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# High humidity alleviates photosynthetic inhibition and oxidative damage of tomato seedlings under heat stress

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## Abstract

This study investigated the effects of high humidity on the growth and photosynthetic and physiology traits of tomato plants under high temperature stress (HT). The results showed that high humidity effectively alleviated the limitation of HT on plant growth and increased the root-to-shoot ratio. In addition, high humidity also increased the chlorophyll content, net photosynthetic rate, and maximum photochemical quantum yield of PSII in tomato seedlings under HT stress, but declined the stomatal limitation value. Moreover, JIP-test showed that increasing air humidity improved the quantum yields and efficiencies of HT-stressed tomato plants and increased the size of functional antenna, while reduced the activity of a portion of reaction centers. Besides, high humidity increased the activity of antioxidant enzymes, but decreased the content of malondialdehyde and hydrogen peroxide in HT-stressed tomato plants. Therefore, high humidity improved the growth and alleviated photoinhibition and oxidative stress of tomato seedlings under heat stress.

*Additional key words*: chlorophyll fluorescence; *Lycopersicon esculentum* Mill.; photosynthesis.

## Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a widely grown vegetable crop in the greenhouse and its optimum growth temperature range is between 18 and 32°C (Berry and Björkman 1980, van der Ploeg and Heuvelink 2005). In summer and autumn greenhouses, the temperature tends to exceed this range, reaching above 35°C, even above 40°C; due to this fact the greenhouse infrastructure is lagging behind and has no precise temperature control systems in plastic greenhouses in northern China (Saeed *et al*. 2007, Pan *et al*. 2018). Therefore, high temperature (HT) is considered to be one of the main environmental limiting factors that negatively affect the growth and productivity of tomato plants, especially in summer and autumn seasons.

HT stress generally means that the air temperature exceeds a certain critical threshold, which causes irreversible damage to the growth and development of plants after a period of time (Yusuf *et al*. 2010, Das *et al*. 2014). HT has an adverse effect on the growth parameters of tomato plants, such as plant height, stem diameter, leaf number, and vegetative biomass. Cruz-Ortega *et al*. (2002) showed that HT stress reduces the leaf emergence rate and

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*Abbreviations*: ABS/RC – apparent antenna size of active PSII RC; AQE – apparent quantum efficiency; CAT – catalase; $C_i$ – intercellular $CO_2$ concentration; $DI_0$/RC – effective dissipation of energy per active RC; $ET_0$/RC – electron transport per active reaction center; $F_0$ – minimal fluorescence yield of the dark-adapted state; $F_m$ – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; $F_v/F_m$ – maximal quantum yield of PSII photochemistry; $g_s$ – stomatal conductance; LCP – light-compensation point; $L_s$ – stomatal limitation value; LSP – light-saturation point; MDA – malondialdehyde; $PI_{abs}$ – performance index on absorption basis; $PI_{total}$ – total performance index; $P_N$ – net photosynthetic rate; $P_{Nmax}$ – maximum photosynthetic rate; POD – peroxidase; RC – reaction center; $RE_0$/RC – electron flux reducing end electron acceptors at the PSI acceptor side per reaction center; SOD – superoxide dismutase; $TR_0$/RC – trapped energy flux per reaction center; $\delta_{R0}$ – efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side; $\varphi_{E0}$ – quantum yield for electron transport; $\varphi_{P0}$ – maximum quantum yield for primary photochemistry; $\varphi_{R0}$ – quantum yield for reduction of end electron acceptors at the PSI acceptor side.
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leaf area index, and also decreases the plant height and stem diameter in tomato plants. Peet *et al*. (1997) demonstrated that under HT stress, the carbon and nitrogen metabolism of tomato plants is imbalanced, and the elongation of petiole and stem consumes too much nutrients, resulting in a decrease in dry matter storage, which in turn affects the quality and yield of tomato. In addition to growth and biomass reduction, HT stress also induces the decline in photosynthesis of tomato plants before other physiological symptoms appear (Murkowski 2001). It is well known that HT stress causes the local electrical responses (LERs) (Sukhov *et al*. 2017) and the propagation of electrical signals (Fromm and Lautner 2007, Sukhov 2016), which, in turn, can affect photosynthesis (Sukhova *et al*. 2018 ), transpiration (Sukhov *et al*. 2015), respiration (Lautner *et al*. 2014), and ATP content (Surova *et al*. 2016) in leaves. Additionally, important photosynthetic processes, such as Chl synthesis, electron transport, and carbon dioxide assimilation process have been found very susceptible to HT stress in tomato plants (Camejo *et al*. 2005, Wu and Kubota 2008). For instance, tomato plants exposed to HT stress have a lower Chl content, cytochrome (Cyt) $b_6/f$, and plastocyanin (PC), as well as lower ATP synthase and Rubisco. Besides, HT stress often leads to excessive production of reactive oxygen species (ROS) and increases membrane lipid peroxidation in plants (Choudhury *et al*. 2013). ROS include molecules such as $H_2O_2$, ions such as $O_2^-$, or radicals such as $OH^•$, which can cause oxidative damage by causing disruption of membrane lipids, denaturation of proteins or destruction of DNA chain reactions, ultimately leading to cell death (Apel and Hirt 2004, Gill and Tuteja 2010). In order to control the effects of excessive ROS production *in vitro* and *in vivo*, plant cells activate photoprotection mechanisms. Antioxidant enzymes, such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), can effectively prevent ROS formation, scavenge the unavoidably formed ROS pool, and reduce the degree of membrane lipid peroxidation, thereby protecting plants from oxidative injury (Pukacka and Ratajczak 2005, Cruz de Carvalho 2008).

Chl fluorescence methods are considered to be one of the noninvasive, rapid, and easy ways for assessing the heat tolerance of plants (Pan *et al*. 2018). Referring to Chl fluorescence parameters, previous studies focused mainly on the maximal quantum yield of PSII photochemistry ($F_v/F_m$) (Li *et al*. 2015) because it represents the photon energy absorbed by PSII for photochemical processes (Zaharieva and Dau 2019). However, the impairment of $Q_A$ energy flow caused by the reduced carboxylation or the decreased pool size of receptors cannot be reflected by $F_v/F_m$. Chl *a* fluorescence rise kinetics, from the initial 'O' to the 'P' (the peak) level, called OJIP kinetics, has become another way to evaluate the photosynthetic activity (Strasser *et al*. 1995). This analysis (Strasser *et al*. 2004, Tsimilli-Michael and Strasser 2008), referred as the 'JIP-test', allows us to obtain information on the structural and functional parameters that quantify the performance/activity of the photosynthetic apparatus (for explanation, *see* 'Materials and methods' section, where the formulae and glossary of terms used by the JIP-test are presented). More generally, the JIP-test has been widely used for evaluation of the effect of different types of abiotic stress, such as high or low temperature, drought, and heavy metals (Yusuf *et al*. 2010). Therefore, Chl fluorescence induction curves become a popular technique for detecting physiological state of plants under abiotic stress conditions.

Although high temperature has many adverse effects on plants, plants can reduce their leaf temperature through vigorous transpiration. The proper relative humidity level (RH) can reduce leaf temperature and maintain stomatal conductance, maintaining the high yield *via* higher photosynthetic efficiency at high temperature (Han *et al*. 2019). Therefore, increasing air humidity is a common defensive measure to alleviate HT stress in facility production. Huang *et al*. (2010) showed that increasing the air humidity under HT conditions can increase the plant height, stem diameter, and leaf area growth of tomato plants, and significantly increase the yield and quality of greenhouse tomatoes in summer. Additionally, Barker (1990) pointed out that higher air humidity in the greenhouse for no more than 24 h significantly increased the stomatal conductance of tomato leaves, thereby improving the heat resistance of tomatoes. Furthermore, Wang *et al*. (2017) have shown that increasing the air humidity to 70% at 38°C significantly reduces the soluble sugar content in the roots and leaves of tomato seedlings, which is beneficial to the growth of tomato seedlings. However, there is little information to elucidate the effects of high humidity on photosynthetic characteristics and reactive oxygen metabolism in tomato plants under HT stress. In this study, we performed experiments (*1*) to compare the growth, biomass partitioning, Chl content, photosynthetic parameters, and antioxidant enzyme activities of tomato seedlings under different temperature regimes, and (*2*) to assess the effect of high humidity for mitigating the negative effect of HT stress, hoping to provide a simple, environmentally friendly, and effective method to alleviate the adverse effects of HT stress in the greenhouse.

## Materials and methods

**Plant material and experimental treatments**: Venlo-type glasshouse of the Agricultural Meteorological Experiment Station, Nanjing University of Information Science and Technology (NUIST), Jiangsu province, China, was used to conduct the experiments. Tomato seedlings (*Lycopersicon esculentum* Mill., cv. 'JinGuan 5') with two leaves were grown in plastic pots filled with a peat:vermiculite mixture of 1:1 (v:v). Plants were drip-irrigated twice a day to prevent water stress with tap water and once every three days with water containing N fertilizer.

Uniform-sized tomato seedlings with eight true leaves were transferred to an artificial climate chamber (*A1000*, *Conviron*, Canada) with a temperature of 25/15°C (day/night), photoperiod of 12/12 h (day/night), relative humidity of 50%, and illumination intensity of 800 μmol(photon) $m^{-2}$ $s^{-1}$ for 3 d to adapt to the environment. Then, all seedlings were divided into four groups with each group containing 40 pots. Two groups were subjected to a temperature of 25/15°C (day/night) with relative humidity of 50 and

70%, respectively [25/15°C + 50% ($T_1H_{50}$, Control) and 25/15°C + 70% ($T_1H_{70}$)]. The other two groups were subjected to HT treatment at 38/28°C, and the relative humidity was set to 50 and 70%, respectively [38/28°C + 50% ($T_2H_{50}$) and 38/28°C + 70% ($T_2H_{70}$)]. The environmental conditions of each artificial climate chamber were as follows: photoperiod of 12/12 h (day/night) and illumination intensity of 800 μmol(photon) $m^{-2}$ $s^{-1}$. Measurements were taken on 3, 6, 9, and 12 d, respectively, and conducted on the fourth to sixth fully mature leaves.

**Morphological index and biomass allocation**: Plant height, stem diameter, and leaf area were measured by ruler, electronic vernier caliper, and leaf area meter (*Model LI-3100A*, *Li-Cor*, *Inc*., Lincoln, NE), respectively.

Tomato seedlings were harvested and partitioned into roots, leaves, and stems. Then plant fractions were oven-dried at 85°C for 48 h and weighed using an electronic balance (*ES-220D*, China).

**Photosynthetic pigment content**: For Chl pigments measurement, leaf samples (0.5 g) were ground in a mortar with 2 ml of 80% chilled acetone, centrifuged, and the supernatant was diluted to a final volume of 10 ml with 80% acetone. Samples were extracted in darkness for 48 h at room temperature until pigments in the leaves were completely extracted. Absorbance values were measured at 645 and 663 nm by using the ultraviolet spectrophotometer (*Cary 50 Conc UV-VIS*, *Varian*, Victoria, Australia). The concentrations of Chl *a* and Chl *b* were determined according to the following formulas (Lu *et al*. 2019): Chl *a* [mg $g^{-1}$] = (12.72 $A_{663}$ – 2.59 $A_{645}$) × V/(1,000 W), Chl *b* [mg $g^{-1}$] = (22.88 $A_{645}$ – 4.67 $A_{663}$) × V/(1,000 W).

**Chl fluorescence and gas-exchange parameters**: Chl fluorescence parameters were measured using a *Handy PEA* fluorimeter (*Hansatech Instruments Ltd*., King's Lynn, UK). Minimal fluorescence ($F_0$), maximum fluorescence ($F_m$), and maximum quantum yield of PSII ($F_v/F_m$) were recorded automatically by exposing the leaves to actinic light of 3,500 μmol(photon) $m^{-2}$ $s^{-1}$ after 25 min of dark adaptation (Maxwell and Johnson 2000).

Then, the same leaves were used to measure gas-exchange parameters using the portable photosynthetic system, *LI-6400* (*LI-COR Inc*., USA), as described previously by Su *et al*. (2017). Net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), and intercellular $CO_2$ concentration ($C_i$) were automatically recorded by the *LI-6400* program (Xu *et al*. 2019). The stomatal limit value ($L_s$) was calculated by the equation $L_s = 1 - C_i/C_a$, where $C_a$ represents the atmospheric $CO_2$ concentration. During the measurement, the light intensity, temperature, and $CO_2$ concentration in the chamber were maintained at 800 μmol(photon) $m^{-2}$ $s^{-1}$, 25°C, and 390 μmol $mol^{-1}$, respectively. The relative humidity used during measurements by *Li-6400* was the same as the humidity of each group to be treated. Each assayed leaf was light-induced with a light intensity of 800 μmol(photon) $m^{-2}$ $s^{-1}$ for 10 min before the measurement.

**Light-response curve, OJIP curve, and JIP-test**: The photosynthetic response curve was determined by using the portable photosynthetic system *LI-6400* (*LI-COR Inc*., USA). During the measurement, the temperature of the leaf chamber was set at 25°C, the concentration of $CO_2$ was maintained at 390 μmol $mol^{-1}$, and the photosynthetic active radiation (PAR) was 1,200; 1,000; 800, 400, 200, 150, 80, 50, 30, and 0 μmol(photon) $m^{-2}$ $s^{-1}$, respectively. The relative humidity used during measurements in leaf chamber was the same as the humidity of each group to be treated.

In order to obtain accurately the parameters on the light-response curve, such as the light-compensation point (LCP), the light-saturation point (LSP), maximum net photosynthetic rate ($P_{Nmax}$), and the apparent quantum yield (AQE), it was necessary to fit the light-response curve. Light-response curves were simulated by a nonorthogonal hyperbolic model using *SPSS 17.0* (*SPSS Inc*., Chicago, IL, USA) as described previously by Farquhar *et al*. (2001).

$$P_N(I) = \frac{\alpha I + P_{max} - \left[(\alpha I + P_{max})^2 - 4\theta\alpha I P_{max}\right]^{0.5}}{2\theta} - R_d$$

where, $P_N$ ($I$) is the net photosynthetic rate, $I$ is the light intensity, θ is the curvature of the curve, and α is the slope of the plant photosynthesis *vs*. light-response curve at $I = 0$, also called initial quantum efficiency, $P_{max}$ is the maximum net photosynthetic rate, and $R_d$ is the dark respiration rate.

The OJIP fluorescence transients (10 μs to 1 s) were monitored by *Handy PEA* fluorimeter as described in detail by Yusuf *et al.* (2010). Tomato leaves were dark-acclimated for 20 min and then received continuous illumination [3,500 μmol(photon) $m^{-2}$ $s^{-1}$] to measure fluorescence transients. The OJIP fluorescence transients were assayed using the JIP-test method (Zeliou *et al*. 2009). The JIP-test is a multiparametric analysis reflecting the structure and function of photosynthetic apparatus. For the detailed meaning of the JIP-test parameters involved in this article were calculated (Yusuf *et al*. 2010): (*1*) performance indexes: $PI_{abs} = (RC/ABS)\cdot[\varphi_{P0}/(1 - \varphi_{P0})]\,[\varphi_0/(1 - \varphi_0)]$, $PI_{total} = PI_{abs}\cdot[RE_0/(ET_0 - RE_0)]$; (*2*) quantum yields and efficiencies: $\varphi_{Po} = TP_0/ABS = [1 - (F_0/F_M)]$, $\varphi_{E0} = ET_0/ABS = [1 - (F_0/F_M)]\,\psi_{E0}$, $\varphi_{R0} = RE_0/ABS = [1 - (F_0/F_M)]\,\psi_{E0}\,\delta_{R0}$, $\psi_{E0} = ET_0/TP_0 = (1 - V_J)$, and $\delta_{R0} = RE_0/ET_0 = (1 - V_I)/(1 - V_J)$; (*3*) energy fluxes: $ABS/RC = M_0\,(1/V_j)\cdot(1/\varphi_{Po})$, $TP_0/RC = M_0\,(1/V_j)$, $ET_0/RC = M_0\,(1/V_j)\,\varphi_0$, and $RE_0/RC = M_0\,(1/V_J)\,\psi_{E0}\,\delta_{R0}$.

**Antioxidant enzyme assays**: The extraction of the enzyme solution was carried out using the method of Dhindsa and Matowe (1981) with slight modifications. Leaf samples of 2 g were ground in an ice bath with 10 ml of phosphate buffer (pH 7.8), and centrifuged at 4,000 × *g* for 25 min at 4°C. The supernatant diluted to a final volume of 35 ml with phosphate buffer (pH 7.8) for the determination of antioxidant enzymes.

Catalase (CAT; EC 1.11.1.6) activity was assayed by potassium permanganate titration according to Huseynova (2012). Peroxidase (POD; EC 1.11.1.7) activity was

measured by guaiacol chromogenic method based on the method of Rai *et al.* (2012). POD activity was expressed as μmol($H_2O_2$ reduced) $min^{-1}$ $mg^{-1}$(protein). Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by nitroblue tetrazolium (NBT) method according to Ansari *et al*. (2018). SOD activity was expressed as U $mg^{-1}$(protein).

**Malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) content**: $H_2O_2$ content was determined using the method of Ansari *et al*. (2018). Leaf tissues (0.2 g) were homogenized in 5 ml of 50 mM sodium phosphate buffer (pH 6.5). Supernatant (3 ml) was mixed with 1 ml of 0.1% (w/v) titanium sulfate in 20% (v/v) $H_2SO_4$ and centrifuged for 25 min at 4,000 × *g*. Absorbances of the supernatant were recorded at 410 nm with an ultraviolet spectrophotometer (*Cary 50 Conc UV-VIS*, *Varian*, Victoria, Australia).

MDA content was assayed by thiobarbituric acid reaction based on the method of Hodges *et al*. (1999) with minor modification. Leaf tissues (0.1 g) were homogenized in 1 ml of 0.1% (v/v) trichloroacetic acid containing 1% (w/v) polyvinylpyrrolidone (PVP), and 0.5% (v/v) butylated hydroxytoluene. The supernatant reacted with 20% thiobarbituric acid, then absorbance values of the supernatant were read at 440, 532, and 600 nm by using the spectrophotometer (*Cary 50 Conc UV-VIS*, *Varian*, Victoria, Australia). MDA content [nmol $g^{-1}$(FM)] was calculated.

**Statistical procedures**: All data were analyzed using the statistical package *SPSS 17.0* (*SPSS*, Chicago, IL, USA). One-way analysis of variance (*ANOVA*) followed by *Duncan*'s multiple range test (at $P$=0.05) was used to assess the differences between treatments. All figures were drawn by using the *GraphPad Prism 7.05* (*GraphPad Software*, San Diego, CA, USA). The results of the measured parameters were presented as the mean ± standard deviation (SD) of five biological replications, with three samples of each replication.

## Results

**Growth parameters**: HT and HH stress were reflected in the morphological indices, the photosynthetic pigments, and the biomass allocation (Table 1S, *supplement*). Compared to those of the CK group, the daily height increment, daily stem diameter increment, and daily leaf area increment of tomato seedlings were inhibited under $T_1H_{70}$. Similar to the $T_1H_{70}$ group, these parameters decreased significantly by 11.9, 35, and 18.5% in $T_2H_{50}$ group, but under $T_2H_{70}$ conditions, these indices improved significantly by 8.1, 46.2, and 15.9%, respectively. Similarly, the contents of Chl *a* and Chl *b* in the $T_1H_{70}$ and $T_2H_{50}$ groups were lower than those of the CK group. However, these two indices increased by 5 and 10%, respectively, under $T_2H_{70}$ group. HT stress also adversely affected the root DM, shoot DM, and root-to-shoot ratio, which decreased by 41.9, 2.8, and 29.4%, respectively, compared to those of nonstressed tomato seedlings, whereas those parameters significantly increased by 36.3, 1.4, and 25%, respectively, under $T_2H_{70}$ conditions.

**Gas-exchange parameters and light-response curves**: HH and HT stress had a negative impact on gas-exchange parameters (Fig. 1). Compared to the control group, the $P_N$, $C_i$, and $g_s$ in $T_2H_{50}$ group were reduced significantly,

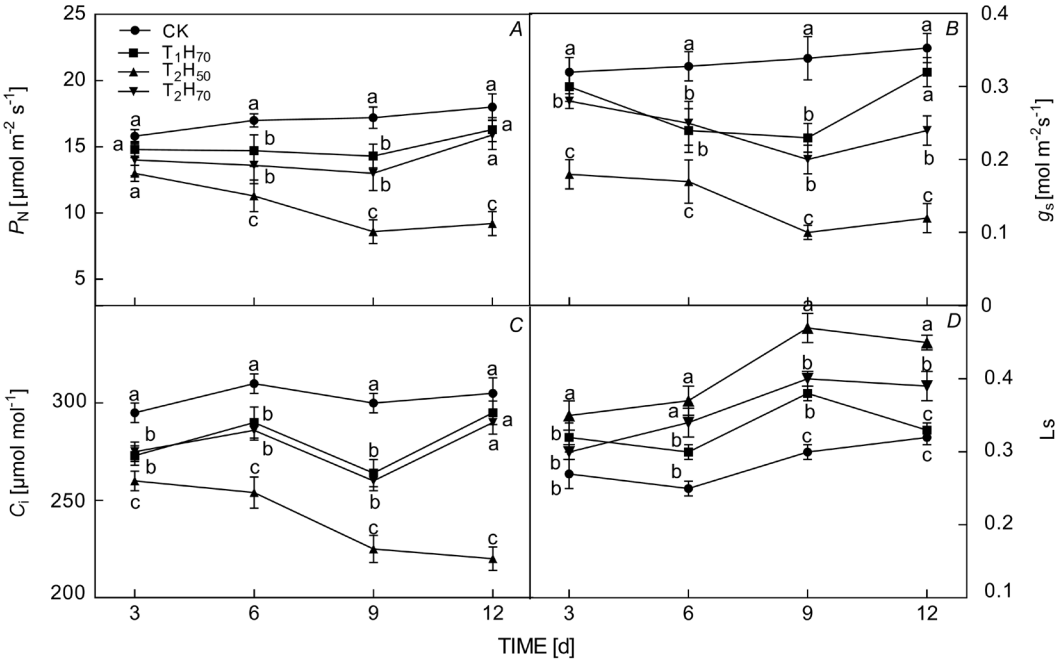


Fig. 1. Effects of high humidity on the net photosynthetic rate ($P_N$) (*A*), stomatal conductance ($g_s$) (*B*), intercellular $CO_2$ concentration ($C_i$) (*C*), and stomatal limitation value ($L_s$) (*D*) in tomato leaves under HT stress. *Different lowercase letters* in the same time represent significant differences at the level of 0.05 by *Duncan*'s multiple range tests. Values are means ± SD, $n$ = 15. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

but the $L_s$ increased significantly. However, high humidity significantly mitigated the effects of high temperatures on gas-exchange parameters, with a marked improvement of $P_N$, $C_i$, and $g_s$ in $T_2H_{70}$ group, and a significant reduction in the value of $L_s$ in $T_2H_{70}$ group.

Furthermore, four rapid light curves of $P_N$ in the range from 0 to 1,400 μmol(photon) $m^{-2}$ $s^{-1}$ were used to investigate the photosynthetic capacity of the tomato plants (Fig. 2). The $P_N$ increased with the increase of PAR. The upper curve ($T_1H_{50}$) showed the highest $P_N$, while $P_N$ under $T_1H_{70}$, $T_2H_{50}$, and $T_2H_{70}$ were 87.5, 62.4, and 81.3% of the control, respectively, when the light intensity reached 1,400 μmol(photon) $m^{-2}$ $s^{-1}$.

HH and HT stress reduced the $P_{Nmax}$, AQE, and LSP of tomato seedlings, which were 15.42 μmol $m^{-2}$ $s^{-1}$, 0.04, and 756.64 μmol $m^{-2}$ $s^{-1}$ in $T_1H_{70}$ group; 11.79 μmol $m^{-2}$ $s^{-1}$, 0.032, and 521.36 μmol $m^{-2}$ $s^{-1}$ in $T_2H_{50}$ group; whereas were 15.25 μmol $m^{-2}$ $s^{-1}$, 0.041, and 764.44 μmol $m^{-2}$ $s^{-1}$ in $T_2H_{70}$ group, respectively (Table 1). In addition, the value of LCP in $T_2H_{50}$ group significantly increased by 21.5% compared to the control, but significantly declined by 14.7% compared with that in the $T_2H_{70}$ group.

**Chl fluorescence parameters, OJIP curve, and JIP-test**: As can be seen from Table 2, the values of $F_v/F_m$ and $F_m$ were significantly reduced by 13.3 and 34.5% in $T_2H_{50}$ group, respectively, but the $F_0$ increased significantly by 30%. High humidity led to a significant increase in $F_v/F_m$ and $F_m$ under heat stress, but still 4.8 and 13.8% lower than that of the control, respectively. Additionally, the $F_0$ significantly decreased by 22.4% under $T_2H_{70}$ conditions compared to the $T_2H_{50}$ group.

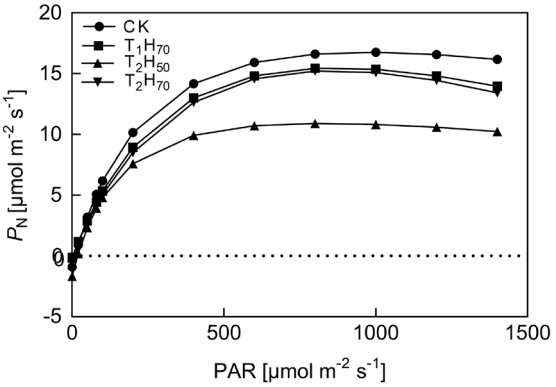


Fig. 2. Effects of high humidity on light-response curves in tomato leaves under HT stress on the 12th day of treatment. $P_N$ – net photosynthetic rate. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

The shape of the OJIP curve was also influenced by HT and HH stress (Fig. 3). The relative variable fluorescence of O–J, J–I, and I–P phases in the $T_2H_{50}$ group was significantly higher than that in the control group. The relative variable fluorescence at the O–J and J–I phases effectively decreased at $T_2H_{70}$ group compared to the $T_2H_{50}$ group, but showed no difference from the $T_2H_{50}$ group in the I–P phase. The JIP-test analysis demonstrated that the values of $\varphi_{P0}$, $\varphi_{E0}$, $\delta_{R0}$, $\varphi_{R0}$, $ET_0/RC$, and $RE_0/RC$ in the $T_2H_{50}$ group were significantly reduced by 13.8, 23.7, 30.6, 42.9, 21.7, and 45.5%, respectively, compared to those in the control group (Table 3). However, those values effectively increased by 4.9, 28.9, 40.0, 58.3, 31.4, and 45.5%, respectively, int $T_2H_{70}$ group. In addition, the values of ABS/RC, $DI_0/RC$, and $TP_0/RC$ also increased by 3.0, 50.0, and 11.1%, respectively, in $T_2H_{50}$ group compared to those in the control group, while those values significantly mitigated by 3.0, 10.0, and 6.2%, respectively, in $T_2H_{70}$ group. Meanwhile, HT stress had an adverse effect on $PI_{abs}$ and $PI_{total}$, which significantly decreased by 56.7 and 74.2%, respectively, compared with the control group. In contrast, high humidity increased them under HT stress 2.07 times and 3.52 times, respectively, compared to the HT stress group.

**Lipid peroxidation assay and ROS metabolism**: HH and HT stress significantly increased the contents of MDA and $H_2O_2$ in leaves of tomato plants (Table 4). Compared to the control group, MDA and $H_2O_2$ contents progressively increased by 100.0 and 91.7%, respectively, in $T_2H_{50}$ group. The content of MDA was significantly reduced by 25% in $T_2H_{70}$ group compared to the HT stress tomato seedlings. MDA content under $T_2H_{70}$ group was 33.3% higher than that of the control group, but no significant differences between them were found. In addition, the content of $H_2O_2$ significantly decreased by 21.7% in $T_2H_{70}$ group compared to that in the $T_2H_{50}$ group.

Furthermore, HT stress increased the activity of SOD, CAT, and POD, which was significantly higher than that in

Table 1. Effects of high humidity on photosynthetic parameters of tomato seedlings under HT stress on the 12th day of treatment. *Different lowercase letters* in the same column represent significant differences at the level of 0.05 by *Duncan*'s multiple range tests. Values are means ± SD, $n$ = 15. $P_{Nmax}$ – maximum photosynthetic rate; AQE – apparent quantum efficiency; LCP – light-compensation point; LSP – light-saturation point. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

| Treatment | $P_{Nmax}$ [μmol $m^{-2}$ $s^{-1}$] | AQE | LCP [μmol $m^{-2}$ $s^{-1}$] | LSP [μmol $m^{-2}$ $s^{-1}$] |
|---|---|---|---|---|
| $T_1H_{50}$, CK | 16.76 ± 0.22$^a$ | 0.049 ± 0.002$^a$ | 8.71 ± 0.19$^a$ | 964.75 ± 21$^a$ |
| $T_1H_{70}$ | 15.42 ± 0.32$^a$ | 0.040 ± 0.001$^b$ | 9.33 ± 0.13$^b$ | 756.64 ± 13$^b$ |
| $T_2H_{50}$ | 8.79 ± 0.20$^c$ | 0.032 ± 0.002$^c$ | 10.58 ± 0.20$^c$ | 521.36 ± 24$^c$ |
| $T_2H_{70}$ | 15.25 ± 0.21$^b$ | 0.041 ± 0.003$^b$ | 9.23 ± 0.15$^b$ | 764.44 ± 20$^b$ |

Table 2. Effects of high humidity on maximal quantum yield of PSII photochemistry ($F_v/F_m$), minimal fluorescence yield of the dark-adapted state ($F_0$), and maximal fluorescence yield of the dark-adapted state ($F_m$) in tomato leaves under HT stress on the 12th day of treatment. *Different lowercase letters* in the same column represent significant differences at the level of 0.05 by *Duncan*'s multiple range tests. Values are means ± SD, $n$ = 15. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

| Treatment | $F_v/F_m$ | $F_0$ | $F_m$ |
|---|---|---|---|
| $T_1H_{50}$, CK | 0.83 ± 0.21$^a$ | 0.45 ± 0.02$^c$ | 2.9 ± 0.13$^a$ |
| $T_1H_{70}$ | 0.81 ± 0.11$^a$ | 0.48 ± 0.01$^c$ | 2.6 ± 0.10$^b$ |
| $T_2H_{50}$ | 0.72 ± 0.12$^b$ | 0.67 ± 0.05$^a$ | 1.9 ± 0.21$^c$ |
| $T_2H_{70}$ | 0.79 ± 0.20$^a$ | 0.52 ± 0.02$^b$ | 2.5 ± 0.12$^b$ |

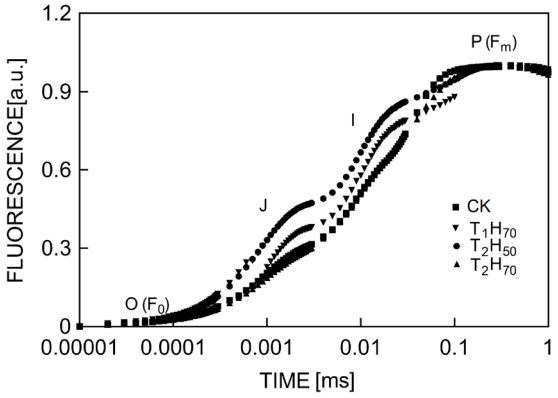


Fig. 3. Effects of high humidity on the performance of chlorophyll *a* fluorescence induction curves in tomato leaves under HT stress on the 12th day of treatment. O – initial fluorescence; J – intermediate level at 2 ms; I – intermediate level at 30 ms; P – peak level from 50 ms to 1s. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

the control group (Fig. 4). However, high humidity led to a significant reduction of SOD, CAT, and POD activities under heat stress compared to the $T_2H_{50}$ group, although the SOD, CAT, and POD activities were still higher than that in the nonstressed tomato seedlings in all the treatments.

## Discussion

The RH plays an important role in plants growth. On one hand, it can interact with other external factors, such as temperature, soil moisture, and soil nutrient. On the other hand, it can affect many physiological processes, such as photosynthesis, transpiration, and the mineral uptake of the plant (Han *et al*. 2019).

In general, under HT stress, the photosynthetic function of plant leaves is reduced, and the transport and distribution of organic matter are in disorder, leading to premature senescence of leaves, resulting in poor growth and development of plants (Krishnan *et al*. 2011). Changes in plant biomass are also responses to adverse stresses (Shu *et al*. 2016). In this study, it was clear that the growth and development of tomato seedlings were inhibited after HT treatments, which was characterized by slower daily increment of height, stem diameter, and leaf area, and lower root-to-shoot ratio, while high humidity treatment could alleviate the symptoms brought by HT stress, indicating that increasing the air humidity maintains the normal transmission and distribution of tomato photosynthetic products, and ensures the growth and development of plants under HT stress. This result can be confirmed by Wang *et al*. (2018); high humidity can increase the soluble sugar content in leaves, stems, and roots of tomato seedlings under HT stress compared with the control.

Chloroplasts are the main site for photosynthetic reactions and are also sensitive to HT stress (Tang *et al*. 2006, Marečková *et al*. 2019). The Chl content often reflects the chloroplast development and photosynthetic performance (Lu *et al*. 2019). In our study, HT stress significantly reduced the contents of Chl *a* and Chl *b*, probably because the high temperature severely damaged the structure and function of Chl, or significantly increased the activity of Chl-degrading enzymes. The results of this study have also been confirmed on spring wheat (Prasad *et al*. 2011), potato plants (Tang *et al*. 2006), *etc*. Moreover, high humidity can effectively alleviate the damage of Chl-containing structure and function by HT stress, and maintain photosynthetic capacity of chloroplasts at high temperatures (Table 1S). Early studies suggested that the decrease in photosynthetic rate was due to the decrease of leaf $g_s$, which hindered the supply of $CO_2$ in chloroplasts caused by stomatal limitation factors (Muraoka *et al*. 2000). However, Su and Liu (2005), Yan *et al*. (2011), and other studies believe that the inhibition of photosynthesis by HT is caused by nonstomatal factors, which is caused by the increase of gas diffusion resistance of mesophyll cells, the decrease of $CO_2$ solubility, and the decrease of Rubisco affinity for $CO_2$. This experiment showed that under HT treatment, $P_N$ decreased in tomato leaves, accompanied by different decline of $g_s$ and $C_i$, and $L_s$ increased significantly (Fig. 1), which indicated that the decrease of $P_N$ in tomato leaves may be the effect of stomatal limitation. However, by increasing the humidity of the air, the $P_N$ of tomato seedlings can be increased and the values of $L_s$ can be reduced under high temperatures. In addition, HT also significantly influenced the photosynthetic curve parameters of tomato seedlings, such as significantly reduced the $P_{Nmax}$, AQE, and LCP, and significantly increased the LSP (Table 1), which was consistent with the research results of Zhou *et al*. (2015), but high humidity can effectively alleviate the influence of HT stress on the characteristic parameters of light-response curve.

The $PI_{total}$ is an index of photosynthetic performance, and is also closely related to the ultimate life state of the plants, such as growth and development under stress conditions (Yusuf *et al*. 2010). A negative value for $PI_{total}$ indicates 'loss' and a positive value indicates 'gain' for energy conservation. HT significantly reduced the value of $PI_{total}$ compared to the control group, but high humidity can alleviate the decrease in the $PI_{total}$ value (Table 3), which means that there was a 'loss' of energy conservation after

Table 3. Effects of high humidity on JIP-test parameters in tomato leaves under HT stress on the 12th day of treatment. *Different lowercase letters* in the same column represent significant differences at the level of 0.05 by *Duncan*'s multiple range tests. Values are means ± SD, $n$ = 15. ABS/RC – apparent antenna size of active PSII per reaction center; $DI_0$/RC – effective dissipation of energy per active RC; $TR_0$/RC – trapped energy flux per reaction center; $ET_0$/RC – electron transport flux per reaction center; $RE_0$/RC – electron flux reducing end electron acceptors at the PSI acceptor side per reaction center; $\varphi_{P0}$ – maximum quantum yield for primary photochemistry; $\varphi_{E0}$ – quantum yield for electron transport; $\delta_{R0}$ – efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side; $\varphi_{R0}$ – quantum yield for reduction of end electron acceptors at the PSI acceptor side; $PI_{abs}$ – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; $PI_{total}$ – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

| Parameter | $T_1H_{50}$, CK | $T_1H_{70}$ | $T_2H_{50}$ | $T_2H_{70}$ |
|---|---|---|---|---|
| ABS/RC | 1.01 ± 0.01$^c$ | 1.03 ± 0.01$^b$ | 1.04 ± 0.01$^a$ | 1.03 ± 0.01$^a$ |
| $DI_0$/RC | 0.40 ± 0.03$^c$ | 0.44 ± 0.01$^b$ | 0.60 ± 0.02$^a$ | 0.45 ± 0.01$^b$ |
| $TR_0$/RC | 0.81 ± 0.01$^c$ | 0.84 ± 0.02$^b$ | 0.90 ± 0.03$^a$ | 0.86 ± 0.02$^b$ |
| $ET_0$/RC | 0.60 ± 0.03$^a$ | 0.58 ± 0.02$^a$ | 0.47 ± 0.05$^b$ | 0.62 ± 0.02$^a$ |
| $RE_0$/RC | 0.22 ± 0.02$^a$ | 0.20 ± 0.01$^a$ | 0.12 ± 0.01$^b$ | 0.22 ± 0.01$^a$ |
| $\varphi P_o$ | 0.94 ± 0.01$^a$ | 0.90 ± 0.02$^b$ | 0.81 ± 0.01$^d$ | 0.85 ± 0.01$^c$ |
| $\varphi E_o$ | 0.59 ± 0.02$^a$ | 0.57 ± 0.01$^a$ | 0.45 ± 0.01$^b$ | 0.58 ± 0.02$^a$ |
| $\delta R_o$ | 0.36 ± 0.03$^a$ | 0.36 ± 0.01$^a$ | 0.25 ± 0.01$^b$ | 0.35 ± 0.02$^a$ |
| $\varphi R_o$ | 0.21 ± 0.03$^a$ | 0.20 ± 0.01$^a$ | 0.12 ± 0.01$^b$ | 0.19 ± 0.01$^a$ |
| $PI_{abs}$ | 11.38 ± 0.46$^a$ | 10.11 ± 0.21$^b$ | 4.96 ± 0.54$^c$ | 10.25 ± 0.67$^b$ |
| $PI_{total}$ | 6.36 ± 0.39$^a$ | 4.93 ± 0.01$^c$ | 1.64 ± 0.36$^d$ | 5.78 ± 0.56$^b$ |

Table 4. Effects of high humidity on malondialdehyde (MDA) and $H_2O_2$ contents in tomato leaves under HT stress on the 12th day of treatment. *Different lowercase letters* in the same column represent significant differences at the level of 0.05 by *Duncan*'s multiple range tests. Values are means ± SD, $n$ = 15. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

| Treatment | MDA content [nmol $g^{-1}$(FM)] | $H_2O_2$ content [μmol $g^{-1}$(FM)] |
|---|---|---|
| $T_1H_{50}$, CK | 0.62 ± 0.21$^c$ | 1.63 ± 0.22$^b$ |
| $T_1H_{70}$ | 0.82 ± 0.22$^c$ | 1.74 ± 0.11$^b$ |
| $T_2H_{50}$ | 1.24 ± 0.14$^a$ | 2.32 ± 0.30$^a$ |
| $T_2H_{70}$ | 0.93 ± 0.24$^b$ | 1.84 ± 0.21$^b$ |

HT stress, and an increase was observed after increasing the air humidity. The Chl fluorescence signal and its calculated parameters were successfully applied to detect the injury of PSII in plants under different environmental stresses (Krause and Weis 1991, Maxwell and Johnson 2000). $F_v/F_m$ is the maximum photochemical quantum yield of PSII, reflecting the intrinsic conversion efficiency of PSII reaction center. Under nonstress conditions, the variation of this parameter is very small, and it is not affected by species and growth conditions, but under stress conditions, the parameter decreases significantly (Baker 2008). In our study, the $F_v/F_m$ decreased significantly at the HT stress, indicating the energy converted into chemical fixation was reduced, while that used for heat dissipation increased (van Kooten and Snel 1990, Baker 2008). In addition, the increase in both $F_0$ and $F_m$ (Table 2), which implied the electron transfer was blocked from the primary acceptor PQ ($Q_A$) to the secondary acceptor PQ ($Q_B$) on the acceptor side of PSII (Baker 2008). ABS/RC is used to measure the size of the apparent antenna (absorption flux of antenna Chl per active RC). Table 3 showed that the value of ABS/RC increased under HT stress, suggesting that either the apparent antenna increased its size or a portion of RCs was inactivated. However, we also observed that the value of $TP_0$/RC increased, indicating that changes took place both in the apparent antenna size and the RCs. Besides, high humidity improved the decline of quantum yields and efficiencies (*e.g.*, $\varphi_{P0}$, $\varphi_{E0}$, $\delta_{R0}$, and $\varphi_{R0}$). This means that high humidity might improve the efficiency of photosynthetic electron transport in PSII under HT stress.

The most important biochemical change that occurs when plants are exposed to HT stress is the production of reactive oxygen species (ROS) (Martínez-Téllez and Lafuente 1997). ROS are highly active and reactive, and they can destroy normal metabolism by oxidative damage to proteins, nucleic acids, and lipids without any protective mechanism (Almeselmani *et al*. 2006). Lipid peroxidation is usually used to detect ROS damage, which clearly reflects the damage of cell membranes caused by abiotic stress (Lu *et al*. 2019). Under stress conditions, the antioxidant defense system of plants is activated to protect cells from the negative effects of ROS (Noctor and Foyer 1998). Our study found that high humidity reduced the contents of MDA and $H_2O_2$ in tomato leaves

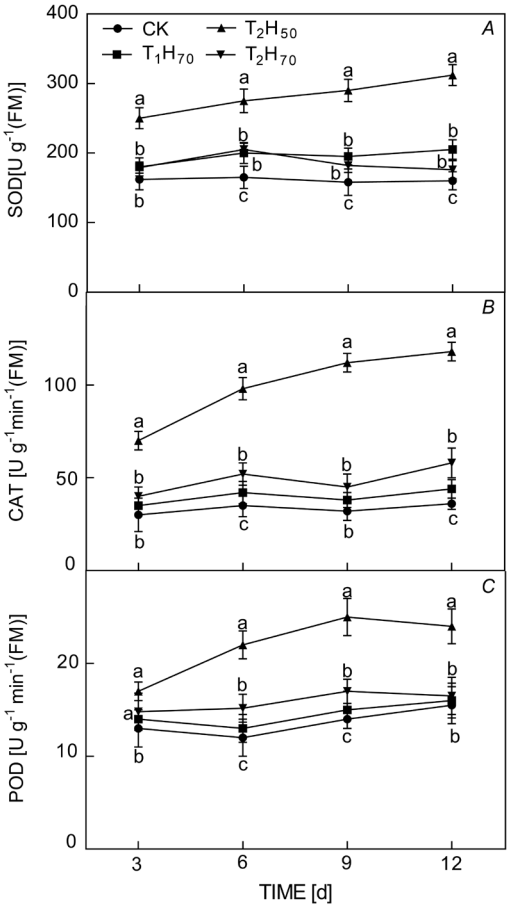


Fig. 4. Effects of high humidity on the activity of catalase (CAT) (*A*), superoxide dismutase (SOD) (*B*), and peroxidase (POD) (*C*) in tomato leaves under HT stress. *Different lowercase letters* in the same time represent significant differences at the level of 0.05 by *Duncan*'s multiple range tests. Values are means ± SD, $n$ = 15. CK – 25/15°C + 50% relative humidity (RH); $T_1H_{70}$ – 25/15°C + 70% RH; $T_2H_{50}$ – 38/28°C + 50% RH; $T_2H_{70}$ – 38/28°C + 70% RH.

compared to the $HT_{50}$ group (Table 4), and also reduced the activity of SOD, POD, and CAT (Fig. 4), indicating that high humidity might help to alleviate cell membrane peroxidation, scavenge excess ROS, and maintain cell metabolism stability under HT stress.

Therefore, we concluded that high humidity improved the growth and alleviated photoinhibition and oxidative stress of tomato seedlings under heat stress.

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