

Full Length Research Paper

Carbohydrate metabolism in tomato (*Lycopersicon esculentum* Mill.) seedlings and yield and fruit quality as affected by low night temperature and subsequent recovery

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In order to clarify carbohydrate content and enzymes activities involved in sugar metabolism in tomato seedling leaves and yield and fruit quality under low night temperature and subsequent recovery, tomatoes (*Lycopersicon esculentum* Mill. cv. Liaoyuanduoli) were grown in different climatic controlled-environment chambers with day/night temperature regimes of 25/15, 25/9 and 25/6°C for 9 days and then were transferred to 25/15°C chamber for 9 days recovery. The results showed that fructose, glucose and sucrose content under low night temperature and starch content at 9°C were higher than that of the control level; only starch content at 6°C decreased and there was no significantly difference in fructose content between 6 and 9°C. Under low night temperature, sucrose phosphate synthase (SPS) activity in tomato leaves significantly increased. NI activity and SS activity decreased after 3 days treatment and soluble acid invertase (SAI) activity increased after 5 days treated. After 9 days recovery, starch content and SAI activity were difficult to return to the level of the control. After fruits were harvested, the marketable yield and single plant yield decreased in the first cluster fruit of the 6°C treated plants. Enhanced SPS activity and sugar accumulation play important roles in tomato leaves under low night temperature stress.

Key words: Low night temperature, *Lycopersicon esculentum*, sucrose metabolism, enzymes related sugar metabolism, yield and quality.

INTRODUCTION

Most warm-adapted plants of the northern part of China are often subjected to low temperature in winter and early spring, especially at night (Jones et al., 1998; Meng et al., 2008). Low temperature is one of the most critical abiotic stresses for plant growth, productivity and energy

distribution (Xiong et al., 2002; Oufir et al., 2008). Carbohydrate metabolism is an important pathway of plants capturing photosynthetic energy and providing the carbon needed for the production of new tissues (Guy et al., 1992; Sivaci, 2006). Low temperature influences the translocation of photosynthetic products and carbohydrate metabolism and then affects plant growth and development, yield and fruits quality.

Many studies have been conducted on carbohydrate metabolism of crops in response to low temperature. Accumulation of carbohydrates including sucrose, glucose, fructose and starch upon cold stress has been shown in tomato, *Arabidopsis*, cucumber and *Spinacia oleracea* Linn. (Brüggemann et al., 1992; Martindale et al., 1997; Taji et al., 2002; Miao et al., 2007). However, sucrose content did not change in chilled tomato plants

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Abbreviations: SAI, Soluble acid invertase; NI, neutral invertase; SPS, sucrose phosphate synthase; SS, sucrose synthase; FW, fresh weight; DDT, dithiothreitol; EDTA, ethylenediamine tetra-acetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazine) ethanesulfonic acid; HPLC, high performance liquid chromatography.

under 10/8°C (day/night) and a strong increase in the reducing sugars and starch content was observed in treated plants compared with the control (Artuso et al., 2000).

Soluble acid invertase (SAI) and neutral invertase (NI) (EC 3.2.1.26) catalyze the hydrolysis of sucrose to glucose and fructose. The SAI activity in quinoa (*Chenopodium quinoa*) decreased at 5°C-low-temperature (Rosa et al., 2004) however, in the course of adaptation to hypothermia, the acid invertase activity in potato leaves was shown to rise (Sin'kevich et al., 2008). Sucrose synthase (SS) (EC 2.4.1.13) catalyzes a reversible reaction of sucrose with UDP to produce UDP-glucose and fructose. Under cold, drought and high salinity stresses, two sucrose synthase genes of *Arabidopsis* were up-regulated (Seki et al., 2001). It also showed a similar trend in three different sensitive potato cultivars leaves under chilling stress (Oufir et al., 2008). Sucrose phosphate synthase (SPS) (EC 2.3.1.14) plays an important role in regulating sucrose synthesis in source leaves (Kerr et al., 1987). In low temperature treated tobacco leaves, SPS activity increased (Shi et al., 2009), in contrast, the SPS activity in vegetative soybean plants decreased after transferring from 26/22 to 18/14°C (Rufty et al., 1985). The effect on flowering time and the first inflorescence fruit setting under low temperature has been reported. The flowering time of tomato was postponed and the first inflorescence fruit setting declined after 10/5°C at 7 to 8 leaves stage. In addition, deformed fruit rate increased under the same temperature treatment at 2 to 3 and 7 to 8 leaves stages as well (Zhou et al., 2008).

It has been reported that the floral bud differentiation could be impacted when young tomato seedlings were subjected to low temperature stress (Li et al., 1997). Sucrose plays a central role in higher plants as a substrate to sustain the heterotrophic growth of sink tissues (Roitsch, 1999).

The changes of carbohydrate metabolism during floral bud differentiation in tomato were less known. The objective of this study was to examine the response of leaves of tomato seedlings to low night temperature and subsequent recovery: (1) changes of carbohydrate content in tomato seedlings; (2) changes of sucrose-metabolism enzymes; (3) changes of yield and fruit quality. We showed that at low night temperature, both SPS activity and sugar content were all increased; but marketable yield and single plant yield decreased in the first cluster fruit at 6°C. After 9 days recovery, starch content and SAI activity were difficult to return to the control level.

MATERIALS AND METHODS

Plant material and experimental conditions

Tomato (*Lycopersicon esculentum* Mill. cv. Liaoyuanduoli) of

'liaoyuanduoli', a very popular cultivar in north of China, was used for experiment. The tomato plants were cultivated in the experimental field of Shenyang Agricultural University, Shenyang, China, from March to July, 2009. Seeds were directly sown in plug tray-50 holes with peat and vermiculite (2:1) in a greenhouse. Seedlings with two fully expanded true leaves were transferred to three growth chambers. 300 seedlings were placed per chamber. Daytime temperature of all the chambers was 25°C, while night temperature was 15, 9 and 6°C, respectively and was 15°C for the control. Relative humidity was 60% and photosynthetic active radiation (PAR) was 800±50 µmol/m²s, for 12 h photoperiod. After 9 days (d) treatment, the chilled plants were set for recovery at 25/15°C for 9 days in the same chamber. Functional leaves were sampled after 0, 1, 3, 5, 7 and 9 days low night temperature and 3, 6 and 9 days after recovery, between 8:00 am and 9:00 am. Tomato leaves were collected and put into liquid nitrogen immediately and then stored at -80°C until analysis. Throughout the experiment, all the samples were collected randomly on fully expanded leaves with three replicates. At the end of the treatment and recovery, the remaining seedlings in the chambers were transplanted to a greenhouse. After 50 to 60 days, 10 uniformed plants of each treatment were selected and the first and second cluster ripe fruits were harvested.

Carbohydrate extraction and analysis

Carbohydrates were extracted as described by Hu et al. (2009) with some modifications. Leaf samples (2 g fresh weight) were used for carbohydrates extraction. 10 ml of 80% (v/v) ethanol was used to fill the tube, then the tube was placed in a boiling water bath for 1 h, cooled and the residues was extracted two additional times with 5 ml 80% (v/v) ethanol. Supernatants from each extraction were retained, combined and evaporated to dryness in a boiling water bath. Samples were re-dissolved in 1 ml distilled water and filtered through an acetate filter (0.45 µm pore size, Nalgene). Waters 600E high performance liquid chromatograph system was used to analyze the soluble sugar (fructose, glucose and sucrose) according to Lu et al. (2010) with some modifications. Carbohydrate compounds were separated on a Dikma NH₂ column at 35°C, with 2410 differential refraction detector. The mobile phase was 80% acetonitrile and ultra water (80:20). The mobile rate was 1.0 ml/min. Water Millennium software was used to interpret the data. Starch was extracted by incubating the residue left after ethanol extraction with 60% HClO₄, Starch content was determined using perchloric acid hydrolyze method according to Sivaci (2006).

Enzyme extraction and activity assays

Enzyme extracts were prepared essentially as described in Lu et al. (2010). 1 g (FW) tissue of tomato seedling leaves were homogenized in 10 ml of ice-cold homogenizing medium (50 mM HEPES-NaOH, pH 7.5) and centrifuged at 12000 g for 20 min at 4°C. The supernatant were dialyzed for approximately 20 h against 5 mM HEPES-NaOH (pH 7.5).

Soluble acid invertase (SAI), neutral invertase (NI), sucrose phosphate synthase (SPS) and sucrose synthase (SS) activities were measured as described by Lu et al. (2010). The SAI activity was assayed in a final volume of 25 ml, containing 0.2 ml of dialyzed enzymatic extract, 0.8 ml of reaction solution (pH 4.8 or 7.2, 0.1 M Na₂HPO₄, 0.1 M sodium citrate an, 0.1 M sucrose for soluble acid invertase and neutral invertase, respectively. The activities were measured by the quantity of reducing sugars released in the assay media with dinitrosalicylic acid. The reducing sugars were revealed by incubation at 100°C for 5 min and read at 520 nm in a Cary 50UV: VIS spectrophotometer (GBC Scientific Equipment Pty Ltd, Heareus, Germany).

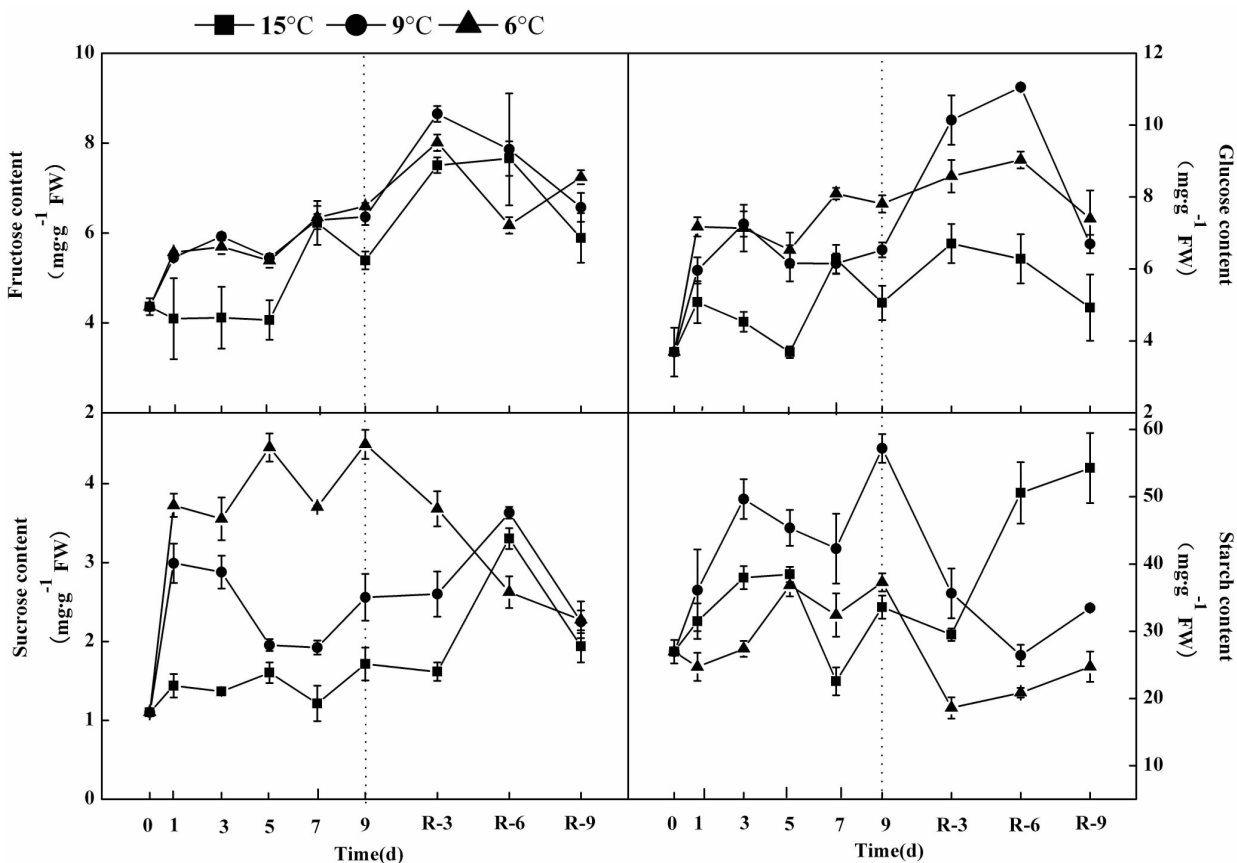


Figure 1: Effects of low night temperature and recovery on the contents of fructose (a), glucose (b), sucrose (c) and starch (d) in tomato seedlings leaves. ■, 15°C; ●, 9°C; ▲, 6°C. The vertical dashed line indicates chilled plants were set for recovery at 25/15°C for nine days in the same chamber. Each data point represents the mean of three biological replicates, and \pm the standard error of mean is shown as a vertical bar.

SS activity was measured by using 0.4 ml reaction solution (0.05 M fructose, 0.82% UDPG, 0.1 M Tris and 10 mM $MgCl_2$), 0.2 ml enzyme, incubation at 37°C for 30 min followed by 1 min at 100°C; 0.6 ml distilled water and 0.1 ml 2 M NaOH was added incubated in a boiling water bath for 10 min and cooled in water to room temperature and then 3.5 ml 30% HCl and 1 ml 0.1% resorcinol were added. Blank controls were obtained by adding the distilled water to the reaction medium containing resorcinol. The reducing sugars were revealed by incubation at 80°C for 10 min and absorbance was read at 480 nm in a Cary 50UV: VIS spectrophotometer (GBC Scientific Equipment Pty Ltd, Heareus, Germany). SPS activity was assayed by measurement of sucrose produced from fructose 6-phosphate plus UDP-glucose. UDPG and F-6-P were purchased from Sigma-Aldrich.

Yield and fruits quality analysis

Marketable productivity (except deformed fruits) rate, fruit setting rate and single fruit weight was investigated, respectively. Total soluble sugar content was measured using anthrone method (Lu et al., 2010). Organic acid content was assayed with 0.1 mol/l NaOH titration (Lu et al., 2010). Vitamin C (VC) content was assayed as described by Yang et al. (2005) with some modifications. 5 g (FW) tissue of tomato fruit were homogenized in 50 ml of homogenizing medium (0.05 mM oxalic acid-0.2 mM EDTA) and centrifuged at

4000 r/min for 15 min. The supernatant was added to 5 ml 0.05 mM oxalic acid-0.2 mM EDTA medium, 0.5 ml metaphosphoric acid-acetic acid and 1 ml H_2SO_4 ($H_2O:H_2SO_4=1:19$) and was shaken. Then 2 ml 5% ammonium molybdate was added and H_2O was added to make it to 25 ml. The extractions were incubation at 30°C for 15 min and absorbance was read at 760 nm in a Cary 50UV: VIS spectrophotometer (GBC Scientific Equipment Pty Ltd, Heareus, Germany).

Statistical analysis

Origin (7.5) and Excel (2003) were used for drawing the figures and tables. Data were subjected to ANOVA with statistical programs DPS (2000).

RESULTS

Carbohydrate contents in tomato seedling leaves

After 1 day low night temperature treatment, the soluble sugar content in tomato leaves was clearly higher than that of the control, especially in the sucrose content ($p < 0.01$) (Figure 1a, b, c). After 3 days treatment, sucrose

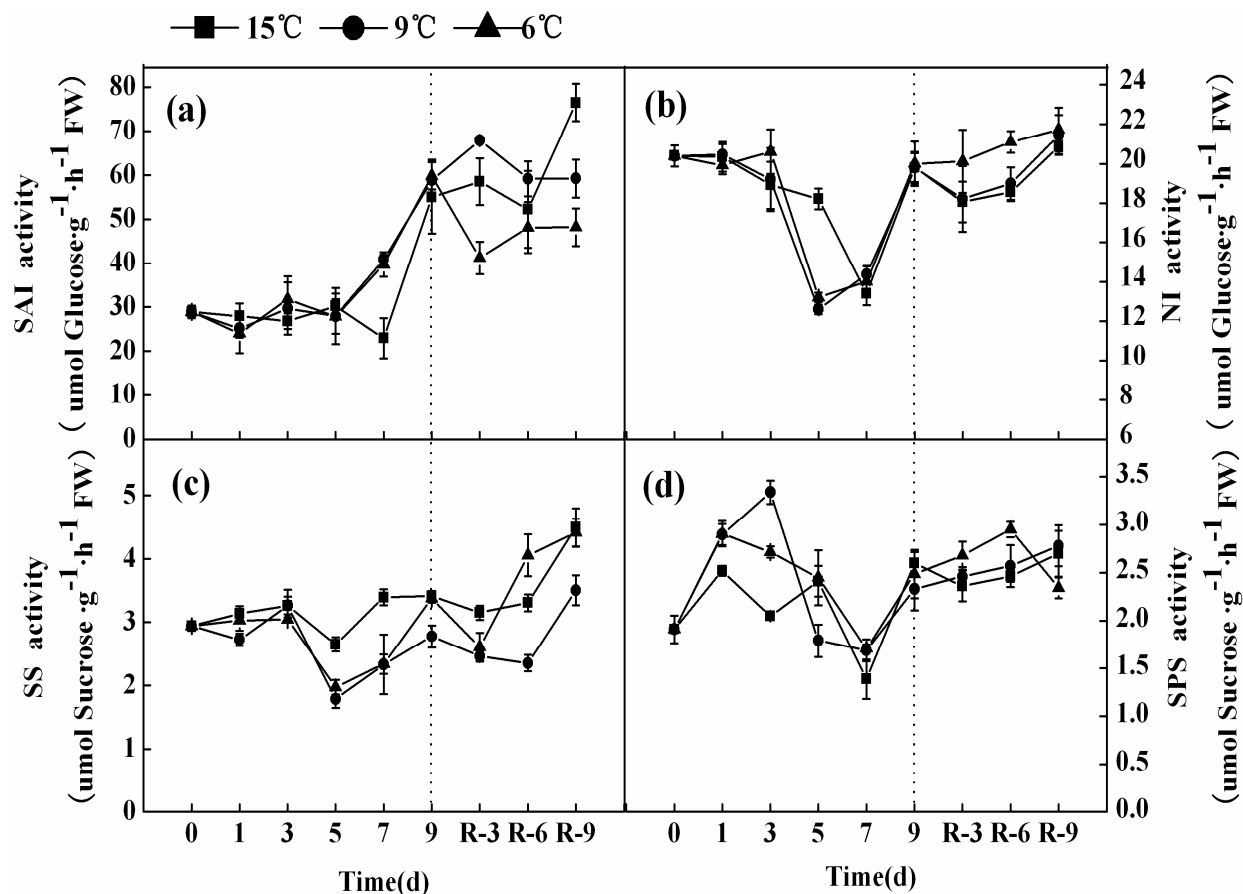


Figure 2. Effects of low night temperature and recovery on the activities of SAI (a), NI (b), SS (c) and SPS (d) in tomato seedlings leaves. ■, 15°C; ●, 9°C; ▲, 6°C. The vertical dashed line indicates that chilled plants were set for recovery at 25/15°C for 9 days in the same chamber. Values are means \pm SD of three different experiments ($n=3$).

content at 6°C was 160.9% greater than that at 15°C ($p < 0.01$) and was 110.99% at 9°C ($p < 0.01$), respectively. Moreover, the highest value at 6°C reached 4.50 mg/ml FW after 9 days of treatment, compared with 2.56 mg/ml at 9°C and 1.71 mg/ml at 15°C (Figure 1c). Following 3 days recovery, sucrose content showed a similar pattern at both 9 and 15°C which increased at first and then decreased after 6 days recovery, whereas sucrose content dropped sharply from the day after the 9 days treatment at 6°C. Soluble sugars except glucose presented no significant difference after 9 days recovery (Figure 1b). Starch content at 9 and 6°C was 87.70 and 43.85% higher than that found at 15°C after 7 days treatment, respectively; but which was lower than that at 15°C after 6 days recovery and then increased until the 9th day of recovery (Figure 1d).

Enzyme activities related to primary carbohydrate metabolism

As shown in Figure 2, no significant difference in SAI, NI and SS activity was observed until the 5th day of

treatment between the treatments (6 and 9°C) and the control seedlings. SAI activity reached 58.56 $\mu\text{mol/g/h}$ after 3 days recovery and then gradually decreased. After 7 days treatment, SAI activity at 9 and 6°C was 78.01% ($p < 0.01$) and 73.02% ($p < 0.01$) higher than that at 15°C, respectively (Figure 2a). Moreover, SAI activity in the control plants reached the highest level (76.49 $\mu\text{mol/g/h}$) after 9 days recovery followed 59.27 $\mu\text{mol/g/h}$ FW at 9°C and 48.17 $\mu\text{mol/g/h}$ FW at 6°C. After 5 days low night temperature treatment, the NI activity of 9°C ($p < 0.01$) and 6°C ($p < 0.01$) treatment declined dramatically compared with that at 15°C, respectively; afterwards, the activity gradually increased up to a similar level between treatments (9 and 6°C) and the control (Figure 2b).

SS activity changed little after 3 days treatment, but declined rapidly subsequently and then increased after 5 days treatment. SS activity in 9 and 6°C plants were lower than that of the control and had no significant difference between 9 and 6°C throughout the experiment; exclusive of the recovery period (Figure 2c). SPS activity at 9°C ($p < 0.01$) and 6°C ($p < 0.01$) after 3 days treatment were over 63.11 and 32.63% compared with that at 15°C,

Table 1. Effects of different night temperature on marketable productivity rate, fruit setting rate, average single fruit weight and single plant yield after low night temperature treatment and subsequent recovery.

Treatment (°C)	Marketable productivity rate (%)		Fruit rate (%)		Fruit weight/per fruit (g)		Yield/per plant (kg)
	1st cluster	2nd cluster	1st cluster	2nd cluster	1st cluster	2nd cluster	
25 /15	86.6 aA	83.5 aA	74.8 aA	86.0 aA	177.93 aA	188.80 aA	2.66 aA
25 /9	70.1 bB	72.3 bAB	74.8 aA	83.36 aA	163.99 bA	191.84 aA	2.50 aA
25 /6	62.9 bB	66.4 bB	78.5 aA	81.00 aA	144.36 cB	188.80 aB	2.36 aB

Values are means \pm SD of three different experiments (n= 10). Capital letters ($p < 0.01$) and lower-case letters ($p < 0.05$) indicate statistical significance.

Table 2. Effects of different night temperature on vitamin C, soluble sugar and organic acid content after low night temperature treatment and subsequent recovery.

Treatment (°C)	Vitamin C (mg/100 g ⁻¹ FW)		Soluble sugar (%)		Organic acid content (%)	
	1st cluster	2nd cluster	1st cluster	2nd cluster	1st cluster	2nd cluster
25 /15	0.276 \pm 0.024 bB	0.312 \pm 0.025 aA	2.94 \pm 0.10 aA	2.28 \pm 0.12 aAB	0.37 \pm 0.03 aA	0.28 \pm 0.02 aA
25 /9	0.355 \pm 0.010 aA	0.316 \pm 0.030 aA	2.64 \pm 0.05 bAB	2.48 \pm 0.17 aA	0.31 \pm 0.05 aA	0.23 \pm 0.03 aA
25 /6	0.297 \pm 0.022 bAB	0.286 \pm 0.016 aA	2.37 \pm 0.14 cB	1.99 \pm 0.13 bB	0.32 \pm 0.05 aA	0.25 \pm 0.04 aA

Values are means \pm SD of three different experiments (n= 3). Capital letters ($p < 0.01$) and lower-case letters ($p < 0.05$) indicate statistical significance.

respectively (Figure 2d). Furthermore, SPS activities declined to the lowest value at the 7th day of treatment in tomato seedlings, then increased again in the recovery period and finally reached equal level at the 9th day recovery.

Yield and fruit quality

As can be noted in Table 1, there were no significant effects on yield and fruit setting rate of tomato under the low night temperature; however, both marketable productivity rate and average single fruit weight of the first cluster decreased significantly at 9°C ($p < 0.05$) and 6°C treatments and in particular at 6°C ($p < 0.01$). No significant influence was observed on the weight of the second cluster of fruits between the treatments and control.

The levels of vitamin C, soluble sugar and organic acid are shown in Table 2. Vitamin C content in ripe tomato fruits exposed to 9 and 6°C were higher than that in the control fruits and a notable difference was seen between the treatments ($p < 0.05$). In contrast to VC content, soluble sugar content reduced extremely under 9 and 6°C ($p < 0.05$). No significant difference was observed in all the plants.

DISCUSSION

Tomato plants begin to flower bud differentiation in the seedling of 2 to 3 true leaves stage and more nutrients are supplied at this stage. As a nutritive substance in source leaves of tomato, sugar content is more important for plants growth and development. Many studies have indicated that starch and sugars accumulated in plants under low temperature stress (Morsy et al., 2007; Shi et al., 2009). Our results showed that the change of sucrose content was consistent with previous studies (Figure 1c).

In this study, no significant difference in SAI, NI and SS activity was observed after 5 days treatment (Figure 2a, b, c), but SPS activity increased (Figure 2d) and it may be as a results of sucrose accumulation. There was also an evidence in the leaves of hardy plant (*Deschampsia Antarctica*), grown in Antarctica (Zúñiga-Feest et al., 2005). However, under saline conditions, increased SPS activity with lower activity of SAI may be responsible for the accumulation of sucrose in plants (Gao et al., 1998). SPS activity could regulate distribution between starch and sucrose content. Higher SPS activity may accelerate the degradation of starch and re-synthesis of sucrose. At 6°C, starch content was converted to sucrose in response to low night temperature (Figure 1d). It was reported that assimilate translocation was inhibited under cold stress

(Paul et al., 1991). Therefore, this is another reason that led to sucrose accumulation in the leaves at 6°C.

Li et al. (1997) indicated that low night temperature in tomato seedling stage increased incidence, average grade and composite index of deformed fruits and the types of deformed fruits, as well; accordingly inferior marketable yields and quality of fruits was observed. Our result was agreed with that mentioned earlier. After 9 days recovery, glucose, starch content, SAI and SS activity could not be recovered to the control level; fruit yields and quality may be influenced by the inhibition of sucrose transportation to apical meristem and roots.

In this experiment, elevated SPS activity and sugar accumulation played an important role in carbohydrate metabolism after low night temperature treated. For further study, we need to verify the specific molecular mechanism for gene expression under low temperature stress.

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