acid first. The tubes were incubated at 37 °C for up to 4 h. The chemical reaction was stopped by adding 2 mL of 1 mol·L⁻¹ sulfuric acid into the tubes. This step was followed by extraction with 10 mL of 95% ethanol for 24 h, which consisted of taking the root in a new tube. The optical density values were recorded at 485 nm.

STATISTICAL ANALYSES. Each of the five physiological indices was analyzed using Proc Mixed in SAS (version 8.0; SAS Institute, Cary, NC) with a variance-covariance structure for the repeated measures analysis of variance. The variance-covariance structure was determined to be compound symmetry structure, and the interactions between treatments and days were significant. Then the treatment comparisons within each day were made. The results presented in the table of each column were the mean of three replications, and means were compared with Duncan's multiple range test ($P < 0.05$) analyzed by SAS (version 8.0).

Results

THE EFFECTS OF PGRs ON LEAF NET PHOTOSYNTHETIC RATE UNDER

SUBOPTIMAL TEMPERATURE STRESS. As shown in Fig. 1, under normal conditions (day 0), the leaf P_n of 'SANTIAM' (8.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ CO₂) was higher than that of 'Zhongshu6' (6.4 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ CO₂). When suffering from suboptimal temperature stress, the leaf P_n of 'SANTIAM' significantly decreased immediately, whereas that of 'Zhongshu6' decreased after the fourth day (Fig. 1). However, exogenous ABA, Put, or EBR applied at appropriate concentrations effectively reduced the decrease in P_n under suboptimal temperature stress (Fig. 1A–C). As shown in Fig. 1A, during the days of stress, only 1 mM ABA significantly reduced the decline in P_n of 'Zhongshu6' and maintained P_n levels that were remarkably higher than those found in the water-treated plants by 51.2%, 30.8%, 109.0%, and 164.0% on days 0, 2, 6, and 8, respectively. For the suboptimal temperature-tolerant cultivar SANTIAM, only 0.6 mM ABA did not show any response, whereas 1 mM provided the greatest effect, with P_n levels higher than those found in the water-treated plants by 18.3%, 141.0%, 62.2%, and 123.0% on days 2, 4, 6, and 8 of stress, respectively.

Table 1. Effects of abscisic acid (ABA), putrescine (Put), and 2,4-epibrassinolide (EBR) on net photosynthetic rate (P_n), leaf chlorophyll content (Chl), proline content, sugar content, and root activity of chilling-sensitive ('Zhongshu6') and chilling-tolerant ('SANTIAM') tomato cultivars on the day 8 of suboptimal temperature. The results presented in each column are the means of three replications.

Cultivar	Treatment ^z		P_n [CO ₂ ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)]	Chl content (mg·g ⁻¹ FW) ^y	Proline content ($\mu\text{g}\cdot\text{g}^{-1}$ FW) ^y	Sugar content (%)	Root activity ($\mu\text{g}\cdot\text{g}^{-1}$ FW per hour)
Zhongshu6	ABA	0.2 mM	2.21 e ^x	0.89 i	95.29 c	2.85 cd	1324.96 cd
		0.6 mM	2.22 e	1.37 c	105.05 bc	3.14 bc	1729.99 ab
		1 mM	4.94 a	1.51 a	113.58 ab	3.40 b	1913.72 a
	Put	0.1 mM	3.67 b	1.39 b	114.99 ab	3.44 b	1783.43 a
		0.5 mM	2.24 e	1.19 f	97.89 bc	2.93 c	1006.80 de
		1 mM	2.84 cd	1.05 h	101.98 bc	3.05 bc	1366.77 bcd
	EBR	0.01 μM	1.63 f	1.24 e	114.94 ab	3.44 b	839.22 e
		0.02 μM	2.96 c	1.27 d	129.13 a	3.86 a	1696.68 abc
		0.1 μM	2.61 d	1.24 e	102.58 bc	3.07 bc	1681.95 abc
	Water		1.87 f	1.09 g	101.81 bc	2.44 d	1199.63 de
SANTIAM	ABA	0.2 mM	2.91 e	1.17 c	103.73 ab	3.10 ab	1548.85 d
		0.6 mM	2.11 f	1.22 bc	95.38 bc	2.85 bc	1309.79 g
		1 mM	5.03 b	1.40 a	94.39 bc	2.82 bc	1725.22 a
	Put	0.1 mM	3.89 c	1.35 a	92.88 bc	2.78 bc	1625.13 c
		0.5 mM	3.77 c	1.25 b	88.34 c	2.64 c	1504.64 f
		1 mM	5.26 a	0.98 d	103.07 abc	3.08 abc	1535.28 e
	EBR	0.01 μM	2.10 f	1.15 c	111.23 a	3.33 a	1158.33 h
		0.02 μM	3.75 c	1.19 bc	97.49 abc	2.92 abc	1710.54 b
		0.1 μM	3.32 d	1.17 c	102.40 abc	3.07 abc	826.25 i
	Water		2.26 f	1.20 bc	104.98 ab	3.11 ab	1311.93 g

^zWater = control samples sprayed with distilled water grown at 16/8 °C (60.8/46.4 °F) (day/night).

^y1 mg·g⁻¹ = 1000 ppm, 1 $\mu\text{g}\cdot\text{g}^{-1}$ = 1 ppm, FW = fresh weight.

^xFor each tomato cultivar, means within a column followed by the same letter are not significantly different via Duncan's multiple range test at $P < 0.05$.

Figure 1B shows that 0.1, 0.5, and 1 mM concentrations of Put could reduce the decrease in P_n in the two cultivars to varying degrees, and 0.1 mM Put showed the most significant effect on Zhongshu6, the P_n of which was effectively increased by 96.3% on day 8 of stress compared with that of water-treated plants (Table 1). The effect of exogenous application of Put on 'SANTIAM' was more effective than it was on 'Zhongshu6'. On day 8 of stress, the P_n of 'SANTIAM' plant treated with 0.1, 0.5, and 1 mM Put was higher than that of the water-treated plants by 72.0%, 67.0%, and 133.0%, respectively (Table 1).

Figure 1C indicates that among the three concentrations, only 0.02 μ M EBR (Fig. 1C) revealed a prominent effect on both 'Zhongshu6' and 'SANTIAM'. Compared with the water-treated plants, the P_n of the treated 'Zhongshu6' increased by 30%, 20%, 28%, 70%, and 58%, and the P_n of the treated 'SANTIAM' increased by 23%, 47%, 50%, 47%, and 66% on days 0, 2, 4, 6, and 8 of stress, respectively.

The effects of the three concentrations of ABA, Put, or EBR on the leaf P_n on day 8 of suboptimal temperature were compared. The results showed that for 'Zhongshu6', 1 mM ABA had the greatest effect (P_n increased by 164.0%) and for 'SANTIAM', 1 mM ABA and 1 mM Put had almost the same best effect (P_n increased by 123.0% and 133.0%, respectively) (Table 1).

THE EFFECTS OF PGRs ON CHLOROPHYLL CONTENT UNDER SUBOPTIMAL TEMPERATURE STRESS. As shown in Fig. 2, the Chl content of 'SANTIAM' [$1.6 \text{ mg} \cdot \text{g}^{-1}$ fresh weight (FW)] was higher than that of 'Zhongshu6' ($1.4 \text{ mg} \cdot \text{g}^{-1}$ FW) under normal conditions. During chilling stress, the Chl content of 'Zhongshu6' appeared more stable than that of 'SANTIAM' before day 6 but decreased more dramatically later. For 'Zhongshu6', except for 0.2 mM ABA and 1 mM Put, all the treatments reduced the decline in the Chl content under suboptimal temperatures, and similar to the results above, treatments with 1 mM ABA, 0.1 mM Put, and 0.02 μ M EBR produced the greatest induced effect (Fig. 2A–C), with the Chl content higher on day 8 than that of the water-treated plants by 37.9%, 27.3%, and 15.6%, respectively (Table 1). For 'SANTIAM', the Chl content

Fig. 2. The effects of exogenous (A) abscisic acid (ABA), (B) putrescine (Put), and (C) 2,4-epibrassinolide (EBR) on the chlorophyll content of the chilling-sensitive tomato cultivar Zhongshu6 and the chilling-tolerant cultivar SANTIAM under suboptimal temperature stress. Water = control samples sprayed with distilled water grown at 16/8 °C (60.8/46.4 °F) (day/night), CK = control samples sprayed with distilled water grown at 28/22 °C (82.4/71.6 °F) (day/night), and FW = fresh weight. Each value is the mean of three replications, and the vertical bars indicate sd ($n = 3$); $1 \text{ mg} \cdot \text{g}^{-1} = 1000 \text{ ppm}$.

was relatively more stable when treated with the exogenous PGRs under suboptimal temperatures; however, the treatments with 1 mM ABA or 0.1 mM Put had a remarkable effect on the increase of Chl content (Fig. 2A–C), which increased on day 8 by 16.9% and 12.7%, respectively (Table 1). Interestingly, the treatments with the three concentrations of EBR were not effective for 'SANTIAM'. Results indicate that an appropriate concentration of ABA, Put, or EBR can reduce the decline in Chl content and that the induced effect was more obvious on 'Zhongshu6' than on 'SANTIAM'.

Comparing the effects of ABA, Put, and EBR treatments on reducing the decline of Chl content on day 8 due to low temperature, we found that 1 mM ABA was the best choice for both 'Zhongshu6' and 'SANTIAM' [Chl content increased by 37.9% and 16.9%, respectively (Table 1)].

THE EFFECTS OF PGRs ON THE PROLINE CONTENT UNDER SUBOPTIMAL TEMPERATURE STRESS. Results showed that under normal conditions (day 0), the proline content of 'SANTIAM' ($40.5 \mu\text{g} \cdot \text{g}^{-1}$ FW) was lower than that of 'Zhongshu6' ($47.8 \mu\text{g} \cdot \text{g}^{-1}$ FW). During the period of suboptimal temperature stress, the proline content of

both cultivars increased; however, that of SANTIAM increased more rapidly, which might be one of the reasons why 'SANTIAM' is more tolerant to chilling. When treated with ABA, Put, or EBR, the proline content of 'Zhongshu6' increased equally during the period of stress, whereas that of 'SANTIAM' reached a maximum on day 6 and then decreased (Fig. 3).

As shown in Fig. 3, exogenous ABA, Put, or EBR applied at 1 mM, 0.1 mM, and 0.02 μ M could significantly improve the proline content of 'Zhongshu6'. Among the three PGRs, 1 mM ABA had the greatest effect on 'Zhongshu6', producing a proline content higher than that of the water-treated plants by 37.1%, 112%, 53.3%, 17.3%, and 11.6% on days 0, 2, 4, 6, and 8, respectively. When treated with 0.1 mM Put or 0.02 μ M EBR, the proline content of 'Zhongshu6' increased by 0%, 59.0%, 34.8%, 36.0%, and 12.9% or by 7.6%, 75.6%, 42.3%, 24.2%, and 26.8%, respectively, on days 0, 2, 4, 6, and 8, respectively (Fig. 3A–C). However, none of the concentrations of these PGRs had any significant effect on promoting the proline content of 'SANTIAM' under suboptimal temperature, except on day 6 of stress, when 1 mM ABA, 0.1 mM Put, and 0.02 μ M EBR significantly increased the proline content by 51.1%, 14.4%, and 22.2%, respectively (Fig. 3A–C).

The results showed that among these treatments, 1 mM ABA had the greatest effect on both 'Zhongshu6' and 'SANTIAM' for increasing the proline content to resist suboptimal temperature stress (Table 1).

THE EFFECTS OF PGRs ON SOLUBLE SUGAR UNDER SUBOPTIMAL TEMPERATURE STRESS. As shown in Fig. 4, the soluble sugar content of 'Zhongshu6' increased gradually over time under suboptimal temperature stress, whereas that of 'SANTIAM' increased quickly, reached a maximum on day 6, and then decreased. The exogenous ABA, Put, or EBR increased the soluble sugar content even further and maintained the higher level at later stages of stress. Similarly, treatments with 1 mM ABA, 0.1 mM Put, and 0.02 μ M EBR produced a significant induced effect on 'Zhongshu6', increasing the sugar content on day 8 over that of the water-treated plants by 47.7%, 49.5%, and 67.9%,

Fig. 3. The effects of exogenous (A) abscisic acid (ABA), (B) putrescine (Put), and (C) 2,4-epibrassinolide (EBR) on the proline content of the chilling-sensitive tomato cultivar Zhongshu6 and the chilling-tolerant cultivar SANTIAM under suboptimal temperature stress. Water = control samples sprayed with distilled water grown at 16/8 °C (60.8/46.4 °F) (day/night), CK = control samples sprayed with distilled water grown at 28/22 °C (82.4/71.6 °F) (day/night), and FW = fresh weight. Each value is the mean of three replications, and the vertical bars indicate sd ($n = 3$); 1 μ g·g⁻¹ = 1 ppm.

respectively (Table 1). Similar to the results with the proline content, the PGRs here produced inferior effects on 'SANTIAM' in increasing soluble sugar content. Except for 1 mM ABA, which increased the soluble sugar content of 'SANTIAM' by 23.6%, 11.3%, 20.8%, and 24.9% on days 0, 2, 4, and 6, respectively (Fig. 4), no treatment had a significant effect in promoting the soluble sugar content of the chilling-tolerant cultivar SANTIAM.

THE EFFECTS OF PGRs ON ROOT 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE-REDUCING ACTIVITY UNDER SUBOPTIMAL TEMPERATURE STRESS. As shown in Fig. 5, under normal conditions (day 0), the root TTC-reducing activity in 'Zhongshu6' was similar to that of 'SANTIAM'. Under suboptimal temperature stress, for all the treatments, the root TTC-reducing activities of the two cultivars first increased, reached a maximum on day 6,

Fig. 4. The effects of exogenous (A) abscisic acid (ABA), (B) putrescine (Put), and (C) 2,4-epibrassinolide (EBR) on the soluble sugar content of the chilling-sensitive tomato cultivar Zhongshu6 and the chilling-tolerant cultivar SANTIAM under suboptimal temperature stress. Water = control samples sprayed with distilled water grown at 16/8 °C (60.8/46.4 °F) (day/night) and CK = control samples sprayed with distilled water grown at 28/22 °C (82.4/71.6 °F) (day/night). Each value is the mean of three replications, and the vertical bars indicate SD ($n = 3$).

and then decreased; however, the activities in 'SANTIAM' reached a higher peak value than that of 'Zhongshu6', which also might be one of the reasons why 'SANTIAM' is more chilling tolerant. We inferred that under suboptimal temperatures, to maintain a normal nutrition metabolism and improve the absorption of inorganic ions to resist damage, the root TTC-reducing activity can increase compensatively. Similarly, exogenous ABA, Put, or EBR treatments applied at the proper concentrations could further increase root TTC-reducing activity in 'Zhongshu6', in which treatments with 1 mM ABA, 0.1 mM Put, and 0.02 μM EBR showed the greatest effect in improving the root activities, and were higher on day 8 than that of the water-treated plants

by 59.5%, 48.7%, and 41.4%, respectively (Fig. 5). For 'SANTIAM', we observed that only 1 mM ABA, 0.1 mM Put, and 0.02 μM EBR could significantly increase the root TTC-reducing activities, which were higher on day 8 than that of the water-treated plants by 31.5%, 23.9%, and 30.4%, respectively (Table 1). The results indicated that foliar spraying of 1 mM ABA, 0.1 mM Put, or 0.02 μM EBR had a significant effect in increasing root TTC-reducing activities under suboptimal temperature on both 'Zhongshu6' and 'SANTIAM', and 1 mM ABA was more effective than the other two (Table 1).

Discussion

The structure and function of cell membranes do not suffer damage

under suboptimal temperature conditions as they do in extreme coldness (van de Dijk and Smeets, 1984); however, suboptimal temperatures can decrease photosynthesis and increase the presence of osmolytes with cryoprotective properties (such as proline and soluble sugars) and root TTC-reducing activity. We have previously reported that exogenous EBR could enhance plant tolerance to weak light (Wang et al., 2010b). Here, we demonstrated that in tomato production, exogenous ABA, Put, or EBR applied in advance at appropriate concentrations can effectively alleviate the damage from suboptimal temperatures.

Previous studies showed that suboptimal temperature stress was not harmful to the structure and function of chloroplasts but could suppress the transport of photosynthate, which ultimately results in a decline in the photosynthetic rate. At the same time, the cold signaling pathway and related genes will be activated to adopt a series of adaptive responses, such as the increase of proline, soluble sugar, and root TTC-reducing activity that increase the osmotic pressure on the plant cells and limit damage from suboptimal temperature stress. During the last decade, many studies on the application of exogenous ABA, Put, or EBR showed these compounds can enhance plant resistance to various abiotic stresses (Dhaubhadel et al., 1999; Hwang and VanToai, 1991; Krishnamurthy, 1991; Masidur et al., 2007; Pospíšilová and Bat'ková, 2004; Rajasekaran and Blake, 1999; Vidya and Seeta, 2003; Wang et al., 2010b). In our current study, the results showed that under suboptimal temperature stress, exogenous ABA, Put, or EBR treatments, especially at 1 mM, 0.1 mM, or 0.02 μM, respectively, can effectively reduce the decline in P_n and Chl content and increase the proline and soluble sugar content and the root TTC-reducing activity of the chilling-sensitive tomato cultivar Zhongshu6 and that the induction effect of 1 mM ABA was the greatest among the three PGRs (Figs. 1–5). Interestingly, we observed that although exogenous ABA, Put, or EBR could significantly alleviate damage from suboptimal temperature stress for the chilling-tolerant tomato cultivar SANTIAM, the induction effects of these PGRs were

Fig. 5. The effects of exogenous (A) abscisic acid (ABA), (B) putrescine (Put), and (C) 2,4-epibrassinolide (EBR) on the root activity of the chilling-sensitive tomato cultivar Zhongshu6 and the chilling-tolerant cultivar SANTIAM under suboptimal temperature stress. Water = control samples sprayed with distilled water grown at 16/8 °C (60.8/46.4 °F) (day/night), CK = control samples sprayed with distilled water grown at 28/22 °C (82.4/71.6 °F) (day/night), and FW = fresh weight. Each value is the mean of three replications, and the vertical bars indicate SD ($n = 3$); 1 $\mu\text{g}\cdot\text{g}^{-1} = 1 \text{ ppm}$.

poorer than that found in ‘Zhongshu6’ (Figs. 1–5).

Comparing the physiological changes in ‘Zhongshu6’, our results indicate that during chilling stress, the soluble sugar content, proline content, and root TTC-reducing activities of ‘SANTIAM’ increased quickly and then reached high peak values; however the leaf P_n in ‘SANTIAM’ decreased quickly, whereas its Chl content was more stable (Figs. 1–5). We inferred that for the chilling-tolerant cultivar SANTIAM, a cellular cold signaling pathway might be highly activated and can produce quick increase in

soluble sugar and proline contents and root TTC-reducing activities under suboptimal temperature stress. The accumulation of soluble sugar, in turn, would quickly suppress the leaf P_n while the Chl content remained more stable, suggesting that there was no link between the leaf P_n and the Chl content. Because of the stronger chilling adaptability of ‘SANTIAM’ itself, the exogenous ABA, Put, or EBR produced a weaker effect in that cultivar.

In conclusion, in tomato production, the foliar application in advance of an appropriate concentration of exogenous ABA (1 mM), Put (0.1

mM), or EBR (0.02 μM) produces a remarkable effect in both the chilling-sensitive cultivar Zhongshu6 and the chilling-tolerant cultivar SANTIAM to reduce the decrease in the P_n and Chl content and further increase the proline content, soluble sugar content, and root TTC-reducing activity in tomato seedlings under suboptimal temperature stress.

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