The Interactive Effects of Daytime High Temperature and Humidity on Growth and Endogenous Hormone Concentration of Tomato Seedlings

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Abstract. High temperature and high relative humidity (RH) are one of the most serious agricultural meteorological disasters that limit the production capacity of agricultural facilities. However, little information is available on the precise interaction between these factors on tomato growth. The objectives of this study were to determine the effect of high temperature under different RH levels on tomato growth and endogenous hormones and to determine the optimal RH for tomato seedling growth under high temperature environment. Two high temperature (38/18 °C, 41/18 °C) and three relative humidity (50 ± 5%, 70 ± 5%, 90 ± 5%) orthogonal experiments were conducted, with 28/18 °C, 50 ± 5% (CK) as control. The results showed that the dry matter accumulation of tomato plants under high temperature environment was significantly lower than that of CK. At 38 °C, the dry matter accumulation with 70% relative humidity was not significantly different from that of CK; at 41 °C, dry matter accumulation with 70% and 90% relative air humidity was significantly greater than that of 50%. The concentrations of soluble sugar and free amino acids in all organs in high temperature-treated plants were significantly higher than that in CK. As relative humidity increased, soluble sugar concentrations of each organ decreased, and the free amino acid concentrations increased. Cytokinin (ZT) and indole acetic acid (IAA) concentrations in tomato buds were significantly lower than in CK under high temperature conditions. The lower the RH, the lower the content of ZT and IAA. The gibberellin (GA₃) and abscisic acid (ABA) concentrations were higher than in CK under high temperatures. GA₃ concentrations decreased and ABA concentrations were augmented with increased humidity. The differences of tomato seedling growth indices and apical bud endogenous hormone concentrations between RHs under high temperature conditions were significant. Raising RH to 70% or higher under high temperature conditions could be beneficial to the growth of tomato plants. The results contribute to a better understanding of the interactions between microclimate parameters inside a Venlo-type glass greenhouse environment, in a specific climate condition, and their effects on the growth of tomato.

Tomato (*Lycopersicon esculentum* Mill.) is native to the western plateau of South America, it adapts to the dry and cool climate of the highland near the equator of the origin, so it is not resistant to high temperature and humidity (Harel et al., 2014). The optimal growth temperature for tomato growth is 18.3 to 32.2 °C, and the relative humidity is 50% to 70% (Shamshiri et al., 2018b), above 35 °C, the growth is slow, and at 40 °C the plants stop growing (Yang et al., 2018a).

Temperature is one of the most important environmental factors affecting tomato growth (Shamshiri et al., 2018b). The Intergovernmental Panel on Climate Change (IPCC) report indicated that global average temperatures had increased by about 1 °C since the preindustrial era and projected that global average warming was likely to reach 1.5 °C between 2030 and 2052 at the current rate of anthropogenic greenhouse gas (GHG) emissions (Intergovernmental Panel on Climate

Change, 2018). Thus, global warming will threaten the production of horticultural tomatoes more frequently and severely. In the late spring and early summer in China, the temperature difference between day and night is large. During the day, the temperature in the greenhouse is higher due to the strong light, but it is low at night, so the phenomenon of high day temperature and normal night temperature occurs (Shamshiri et al., 2017b, 2018a, 2020). Research has shown that the temperature and RH outside the greenhouse were between 28 and 33 °C and 70% to 85%, but the internal microclimate reached 68 and 70 °C and RH = 20% to 35%, resulting in air vapor pressure deficit (VPD) between 18 and 21 kPa (Shamshiri et al., 2017a). The excess heat imposed by direct solar radiation causes substantial increase in inside air temperature, 20 to 30 °C higher than the outside (Shamshiri et al., 2017a). At this time, high temperature has become an important limiting factor for horticultural tomatoes.

High temperature stress causes poor pollination of tomato plants and reduces seed setting rate (Cruz-Ortega et al., 2002; Singh et al., 2015), plant dwarfing, aging (Rohn et al., 2002; Wang et al., 2018; Zhang et al., 2005) and fruit quality decline (Mulholland et al., 2003) and also corresponds to a low optimality degree and comfort ratio of the microclimate (Shamshiri et al., 2020). Although high temperature stress has many adverse effects on plants, we can reduce plant transpiration by increasing RH accordingly alleviate damage caused by high temperature stress (Peet et al., 2003; Shamshiri et al., 2018b; Wang et al., 2017, 2018; Xue et al., 2010; Yang et al., 2018a, 2018b; Zhao et al., 2019). However, temperature and humidity exist at the same time, affecting and interacting with each other. Excessive temperature and humidity are likely to cause early plant senescence, shorten the growth period, increase vulnerability to pests and diseases, and affect fruit yield and quality (Li, 2014).

Carbon and nitrogen metabolism are the most basic metabolic pathways of plants, and their intensity and coordination degree have a significant impact on plant growth and development (David, 2002). Soluble sugar is the first product of plant photosynthesis; it can also be used as an osmotic adjustment substance when the plant is under stress, adjust the concentration of cell fluid, and enhance the resistance (Barickman et al., 2016; Zhang et al., 2005). Free amino acid is an intermediate product of nitrogen metabolism. Its content increases rapidly under adverse conditions, and it accumulates in large amounts without destroying the biochemical reaction in the cell (Parida and Das, 2005). The increase is positively correlated with heat resistance (Jin, 2011). Endogenous hormones (ABA, IAA, GA₃, and ZT) are more sensitive to temperature changes and respond quickly to high temperature stress, and thus they are seen as an important signal substance for plants to adapt to adversity (Zou et al., 2019). Zhang et al. (2013) showed

that under high temperature treatment, the content of GA₃ and IAA in rice anthers decreased, leading to the reduction of pollen-promoting substances in pollen and the potential for pollen germination, while the increase of ABA level in anthers increases the adaptability of anthers to high temperature conditions.

Future climate scenarios predict that surface temperature and precipitation will rise in East Asia (Chevuturi et al., 2018). Although many previous studies have reported the effect of high temperature and RH on tomato yield and quality, little information is available on the precise interaction between them on tomato growth. Therefore, this study aimed to 1) evaluate the effects of high temperature and three levels of RH on tomato external morphology, dry matter accumulation, and soluble sugar and free amino acid content and 2) explore the interaction of high temperature and humidity on the content of tomato endogenous hormones (ABA, IAA, GA₃, ZT) and screen out the optimal humidity for tomato growth under high temperature environment. The results contribute to a better understanding of the interactions between microclimate parameters inside a Venlo-type glass greenhouse environment in a specific climate condition, and their effects on the growth of tomato.

Material and Methods

Experimental design. The experiment was carried out from Apr. to Sept. 2017 in a Venlo-type glass greenhouse and in an intelligent artificial climate chamber at Nanjing University of Information Science and Technology. The experiment materials were 'Jinfen5' tomato seedlings (*Lycopersicon esculentum* Mill. 'Jinfen 5'). Seedlings were grown in a greenhouse in early April at a temperature of 22 to 28 °C and RH of 45% to 55%. When the tomato seedlings reached the three-leaf center, a tomato plant with good growth and uniformity was chosen and transplanted into a 28 cm (high) × 34 cm (internal diameter) pot. The contents of soil organic carbon, nitrogen, available phosphorus, and available potassium in the pot were 11,600, 1190, 29.3, and 94.2 mg·kg⁻¹, respectively. Soil pH was 6.8 and soil texture was medium loam. When the seedlings reached 15 cm in height, the potted plants were placed in an artificial climate box (TPG1260; Thermo-line, NSW, Australia). Two high-temperature

treatment conditions were set in the test: 38/18 °C (H₁), 41/18 °C (H₂) (day/night temperature); three air RH treatments were set under each high temperature condition (the error is controlled at 5%): 50% (RH_{50%}), 70% (RH_{70%}), and 90% (RH_{90%}). Conditions of 28/18 °C and 50% relative humidity were used as controls (CK). Six treatments were set up in an orthogonal experiment design, with 28/18 °C, and 50 ± 5% (CK) as control (Table 1). During the experiment, the temperature change in the artificial climate box is shown in Fig. 1, and the RH during the day was set to 50%/70%/90% and at night to 80%. The photoperiod of the artificial climate box was set to 12 h/12 h (day/night), and photosynthetically active radiation was set to 800 μmol·s⁻¹·m⁻² from 0600 to 11:00 HR, 1000 μmol·s⁻¹·m⁻² from 1100 to 1400 HR, 800 μmol·s⁻¹·m⁻² from 1400 to 1800 HR, and 0 μmol·s⁻¹·m⁻² from 1800 to 0600 HR. Water and nutrient conditions in the potted soil were consistent during the experiment.

The temperature, RH, and the solar radiation inside the greenhouse were measured by thermistor thermometer and relative humidity probes (CR3000; Campbell Scientific, Logan, UT) with respective accuracies of ±0.3 °C and ±5% RH. The data were automatically recorded at 10-min intervals. During the test, air temperature ranged from 13.3 to 33.3 °C, RH was between 45% and 75%, and daily solar radiation was 7.3–205.4 W·m⁻².

Measurements and samples were taken before treatment (day 0) and at 3, 6, 9, and 12 d of each treatment. We picked a whole tomato seedling and measured the dry matter weight of each organ separately. Then we selected another tomato seedling in the same treatment, took the main stem top bud to measure the endogenous hormone content, and used the remaining roots, stems, and leaves to measure the soluble sugar and free amino acid content. Because the cells in the top bud grow rapidly and are metabolized vigorously, and it is the most sensitive and easiest to measure under high temperature, we chose the top bud of tomato seedlings to measure the endogenous hormone.

Determination of plant morphological indicators. The heights of the tomato seedlings (from the base to the growth point) were measured using a ruler (centimeters). The diameters of the base of the stems were measured using a vernier caliper (millimeters), and the leaf areas were measured using a LI-3100C (LI-COR Biosciences, Lincoln, NE) leaf area meter (square centimeters).

Dry matter weight. We selected the whole tomato seedlings under CK and high temperature treatment, dug them out of the pots, and cleaned them. The different organs (roots, stems, leaves) were put in an oven at 105 °C for 5 min and then dried to a consistent weight in an 85 °C oven. A one-thousandth electronic balance (ES-220D; Shanghai Xinheng Electronics, Shanghai, China) was used to determine the dry weight of each organ, and three replicates for each treatment were taken.

Table 1. Experimental design table.

Treatments	Temperature (°C)	Humidity (%)
CK	28/18	50 ± 5
H ₁ RH _{50%}	38/18	50 ± 5
H ₁ RH _{70%}	38/18	70 ± 5
H ₁ RH _{90%}	38/18	90 ± 5
H ₂ RH _{50%}	41/18	50 ± 5
H ₂ RH _{70%}	41/18	70 ± 5
H ₂ RH _{90%}	41/18	90 ± 5

Determination of soluble sugars and free amino acids. Soluble sugar concentrations of different organs (root, stem, leaf) were determined using the anthrone-sulfuric acid colorimetric method and free amino acid concentrations were determined using ninhydrin calorimetry (Zheng et al., 2016).

Determination of concentration of endogenous hormones. The concentration of endogenous hormones (IAA, GA₃, ABA, and ZT) was determined based on the method of with minor improvement (Hou et al., 2008). A 1.00-g top bud sample was ground to a fine powder in the presence of liquid nitrogen and then extracted with 20 mL of an 80% v/v methanol solution containing 20 μg/L of antioxidant BHT at 4 °C for 12 h. The extract was centrifuged at 13,000 r/min for 15 min to obtain a supernatant. The residue was added to 8 mL of 80% methanol reextracted twice in the same manner. The whole filtrate was concentrated under reduced pressure at 35 °C to the original volume of 1/3, and then adjusted to a pH of 8.0 with 1 mol/L Na₂HPO₄. Decolorization was carried out three to five times by adding the same volume of petroleum ether. After discarding the ester phase, 0.1 g of polyvinylpyrrolidone (PVPP) was added to absorb the phenolic substance, and the shaker was shaken at room temperature for 30 min. The PVPP was discarded by filtration, and the filtrate was adjusted to a pH of 3 with 2 mol/L citric acid. Equivalent volume of ethyl acetate was extracted three to five times, and the ester phase was combined and concentrated to dryness under reduced pressure at 35 °C. The residue was dissolved in 3 mL of pH 3.5 phosphate buffer and passed through a pre-conditioned C18 SPE column (Sep-Pak; Waters, Milford, MA, 500 mg, 6 mL) to further purify GA₃, IAA, ABA, and ZT. The reconstituted eluate was filtered using a 0.45 μm microfiber filter before high performance liquid chromatography analysis (Shao et al., 2016). A column thermostat was set at 30 °C, and the flow rate was 1.0 L·min⁻¹ throughout the separation.

Data Processing and Analysis. The data reported in all tables and figures are expressed as the average of three repeated observations. Analysis of variance was employed with the high temperature, RH, and treatment days as the three fixed factors and was used to assess variations in the external morphological indicators, dry matter accumulation, soluble sugar content, free amino acid content, and endogenous hormone content. Differences between all treatments were detected using Duncan's multiple

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range test at the 0.05 significance level using the SPSS 19.0 software (SPSS Inc., Chicago, IL). All of the figures were generated using Origin Pro 8.0 software (OriginLab, Northampton, MA).

Results

External form indicator. There were striking differences in the responses of seedling height to RH at different temperatures (Table 2). The plant height under RH_{70%} and RH_{90%} was significantly greater than that of CK, but the difference in stem diameter under all treatments was generally small; the RH_{70%} and RH_{90%} treatment in particular was not significantly different from CK, and the leaf area treated with H₂RH_{90%} was significantly larger than that of CK. However, in the high temperature environment, plant height, stem diameter, and leaf area of tomato seedlings under RH_{50%} were less than CK, which becomes more significant as the treatment time was extended. Under the same high temperature treatment, the plant height and

stem diameter were the highest in RH_{90%}, and the RH_{70%} and RH_{50%} treatments decreased sequentially. At the end of the treatment (12 d), the increase in stem diameter and leaf area under H₁RH_{70%} was greater than that before the treatment (0 d), with an increase of 0.8 mm and 207.73 cm², respectively.

Dry matter accumulation. The interaction of daytime high temperature and RH has different effects on the dry matter accumulation of tomato plants (Fig. 2). Under normal temperature and humidity conditions (CK), tomato seedlings had the largest dry matter, which increased with time. Under 38 °C, the dry matter accumulation at 3 d was not significantly different from that of CK. After 6 d of treatment, the RH_{50%} and RH_{90%} were significantly less than CK. At the end of the treatment, the dry matter accumulation under RH_{70%}, RH_{90%}, and RH_{50%} were 14.755%, 31.554%, and 35.723% lower than CK, respectively. The difference between RH_{70%} and CK was not significant, which was significantly greater than RH_{50%} and RH_{90%}. At

41 °C, the dry matter accumulation after 6 d of treatment was significantly less than CK, and as the processing time increased, the gap became more obvious. After 12 d of treatment, the dry matter accumulation under RH_{90%}, RH_{70%}, and RH_{50%} was 27.294%, 35.232%, and 42.562% lower than that of CK, respectively. In the three humidity treatments, RH_{70%} and RH_{90%} were significantly greater than RH_{50%}. In general, the dry matter accumulation under H₁ was slightly greater than that of H₂.

Soluble sugar concentrations. Under normal temperature and humidity conditions (CK), the content of soluble sugar in the leaves, stems and roots of tomatoes had a small range of variation, floating between 20.3 and 25.0 mg·g⁻¹, while in the high temperature environment, the content increased rapidly. With extension of the treatment time, the content showed a significant increase trend, significantly higher than the control CK (Fig. 3). Under the same high temperature treatment, RH_{50%} treatment (normal humidity) had the highest soluble sugar content, which was significantly higher than RH_{70%} and RH_{90%}. At the same RH (RH_{50%} and RH_{70%}), the soluble sugar content under the high temperature treatment of 41 °C was higher than that of 38 °C. It is worth noting that at 38 °C, the soluble sugar content under RH_{70%} treatment was not significantly different from RH_{90%} except for 12-d treatment of leaves. At 41 °C, the soluble sugar content under RH_{70%} treatment was higher than RH_{90%}, and with the extension of treatment time, this gap became increasingly significant, especially in leaves and roots.

Free amino acid concentrations. Under the conditions of high temperature and different RH conditions, the content of free amino acids in the leaves, stems, and roots of tomatoes was consistent (Fig. 4). The content of free amino acids under high temperature was significantly higher than that of CK, and with the prolongation of the

Fig. 1. Temperature changes in the artificial climate box.

Table 2. Effects of different treatments on the growth of tomato plants.

		Treatment days				
	Treatment	0	3	6	9	12
Plant height (cm)	CK	17.30 a	18.10 bc	20.03 c	21.50 c	22.45 c
	H ₁ RH _{50%}	17.25 a	17.90 c	18.51 c	19.65 cd	20.186 d
	H ₁ RH _{70%}	17.35 a	19.35 a	21.15 b	22.52 b	24.05 a
	H ₁ RH _{90%}	17.30 a	18.84 ab	21.33 b	22.60 b	23.45 b
	H ₂ RH _{50%}	17.20 a	17.80 c	18.45 c	19.25 d	19.71 d
	H ₂ RH _{70%}	17.40 a	18.60 b	21.42 ab	22.75 ab	23.75 bc
	H ₂ RH _{90%}	17.55 a	19.5 a	22.15 a	23.90 a	25.00 a
Stem diameter (mm)	CK	5.63 ab	5.83 ab	6.02 ab	6.13 bc	6.27 a
	H ₁ RH _{50%}	5.60 b	5.78 c	5.90 ab	6.00 c	6.12 bc
	H ₁ RH _{70%}	5.65 ab	6.02 a	6.25 a	6.38 a	6.47 a
	H ₁ RH _{90%}	5.64 a	5.95 ab	6.15 ab	6.30 a	6.37 ab
	H ₂ RH _{50%}	5.60 ab	5.70 c	5.85 b	5.99 c	6.05 c
	H ₂ RH _{70%}	5.64 ab	6.00 a	6.15 ab	6.27 ab	6.38 a
	H ₂ RH _{90%}	5.65 ab	5.95 bc	6.10 ab	6.25 ab	6.41 a
Leaf area (cm ²)	CK	164.74 ab	198.14 b	232.35 b	270.72 d	312.03 b
	H ₁ RH _{50%}	165.26 b	179.78 d	205.09 d	244.46 e	288.48 c
	H ₁ RH _{70%}	166.04 ab	202.56 b	255.11 b	298.31 bc	373.77 a
	H ₁ RH _{90%}	164.78 ab	199.00 bc	240.65 bc	286.28 b	352.23 a
	H ₂ RH _{50%}	167.01 a	188.14 c	212.12 d	250.14 e	287.43 c
	H ₂ RH _{70%}	164.35 b	207.37 ab	243.07 b	289.71 c	344.28 b
	H ₂ RH _{90%}	163.65 ab	219.18 a	272.53 a	311.69 a	362.62 a

Different letters ($P < 0.05$) indicate significant differences within each column according to the Duncan's multiple range test.

Dry matter accumulation (g)

Fig. 2. Effects of different treatments on dry matter accumulation of tomato seedlings. Values with different letters show significant differences at $P < 0.05$ between treatments. Values are means $\pm$ SD, $n = 3$.

treatment time, the content showed a clear increase trend under each humidity condition, especially RH_{90%} and RH_{70%}. Under the same humidity, the content of free amino acids treated at 41 °C was slightly higher than that at 38 °C. At the same high temperature, the content of free amino acids under RH_{90%} treatment was the highest, followed by RH_{70%} treatment, and RH_{50%} treatment was the smallest.

ABA concentrations. Under CK treatment, the content of ABA in tomato seedlings was the lowest, and the content under high temperature environment was significantly higher than CK after 3 d of treatment (Fig. 5A). With the increase of the treatment time, the ABA content increased significantly, and by 12 d of treatment, H₁M and H₂H were 91.49% and 94.68% higher than CK, respectively. At 38 °C, the ABA content

of RH_{70%} was the highest, which was close to the RH_{90%}, whereas at 41 °C, the ABA content of RH_{90%} was the highest, which was significantly higher than that of RH_{70%} and RH_{50%}.

IAA concentrations. Under CK treatment (normal temperature and humidity conditions), the IAA content in the top shoots of tomato seedlings was the highest, and the content under high temperature environment

Fig. 3. Effects of different treatments on soluble sugar content in various organs of tomato. Values with different letters show significant differences at $P < 0.05$ between treatments. Values are means $\pm$ SD, $n = 3$.

was significantly lower than that of CK (Fig. 5B). With the increase of the treatment time, the IAA content decreased significantly, and by 12 d of treatment, H₁L and H₂L were 48.28% and 51.47% lower than CK, respectively. At 38 °C, IAA content was the highest under RH_{90%}, and RH_{50%} was the lowest, whereas at 41 °C, IAA content of

RH_{70%} was the highest, which was 1.23 $\mu\text{g}\cdot\text{g}^{-1}$ higher than RH_{90%}.

GA₃ concentrations. Under CK treatment (normal temperature and humidity conditions), the content of GA₃ in tomato seedlings was the lowest, and the content under high temperature environment was significantly higher than CK after 3 d of treatment

(Fig. 5C). With the increase of the treatment time, the GA₃ content increased significantly, and by 12 d of treatment, H₁L and H₂L were 39.59% and 44.64% higher than CK, respectively. Under the high temperature treatment, GA₃ content at RH_{50%} humidity treatment was the highest, and there was no significant difference between RH_{70%} and RH_{90%}.

ZT concentrations. The ZT content in the top buds of tomato seedlings under normal temperature and humidity (CK) was the highest (Fig. 5D). Under high temperature treatment, the ZT content was significantly lower than that of CK. With the increase of the treatment time, the content decreased significantly. By 12 d of treatment, H₁L and H₂L were 47.03% and 60.37% lower than CK, respectively. Under the same high temperature environment, the ZT level was the highest under RH_{90%} humidity treatment, followed by RH_{70%}, and under the same humidity environment, the ZT level under the treatment of 38 °C was higher than that of 41 °C.

Analysis of variance. After 12 d of treatment, the index values of tomato seedlings were analyzed by variance (Table 3). The results showed that the effects of varied high temperatures on plant height and stem diameter of tomato were not significant, whereas there were significant effects on leaf area and stem soluble sugars ($P < 0.05$). In addition, high temperatures had significant effects on the dry matter accumulation, endogenous hormones in the top buds, and free amino acid and soluble sugar concentrations in leaves and roots ($P < 0.01$). The effects of varied humidity levels on tomato plant growth and hormone concentrations were significant ($P < 0.01$). The influence of temperature and humidity on stem diameter, soluble sugar, and free amino acid concentrations in leaves were not significant, whereas the influence on the dry matter accumulation, free amino acids, and ABA was significant ($P < 0.01$).

Discussion

Previous studies have reported that high temperatures are detrimental to the growth and development of greenhouse tomatoes (Giri et al., 2017; Sang et al., 2016; Van Ploeg and Heuvelink, 2005). In this study, at 38 and 41 °C, the dry matter accumulation of tomato seedlings was significantly lower than that of CK (Fig. 2), indicating that the high temperature environment exerted stress on the growth of tomato plants, and the dry matter accumulation decreased, which is consistent with the results of Yang et al. (2020). In the short term (9 d), under the 38 °C high temperature and RH_{70%}, the plant dry matter accumulation was not significantly different from CK, indicating that the temperature and humidity environment has no obvious stress on plant growth; the high temperature of 41 °C obviously caused heat stress to the plants. RH_{70%} and RH_{90%} were significantly higher than RH_{50%}, indicating that under 41 °C high temperature increasing

Free amino acid content (mg·100g⁻¹)

Fig. 4. Effect of different treatments on free amino acid content in various organs of tomato. Values with different letters show significant differences at $P < 0.05$ between treatments. Values are means $\pm$ SD, $n = 3$.

the RH to above 70% can alleviate high temperature stress. In summary, increasing the RH to 70% in a high-temperature environment can effectively alleviate high-temperature stress. In our study (Table 2), the tomato plant height, stem diameter, and leaf area measured after high temperature

were higher than CK, mainly due to the stress compensation effect of the plant.

Soluble sugar can be used as an osmotic adjustment substance when the plant is under adverse conditions, it can adjust the concentration of cell fluid and enhance the stress resistance of crops (Barickman et al., 2016;

Zhang et al., 2005). Du et al. (2010) showed that the soluble sugar content of thread pepper leaves increases significantly at high temperatures. Cao et al. (2011) found that increasing air humidity at high temperatures can significantly reduce the soluble sugar content in the roots and leaves of seedlings. The results of this study showed that the changes of soluble sugar content in various organs of tomato seedlings treated with three kinds of air humidity at high temperature were basically the same, all of which showed that the soluble sugar content of leaves, stems and roots of tomato seedlings was higher than CK under each treatment, this is consistent with the results obtained by previous studies (Huang et al., 2010) that increased the content of soluble sugar in plants without humidification at high temperature. Under high temperature, when the RH was increased, its content decreased significantly ($P < 0.05$), and the higher the treatment temperature, the higher the soluble sugar content. Wang et al. (2006) found that at 28 to 44 °C, with the increase of high temperature stress, the soluble sugar content of tomato increased, but if the variety has strong heat resistance, the increase was small, indicating that the degree of damage is negligible. Under the high temperature environment, with the extension of the treatment time, the soluble sugar content in tomato organs under RH_{70%} and RH_{90%} increased slowly, whereas the soluble sugar content under RH_{50%} treatment increased sharply (Fig. 3), indicating that 50% of the RH environment during the high temperature treatment exerts severe stress on tomato plants, and the higher RH can effectively alleviate the high temperature stress. It can be seen that increasing the air humidity can reduce the soluble sugar content of tomatoes so that they are more used for growth rather than as a stress osmotic adjustment substance.

Free amino acids are also a key osmotic substance in plants; their concentration is low under normal conditions, but the concentration in adverse conditions will increase rapidly, and the amplitude is positively related to heat resistance (Karami Mehrian et al., 2015; Parida and Das, 2005). Our research showed that under high temperature conditions, the content of free amino acids in leaves, stems, and roots of tomato seedlings increased significantly, and the this increased further with temperature. The content was highest under RH_{90%} and lowest under RH_{50%}, indicating that under high temperature treatment, free amino acids in tomato seedlings act as osmotic adjustment substances and participate in resisting adversity. The higher the temperature, the greater the content of free amino acids in the plant, which means the more severe the stress on the plant. Under RH_{90%}, the content of free amino acids was significantly higher than that of RH_{50%} and RH_{70%}, indicating that under this humidity treatment, a large number of amino acids were produced in tomato seedlings to resist stress, which means the stress resistance of plants under this humidity was significantly improved.

The content of free amino acids in the roots of tomato seedlings was obviously higher than that in leaves and stems, mainly because the roots are the main nitrogen absorbing organs, which are in direct contact with the soil and are important nitrogen reservoirs for plants, so the content of free amino acids is higher than that of the ground growth parts. The free amino acids content in the root system increased more than that of leaves and stems with the extension of treatment time, indicating that under high temperature stress, the root system absorbed or produced more free amino acids to resist adversity.

As a stress hormone, ABA is an important signal substance for plants to respond to stress (Leung and Giraudat, 1998; Nambara and Marion-Poll, 2005), and it will regulate plants to respond to many multiple environmental stresses. ABA is a signal substance that responds to heat stress (Larkindale, 2002). By activating the synthesis of ABA, inhibiting its degradation, or unlinking the conjugated form, it increases the ABA content in stressed plants (Boursiac et al., 2013; Song et al., 2014). Adversity stress can induce a large amount of ABA accumulation in plants by adjusting the metabolic balance,

improving the plant's adaptability to stress and resistance to stress. Mao et al. (2005) found that under high temperature stress, the ABA content of heat-tolerant tomato varieties increased more than that of heat-sensitive varieties, and the ABA content in floral organs of heat-tolerant varieties is higher than that of heat-sensitive varieties regardless of whether they are at normal or high temperature. In this study, the ABA concentration in the top buds of tomato plants also showed an increasing trend at high temperatures, and the ABA concentration increased significantly when the humidity increased, indicating that increasing humidity can enhance the heat resistance of the plants. Under high temperature conditions, the ABA content was the lowest under RH_{50%}. It may be that the self-regulating ability of the plants under this temperature and humidity condition has been damaged, and it has already caused extremely severe stress. The increase in the ABA content of tomato plants not only protects against light inhibition and induces or increases the expression of resistance genes to enhance the plant's ability to adapt to high temperatures (Alamillo and Bartels, 2001), it can also increase the activity of

antioxidant enzymes such as SOD, POD, and CAT, and reduce the damage to the cell membrane system caused by oxygen free radicals produced by stress (Li et al., 2006).

IAA is involved in regulating almost all growth and development processes of plants and has the functions of regulating the growth rate of stems, inhibiting lateral buds, and promoting rooting (Celis-Arámbaro et al., 2011; Sunita et al., 2011). At present, there are few studies on the effects of IAA under high temperature stress; however, the current research results indicate that high temperature stress changes the response of IAA by changing its biosynthesis (Li et al., 2012). When the plant is subjected to high temperature stress, growth and development are ended or cell death, which causes the content of IAA to decrease. Duan (2010) found that under high temperature stress, the content of IAA in heat-tolerant varieties was higher than that of heat-sensitive varieties. It can be said that the IAA content is positively correlated with its heat resistance (Shen et al., 2006). In this study, the concentration of IAA in the top bud decreased at high temperature, consistent with the conclusions mentioned earlier. Under high temperature environment, IAA content under RH_{50%} was significantly less than that of RH_{70%} and RH_{90%}. This indicates that when the RH is 50% under high temperature conditions, the self-regulation ability of tomato plants is destroyed, which causes extremely severe stress on tomatoes. Increasing the RH to above 70% can effectively alleviate high-temperature stress.

GA₃ can promote plant flower bud differentiation, induce flowering, and promote fruit ripening (Sharmshiri and Singh, 2009). It is mostly found in vigorous plant organs and is an important hormone for regulating plant height (Wolbang et al., 2004). GA₃ can affect the stress resistance of plants by regulating the content of free water and bound water in plants. Studies have found that plants with low GA₃ content have better tolerance (Sarkar et al., 2004). In this study, the GA₃ content of tomato plants increased under high temperature, and the content under RH_{70%} and RH_{90%} was significantly less than that of RH_{50%}, indicating that under high temperature conditions, RH above 70% has better heat resistance. However, some studies have found that GA₃ content shows a downward trend at high temperatures (Teng et al., 2010; Wang and Xiong, 2016). This demonstrates that the dynamic change of plant GA₃ under high temperature stress is closely related to the nature of the plant itself. Therefore, the role of GA₃ in tomato plants under high temperature stress remains to be further explored.

endogenous hormone content ($\mu\text{g}\cdot\text{g}^{-1}$)

Fig. 5. Effect of different treatments on endogenous hormone content in top bud of tomato. Values with different letters show significant differences at $P < 0.05$ between treatments. Values are means $\pm$ SD, $n = 3$.

Table 3. Analysis of variance table.

	Plant ht	Stem diam	Leaf area	ZT	GA ₃	IAA	ABA	Leaf SS content	Stem SS content	Root SS content	Leaf free AA content	Stem free AA content	Root free AA content	Dry matter accumulation
Temperature	NS	NS	*	**	**	**	**	**	*	**	**	**	**	**
Humidity	**	**	**	**	**	**	**	**	**	**	**	**	**	**
TxH	**	NS	**	**	**	**	*	NS	**	**	NS	*	**	**

AA = amino acid; ABA = abscisic acid; GA₃ = gibberellin; IAA = indole acetic acid; SS = soluble sugar; ZT = cytokinin.

NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01 , respectively.

ZT is an important phytohormone, mainly produced in roots, involved in various physiological processes such as plant cell division, chlorophyll synthesis, leaf and shoot formation, and leaf senescence (Binns, 1994). ZT is involved in the development of chloroplast and thylakoid lamina, thereby delaying the senescence of leaves under stress (Hare et al., 1997). It can also increase the activity of membrane protective enzymes, such as SOD; directly or indirectly scavenge free radicals; and reduce the ratio of lipid peroxidation and membrane fatty acid composition to protect the integrity of cell membrane structure and function (Wang, 2000). In this study, under high temperature, the ZT content of tomato seedling top buds showed a downward trend, and the ZT content under RH_{50%} was significantly lower than RH_{70%} and RH_{90%}, indicating that 50% RH at high temperature can accelerate the senescence of tomatoes. Increasing the RH can increase the ZT content of tomato plants, help to reduce the content of free water, delay the yellowing of leaves and maintain the stability of cell membranes, thereby improving the high temperature resistance of tomatoes.

Conclusion

This study investigated the effects of the interactions between daily maximum temperatures of 38 and 41 °C and various relative humidity levels on the growth and endogenous hormone concentrations in tomato seedlings. It was observed that increasing humidity to ≈70% in a high temperature environment was beneficial to the growth of tomato. Generally, in humid environments with ≥70% of relative humidity, plant growth at 38 °C/18 °C was slightly better than that at 41 °C/18 °C. This suggests that increasing humidity could enhance heat resistance in tomato plants within a certain temperature range. The results contribute to a better understanding of the interactions between microclimate parameters inside a Venlo-type glass greenhouse environment, in a specific climate condition, and their effects on the growth of tomato. Now, because Venlo-type greenhouses are becoming popular for urban farming as rooftop greenhouses (Shamshiri et al., 2018b), this study is a step toward engineering such greenhouses under the specific climate conditions. These results can be directly incorporated in updating databases of decision support systems and adaptive analysis frameworks (Shamshiri et al., 2017c) that are interfaced with yield prediction models for dynamic assessment of microclimate and control (Shamshiri et al., 2017d, 2020). In addition, this study indirectly contributes to reducing risks of greenhouse tomato production and increasing profit.

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