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Effects of different levels of water stress on leaf photosynthetic characteristics and antioxidant enzyme activities of greenhouse tomato

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Abstract

Two greenhouse experiments were conducted in order to investigate the effects of different levels of water stress on gas exchange, chlorophyll fluorescence, chlorophyll content, antioxidant enzyme activities, lipid peroxidation, and yield of tomato plants (*Solanum lycopersicum* cv. Jinfen 2). Four levels of soil water content were used: control (75 to 80% of field water capacity), mild water stress (55 to 60%), moderate water stress (45 to 50%), and severe water stress (35 to 40%). The controlled irrigation was initiated from the third leaf stage until maturity. The results of two-year trials indicated that the stomatal conductance, net photosynthetic rate, light-saturated photosynthetic rate, and saturation radiation decreased generally under all levels of water stress during all developmental stages, while compensation radiation and dark respiration rate increased generally. Water stress also declined maximum quantum yield of PSII photochemistry, electron transfer rate, and effective quantum yield of PSII photochemistry, while nonphotochemical quenching increased in all developmental stages. All levels of water stress also caused a marked reduction of chlorophyll *a*, chlorophyll *b*, and total chlorophyll content in all developmental stages, while activities of antioxidant enzymes, such as superoxide dismutase, peroxidase, and catalase, and lipid peroxidation increased.

Additional key words: drought stress; malondialdehyde; nonstomatal limitation; P_N/PPFD response curve; stomatal limitation.

Introduction

Drought is considered as the main environmental factor limiting plant development and yield worldwide and it becomes increasingly severe in some regions due to the changes in the global climate. Over the past twenty years, considerable achievements have been made in research related to water physiology of crops and fruits (Plaut 1995, Tezara *et al.* 1999, Baquedano and Castillo 2006, Elsheery and Cao 2008). However, most water stress research was performed during short-term periods and did not pay enough attention to developmental stages during which water stress occurred. At present, influence of long-time water stress on biochemical responses of tomato, including gas exchange, chlorophyll (Chl) fluorescence, Chl content, and antioxidant enzymes during different developmental stages still remains poorly understood. As a matter of fact, the responses of plants to water stress have been reported to depend on duration and severity of water deficit, on a stage of development, *etc*. (Bray 1997). Tomato is one of the most important crops in China, planted mainly in semiarid and arid regions. Tomato is sensitive to drought at various growth stages and the yield is easily affected by this stress. Therefore, it is necessary to investigate the effects of different levels of water stress on leaf photosynthetic characteristics

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Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; Chl – chlorophyll; CAT – catalase; CK – control; ETR – electron flow rate; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; I_c – compensation irradiance; I_s – saturation irradiance; k – curve convexity; L_s – stomatal limitation value; MiWS – mild water stress; MoWS – moderate water stress; MDA – malondialdehyde; NPQ – nonphotochemical quenching; P_{max} – light-saturated photosynthetic rate; P_N – net photosynthetic rate; POD – peroxidase; R_D – dark respiration rate; ROS – reactive oxygen species; SeWS – severe water stress; SOD – superoxide dismutase; α – apparent photosynthetic quantum yield; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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and antioxidant enzyme activities of tomato at different development stages in order to understand the mechanisms utilized by tomato plants to tolerate drought stress and to improve the yield.

Photosynthesis inhibition is well known as one of the primary physiological consequences of drought (Chaves 1991, Cornic 1994, Lawlor 1995). Photosynthetic pigments also play an important role in the adaptation and survival of plants under drought, because they control the absorption of energy through Chl and dissipate excess energy through carotenoids (Baquedano and Castillo 2006). Fluorescence can provide insight into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses damage the photosynthetic apparatus (Fracheboud *et al.* 1999, Maxwell and Johnson 2000). Drought stress usually induces the accumulation of reactive oxygen species (ROS) (Smirnoff 1993) and excessive ROS are detrimental because they damage membranes, proteins, Chl, and nucleic acids (Ma *et al*. 2013). To reduce this oxidative damage, plants use complex defense mechanisms, including enzymatic antioxidants, such as superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), and catalase (CAT, EC 1.11.1.6). SOD is a major scavenger of $O_2^{\bullet-}$ by converting it into O_2 and H_2O_2. The latter is then converted by CAT into H_2O and O_2, while POD decomposes H_2O_2 by oxidation of substrates. Plants with lower ROS production and greater activity of antioxidant enzymes potentially better resist to drought stress. Malondialdehyde (MDA) is a marker for lipid peroxidation and shows greater accumulation under environmental stresses (Cakmak and Horst 1991).

In our study, two long-time field experiments were carried out to investigate the effects of different levels of water stress on the leaf gas exchange and Chl fluorescence parameters of tomato in all developmental stages, from seedling to maturation. We measured light response curves to quantify the physical and biochemical limitations to the leaf gas exchange in response to water stress. Leaf Chl content, antioxidant enzyme activities, and lipid peroxidation were also analyzed in different developmental stages under different levels of water stress. Two hypotheses were tested: (*1*) based on the fact that photosynthesis was hampered under drought conditions, net photosynthetic rate (P_N) and photosynthetic rate at light saturation (P_{max}) should decrease with the increased level of water stress in all developmental stages; and (*2*) under different levels of water stress, the reason why P_N decrease would be different.

Materials and methods

Plants and experimental design: The greenhouse experiments were carried out at the greenhouse of Nanjing University of Information Science and Technology, Nanjing City, from September to December in 2012 and repeated from April to July in 2013. Seeds of tomato (*Solanum lycopersicum* cultivar 'Jinfen 2') were germinated in pots containing sand and peat. The experimental plots were randomly designed. Each water treatment was applied to tomato planted in three plots, covering the area of 1.5 × 2.0 m, with two replicated plots, resulting in a total of 12 plots. Concrete curbs (depth of 1.5–2.0 m) and dikes were constructed around each plot to prevent lateral spread of water. The soil was medium loam. The soil water content was adjusted to four levels: control (CK, 75–80% of field capacity), mild water stress (MiWS, 55–60% of field capacity), moderate water stress (MoWS, 45–50% of field capacity), and severe water stress (SeWS, 35–40% of field capacity). The timing of irrigations (frequency and duration) was carried out according to the soil water potential monitored in each treatment using two granular matrix sensors (*EM50*, *Decagon*, USA) at 15-cm depth. Sensors were read hourly, and irrigation started when soil water potential dropped below the target values. The controlled irrigation was initiated from the third leaf stage until maturity, about 120 d. Measurements of gas exchange, Chl fluorescence, Chl content, antioxidant enzyme activities, and MDA content were made on September 28–30, October 15–18, October 25–26, and November 8–10 in 2012, as well as May 9–11, May 23–25, June 13–14, and June 25–26 in 2013, which represented the seedling stage, stage of anthesis, young fruit stage, and maturation stage of tomato plants, respectively. During the experimental period, the average air temperature in the greenhouse was 26.5 ± 4.3°C in 2012 and 21.2 ± 3.5°C in 2013, with the maximum air temperature of 31.8 ± 4.6°C in 2012 and 26.8 ± 4.7°C in 2013, and the minimum air temperature of 20.5 ± 3.4°C in 2012 and 17.0 ± 3.4°C in 2013, and the average relative humidity (RH) was 71.1 ± 10.9% in 2012 and 69.2 ± 8.2% in 2013. The volumetric soil water content of CK varied between 30–32%, while it varied between 22–24, 18–20, and 13–16% under MiWS, MoWS, and SeWS, respectively (Fig. 1).

Gas exchange and P_N/PPFD response curve: P_N, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and ambient CO_2 concentration (C_a) were measured using a portable photosynthesis measurement system (*LI-6400*, *LI-COR Bioscience*, Lincoln, NE, USA). The measurements were made of three plants per treatment. For each measurement, the third fully expanded leaf (from the apex), which was free of chlorosis and/or disease symptoms, was exposed to 1,000 μmol(photon) m^{-2} s^{-1} PPFD, chamber temperature of 25°C, CO_2 concentration of 380 ± 10 μmol(CO_2) mol^{-1}, and RH of 60–70%. The stomatal limitation value (L_s) was then calculated using the following formula: $L_s = 1 - C_i/C_a$ according to Berry and Downton (1982). The P_N/PPFD curves were measured under the conditions inside the sample chamber maintained at 25°C,

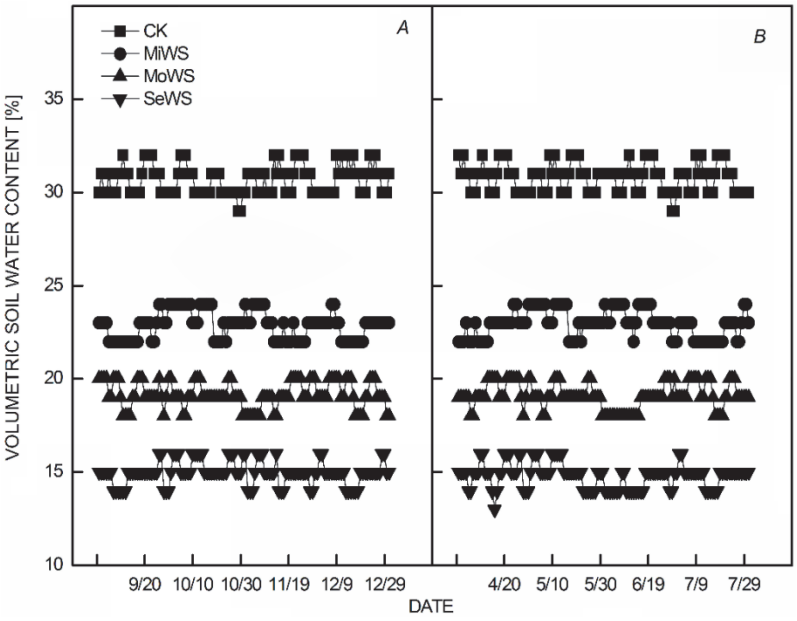

Fig. 1. Variation of volumetric soil water content of the greenhouse soil during experimental period in 2012 (*A*) and in 2013 (*B*). CK – control; MiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

CO_2 concentration of 380 ± 10 μmol(CO_2) mol^{-1}, and 60–70% RH. The light intensity at each point of the curves were 0, 50, 100, 200, 300, 400, 600, 800; 1,000; 1,200; 1,400; 1,600; 1,800; and 2,000 μmol(photon) m^{-2} s^{-1}, respectively. Measurement of photosynthesis was taken after 3–5 min of light exposure, and repeated in triplicate for each treatment. P_N/PPFD curves were modeled by fitting nonrectangular hyperbola to data as described by Prioul and Chartier (1977):

$$P_N = \frac{\alpha \mathrm{PPFD} + P_{max} - \left[(\alpha \mathrm{PPFD} + P_{max})^2 - 4k\alpha \mathrm{PPFD} P_{max} \right]^{0.5}}{2k} - R_D$$

where α is the initial slope or apparent photosynthetic quantum yield (P_N/PPFD at low PPFD); PPFD is the photosynthetic photon flux density [μmol(photon) m^{-2} s^{-1}]; P_{max} is the photosynthetic rate at light saturation [μmol(CO_2) m^{-2} s^{-1}]; k is the curve convexity (dimensionless); and R_D is the dark respiration rate [μmol(CO_2) m^{-2} s^{-1}].

Chl fluorescence parameters were recorded on the fully expanded, penultimate leaf with a portable fluorimeter (*FMS 2*, *Hansatech*, U.K.). Leaflets were acclimated to darkness for 15 min (Murkowski 2001). The minimal fluorescence level (F_0) was measured with the modulated light (0.1 μmol m^{-2} s^{-1}) in the completely dark-adapted state, and the maximal fluorescence level (F_m) was determined after a 0.8 s saturating flashes at 8,000 μmol m^{-2} s^{-1} in dark-adapted leaves. The leaves were then continuously illuminated with white actinic light (600 μmol m^{-2} s^{-1}) for 3 min; the steady state value of fluorescence (F_s) was recorded. A second saturating pulse at 8,000 μmol m^{-2} s^{-1} was imposed to determine maximal fluorescence level in the light-adapted state (F_m'). Using both light and dark fluorescence parameters, the following calculations were made: (*1*) $F_v/F_m = (F_m - F_0)/F_m$; (*2*) $\Phi_{PSII} = (F_m' - F_s)/F_m'$; (*3*) ETR = Φ_{PSII} × PPFD × 0.84 × 0.5; (*4*) NPQ = $(F_m - F_m')/F_m'$, where F_v/F_m is maximum quantum yield of PSII photochemistry, Φ_{PSII} is effective quantum yield of PSII photochemistry, ETR is electron flow rate, and NPQ is nonphotochemical quenching.

Chl content: A known mass of fresh leaves (FM) of the third fully expanded leaf (from the apex) was extracted with a mixture of acetone:ethanol:water (4.5:4.5:1, v/v/v). The exact concentrations of Chl *a* and Chl *b* were measured using spectrophotometry (*Cary 50 UV-VIS*, *Varian*, Victoria, Australia) and the absorbance was measured at 664 and 647 nm. Chlorophyll concentrations were calculated using the equation proposed by Wellburn (1994).

Chl $a = 11.65A_{664}$-$2.69A_{647}$
Chl $b = 20.81A_{664}$-$4.53A_{647}$

where (A_{664}) and (A_{647}) represent absorbance values read at 663 and 645 nm, respectively. Chl *a*, Chl *b*, and Chl (*a*+*b*) contents were expressed as mg g^{-1}(FM). The pigment measurements were performed on the same leaf used for the photosynthesis and Chl fluorescence measurements.

Antioxidant enzyme assays: SOD activity was analyzed according to Beauchamp and Fridovich (1971) with some modifications. A known mass of fresh leaves (FM) was homogenized in the extraction buffer consisting of 50 mM sodium phosphate buffer, pH 7.8, 0.1% (w/v) ascorbate, and 0.05% (w/v) β-mercaptoethanol. The assay mixture of 3 ml contained 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 0.025% (w/v) nitroblue tetrazolium (NBT), and 0.0044% (w/v) riboflavin. The photoreduction of NBT (formation of purple formazan) was measured at 560 nm

(*UV-1800*, *Shimadzu*, Japan). One unit of SOD activity was defined as extract volume that caused 50% inhibition of the photoreduction of NBT. POD activity was assayed by the oxidation of guaiacol in the presence of H_2O_2 (Ghanati *et al.* 2002). The increase in absorbance was recorded at 470 nm. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.3 mM guaiacol, and the enzyme extract. Activity was determined using the extinction coefficient of 6.39 mM^{-1} cm^{-1}. The activity was expressed in $mmol^{-1}$(guaiacol) min^{-1} g^{-1}(FM) of a leaf tissue. CAT activity was measured by monitoring the disappearance of H_2O_2. CAT activity was assayed as described in Díaz-Vivancos *et al.* (2008) by measuring the decrease in absorbance at 240 nm in a reaction mixture consisting of 1.5 ml of 50 mM sodium phosphate buffer (pH 7.8), 0.3 ml 100 mM H_2O_2, and 0.2 ml of the enzyme extract. Activity was determined using the extinction coefficient of 39.4 mM^{-1} cm^{-1}.The activity was expressed as $mmol^{-1}(H_2O_2)$ min^{-1} g^{-1}(FM). Each result of SOD, POD and CAT was the mean of three replications.

Lipid peroxidation was estimated in terms of MDA content. The level of lipid peroxidation was measured with the method of Zheng *et al.* (2006) with slight modifications. Fresh leaves (1.0 g) were grinded in 10% trichloroacetic acid and then centrifuged at 3,000 rpm for 10 min. The supernatant (2 ml) was mixed with 2 ml of 0.6% thiobarbituric acid (TBA) and incubated for 30 min at 100°C to develop the (TBA)2-MDA adduct. The mixture was cooled rapidly in an ice bath. After centrifugation at 5,000 rpm for 10 min, the absorbance was measured at wavelength of 450, 532, and 600 nm. Lipid peroxidation was expressed as μmol g^{-1}(FM) by using the following formula:
$6.45(A_{532} - A_{600}) - 0.56A_{450}$, where A_{532}, A_{600}, A_{450} refers to the absorbance measured at wavelength of 450, 532, and 600 nm, respectively.

Yield: On the harvest day, the fruit number of three tomato plants, which were chosen randomly from each water treatment, was counted. The fresh mass (FM) of tomato fruits was obtained instantly, while the dry mass (DM) was measured after oven drying at 80°C until the constant mass was reached during a period of 72 h.

Statistical analysis: Differences among different levels of water stress for the photosynthetic parameters, Chl fluorescence parameters, Chl content, and antioxidant enzyme activities were tested by a one-way analysis of variance (*ANOVA*) using the statistical software *SPSS 16.0* (*SPSS Inc*., Chicago, IL, USA); the treatment means were compared by using *Duncan*'s multiple range test (DMRT) at $P \leq 0.05$. Data were expressed as mean ± standard error (SE). The P_N/PPFD response curves were fitted using linear regression and nonlinear regression.

Results

Gas exchange and P_N/PPFD response curve: P_N and g_s decreased in response to water stress in all developmental stages; the decline was more pronounced with the increasing level of water stress (Table 1). From the seedling stage to maturation stage, P_N and P_{max} increased with the advancing development under all water treatments (Tables 1, 2). C_i decreased, therefore L_s increased under MiWS in all developmental stages. However, under MoWS and SeWS, C_i increased in all developmental stages, causing L_s decline (Table 1). Compensation irradiance (I_c) increased with the increasing water stress in almost all developmental stages (except for the maturation stage in 2012 and the young fruit stage in 2013). Except for the seedling stage in 2012 and the anthesis stage in 2013, saturation irradiance (I_s) decreased under all levels of water stress in all developmental stages and showed the tendency to drop even more under more severe water stress. R_D generally increased with the increasing water stress in all developmental stages (Table 2).

Chl fluorescence parameters: F_v/F_m declined under water stress in all developmental stages (Figs. 2, 3). As water stress became more severe, it dropped more. Similarly, ETR and Φ_{PSII} decreased under water stress. They declined less under MiWS, while they declined sharply under MoWS and SeWS (Figs. 2, 3). With plant developmental advancing, both parameters increased under almost all water treatments. Contrary to ETR and Φ_{PSII}, NPQ increased under all levels of water stress in all developmental stages and it generally decreased with developmental stage advancing under all levels of water-stressed treatments (Figs. 2, 3).

Chl content: Chl *a*, Chl *b*, and Chl (*a*+*b*) decreased significantly under MoWS and SeWS in all developmental stages (Table 3). With increasing water stress, they decreased more. From the seedling to maturation stage, Chl *a*, Chl *b*, and Chl (*a*+*b*) increased with proceeding age. Similar to Chl *a*, Chl *b*, and Chl (*a*+*b*), the ratio of Chl *a*/*b* also generally decreased under water stress treatments.

Antioxidant enzyme activities and lipid peroxidation: Significant differences among different water treatments were observed in the enzyme activities in all the developmental stages in dependence on water deficit (Table 4). SOD activity was much higher in water stress-treated plants than that of control during all developmental stages; it increased with the increasing degree of water stress. POD and CAT activity changed in the same way as SOD under the water stress treatments. From the seedling to anthesis stage, with the duration of drought prolonged, antioxidant enzyme activities of SOD, POD, and CAT increased. However, all of them dropped sharply at the maturation stage. The MDA content increased under water

Table 1. Effects of water stress on net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and stomatal limitation value (L_s). Data are mean values ± SD (n = 3). Values not followed by *the same letter* within a column indicate significant differences between treatments at $P \leq 0.05$, based on *Duncan*'s means tests. CK – control; WiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

Developmental stage	Treatment	P_N [μmol m^{-2} s^{-1}]		g_s [mol m^{-2} s^{-1}]		C_i [μmol m^{-2} s^{-1}]		L_s	
		2012	2013	2012	2013	2012	2013	2012	2013
Seedling	CK	4.70 ± 0.18^a	4.33 ± 0.22^a	0.21 ± 0.03^a	0.18 ± 0.03^a	330.12 ± 4.03^b	338.18 ± 4.58^b	0.081 ± 0.002^b	0.071 ± 0.001^b
	MiWS	4.11 ± 0.15^a	3.98 ± 0.18^a	0.17 ± 0.02^a	0.15 ± 0.02^b	292.36 ± 3.48^c	322.24 ± 3.18^c	0.182 ± 0.015^a	0.115 ± 0.009^a
	MoWS	2.85 ± 0.09^b	2.99 ± 0.14^b	0.12 ± 0.02^b	0.11 ± 0.02^b	348.42 ± 4.85^b	364.28 ± 3.71^b	0.014 ± 0.001^c	0.013 ± 0.001^b
	SeWS	2.34 ± 0.06^c	1.92 ± 0.07^c	0.08 ± 0.01^c	0.07 ± 0.01^c	372.68 ± 4.61^a	382.38 ± 4.12^a	–0.051 ± 0.001^d	–0.06 4± 0.002^c
Anthesis	CK	14.22 ± 2.43^a	6.70 ± 1.63^a	0.28 ± 0.03^a	0.25 ± 0.03^a	328.53 ± 4.72^b	336.89 ± 3.55^b	0.062 ± 0.002^b	0.085 ± 0.002^b
	WiWS	12.37 ± 2.14^a	5.99 ± 1.34^a	0.22 ± 0.02^a	0.19 ± 0.02^b	306.21 ± 3.83^c	308.74 ± 3.13^c	0.134 ± 0.011^a	0.162 ± 0.011^a
	MoWS	7.51 ± 1.76^b	5.46 ± 1.17^b	0.15 ± 0.02^b	0.14 ± 0.02^b	343.54 ± 4.55^b	353.29 ± 3.83^b	0.023 ± 0.001^b	0.028 ± 0.001^b
	SeWS	5.04 ± 1.12^c	4.67 ± 1.12^c	0.10 ± 0.02^c	0.09 ± 0.01^c	375.46 ± 4.82^a	375.17 ± 4.14^a	–0.076 ± 0.001^c	–0.064 ± 0.002^c
Young fruit	CK	18.69 ± 2.87^a	10.81 ± 1.85^a	0.31 ± 0.03^a	0.29 ± 0.03^a	323.12 ± 4.41^b	323.35 ± 2.95^b	0.061 ± 0.001^b	0.101 ± 0.011^b
	WiWS	15.11 ± 2.34^a	10.06 ± 1.67^a	0.24 ± 0.02^b	0.23 ± 0.02^a	303.35 ± 3.74^c	301.47 ± 2.62^c	0.144± 0.015^a	0.166 ± 0.013^a
	MoWS	11.93 ±1.94^b	9.28 ± 1.18^b	0.19 ± 0.02^b	0.15 ± 0.02^b	345.24 ± 4.28^b	354.59 ± 3.64^b	0.035 ± 0.002^b	–0.017 ± 0.001^c
	SeWS	8.81 ± 1.38^c	8.88 ± 1.06^c	0.13 ± 0.01^c	0.10 ± 0.01^c	386.47 ± 4.79^a	386.43 ± 3.72^a	–0.104 ± 0.01^c	–0.105 ± 0.008^d
Maturation	CK	21.79 ± 2.85^a	15.79 ± 1.69^a	0.35 ± 0.04^a	0.32 ± 0.03^a	334.18 ± 4.51^b	326.13 ± 3.73^b	0.071 ± 0.001^b	0.113 ± 0.012^b
	WiWS	19.30 ± 2.42^a	14.26 ± 1.26^a	0.29 ± 0.03^b	0.26 ± 0.03^a	303.35 ± 3.67^c	303.18 ± 2.43^c	0.164 ± 0.012^a	0.184 ± 0.014^a
	MoWS	17.81 ± 2.14^b	13.04 ± 1.32^b	0.18 ± 0.02^c	0.18 ± 0.02^b	366.47 ± 4.64^b	367.46 ± 3.84^b	–0.032 ± 0.001^b	–0.035 ± 0.001^c
	SeWS	15.50 ± 1.23^c	11.78 ± 1.03^c	0.15 ± 0.02^c	0.10 ± 0.01^c	384.16 ± 4.74^a	393.57 ± 3.56^a	–0.083 ± 0.001^c	–0.092 ± 0.002^d

Table 2. Effects of water stress on light-saturated photosynthetic rate (P_{max}), compensation irradiance (I_c), saturation irradiance (I_s), and dark respiration rate (R_D). Data are mean values ± SD (n = 3). Values not followed by *the same letter* within a column indicate significant differences between treatments at $P \leq 0.05$, based on *Duncan*'s means tests. CK – control; WiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

Developmental stage	Treatment	P_{max} [µmol m^{-2} s^{-1}]		I_c [µmol m^{-2} s^{-1}]		I_s [µmol m^{-2} s^{-1}]		R_D [µmol m^{-2} s^{-1}]	
		2012	2013	2012	2013	2012	2013	2012	2013
Seedling	CK	5.33 ± 0.15^{a}	6.45 ± 0.13^{a}	12.84 ± 1.16^{c}	44.12 ± 2.07^{c}	356.32 ± 8.68^{c}	420.57 ± 5.18^{a}	0.49 ± 0.02^{c}	1.94 ± 0.28^{b}
	MiWS	5.08 ± 0.25^{a}	5.79 ± 0.16^{b}	13.15 ± 1.05^{c}	56.32 ± 2.64^{b}	420.16 ± 6.75^{a}	484.53 ± 6.27^{a}	0.46 ± 0.02^{c}	1.74 ± 0.32^{b}
	MoWS	4.16 ± 0.23^{b}	4.92 ± 0.19^{b}	28.32 ± 2.25^{b}	72.49 ± 3.78^{a}	428.02 ± 4.18^{a}	220.43 ± 4.83^{b}	0.68 ± 0.01^{b}	3.39 ± 0.32^{a}
	SeWS	3.50 ± 0.17^{c}	3.49 ± 0.11^{c}	40.47 ± 3.15^{a}	76.53 ± 4.35^{a}	408.09 ± 3.18^{b}	140.28 ± 3.15^{c}	0.72 ± 0.03^{a}	3.09 ± 0.42^{a}
Anthesis	CK	17.64 ± 2.15^{a}	8.68 ± 0.48^{a}	20.12 ± 1.85^{c}	26.21 ± 1.85^{d}	868.32 ± 8.85^{a}	644.35 ± 10.17^{a}	1.43 ± 0.05^{b}	1.03 ± 0.12^{c}
	MiWS	14.69 ± 1.95^{b}	8.28 ± 0.53^{a}	24.57 ± 2.15^{c}	32.59 ± 2.67^{c}	852.54 ± 6.15^{a}	552.74 ± 9.15^{b}	1.16 ± 0.04^{c}	1.32 ± 0.33^{b}
	MoWS	12.75 ± 1.65^{b}	8.13 ± 0.47^{a}	52.36 ± 4.15^{b}	56.38 ± 3.29^{b}	740.89 ± 5.15^{b}	472.69 ± 6.74^{c}	2.46 ± 0.09^{a}	2.45 ± 0.23^{a}
	SeWS	10.15 ± 0.95^{c}	7.27 ± 0.36^{b}	88.12 ± 6.15^{a}	72.16 ± 4.14^{a}	832.56 ± 4.15^{a}	576.32 ± 6.37^{b}	2.66 ± 0.09^{a}	2.33 ± 0.42^{a}
Young fruit	CK	24.85 ± 3.45^{a}	11.69 ± 1.46^{a}	10.47 ± 0.95^{c}	28.27 ± 3.15^{b}	1,112.23 ± 10.75^{a}	520.28 ± 6.73^{a}	1.32 ± 0.03^{c}	1.08 ± 0.31^{c}
	MiWS	22.09 ± 2.65^{a}	10.87 ± 1.58^{a}	12.36 ± 1.35^{c}	24.23 ± 2.78^{b}	1,072.42 ± 9.88^{a}	492.37 ± 8.28^{b}	1.51 ± 0.03^{c}	1.15 ± 0.33^{b}
	MoWS	16.61 ± 1.85^{b}	9.74 ± 0.87^{b}	52.49 ± 5.75^{b}	16.79 ± 1.15^{c}	916.26 ± 9.48^{b}	500.19 ± 8.42^{b}	2.22 ± 0.14^{b}	1.21 ± 0.12^{b}
	SeWS	14.53 ± 1.15^{b}	9.13 ± 0.96^{b}	84.63 ± 7.35^{a}	40.46 ± 2.91^{a}	920.13 ± 7.65^{b}	480.35 ± 9.84^{b}	2.63 ± 0.18^{a}	1.56 ± 0.33^{a}
Maturation	CK	32.64 ± 4.15^{a}	22.32 ± 2.35^{a}	16.84 ± 1.32^{c}	36.17 ± 2.66^{d}	1,544.85 ± 12.62^{a}	1,100.47 ± 12.45^{a}	1.05 ± 0.24^{c}	2.86 ± 0.34^{b}
	MiWS	29.69 ± 3.15^{a}	18.08 ± 1.88^{b}	28.56 ± 2.87^{b}	44.43 ± 2.19^{c}	1,428.29 ± 11.86^{a}	936.87 ± 10.67^{b}	1.64 ± 0.12^{b}	2.16 ± 0.32^{b}
	MoWS	23.16 ± 2.75^{b}	18.24 ± 1.75^{b}	18.47 ± 1.64^{c}	64.42 ± 3.88^{b}	1,264.74 ± 10.52^{b}	888.62 ± 9.66^{c}	1.84 ± 0.01^{b}	3.96 ± 0.55^{a}
	SeWS	20.84 ± 2.45^{b}	16.18 ± 1.15^{c}	44.52 ± 3.76^{a}	72.64 ± 4.06^{a}	1,156.39 ± 10.68^{c}	812.41 ± 8.71^{c}	3.49 ± 0.22^{a}	3.49 ± 0.62^{a}

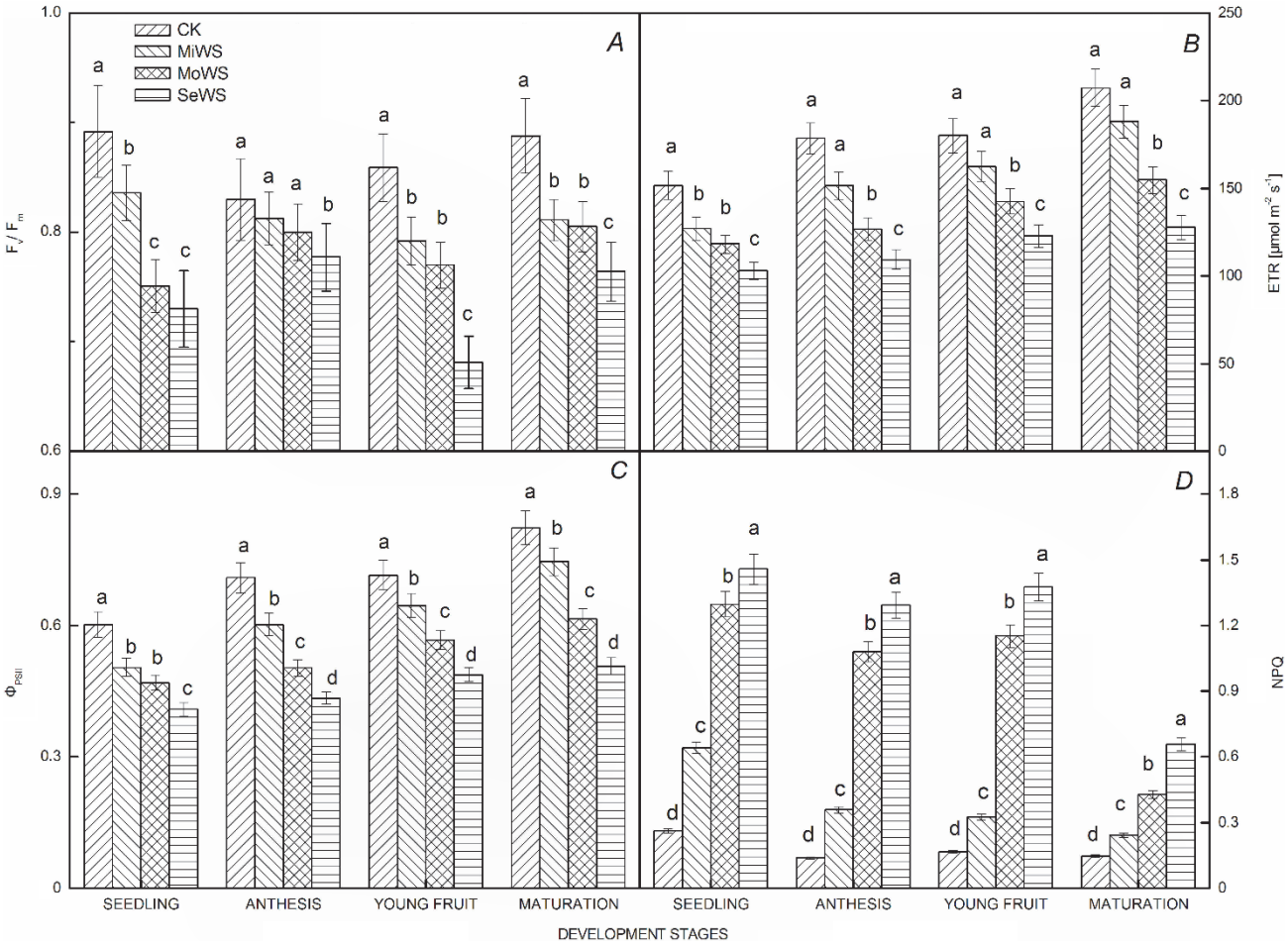

Fig. 2. Effect of different levels of water stress on maximum quantum yield of PSII photochemistry (F_v/F_m) (*A*), electron flow rate (ETR) (*B*), effective quantum yield of PSII photochemistry (Φ_{PSII}) (*C*), and nonphotochemical quenching (NPQ) of tomato at different developmental stages during 2012 experimental period. Values are mean ± SE ($n = 3$) and differences between the means were compared by *Duncan*'s multiple range test. *Different letters* indicate significant differences with control groups at $P \leq 0.05$. CK – control; MiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

stress, especially under MoWS and SeWS, in all developmental stages compared with control. From the seedling to maturation stage, it increased with the advanced plant age.

Yield: Significant differences among different water stress treatments were observed in the yield of tomato plants. In 2012, the fruit number per plant decreased by 16.3, 37.2, and 57.0% under MiWS, MoWS, and SeWS compared with CK, respectively. The fresh mass per fruit was reduced by 10.0, 29.1, and 47.8% and the dry mass per fruit decreased by 9.8, 24.2, and 32.6% under MiWS, MoWS, and SeWS compared with CK, respectively. The fresh fruit mass per plant declined by 24.4, 55.4, and 77.6% and the dry fruit mass per plant decreased by 19.5, 49, and 70.4% under MiWS, MoWS, and SeWS compared with CK, respectively (Table 5). The experiment in 2013 showed the same trend as that in 2012.

Discussion

Water stress is an important environmental factor that could influence the physiological and biochemical characteristics of plants (Ren *et al.* 2007). Photosynthesis is a highly regulated and integrated process. Photosynthetic machinery evolved to maximize the use of light, optimize the use of carbon resources, and minimize the damaging effects of excessive energy. The photosynthetic process is highly sensitive to any change in the environment (Yin *et al.* 2006). In our experiment, P_N, P_{max}, g_s, and I_s decreased, while I_c and R_D increased under all levels of water stress in all developmental stages in accordance with Yin *et al.* (2006), Sofo *et al.* (2009) and Peri *et al.* (2011). Stomatal conductance is the first parameter affected by water stress because plant hormones, especially abscisic acid, could act as root-to-shoot signals triggering stomatal closure (Cornic 2000). Under MiWS, C_i decreased and L_s

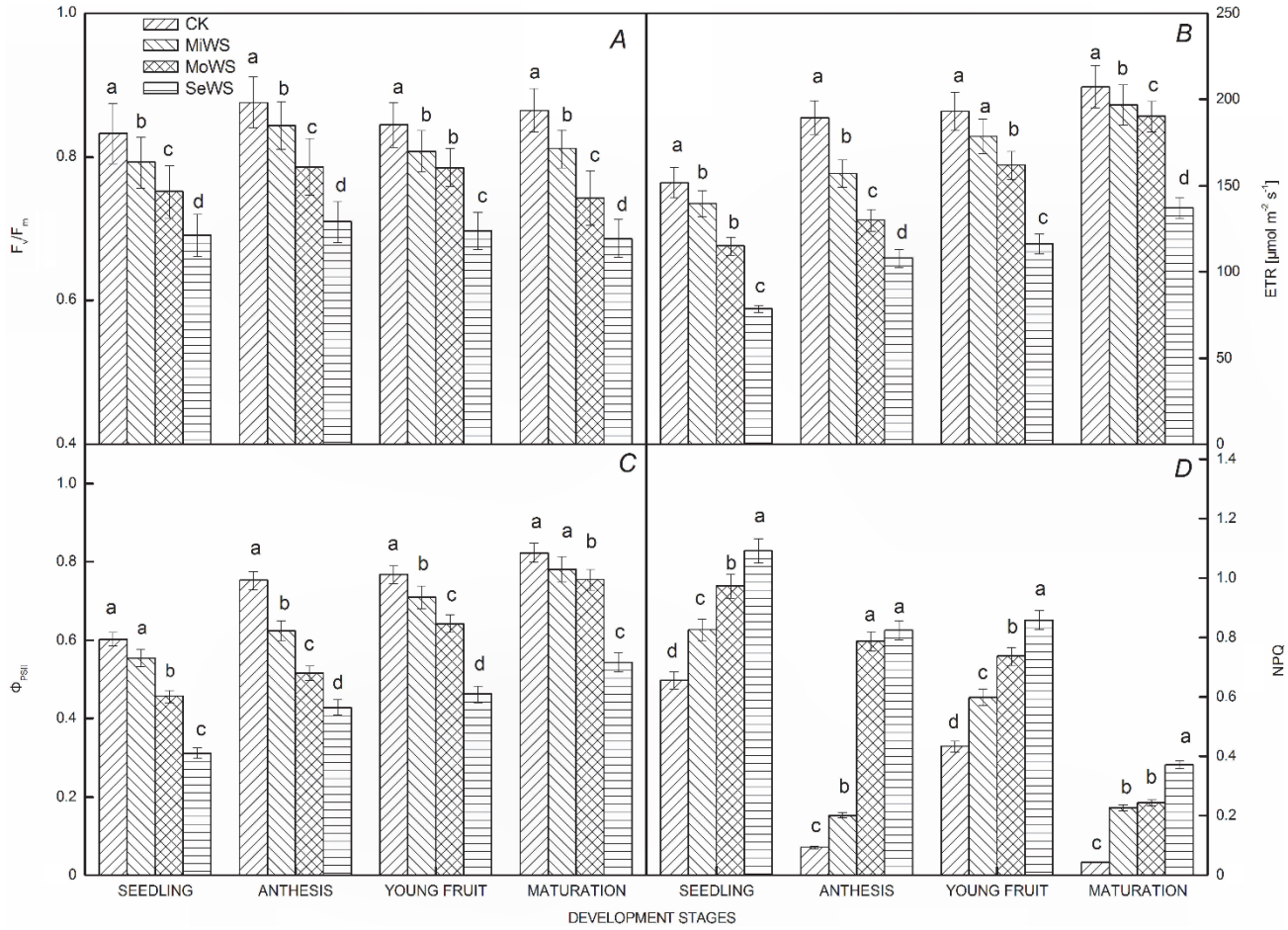

Fig. 3. Effect of different levels of water stress on maximum quantum yield of PSII photochemistry (F_v/F_m) (*A*), electron flow rate (ETR) (*B*), effective quantum yield of PSII photochemistry (Φ_{PSII}) (*C*), and nonphotochemical quenching (NPQ) (*D*) of tomato at different developmental stages during 2013 experimental period. Values are mean ± SE ($n = 3$) and differences between the means were compared by the *Duncan*'s multiple range test. *Different letters* indicate significant differences from control groups at $P \leq 0.05$. CK – control; MiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

increased, thus, stomatal limitation was the main reason of the P_N decline. However, C_i increased and L_s decreased under MoWS and SeWS, therefore the main reason of the decrease in P_N was nonstomatal limitation under MoWS and SeWS; it resulted mainly from photosystem damage, inhibition of Rubisco and other enzyme activities (Cornic and Fresneau 2002, Vandoorne *et al.* 2012), and the increasing R_D and photorespiration. Increasing I_c and decreasing I_s would reduce the amount of available light energy for phosynthesis, and increasing of R_D would make plants consume more photosynthetic products. With the advancing plant development, P_N and P_{max} increased significantly under the same level of water stress. The reason might be that plants in advanced developmental stages have higher photosynthetic capacity. R_D also increased in advanced developmental stages (the young fruit and maturation stage).

F_v/F_m showed the proceeding decrease with intensifying water stress, which was in agreement with Baquedano and Castillo (2006) and Galmés *et al.* (2007). The reason might be in inhibited activity of PSII that caused the reduction in Φ_{PSII} in agreement with Baquedano and Castillo (2006) and Xu and Zhou (2006). Many previous studies used a decline in F_v/F_m and Φ_{PSII} as reliable indicators of photoinhibition in response to stress (Wagner and Dreyer 1997, Lu and Zhang 1998). ETR decreased under water stress in all the developmental stages. It has been assumed that downregulation of PSII efficiency might be a strategy, by which the stressed plants down-regulate the photosynthetic electron transport to keep the production of adenosine triphosphate and nicotinamide adenine dinucleotide phosphate in equilibrium with the decreased CO_2 assimilation capacity (Baker and Rosenqvist 2004). It corresponded with the findings of Flexas *et al.*(2004). NPQ increased under water stress in all developmental stages in agreement with other studies (Medrano *et al.* 2002, Tezara *et al.* 2005, Wu *et al.* 2008, Elsheery and Cao 2008). Higher NPQ under water stress indicates that plants efficiently dissipate energy trapped at PSII in the form of heat (Wu *et al.* 2008) and it protects

Table 3. Effects of water stress on content of chlorophyll (Chl) *a*, Chl *b*, Chl (*a*+*b*), and the ratio Chl *a*/*b*. Data are mean values ± SD (n = 3). Values not followed by *the same letter* within a column indicate significant differences between treatments at $P \leq 0.05$, based on *Duncan*'s means tests. CK – control; MiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

Developmental stage	Treatment	Chl *a* [mg g^{-1}(FM)]		Chl *b* [mg g^{-1}(FM)]		Chl (*a*+*b*) [mg g^{-1}(FM)]		Chl *a*/*b*	
		2012	2013	2012	2013	2012	2013	2012	2013
Seedling	CK	7.13 ± 0.85^{a}	6.61 ± 0.54^{a}	2.96 ± 0.54^{a}	2.53 ± 0.68^{a}	10.09 ± 0.88^{a}	9.14 ± 0.66^{a}	2.41 ± 0.33^{a}	2.61 ± 0.41^{a}
	MiWS	6.91 ± 0.82^{a}	5.54 ± 0.76^{b}	3.01 ± 0.25^{a}	2.40 ± 0.46^{a}	9.92 ± 1.02^{a}	7.95 ± 0.13^{b}	2.30 ± 0.12^{a}	2.31 ± 0.22^{b}
	MoWS	5.57 ± 0.92^{b}	5.29 ± 2.48^{b}	2.45 ± 0.22^{b}	2.30 ± 0.99^{b}	8.03 ± 1.02^{b}	7.59 ± 0.23^{b}	2.27 ± 0.64^{b}	2.30 ± 0.44^{b}
	SeWS	4.93 ± 0.71^{c}	5.04 ± 1.33^{b}	2.29 ± 0.12^{c}	1.88 ± 0.63^{c}	7.23 ± 1.89^{c}	6.92 ± 0.25^{c}	2.15 ± 0.32^{b}	2.68 ± 0.24^{a}
Anthesis	CK	7.75 ± 0.90^{a}	6.32 ± 0.75^{a}	2.88 ± 0.513^{a}	2.86 ± 0.52^{a}	10.63 ± 1.25^{a}	9.18 ± 0.66^{a}	2.69 ± 0.32^{a}	2.47 ± 0.14^{a}
	MiWS	7.38 ± 0.64^{a}	5.88 ± 0.11^{b}	2.67 ± 0.24^{b}	2.55 ± 0.24^{b}	10.06 ± 1.54^{a}	8.43 ± 0.15^{b}	2.76 ± 0.43^{a}	2.31 ± 0.42^{b}
	MoWS	6.33 ± 0.91^{b}	5.65 ± 0.92^{c}	2.99 ± 0.23^{b}	2.47 ± 0.22^{b}	9.33 ± 1.37^{b}	8.13 ± 0.65^{b}	2.12 ± 0.17^{b}	2.29 ± 0.24^{b}
	SeWS	5.59 ± 0.82^{c}	5.13 ± 0.21^{d}	1.95 ± 0.12^{c}	2.23 ± 0.12^{c}	7.54 ± 1.23^{c}	7.36 ± 0.29^{c}	2.87 ± 0.25^{a}	2.30 ± 0.46^{b}
Young fruit	CK	9.03 ± 0.78^{a}	8.56 ± 0.74^{a}	2.52 ± 0.12^{a}	2.95 ± 0.61^{a}	11.55 ± 1.45^{a}	11.50 ± 1.25^{a}	3.58 ± 0.83^{a}	3.23 ± 0.44^{a}
	MiWS	8.00 ± 0.61^{b}	8.32 ± 0.16^{a}	2.92 ± 0.55^{b}	2.63 ± 0.22^{b}	10.92 ± 1.69^{b}	10.95 ± 0.99^{b}	2.74 ± 0.54^{b}	3.16 ± 0.45^{a}
	MoWS	7.16 ± 0.92^{c}	7.84 ± 0.72^{b}	2.53 ± 0.23^{b}	2.52 ± 0.21^{b}	9.69 ± 0.99^{b}	10.37 ± 1.02^{b}	2.83 ± 0.36^{b}	3.11 ± 0.37^{b}
	SeWS	6.27 ± 0.93^{d}	6.32 ± 0.21^{c}	1.65 ± 0.25^{c}	2.25 ± 0.12^{c}	8.21 ± 1.24^{c}	8.57 ± 0.86^{c}	3.23 ± 0.95^{a}	2.95 ± 0.41^{b}
Maturation	CK	10.25 ± 0.83^{a}	9.58 ± 0.76^{a}	3.37 ± 0.20^{a}	2.73 ± 0.56^{a}	13.64 ± 0.89^{a}	12.32 ± 1.24^{a}	3.04 ± 0.62^{b}	3.51 ± 1.23^{a}
	MiWS	9.51 ± 0.60^{b}	9.08 ± 0.12^{b}	3.24 ± 0.24^{b}	2.68 ± 0.24^{b}	12.76 ± 1.28^{b}	11.76 ± 0.98^{b}	2.94 ± 1.13^{a}	3.39 ± 0.98^{a}
	MoWS	8.57 ± 0.92^{c}	8.73 ± 0.92^{c}	3.21 ± 0.22^{b}	2.53 ± 0.22^{b}	11.78 ± 1.86^{b}	11.26 ± 1.54^{b}	2.67 ± 0.21^{b}	3.45 ± 0.88^{a}
	SeWS	6.80 ± 0.85^{d}	6.84 ± 0.21^{d}	2.38 ± 0.66^{c}	2.33 ± 0.12^{c}	9.17 ± 1.45^{c}	9.17 ± 1.21^{c}	2.86 ± 0.46^{b}	2.94 ± 0.63^{b}

Table 4. Effects of water stress on superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA). Data are mean values ± SD (n = 3). Values not followed by *the same letter* within a column indicate significant differences between treatments at $P \leq 0.05$, based on *Duncan*'s means tests. CK – control; MiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

Developmental stage	Treatment	SOD [units g^{-1}(FM)]		POD [$mmol^{-1}$(guaiacol) $min^{-1}g^{-1}$(FM)]		CAT [$mmol^{-1}(H_2O_2)$ $min^{-1}g^{-1}$(FM)]		MDA [µmol g^{-1}(FM)]	
		2012	2013	2012	2013	2012	2013	2012	2013
Seedling	CK	152.64 ± 4.25^a	182.64 ± 4.65^a	61.59 ± 4.34^a	71.89 ± 5.71^a	63.68 ± 4.54^a	77.71 ± 5.09^a	0.009 ± 0.001^a	0.011 ± 0.003^a
	MiWS	187.66 ± 3.42^b	213.66 ± 3.64^b	65.78 ± 4.62^a	77.84 ± 5.94^a	75.02 ± 5.31^b	94.58 ± 6.14^b	0.012 ± 0.002^b	0.014 ± 0.005^a
	MoWS	232.13 ± 4.64^c	242.13 ± 3.75^c	77.34 ± 5.47^b	94.00 ± 6.61^b	95.85 ± 6.49^c	121.83 ± 6.87^c	0.015 ± 0.002^c	0.019 ± 0.007^b
	SeWS	274.62 ± 3.38^d	294.62 ± 2.33^d	88.19 ± 6.73^c	114.50 ± 7.46^c	102.05 ± 6.56^c	144.97 ± 7.13^d	0.017 ± 0.003^c	0.021 ± 0.006^c
Anthesis	CK	186.35 ± 4.31^a	238.35 ± 4.51^a	71.58 ± 5.29^a	82.69 ± 5.91^a	95.92 ± 6.57^a	199.54 ± 9.53^a	0.016 ± 0.002^a	0.021 ± 0.002^a
	MiWS	254.54 ± 3.42^b	267.54 ± 3.62^b	82.54 ± 6.53^a	94.15 ± 6.41^a	191.40 ± 8.63^b	237.69 ± 9.88^b	0.019 ± 0.001^b	0.028 ± 0.003^a
	MoWS	298.17 ± 2.54^c	311.17 ± 3.78^c	100.11 ± 7.47^b	123.33 ± 7.51^b	327.33 ± 12.64^c	370.86 ± 12.86^c	0.021 ± 0.002^c	0.034 ± 0.004^b
	SeWS	325.54 ± 3.67^c	409.54 ± 3.43^c	134.99 ± 8.77^c	163.26 ± 8.29^c	417.06 ± 13.73^d	506.27 ± 15.61^d	0.022 ± 0.003^c	0.044 ± 0.005^c
Young fruit	CK	220.83 ± 3.18^a	278.85 ± 3.36^a	96.57 ± 7.05^a	139.49 ± 7.37^a	156.32 ± 8.49^a	312.33 ± 10.83^a	0.017 ± 0.002^a	0.023 ± 0.004^a
	MiWS	273.37 ± 2.47^b	313.37 ± 3.27^b	145.59 ± 8.71^b	158.46 ± 8.56^b	338.46 ± 12.61^b	407.44 ± 13.74^b	0.026 ± 0.003^b	0.029 ± 0.008^a
	MoWS	309.54 ± 3.64^c	369.54 ± 2.47^c	192.03 ± 9.63^c	219.95 ± 10.61^c	390.78 ± 13.43^c	506.42 ± 15.69^c	0.038 ± 0.002^c	0.045 ± 0.009^b
	SeWS	365.39 ± 4.78^d	452.36 ± 2.57^d	224.80 ± 11.46^d	269.98 ± 11.74^d	562.26 ± 15.67^d	664.15 ± 16.65^d	0.046 ± 0.003^d	0.066 ± 0.013^c
Maturation	CK	187.26 ± 3.55^a	210.46 ± 3.69^a	86.48 ± 5.67^a	113.18 ± 6.59^a	124.74 ± 6.53^a	181.47 ± 8.64^a	0.022 ± 0.003^a	0.027 ± 0.005^a
	MiWS	198.59 ± 4.64^a	238.32 ± 3.64^a	100.16 ± 7.84^a	128.63 ± 6.85^a	129.67 ± 6.81^a	220.74 ± 9.39^b	0.032 ± 0.004^b	0.043 ± 0.006^b
	MoWS	228.02 ± 2.67^b	278.19 ± 2.41^b	111.26 ± 8.52^b	145.04 ± 7.49^b	172.59 ± 7.53^b	282.47 ± 10.62^c	0.048 ± 0.009^c	0.054 ± 0.007^c
	SeWS	239.54 ± 3.74^b	319.43 ± 2.37^b	123.83 ± 8.74^c	180.37 ± 7.81^c	215.21 ± 8.72^c	400.34 ± 14.59^d	0.054 ± 0.012^c	0.071 ± 0.009^d

Table 5. Effects of water stress on mass of per fruit and mass of fruits per plant. Data are mean values ± SD (n = 3). Values not followed by *the same letter* within a column indicate significant differences between treatments at $P \leq 0.05$, based on *Duncan*'s means tests. CK – control; MiWS – mild water stress; MoWS – moderate water stress; SeWS – severe water stress.

Treatment	Fruit number per plant		Mass per fruit [g] Fresh mass		Dry mass		Mass of fruits per plant [g] Fresh mass		Dry mass	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
CK	9.32 ± 0.53^a	8.61 ± 0.45^a	245.64 ± 2.57^a	231.64 ± 2.45^a	25.05 ± 0.12^a	23.86 ± 0.35^a	2,284.23 ± 4.25^a	1,986.47 ± 4.94^a	231.64 ± 4.38^a	200.25 ± 3.64^a
MiWS	7.45 ± 0.62^b	6.24 ± 0.36^b	221.73 ± 2.87^b	208.52 ± 1.89^b	22.52 ± 0.34^b	21.17 ± 0.24^a	1,855.47 ± 4.12^b	1,502.63 ± 4.65^b	186.45 ± 3.65^b	151.84 ± 2.78^b
MoWS	6.28 ± 0.48^b	5.46 ± 0.41^b	187.31 ± 3.64^c	164.38 ± 2.78^c	18.91 ± 0.42^c	15.84 ± 0.62^b	1,160.27 ± 3.74^c	885.57 ± 3.84^c	118.14 ± 2.38^c	90.54 ± 2.31^c
SeWS	4.44 ± 0.39^c	3.77 ± 0.63^c	153.46 ± 3.31^d	120.81 ± 2.32^d	16.85 ± 0.61^c	11.61 ± 0.43^c	673.68 ± 3.09^d	444.42 ± 3.24^d	68.56 ± 1.78^d	46.35 ± 1.88^d

photosynthetic apparatus against photoinhibition.

Chl *a*, Chl *b*, and Chl (*a*+*b*) decreased under water stress in all developmental stages in accordance with Loggini *et al.* (1999), Younis *et al.* (2000), and Elsheery *et al.* (2008). Some authors have suggested that the decrease in pigment contents in stressed plants could be related to pigment photooxidation because of excess energy absorbed (Powles 1984, Krause 1988), while others have proposed that it could be an adaptive mechanism to prevent the absorption of excessive energy (Elvira *et al.* 1998).

SOD activity increased under drought stress in all developmental stages when compared with control plants. Similar findings were presented under drought stress in higher plants, such as rice (Wang *et al.* 2005), wheat (Shao *et al.* 2005), and maize (Jiang and Zhang 2002). POD also increased under water stress in all developmental stages when compared with control plants. POD plays a role in decreasing H_2O_2 content, eliminating peroxidation of membrane lipids, and maintaining cell membrane integrity (Jaleel *et al.*2008). Increased POD activity was also reported in drought stressed soybean (Zhang *et al.* 2006) and chives plants (Egert and Tevini 2002). Similarly to SOD and POD, the activity of CAT also increased in all developmental stages under drought stress when compared with control plants. Similar results were reported under drought stress in wheat (Shao *et al.* 2005) and *Phaseolus acutifolius* (Türkan *et al.* 2005). MDA content increased under drought stress in all developmental stages when compared with control. Similar findings were presented under drought stress in rice (Farooq *et al.* 2009). It is well known that peroxidation of lipid membranes of higher plants reflects free radical-induced oxidative damage at the cellular level under abiotic stress (Hernández *et al*. 1995, Nouairi *et al.* 2009). Therefore, water stress caused cellular damage to tomato plants, especially under MoWS and SeWS as the increased MDA content showed.

Water stress resulted in a reduction of tomato yield, both fresh mass and dry mass, because P_N decreased, while R_D and photorespiration increased in response to all levels of water stress. Therefore, assimilation decreased and dissimilation increased, resulting in the lower productivity.

In conclusion, our results proved that water stress decreased g_s, P_N, P_{max}, Chl content, and I_s, and increased I_c and R_D in all developmental stages of tomato plants. L_s increased under MiWS and decreased under MoWS and SeWS in all developmental stages, suggesting that stomatal limitation to photosynthesis was dominating under MiWS, but nonstomatal limitation was dominating under MoWS and SeWS. Water stress also decreased F_v/F_m, Φ_{PSII}, and ETR, while increased NPQ during all developmental stages. Under water stress, antioxidant enzyme activities, such as SOD, POD, and CAT, increased in order to eliminate the increasing free radicals and ROS. Water stress caused membrane injuries as the MDA content increased.

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