Effects of Drought Stress on Photosynthetic and Physiological Parameters of Tomato

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ABSTRACT. Drought has become an important factor limiting crop yields in China. As an important greenhouse horticultural crop in China, the research of tomato (*Solanum lycopersicum* L. cv. Jinpeng No.10) is of great theoretical and practical significance. In the study, four different relative soil moisture contents (74% to 80%, 55% to 61%, 47% to 52%, and 25% to 30%) were used to induce drought stress. We investigated changes in photosynthetic gas exchange, chlorophyll fluorescence, and other related physiological parameters in response to different relative soil moisture contents. Drought inhibited the photosynthesis of tomato significantly, as shown by a clear decline in the net photosynthetic rate. Our results indicated stomatal limitation and nonstomatal limitation were responsible for the photosynthesis reduction.

Recently, under the influence of global climate change, drought has occurred more frequently and seriously, with most countries and regions being threatened by drought to varying degrees (Fullana-Pericàs et al., 2018; Lehner et al., 2006; Lesk et al., 2016). The rational use of water resources has become a worldwide problem (Cominelli et al., 2009; Yan and Wang, 2013). China is one of the countries suffering the shortage of water resources (Feng and Liu, 2006; Jiang, 2009). Due to the shortage and uneven distribution of water resources, water has become an important factor limiting crop yields in China (Miao et al., 2017).

Crop losses caused by drought are the highest among losses caused by all abiotic stresses (Placide et al., 2014). Drought can influence water metabolism in plants and cause significant changes in plant morphology, physiology, and biochemistry (Torres-Ruiz et al., 2015). Therefore, more research into the responses and adaptation mechanisms of crops to drought stress is needed to develop tolerant and high water-use-efficiency crop systems in agriculture.

Drought stress restricts plant growth by decreasing photosynthetic rate (Kebbas et al., 2015; Zhang et al., 2018). The main factors responsible for decreased photosynthesis could be stomatal (stomatal closure due to decreased CO₂), nonstomatal (decreased photosynthetic activity in mesophyll tissue), or both (Ghotbi-Ravandi et al., 2014; Varone et al., 2012). These factors (stomatal, nonstomatal, or both) depend on experimental treatment as well as species, cultivar, age, and development

status of the plants being studied (Liang et al., 2018; Liu et al., 2017).

Under stress, plants can reduce the production of excess electrons through energy dissipation mechanisms during photosynthetic electron transfer. Under drought stress, energy dissipation mechanisms, such as heat dissipation or photorespiration, play roles in the drought tolerance of plants (Flexas and Medrano, 2002). Regarding photosynthesis in leaves, gas exchange index reflects its “apparent” characteristics, while chlorophyll fluorescence reflects its “intrinsic” characteristics (Jiang et al., 2006; Long and Bernacchi, 2003). Therefore, these two parameters have been widely used in plant stress-tolerance physiology, crop breeding, and agronomy (Paleg and Aspinall, 1982).

Drought tolerance in plants is mainly manifested by effects such as the accumulation of osmotic substances, changes in membrane lipid composition, scavenging of reactive oxygen species (ROS), and regulation of protein induction and hormonal balance (Cumming et al., 2007; Martinez et al., 2004). Drought stress causes an imbalance between the production and scavenging of ROS in plants (Ajithkumar and Panneerselvam, 2014). ROS accumulation causes membrane damage and membrane lipid peroxidation (Xu et al., 2006). Several antioxidant enzymes and osmotic substances (including soluble sugars, proteins, free polyols, and so forth) in plants constitute the antioxidant enzyme systems (Malviya, 2015; Sairam et al., 2011), which scavenge these ROS and protect macromolecules in plant cells (Ennajeh et al., 2009).

Tomato is an important greenhouse horticultural crop in China, with a wide distribution and high economic value. Moreover, tomato is a model plant of theoretical and practical significance in research (Arie et al., 2007). Although some studies have been carried out on the photosynthesis of tomato under drought stress (Brix, 2010; Jangid and Dwivedi, 2016), these studies are not systematic and comprehensive. Thus, in this study, the combination measurement of photosynthetic gas exchange, chlorophyll fluorescence, and antioxidant enzyme activities were used to identify key regulatory photosynthesis circuits under drought stress. The objective of this study was to

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provide valuable information regarding drought resistance mechanisms of plant.

Materials and Methods

TEST MATERIALS AND PROCESSING. All experiments were conducted in the greenhouse of Shandong Facility Horticultural Laboratory, Weifang, Shandong Province, China. The temperature in the greenhouse was 12 to 30 °C. The maximum illumination in sunny days is 518.4 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Tomato seeds were planted in plug plate (Wei Nong Co., Taizhou, China). Tomato special matrix (Organic Nutrient Soil Co., Weifang, China) and vermiculite were mixed in a 3:1 ratio, the organic matter was 14% to 18%, the total nitrogen was 0.4%, the total phosphorus was 0.12%, the available nitrogen was 0.0033%, and the available phosphorus was 0.012%. When plants reached the fifth true leaf stage, they were transplanted (one seedling per pot) into plastic flowerpots in early Mar. 2018. Each pot (30 cm diameter, 14 cm height) was spaced 1 m apart. Loam soil was collected from the greenhouse from areas that were planted with tomato, with 5 kg soil being placed in each flowerpot. Soil bulk density was 1.27 $\text{g}\cdot\text{cm}^{-3}$, pH was 6.6 to 7.2, soil organic content was 1.23%, available nitrogen was 45.12%, available phosphorus was 13.18%, and available potassium was 0.008%. Plants were watered regularly during the drought treatment period, but no additional nutrients were applied.

The experimental treatments were applied 1 week after transplanting. Field soil capacity was 36.2%. In total, 40 well-developed plants with similar heights and basal diameters were selected as test plants and divided into four groups (10 plants per group). Soil water content (SWC) was determined using a soil moisture tester (TDR FS6440; Spectrum Technologies, Aurora, IL) with a P3-type soil moisture probe. According to the equation, SWC was calculated as the product of field capacity (FC) and relative soil water content (RSMC). RSMC of the control (CK) was controlled at 74% to 80%, that of treatment 1 (T_1) at 55% to 61%, that of treatment 2 (T_2) at 47% to 52%, and that of treatment 3 (T_3) at 25% to 30%. After 15 d of treatment, 9 of the 10 plants from each treatment condition were selected (the height for CK, T_1 , T_2 , and T_3 was 45, 39, 36, and 30 cm, respectively), and they were divided into three groups and each group was considered as a biological replicate. The third leaves from the stem end of the test plants were chosen to examine appropriate physiological and biochemical indices. Each group was measured once per day for 3 d.

MEASUREMENT OF PHOTOSYNTHETIC GAS EXCHANGE PARAMETERS. A portable photosynthesis system (CIRAS-2; PPS Systems, Amesbury, MA) was used to measure the photosynthetic gas exchange parameters of the sampled leaves at 0830–1130 HR. The environment temperature was set at 27 ± 3 °C. During measurements, CO_2 concentration, relative humidity, and air temperature in the leaf chamber were 365 ± 5 $\mu\text{mol}\cdot\text{m}^{-2}$, 60% $\pm$ 4.0%, and 26 ± 1.5 °C, respectively. A gradient of 14 photosynthetic active radiation (PAR) settings (2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, and 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was supplied via a cold light-emitting diode (LED) irradiation source (CIRAS-2 LED; range 0 to 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Photosynthetic parameters were measured at each PAR setting for 120 s, repeating each measurement three times. The automatic portable photosynthesis system recorded PAR , transpiration rate (E), net photosynthetic rate (P_N), stomatal conductance (g_s), the air CO_2 concentration (C_a), and intercellular CO_2 concentration

(C_i). Water-use efficiency (W_{UE}) was calculated as $W_{UE} = P_N/E$, and stomatal limitation (L_s) was calculated as $L_s = 1 - C_i/C_a$ (Nijs et al., 1997).

MEASUREMENT OF CHLOROPHYLL FLUORESCENCE PARAMETERS. A portable pulse amplitude-modulated chlorophyll fluorometer (FMS-2; Hansatech, King's Lynn, UK) was used to measure chlorophyll fluorescence parameters, such as minimum chlorophyll fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_M), steady-state fluorescence yield (F_S), minimum fluorescence of the light-adapted state (F_0'), and maximal fluorescence yield of the light-adapted state (F_M'). All measurements were taken three times. Under 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light, the leaves of each treated plant reached steady state after photochemistry, F_S was measured; then under saturated pulsed light (12,000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), F_M' was measured. Then the action light was closed and the far red light was turned on immediately; F_0' was measured after 2 s. After that, dark treatment was carried out for 30 min with a dark adaptation clip; F_0 and F_M were measured.

Other chlorophyll fluorescence parameters were calculated according to the following equations: maximal quantum yield of photosystem II (PSII) photochemistry: $F_v/F_M = (F_M - F_0)/F_M$; photochemical quenching coefficient: $q_p = (F_M' - F_S)/(F_M' - F_0')$; nonphotochemical quenching: $NPQ = (F_M - F_M')/F_M'$; and effective quantum yield of PSII photochemistry: $\Phi_{PSII} = (F_M' - F_S)/F_M'$ (Liang et al., 2017).

MEASUREMENT OF ANTIOXIDANT ENZYME ACTIVITIES AND MALONDIALDEHYDE CONCENTRATION. The antioxidant enzyme activities [(superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)], and malondialdehyde (MDA) concentration were measured with the corresponding reagent kits for plant tissue (Jiancheng, Nanjing, China), following the manufacturer's protocols. SOD activity was determined by xanthine oxidase method (Xu et al., 2011), POD activity was measured by guaiacol method (Cazenave et al., 2006), CAT activity was determined by potassium permanganate titration (Jiang et al., 2009), MDA content was measured by thiobarbituric acid (TBA) method (Schmedes and Hølmér, 1989). An ultraviolet-visible spectrophotometer (ultraviolet-265; Shimadzu, Kyoto, Japan) was used to measure the light absorption value.

PHOTOSYNTHESIS-LIGHT RESPONSE CURVES. Ye and Yu (2007) demonstrated that response curves of P_N to light intensity could be accurately fitted by the rectangular hyperbolic correction model. The light response parameters were analyzed using SPSS (version 17.0; IBM Corp., Armonk, NY) software. Equations for the rectangular hyperbolic correction model were as follows:

$$P_N = \Phi \frac{1 - \beta P_{AR}}{1 + \gamma P_{AR}} P_{AR} - R_D \quad [1]$$

$$L_{SP} = \frac{\sqrt{(\beta + \gamma)/\beta} - 1}{\gamma} \quad [2]$$

$$P_{Nmax} = \Phi \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right)^2 - R_D \quad [3]$$

Equations for quantum yield at the light compensation point (Φ_C), intrinsic quantum yield (Φ_0), and the absolute value of the

Table 1. Responses of apparent quantum yield (AQY), light saturation point (LSP), light-saturated net photosynthetic rate (P_{Nmax}), light compensation point (LCP), and respiration rate (R_D) of tomato under different soil drought stress.

Light response model	Treatment ^z	Φ_0^y	Φ_C^x	Φ_{C0}^w	LSP	P_{Nmax}	LCP	R_D	R^2^v
Rectangular hyperbolic modified model	74% to 80%	0.052095	0.046162	0.046160	1229	7.55	29.61	1.367	0.9991
	55% to 61%	0.048619	0.040738	0.040738	1157	7.09	51.97	2.117	0.9991
	47% to 52%	0.047904	0.038821	0.038822	1153	6.64	62.94	2.444	0.9992
	25% to 30%	0.046764	0.035937	0.035936	1050	5.70	80.15	2.880	0.9996

^zControl relative soil water content (RSMC) = 74% to 80%, Treatment 1 (T_1) RSMC = 55% to 61%, Treatment 2 (T_2) RSMC = 47% to 52%, Treatment 3 (T_3) RSMC = 25% to 30%.

^yIntrinsic quantum yield (Φ_0).

^xQuantum yield at the light compensation point (Φ_C).

^wThe absolute value of the slope of the light response curve between $PAR = 0$ and $PAR = I_c$ (Φ_{C0}).

^vCoefficient of determination (R^2).

Fig. 1. Photosynthetic rate-light response curves [photosynthetically active radiation (PAR), net photosynthetic rate (P_N)] of tomato under different soil drought stress. Values are the means of three replicates $\pm$ SD. Control (CK) relative soil water content (RSMC) = 74% to 80%, Treatment 1 (T_1) RSMC = 55% to 61%, Treatment 2 (T_2) RSMC = 47% to 52%, Treatment 3 (T_3) RSMC = 25% to 30%.

slope of the light response curve between $PAR = 0$ and $PAR = I_c$ (Φ_{C0}) were as follows:

$$\Phi_C = P'_N(P_{AR} = I_c) = \alpha \frac{1 + (\gamma - \beta)I_c - \beta\gamma I_c^2}{(1 + \gamma I_c)^2} \quad [4]$$

$$\Phi_0 = P'_N(P_{AR} = 0) = \Phi \quad [5]$$

$$\Phi_{C0} = |R_D/I_c| \quad [6]$$

where, P'_N is one-step derivatives of P_N ; Φ is apparent quantum yield (AQY) ($\text{mol} \cdot \text{mol}^{-1}$); P_{AR} is the photosynthetic active radiation intensity ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); L_{SP} is the light saturation point ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); R_D is respiration rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); β and γ are model coefficients; P_N is net photosynthetic rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); P_{Nmax} is maximum net photosynthetic rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); and I_c is the light compensation point (LCP) ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

DATA PROCESSING. SPSS 17.0 software and Excel (2016 for Windows; Microsoft, Redmond, WA) were employed for one-way analysis of variance and Fisher's protected least significant difference method for multiple pairwise comparisons. $P < 0.05$

was considered significant. The photosynthetic light response parameters of tomato to different degrees of drought stress were based on gas exchange data, which were obtained under different light intensity and RSMC treatments (Ye and Yu, 2007).

Results

LIGHT RESPONSE PARAMETERS. Light response parameters of tomato are listed in Table 1. For the rectangular hyperbolic correction model between photosynthetic rate and PAR , R^2 was > 0.999 , which showed that the model closely simulated the response of the photosynthetic rate to light intensity (Liang et al., 2017).

Table 1 and Fig. 1 describe changes in light response parameters of tomato under different soil drought stress treatments. With the increase in drought stress severity, P_{Nmax} , L_{SP} , and AQY all decreased, while R_D and LCP increased.

Under a stable PAR ($1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), P_N , E , g_s , C_i , W_{UE} , and L_s all significantly changed with increasing drought stress. P_N , E , and g_s all declined, with significant differences being detected among different drought treatments. W_{UE} decreased with increasing drought stress, suggesting that drought stress directly affected the W_{UE} of tomato. L_s gradually increased, showing the opposite response of decreasing C_i , while P_N declined under increasing drought stress severity (Table 2).

CHLOROPHYLL FLUORESCENCE PARAMETERS. F_v/F_m and Φ_{PSII} decreased while $1-q_P$ significantly increased with increasing severity of drought stress (Table 3). However, NPQ increased initially—then decreased as drought stress increased (Table 3).

ANTIOXIDANT ENZYME ACTIVITIES (SOD, POD, AND CAT) AND MDA CONCENTRATION. With increased severity of drought stress, the activities of the key antioxidant enzyme, namely SOD, POD, and CAT, increased initially and then decreased at low drought stress (Table 4), while MDA increased gradually but steadily as RSMC decreased and drought stress increased (Table 4).

Discussion

With the increase of the world population, the need for more crops is also increasing. However, drought stress was one of the main reasons causing the crop losses all over the world, which has aroused the attention of the scholars worldwide (Li et al., 2017). As one of the main basics for plant growth, photosynthesis is a hot topic in the study of plant responses under abiotic stress (Gururani et al., 2015; Liu et al., 2017).

Table 2. Response of photosynthetic parameters of tomato to different soil drought stress severities under the same photosynthetically active radiation value ($1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Values are means of three replications.

Treatment ^z	P_N^y ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	E^x ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	g_s^w ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	C_i^v ($\mu\text{mol}\cdot\text{mol}^{-1}$)	L_s^u	WUE^t ($\mu\text{mol}\cdot\text{mmol}^{-1}$)
	(mean $\pm$ SD)					
74% to 80%	7.59 ± 0.25 a ^s	2.05 ± 0.05 a	53.42 ± 3.09 a	275.72 ± 2.33 a	0.24 ± 0.06 c	3.70 ± 0.04 a
55% to 61%	7.07 ± 0.26 b	1.96 ± 0.06 ab	49.57 ± 0.45 b	271.00 ± 1.23 ab	0.25 ± 0.03 bc	3.61 ± 0.03 b
47% to 52%	6.62 ± 0.24 b	1.87 ± 0.06 b	47.18 ± 2.32 b	260.89 ± 5.39 c	0.29 ± 0.14 a	3.55 ± 0.02 c
25% to 30%	5.60 ± 0.22 c	1.61 ± 0.06 c	38.28 ± 1.20 c	269.82 ± 1.24 b	0.26 ± 0.03 b	3.49 ± 0.01 d

^zControl relative soil water content (RSMC) = 74% to 80%, Treatment 1 (T₁) RSMC = 55% to 61%, Treatment 2 (T₂) RSMC = 47% to 52%, Treatment 3 (T₃) RSMC = 25% to 30%.

^yNet photosynthetic rate (P_N).

^xTranspiration rate (E).

^wStomatal conductance (g_s).

^vIntercellular CO₂ concentration (C_i).

^uStomatal limitation (L_s).

^tWater-use efficiency (WUE).

^sMeans in columns under different soil drought stress followed by different letters are significantly different (LSD test, $P < 0.05$).

Table 3. Effects of soil drought stress on chlorophyll fluorescence parameters of tomato. Values are means of three replications.

Treatment ^z	F_v/F_m^y	$1-q_p^x$	NPQ ^w	Φ_{PSII}^v
	(mean $\pm$ SD)			
74% to 80%	0.843 ± 0.009 ab ^u	0.239 ± 0.013 b	0.701 ± 0.009 d	0.752 ± 0.008 a
55% to 61%	0.839 ± 0.04 abc	0.281 ± 0.059 ab	0.787 ± 0.007 c	0.623 ± 0.006 b
47% to 52%	0.821 ± 0.005 bc	0.333 ± 0.011 a	0.893 ± 0.004 a	0.515 ± 0.003 c
25% to 30%	0.780 ± 0.003 d	0.364 ± 0.070 a	0.824 ± 0.003 b	0.428 ± 0.005 d

^zControl relative soil water content (RSMC) = 74% to 80%, Treatment 1 (T₁) RSMC = 55% to 61%, Treatment 2 (T₂) RSMC = 47% to 52%, Treatment 3 (T₃) RSMC = 25% to 30%.

^yMaximal quantum yield of PSII (F_v/F_m).

^xPhotochemical quenching coefficient (q_p).

^wNonphotochemical quenching (NPQ).

^vEffective quantum yield of PSII photochemistry (Φ_{PSII}).

^uMeans in columns within different soil water contents followed by different letters are significantly different (LSD test, $P < 0.05$).

Table 4. Changes in superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities, as well as malondialdehyde (MDA) content in tomato under soil drought stress. Values are means of three replications.

Treatment ^z	SOD ($\text{U}\cdot\text{g}\cdot\text{min}^{-1}$)	POD ($\text{U}\cdot\text{g}\cdot\text{min}^{-1}$)	CAT ($\text{U}\cdot\text{g}\cdot\text{min}^{-1}$)	MDA ($\mu\text{mol}\cdot\text{kg}^{-1}$)
	(mean $\pm$ SD)			
74% to 80%	97.505 ± 0.257 d ^y	90.037 ± 0.037 d	75.760 ± 0.845 cd	4.526 ± 0.019 d
55% to 61%	139.127 ± 1.160 c	240.032 ± 5.808 c	82.245 ± 2.880 bc	7.962 ± 0.064 c
47% to 52%	286.385 ± 0.795 a	330.071 ± 6.317 a	101.570 ± 1.154 a	10.641 ± 0.173 b
25% to 30%	191.911 ± 0.104 b	270.816 ± 3.148 b	78.453 ± 0.520 bcd	14.593 ± 0.039 a

^zControl relative soil water content (RSMC) = 74% to 80%, Treatment 1 (T₁) RSMC = 55% to 61%, Treatment 2 (T₂) RSMC = 47% to 52%, Treatment 3 (T₃) RSMC = 25% to 30%.

^yDifferent letters indicate significant differences (LSD test, $P < 0.05$).

In this study, with the increase of drought stress, a decrease in $P_{N\text{max}}$ and a simultaneous increase in R_D imply lower photosynthetic rates and higher respiration rates. Increases in LCP and decreases in L_{SP} indicate that light-use efficiency (under both weak and strong light) declined as RSMC decreased. Decreasing Φ (AQY) suggested a decline in the use and transformation efficiency of low-light energy (Farquhar and Sharkey, 1982). It was apparent that photosynthetic rate and light-use efficiency were both inhibited by drought stress.

Changes in photosynthetic parameters under different levels of drought stress are mainly caused by stomatal or nonstomatal factors, which are reflected in changes in C_i and/or L_s , as C_a is

relatively stable. When decreased P_N as a result of drought stress is accompanied by increased (or unchanged) C_i and decreased L_s , non-stomatal factors are the main cause of reduced photosynthetic rate. In contrast, when decreased P_N is accompanied by unchanged or increased L_s , while C_i is unchanged or decreased, stomatal factors are the main cause (Farquhar and Sharkey, 1982). In this study, when the RSMC was <47% to 52%, any increase in drought stress and corresponding decrease in P_N were associated with decreased C_i and increased L_s , suggesting that stomatal limitation was responsible for reduced P_N in tomato leaves. However, when RSMC was >52%, any increase in drought stress and corresponding decrease in P_N were accompanied by increased C_i and decreased L_s , indicating that non-stomatal limitation was responsible for reduced P_N . Under these conditions, the photosynthetic apparatus was damaged.

Changes in W_{UE} by plants are regulated by coordinating the association between carbon assimilation

and water consumption, which is an important strategy used by plants to withstand drought. In this study, P_N , E , and W_{UE} of tomato leaves all showed similar responses to soil moisture deficits, each parameter decreasing as RSMC decreased (Table 2).

During plant photosynthesis, light energy absorbed by chloroplasts is dissipated through three reciprocally related pathways: photosynthetic electron transport, chlorophyll fluorescence, and heat dissipation (Hendrickson et al., 2004). F_v/F_m reflects the ability of antenna pigments to absorb and convert light energy to PSII (Mckew et al., 2013). Representing the proportion of light energy is Φ_{PSII} , which is used for the

photochemical reaction (Ma and Guo, 2014); and NPQ represents the heat dissipation in PSII and reflects the amount of heat dissipated from light energy absorbed by the PSII antenna pigments (Stefanov and Terashima, 2008). The excitation energy used in the PSII reaction center, q_p , reflects the electron transfer activity of the PSII center (Yokono et al., 2015).

In the current study, with an increase in the degree of drought stress, F_v/F_m , Φ_{PSII} , and q_p gradually decreased, suggesting that drought initiated the closure of the PSII reaction center, limiting electron transfer and reducing the light energy available for actual photochemical reactions in the PSII reaction center (Stefanov and Terashima, 2008). Maximum F_v/F_m is reflective of potential maximum photosynthetic capacity of plant leaves under nonstressful conditions after a sufficient dark adaptation. F_v/F_m reflects the potential maximum photosynthetic capacity of plant leaves after a sufficiently dark adaptation (Sharma et al., 2015). F_v/F_m is relatively constant, generally between 0.8 and 0.85; but it also varies with plant cultivars (Xu, 2002). When plants are stressed, F_v/F_m decreases, depending on the plant cultivar and growth status (Xu, 2002). In this experiment, with the increase of drought stress, F_v/F_m decreased. In the range of RSMC 47% to 80%, the differences between the values of F_v/F_m is not significant. However, when the RSMC was in the range of 25% to 30%, F_v/F_m was decreased significantly. NPQ initially increased as drought stress increased and then decreased; this finding suggests that tomato leaves dissipated excess excitation energy by heat dissipation under mild drought stress, which effectively released photoinhibition and photooxidation triggered by excessive energy absorbed by the PSII reaction center. As drought stress increased, the heat dissipation capacity reduced, and PSII was damaged (Hendrickson et al., 2004).

As the key enzymes scavenging ROS, SOD, POD, and CAT play important roles in regulating ROS accumulation. In the current study, changes in SOD, POD, and CAT activities and associated changes in MDA concentrations in tomato leaves under drought stress were analyzed. With an increase in drought stress, MDA concentration increased; this result indicated that under drought stress, the balance of the antioxidant enzyme system in plants was disrupted, resulting in ROS accumulation and increased peroxidation of membrane lipids, eventually causing membrane damage. Above a certain level of stress, which coincided with the maximum level of tolerance to drought stress of tomato seedlings (corresponding to RSMC = 47% to 52%), drought stress damaged the antioxidant enzyme system and reduced enzyme activity. These results are in consistent with the result of NPQ, which indicate that with the increase of drought stress, the PSII reaction center was damaged by ROS. Several studies have reported on the association between the activity of the antioxidant enzyme system and drought tolerance in plants but with variable and inconsistent results (Li et al., 2010, 2017). This variation may be caused by the activity of the antioxidant enzyme system and antioxidant concentration, among many other factors.

In conclusion, drought stress affects photosynthetic activity by disrupting the energy balance between the capacity to absorb and use light energy during photosynthesis (Pinheiro and Chaves, 2011). In this study, the response of tomato photosynthesis to drought stress transitioned from stomatal limitation to nonstomatal limitation as the stress severity increased. Due to stomatal closure, photosynthesis is decreased and the demand for NADPH in Calvin cycle reduces, which leads to the

excessive reduction of photosynthetic electron transport chains and the blockage of photosynthetic electron transport. In this way, the photosynthetic electron transport branch reaction (with molecular oxygen as the acceptor) is enhanced, resulting in the formation of destructive singlet oxygen (1O_2). Superoxide anion free radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are two kinds of ROS produced during the metabolism of organisms, which have strong oxidative damage to cell membrane lipids. SOD, POD, and CAT are key enzymes for scavenging ROS in cells and play an important role in scavenging ROS. Under normal environmental conditions, the metabolism of reactive oxygen species in plants is in a dynamic balance of continuous production and elimination, which does not easily lead to membrane lipid peroxidation. When drought stress is aggravated, a large amount of reactive oxygen species such as 1O_2 , $O_2^{\cdot-}$ and H_2O_2 accumulated. These reactive oxygen species can be converted into more active $\cdot OH$ via the Haber–Weiss reaction and Fenton reaction. Thus, membrane lipid peroxidation was aggravated, and MDA content of tomato seedlings increased significantly. At the same time, the chloroplast membrane was damaged and the PSII reaction center was damaged, resulting in a significant decrease in photosynthetic rate. The nonstomatal limitation occurred. The decrease of photosynthetic rate will further aggravate the imbalance of reactive oxygen species metabolism.

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