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Full Length Research Paper

Effect of moderately-high temperature stress on photosynthesis and carbohydrate metabolism in tomato (*Lycopersico esculentum* L.) leaves

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Changes in the production and metabolism of photosynthate in tomato leaves were investigated after plants were treated to moderately high temperature stress for 8 h. Plants grown continuously at 25°C served as the controls. Compared to the controls, photosynthetic activity decreased in plants exposed to 35°C for ≥2 h. The net rate of photosynthetic (P_n) and the limitation of stomatal conductance (L_s) decreased, but stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and the rate of transpiration (T_r) increased. These results suggested the decreased in P_n under 35°C stress was caused mainly by non-stomatal restriction. In parallel with the decline in photosynthesis, the activities of sucrose-metabolizing enzymes and the contents of carbohydrate of plants that had been exposed to 35°C also changed. The invertase activities increased first then decreased but the activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS) increased continuously in stressed plants. The contents of fructose and glucose decreased but sucrose content increased compared with controls. It may result from the effects of 35°C on photosynthesis and sucrose-metabolizing enzymes activities that provoked the changes in carbohydrate contents in tomato leaves.

Key words: Photosynthesis, carbohydrate, sucrose-metabolizing enzymes.

INTRODUCTION

Tomato is one of the most common vegetables grown in protected horticulture. Plants synthesize carbohydrate through photosynthesis in mature leaves. Sucrose is the mainly end product of photosynthetic substance and it is also the major format that be transported from leaves to other sink organs by phloem to supply carbon and energy for the growth and development of plants (ap Rees, 1987). Photosynthetic end products are synthesized by several related enzymes in leaves. The ability of plants to synthesize and accumulate sucrose in leaves is mainly determined by the concerted action of sucrose-metabolizing enzymes including sucrose phosphate synthase (SPS; EC 2.3.1.14), sucrose synthase (SS; EC2.4.1.13) and invertases (acid invertase, AI, EC 3.2.1.26; neutral invertase, NI, EC 3.2.1.27) (Miguel et al., 2007). Sucrose synthase and sucrose phosphate synthase in tomato leaves mainly catalyse the synthesis of glucose and fructose to sucrose, while acid invertase and neutral invertase catalyse the hydrolysis of sucrose to glucose and fructose (Qi et al., 2004; Miguel et al., 2007).

Many studies have been carried out on the adverse effects of environmental alterations such as high or low temperature stress, water stress, or salinity on the growth and development of tomato (Camejo et al., 2005; Karim

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et al., 1999, 2000; Miao et al., 2007; Krzysztof and Gabriela, 2007; Li WD et al., 2007). High temperature stress causes severe damage to the photosynthetic apparatus. Photosynthesis is one of the most heat-sensitive processes in plants and it can be completely inhibited by high temperatures before any other symptoms are detected (Berry and Bjorkman, 1980; Camejo et al., 2005). Li WD et al. (2007) thought the photosynthetic apparatus of peach may be damaged when leaf temperature exceeds a critical temperature and the Pn decreased. And high temperature also had adverse effects on the carbohydrate metabolism of plants (Lorenzen and Lafta, 1996; Ebrahim et al., 1998) and so on.

Associated with the reduction of photosynthesis under high temperature, the production of photosynthate inevitably decreased. Efficient carbohydrate metabolism as a source of energy and of carbon skeletons is the basis of survival strategies of plants subjected to environmental influence including high temperature (Krzysztof and Gabriela, 2007). As we all know, high temperature can alter the activity of enzyme, and the changes of sucrose-metabolizing enzymes activities also modify the sucrose metabolism in leaves. But until now there is no consistent conclusion on the effects of heat stress on sucrose metabolism, and different studies have got dissimilar conclusions.

The optimum range of daytime temperatures for the growth and development of tomato plants has been reported to be between 25 - 30°C, with an upper limit of 35°C (Zhang, 2010). Studies on high-temperature conditions have focused on extremely high temperatures (Wu et al., 2001; Daymi et al., 2005; Mao et al., 2005). However, little information is available on the effects of moderately-high temperatures (Zhang et al., 2005, 2011) on photosynthesis and carbohydrate metabolism of tomato leaves.

We choose a day time temperature of 35°C as a moderately-high temperature for tomato, as this has been shown to be detrimental to the growth and development of plants and to fruit set (Zhang et al., 2005). In the present study, tomato plants were exposed to a moderately-high temperature of 35°C for 8 h. The indexes of gas-change, carbohydrate contents and activities of sucrose-metabolizing enzymes in tomato leaves were determined at various times. Our objective was to study the effects of a short exposure to a moderately-high temperature on photosynthetic activity and carbohydrate metabolism in tomato leaves. Based on this physiological comparison with control, the mechanism responsible in tomato leaves after a short period of heat stress was investigated.

MATERIALS AND METHODS

Plant material and treatments

Plants of the tomato cultivar Liaoyuanduoli were used in these experiments. Seedlings were sown in plastic pots filled with a 2:1 (v/v) peat-vermiculite of mixture and grown in a greenhouse at Shenyang Agricultural University, P.R.China. Plants were transferred to 15 cm × 15 cm plastic pots when they had five true leaves and were watered daily and fertilized each week with Hoagland's nutrient solution. Plants were grown in an artificial climate box (TPG-1260, Austrulia), and were grown at (25±1)°C under a 12 h photoperiod with a 12 h dark period at 15±1°C. The irradiance was 360 $\mu molm^{-2}s^{-1}$ and the relative humidity was 70%.

When the first inflorescence opened, groups of plants (n=18) were transferred to another artificial climate box (TPG-1260, Austrulia) and exposed to a 35±1°C temperature stress for 8 h. A group of plants (n= 18) maintained at 25°C served as the controls. Mature leaves of the first node under the 1st inflorescence of plants were measured every 2 h after plants were treated at 35°C. These leaves were analyzed for gas-exchange, carbohydrate contents and enzymes activities.

Gas-exchange measurements

Gas-exchange measurements, including the rate of net photosynthesis (P_n), stomatal conductance (G_s), the intercellular CO_2 concentration (C_i), and the rate of transpiration (T_r), were made using the middle part of the first leaf below the 1st inflorescence using a portable Li-6400 meter (LI-COR Inc., Lincoln, NE, USA) every 2 h after plants were treated by the moderately-high temperature. The light intensity during the measurements was 600 $\mu mol.m^{-2}.s^{-1}$. The limitation of stomatal conductance (L_s) was calculated using the equation: $L_s = 1 - C_i / C_a$ (Farquhar and Sharkey, 1982).

Carbohydrate analysis

Carbohydrates were analyzed essentially as described by Pukacka and Pukacki (1997) and Miao et al. (2007). Briefly, 0.5 g (FW) of leaf tissues was homogenized using 5 mL 80 % (v/v) ethanol. The powdered material was shaken and incubated at 80 °C for 60 min, then centrifuged at 12,000× g for 10 min. The pellets were extracted further two times with ethanol. The extracts were pooled and evaporated to dryness. The residues were redissolved in 1 mL distilled water, and deionized on coupled columns of Dowex 50 × 8 and Dowex 1 × 8. The column was eluted with 20 mL distilled water, and the solution was taken to dryness and resuspended in 1 mL distilled water for a HPLC (Waters 600E, USA) system equipped with refractometric detector and column Sugar Pack 1 (Waters) analysis. The mobile phase was acetonitrile: distilled water = 4:1 (v/v) and the flow rate was 1.0 $mLmin^{-1}$. Sucrose, glucose and fructose were identified by comparison of retention times of known standards (Sigma) and quantified by Waters Millennium system.

Enzyme extraction and assay

Enzymes were extracted according to the methods described by Copeland and Lima (1992) and Gao et al. (1999) with a few modifications.

1 g (FW) of leaf tissues was frozen by liquid N_2 and stored in ultra low temperature freezer. Frozen leaf tissues were ground in a cold mortar and pestle using a suitable amount of polyvinylpolypyrrolidone with 10 mL extraction buffer at pH 7.5 with final concentrations 50 mM HEPES-NaOH, 1 mM EDTA, 10 mM $MgCl_2$, 2.5 mM dithiothreitol (DTT), 10 mM ascorbic acid. After centrifugation at 15,000 × g for 30 min (4°C), the supernatant was gel-filtered through Sephadex G-25 columns equilibrated with extraction buffer.

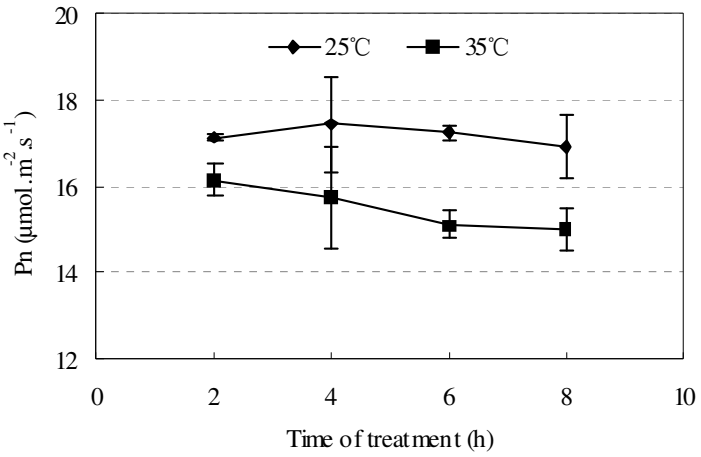

Figure 1. Effect of a moderately-high temperature stress on the net rate of photosynthesis (P_n) in tomato leaves. All values are means ± SE (n=3). 25℃, the control plants that grown continuously at 25℃; 35℃, plants that exposed to 35℃ for 8 h.

Table 1. Effects of moderately-high temperature on stomatal conductance (Gs), intercellular CO2 concentration (Ci), the rate of transpiration (Tr) and stomatal limitation (Ls) in tomato leaves.

Item	Treatment (℃)	Time /h			
		2	4	6	8
G_s /mol.m^{-2}.s^{-1}	25	0.22±0.09	0.36±0.04	0.42±0.00	0.40±0.05
	35	0.55±0.04	0.55±0.08	0.59±0.05	0.56±0.03
C_i /μmol.mol^{-1}	25	257±19	274±3	277±2	310±6
	35	315±12	292±12	296±4	326±4
T_r /mmol.m^{-2}.s^{-1}	25	3.40±0.41	4.36±0.16	6.40±0.18	6.52±0.24
	35	6.56±0.11	6.42±0.72	7.73±0.20	7.42±0.19
L_s	25	0.219	0.315	0.279	0.279
	35	0.158	0.251	0.239	0.229

Sucrose-phosphate synthase (SPS) was assayed according to the methods described by Doehlert and Huber (1983) and Sidney et al. (2006). Sucrose synthase (SS) and invertase (AI and NI) were assayed as described by Miron and Schaffer (1991).

Data analysis

Gas-exchange, carbohydrates contents and enzymes activities results were expressed as the means of three independent measurements in each treatment. Data were examined using analysis of variance and tested for significant difference by the Student–Newman–Keuls test.

RESULTS

Compared with the controls, the net rate of assimilation CO_2 decreased gradually in plants stressed at 35℃ after 2 h to 8 h of exposure. Within 8 h of exposure, the P_n of controls kept the linear condition, while P_n was lowered by 5.8 and 11.2% compared to the control after plants had been treated at 35℃ for 2 h and 8 h, respectively (Figure 1).

Changes of P_n in stressed plants were accompanied by alterations in the values of G_s, C_i, T_r and L_s. The changes of G_s, T_r and C_i were similar, but there was a reverse trend for L_s. G_s, T_r and C_i were significantly higher than the control during the time that plants were exposed to 35℃ (Table 1). On the other hand, an obviously decrease in L_s was shown in stressed plants, and L_s decreased 27.85 % after 2 h of 35℃ compared to controls.

Moderately-high temperature stress altered the activities of sucrose-metabolizing enzymes including AI, NI, SS and SPS in tomato leaves (Figure 2). Compared with the controls, the activities of AI and NI in plants stressed at 35℃ apparently increased within the first 4 h

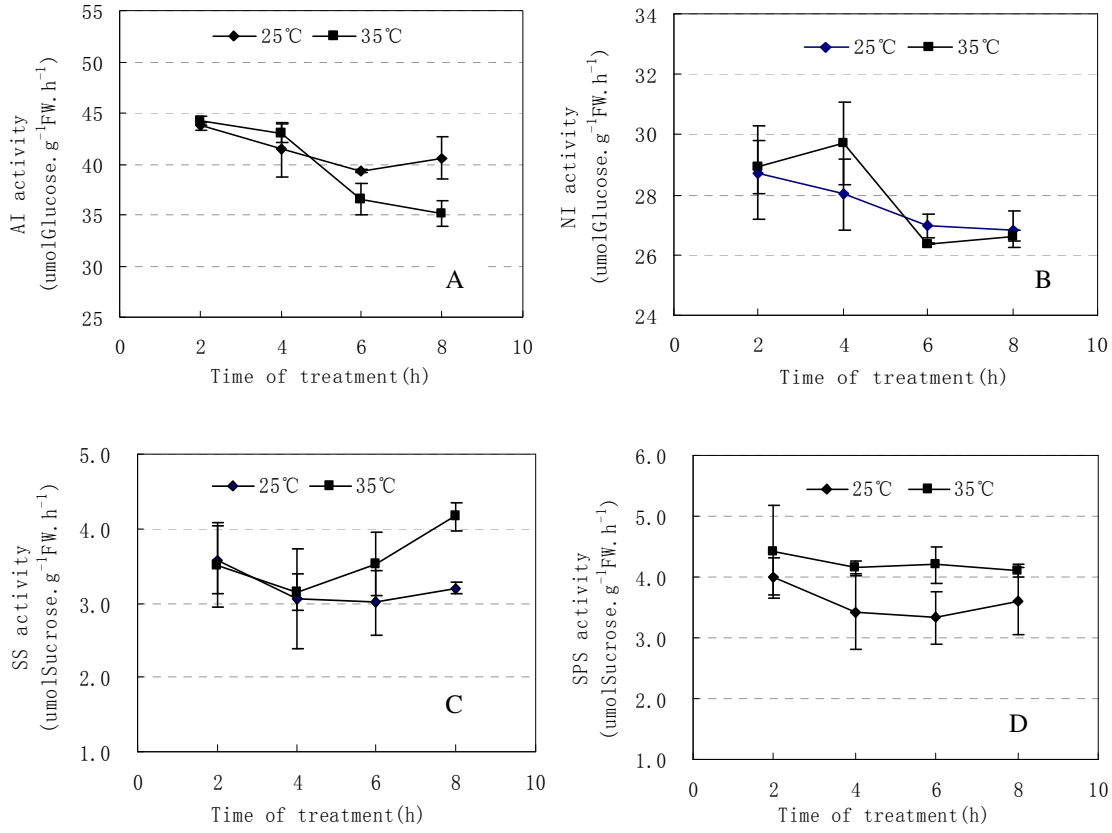

Figure 2. Effect of a moderately-high temperature stress on the activities of acid invertase (AI; Panel A), neutural invertase (NI; Panel B) , sucrose synthase (SS; Panel C) and sucrose phosphate synthase (SPS; Panel D) in tomato leaves. All values are means ± SE (n=3). 25℃, the control plants that grown continuously at 25℃; 35℃, plants that exposed to 35℃ for 8 h.

of stress condition, then decreased even lower than those of controls.

The activities of SS and SPS significantly increase in the stressed plants within 8 h of treatment, and enhanced by 30.4 % and 13.9 % individually after 8 h of 35℃ (Figure 2). According to the statistical analysis, the activities of SS were significantly different from the controls (P< 0.01). The contents of fructose, glucose and sucrose also changed obviously in tomato leaves stressed at 35℃ (Figure 3). A significant (P<0.05 or 0.01) decrease in the contents of fructose and glucose in stressed plants were found during 8 h of treatment. The contents of fructose and glucose were lowered by 23.78 and 20.13%, respectively, but the sucrose content increased by 30.36% (Figure 3) after 8 h of 35℃ stress. The contents of fructose, glucose and sucrose were significantly different from the controls at 8 h of 35℃ (Figure 3).

DISCUSSION

The temperature 35℃ might be the upper limit temperature for tomato plants (Zhang, 2010). P_n decreased within 2 h of 35℃ stress, and continued to fall during 8 h of treatment (Figure 1). Reduction of photosynthesis would directly affect the production and accumulation of photosynthate.

In our case, an increase in G_s, C_i, T_r and a decrease in L_s (Table 1) were observed in stressed plants relative to controls, suggesting the relationship between temperature and G_s,T_r,C_i (Leonardos et al., 1996; Daymi et al., 2005). The increased G_s, C_i, T_r that were observed in stressed plants indicated that the reduction in CO_2 assimilation during the moderately-high temperature stress was mainly not limited by stomatal closure. Our results found that the decrease in P_n in stressed plants was mainly caused by non-stomatal limitation and the alterations on mesophyll capacity, which depend on the activity of Rubisco and on the capacity of photosynthetic electron transport to regenerate Rubisco (Crafts et al., 1997; Feller et al., 1998; Daymi et al., 2005).

The effects of environmental stress on carbohydrate metabolism have been investigated in various species. However, there is no conforming conclusion in different crops. Abbas et al. (1995) concluded that glucose content had no change but sucrose content increased in potato leaves under high temperature treatment. Ebrahim et al.

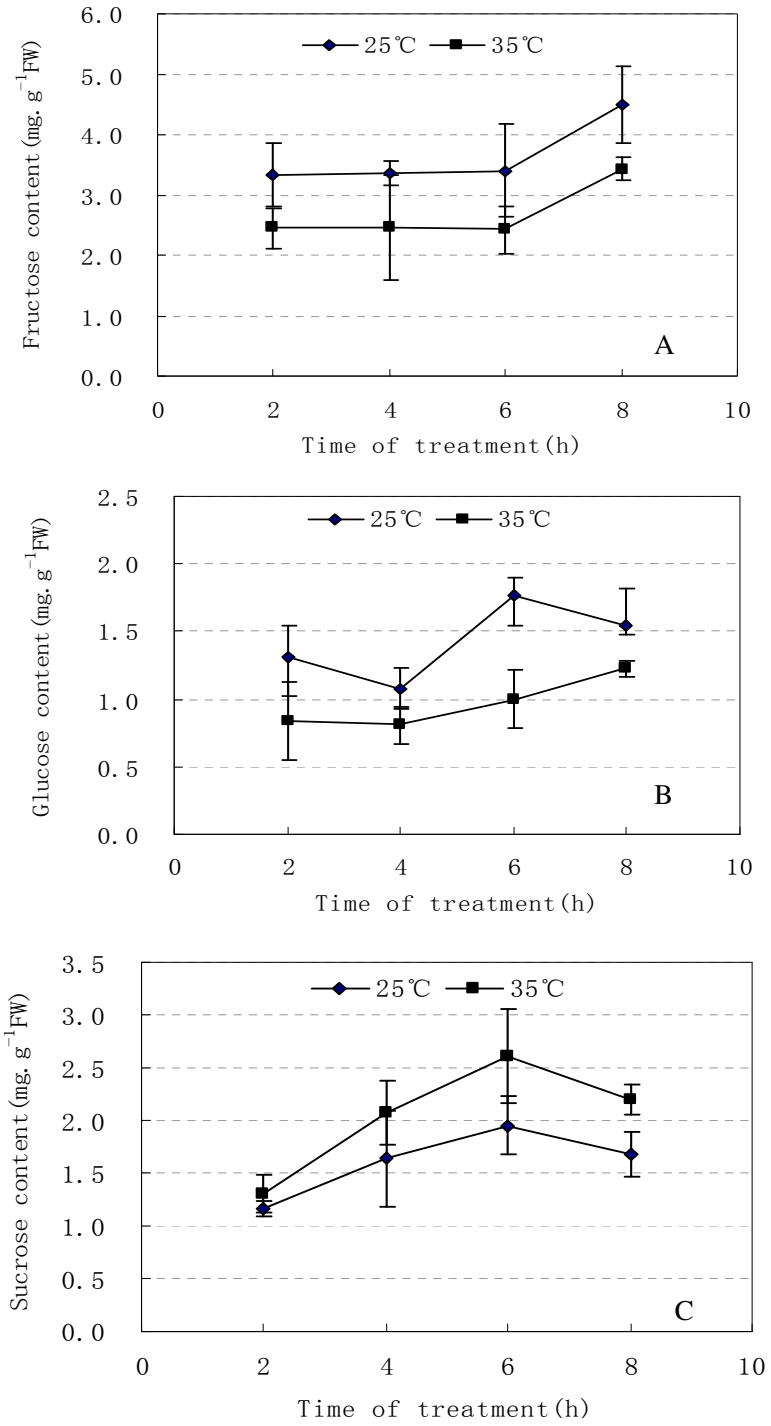

Figure 3. Effect of a moderately-high temperature stress on fructose content (Panel A), glucose content (Panel B) and sucrose content (Panel C) in tomato leaves. All values are means ± SE (n=3). 25℃, the control plants that grown continuously at 25℃; 35℃, plants that exposed to 35℃ for 8 h.

(1998) thought the activities of sucrose synthase, sucrose phosphate synthase and invertase all decreased in sugarcane leaves under high temperature stress accompanied with the sucrose content reduced. In our experiment, fructose content and glucose content in stressed plants leaves decreased but sucrose content increased (Figure 3), and the activities of sucrose synthase, sucrose phosphate synthase both increased during 8 h of 35℃ treatment. Miguel et al. (2007) reported that the increase invertase activity in tomato

leaves exposure to stress was accompanied by greater glucose and fructose formation. But in our case, the increase invertase activity was occurred during the first 4 h of 35℃ treatment then decreased (Figure 2), while both fructose content and glucose content reduced in stressed plants (Figure 3). We suggested it was mainly the decrease photosynthesis (Figure 1) and the increase activities of sucrose phosphate synthase (Figure 2) that inhibited glucose and fructose formation and induced the slightly elevated of sucrose in stressed plants leaves.

Conclusions

In conclusion, exposure to a moderately-high temperature stress of 35℃ for 8 h provoked important changes in photosynthesis activity and carbohydrate metabolism in tomato leaves. The decrease in net rate of CO_2 assimilation was mainly caused by non-stomatal limitation. The decrease in photosynthesis modified carbohydrate contents in stressed plants leaves, on the other hand, the changes of sucrose-metabolizing enzymes activities altered carbohydrate metabolism and changed the contents of fructose, glucose and sucrose in stressed plants.

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