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Full Length Article

Effects of Water Stress on Growth, Photosynthesis, Root Activity and Endogenous Hormones of *Cucumis sativus*

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Abstract

A greenhouse experiment was conducted to investigate the effects of water stress on growth, photosynthesis, root activity and endogenous hormones of cucumber (*Cucumis sativus* L. var. Boxin 525). Three water stress levels were involved in the experiment: mild water stress (T1, relative soil water content (RSWC) was 60–70%), moderate water stress (T2, RSWC was 50–60%), and severe water stress (T3, RSWC was 35–45%); cucumber plants being irrigated adequately served as the control (CK, RSWC was 70–80%). The results suggested that the plant height, leaf area per plant, root length and root dry weight decreased with increasing water stress levels. The plant height at 60 days after transplanting (DAT) decreased by 5.00%, 25.13%, and 49.75% under T1, T2 and T3, respectively, compared to CK. The leaf area per plant under CK was more than double of that under severe water stress. The average decreasing amplitude of root length was 26.26, 42.91 and 66.92% with increasing water stress levels, compared to CK. In the same way, root dry weights were decreased on average by 13.89, 30.88 and 47.52%, respectively. Water stress reduced the net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (E) at 20, 30, 40, 50 and 60 DAT under T1, T2, and T3. Compared to CK, the average Pn of cucumber leaves over the entire growth stage decreased by 9.27%, 27.86% and 42.37%, respectively. The gs values under CK were almost 1.18, 1.20 and 1.36 times of that under T1, T2 and T3, respectively. The average E in CK plants was 10.23 mmol (H_2O) m^{-2} s^{-1}, while it ranged from 5.83 to 9.22 mmol (H_2O) $m^{-2}s^{-1}$ Under different water stress conditions. The root activity of cucumber plants declined by 13.04%, 26.57% and 34.41% with increasing water stress levels, respectively. The zeatin nucleoside (ZT) in cucumber leaves decreased with the aggravation of soil water stress, with the average reduction amplitude being 14.65%, 28.93% and 38.62% compared to CK, respectively, for T1, T2 and T3, while the abscisic acid (ABA) contents in cucumber leaves rose as the water stress increased at 20, 30, 40, 50 and 60 DAT. Compared with CK, the ABA contents were elevated by 58.45 and 107.66%, respectively, under moderate water stress and severe water stress. Mild water stress reduced the gibberellin (GA_3) concentration, except at 20 DAT. The GA3 levels reached their peaks at 60 DAT for all treatments, with the peak value for T1 rising by 5.73%, and those for T2 and T3 reducing by 21.53 and 36.21%, respectively, compared to CK. The auxin (IAA) contents increased under both mild water stress and moderate water stress over the whole stage, while those under severe water stress were the lowest among all treatments. Water stress reduced the ZT/ABA, IAA/ABA, ZT/IAA, and (GA_3 + ZT + IAA)/ABA ratios in cucumber leaves. Compared with CK, the GA_3/ABA ratio decreased under moderate water stress and severe water stress, while no significant difference in the GA_3/ABA ratio was found between mild water stress and CK, with an exception at 20 DAT. Water stress decreased growth traits and root activity of cucumber plants, affected leaf gas exchange parameters. The changes of endogenous hormone and the ratio between different endogenous hormones could resist the adversity of water stress and affect the growth and development of crops.

Keywords: Water stress; Photosynthetic parameters; Root system; Endogenous hormone; Dynamic balance

Introduction

Drought is one of the most common natural disasters around the world due to climate change and the spatial and temporal maldistribution of water resources (Chaves and Oliveira, 2004). These threats have led to considerable research on water availability. Water stress changes the morphological structure and function of plants, and inhibits plant growth and crop yield. Therefore, it is important to investigate plants' responses and adaptions to water stress (Wilkinson and Davies, 2010).

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Growth is the macroscopic response of plants to water stress; most studies have indicated that water stress is adversely affected plant growth (Xie *et al*., 2003; Irving and Drost, 2015). Sacała *et al.* (2008) reported that water stress decreased seedling shoot and root dry mass of cucumber. Under drought conditions, water stress led to less dry matter being allocated to red beet root, and decreased the relative root and leaf growth rates (Stagnari *et al*., 2014). The weights of cotton lint and seeds were also reduced as the level of irrigation decreased (Davis *et al*., 2014). Approximately 90% of the dry matter accumulation of plants is derived from photosynthesis (An *et al*., 2000). Water stress impairs the physiological properties of plants, which leads to a reduction in photosynthetic capacity.

Numerous studies investigated that water stress affects plant photosynthesis (Li *et al*., 2007; Lang and Wang, 2015; Samancioglu *et al.*, 2016). Jaiphong *et al.* (2016) suggested that drought conditions reduced CO_2 assimilation and transpiration rate (through stomatal closure) in sugarcane plants. Liu *et al.* (2017) indicated that when the soil water content (RWC) was between 38.9 and 70.5%, stomatal limitation was responsible for the reduced net photosynthetic rate (P_N). When RWC was lower than 38.9%, nonstomatal limitation played a key role in restricting P_N. Lawlor and Cornic (2002) indicated that reducing the relative soil water content (RSWC) from 100% to 75% did not affect the net photosynthetic rate (P_N) in mesophytic C_3 plant leaves, but the stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) decreased. When RSWC fell below 75%, P_N and g_s declined, whereas Ci increased. g_s played a key role in P_N at the start, but as the RSWC fell, metabolic limitations became more important. It has also been reported that gas exchange parameters were more affected in drought-sensitive wheat cultivars (Wu and Bao, 2011). Root activity reflects the ability of root absorption and metabolism, and directly influences stress resistance and the growth of plants (Kramer and Boyer, 1995). Most research has indicated that water stress significantly affects root activity (Li *et al*., 2010; Wang *et al*., 2012).

Endogenous hormones have been shown to play roles in controlling the developmental processes of plants. Zeatin nucleoside (ZT) and auxin (IAA) are produced during active cell division. ZT is a cytokinin that can promote cell division and expansion, and has a great effect on delaying senescence (Kokubun *et al*., 2004). IAA promotes the growth of top plant tissues, leaf synthesis, and root elongation, which can be advantageous for the response of plants to water stress (Zhao *et al*., 2007). Gibberellin (GA_3) is known to be conductive to stem elongation and flower bud differentiation. Abscisic acid (ABA) is an important internal regulation phytohormone, which can promote stomatal closure and thus play a crucial role in drought resistance (Wilkinson and Davies, 2002; Jiang and Hartung, 2008). To date, most studies have indicated that ZT decreases and ABA increases under water stress (Iqbal *et al*., 2012; Saeedipour, 2012). However, IAA and GA_3 show no consistent changes (Koshita and Takahara, 2004; Liu and Ye, 2016).

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae, which is suitable for mild, wet environments and is sensitive to drought and water logging (Sun *et al*., 2003; Zhang *et al*., 2017), therefore, scientific water management is the key to determining production yield. Studies have shown that, ABA, IAA, GA_3 and ZT can affect the cucumber plant antioxidant system, photosynthesis and root growth to reduce drought damage to cucumber and improve plant tolerance to water stress (Boldaji *et al*., 2012; Karataş *et al*., 2014). There for, the system research in the changes and equilibrium of plant hormones under water stress and its relationship with the plant growth is of great significance to adjust the cucumber growth, improve the yield and quality under water stress. In this study, we conducted a water stress experiment on cucumber plants to study the effects of water stress on cucumber growth, photosynthesis, root activity, and endogenous hormones, aiming to reveal the response mechanism of cucumber to water stress in a microcosmic way. This study was expected to provide a scientific basis for water management and disaster reduction in greenhouse cucumber production, thereby improving cucumber yield and quality.

Materials and Methods

Plant Materials and Experimental Design

In order to control the relative soil water content and the weather conditions, the experiment was conducted from October 1 to December 1, 2016 in the greenhouse at Nanjing University of Information Science & Technology (32°12′N, 118°42′E), Jiangsu Province, China. The soil contained 189 g (clay) kg^{-1}, 716 g (silt) kg^{-1}, and 95 g (sand) kg^{-1}, with its bulk density being 1.32 g cm^{-3}. The basal soil fertility was as follows: 18.4 g (organic matter) kg^{-1}, 0.79 g (total nitrogen) kg^{-1}, 80.5 mg (available nitrogen) kg^{-1}, 0.75 g (total phosphorus) kg^{-1}, 27.6 mg (available phosphorus) kg^{-1}, and 125.2 mg (available potassium) kg^{-1}. The field water capacity was 32.45 m^3 m^{-3} and the wilting coefficient was 11.35 m^3 m^{-3}. The cucumber (*Cucumis sativus* L. var. Boxin 525) seeds were sown in a feeding block on September 1 and then transplanted to three water controlled cement pool seedbeds (length 12 m, width 1 m) on September 20 when the cucumber plants had two mature leaves and one interior leaf. Every seedbed was divided into four plots. To prevent water leakage, a waterproof carpet was used to separate different plots. The water stress experiment commenced after the seedlings had recovered from transplanting. Three water stress levels were included in the experiment: mild water stress (T1, RSWC was 60–70%), moderate water stress (T2, RSWC was 50–60%), and severe water stress (T3, RSWC was 35–45%); cucumber plants with adequate irrigation served as

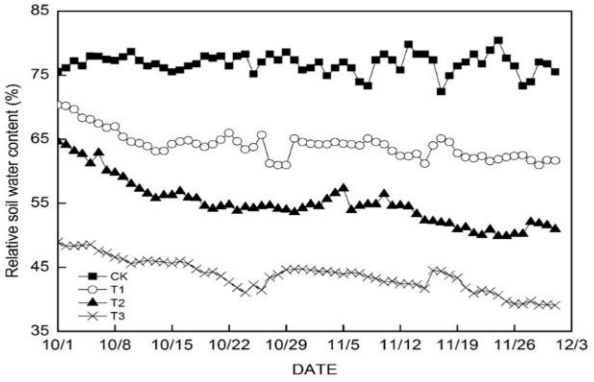

Fig. 1: Comparison of relative soil water content (RSWC) under different water conditions. CK – normal irrigation; T1 – mild water stress; T2 – moderate water stress; T3 – severe water stress

the control (CK, RSWC was 70–80%). The experiment was arranged as a completely randomised design, with three replications per treatment. There were 12 independent plots in total and each plot consisted of seven plants with a plant spacing of 40 cm. Before transplanting, fertiliser (N: P: K= 10:9:8) was applied at a level of 70 g per plot. Soil moisture sensors (ECH$_2$O, Decagon Devices Inc., USA) were used in each plot to measure the soil water content as volumetric water content (m^3 m^{-3}); the data were collected and stored by a portable data logger (EM50, Decagon Devices Inc., USA) every 30 min. The measured volumetric water content of each treatment was then converted to RSWC (%) (Fig. 1). The max and min temperature of greenhouse during the course of study was controlled at 27.0°C and 15.0°C, respectively. Based on the set point of soil water content and the measured soil water content, the water demand of each plot was calculated and daily water compensation was implemented to control the water content within the treatment ranges using drip irrigation. Independent trials were carried out in each plot.

Determinations of Growth Traits

Plant height, leaf area per plant, root length, and root dry weight were measured in the growth analysis. Three plants per plot were sampled at ten day intervals (10, 20, 30, 40, 50, and 60 days after transplanting (DAT)). Plant height was considered to be the natural height from the base to the top of the plant. Leaf area was determined by a Leaf Area Meter (LI-3000, LI-COR Inc., USA). Root image capture system (BTC7 ICAP, Bartz Technology Corporation, USA) was used to take root photographs, then root system analyser (BTC7 ICAP, Bartz Technology Corporation, USA) was applied to quantitatively analyse root length. Root dry weights were determined after oven-drying at 80°C until a constant weight was reached.

Leaf Gas Exchange Parameters

Three cucumber plants per each plot were selected to determine leaf gas exchange parameters at 20, 30, 40, 50 and 60 DAT, respectively. Leaf gas exchange parameters were measured by the LI-6400 portable photosynthesis system (LI-COR Inc., USA). The measured leaves were selected from among the 5th–7th functional leaves at the top of each plant. The air temperature and light intensity inside the leaf chamber were controlled at 25°C and 800 μmol (photon) m^{-2} s^{-1}, respectively, The CO$_2$ concentration in the reference chamber was maintained at 400 μmol(CO$_2$) mol^{-1}. The net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO$_2$ concentration (C_i), and transpiration rate (E) were measured according to Guo *et al.* (2005).

Root Activity

Root activity was measured by the triphenyl tetrazolium chloride (TTC) method (Bai *et al*., 1994). The specific method: 0.5 g root tips of each sample were dipped in the mixed solution, containing 5 mL of 0.4% TTC and 5 mL of 0.1 mol L^{-1} phosphate buffer (pH 7.0), and incubated at 37°C for 1 h. Then, 2 mL of 1 mol L^{-1} H$_2$SO$_4$ was added to the mixture to stop the reaction, and the root tips were removed. Next, the root tips were ground with 10 mL of ethyl acetate and a little silica sand. The extracting solution was recorded at the wavelength of 485 nm.

Determination of Endogenous Hormone Contents

The contents of endogenous hormones in cucumber leaves were determined using high performance liquid chromatography (HPLC) (López-Carbonell *et al*., 2009; Zhang *et al*., 2013). One sample from the youngest cucumber leaves per replicate plot were selected at 20, 30, 40, 50 and 60 DAT, respectively, and immediately frozen in liquid nitrogen for 20 min. Then, the samples were stored in a low temperature freezer (–20°C). A leaf sample of 1 g was ground together with 10 mL of methanol solution (the concentration is 80% (v/v)) at 4°C until the mixture became a homogenate, which was then sealed using preservative film. After extraction for 16 h at 4°C, the homogenate was centrifuged at 10,000 *g* under 4°C for 10 min, and then the supernatant was decanted. The residues were re-extracted twice with 10 mL of cold methanol (the concentration is 80% (v/v)), and the supernatants from the three extractions were combined. The supernatants were decoloured three times with 20 mL petroleum ether and the products were reduced to the aqueous phase by removing the ether phase. Then the aqueous phase products were evaporated to a quarter of the original volume under reduced pressure at 37°C (RE-206B rotary evaporators, SHZ-D (III) vacuum pump, Keer Inc., Nanjing, China). The pH values of the

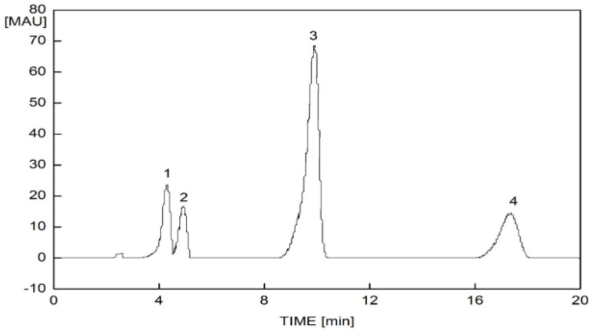

Fig. 2: Chromatograms of mixed standard sample. 1 – zeatin nucleoside (ZT), 2 –gibberellin (GA_3), 3 – auxin (IAA), 4 – abscisic acid (ABA)

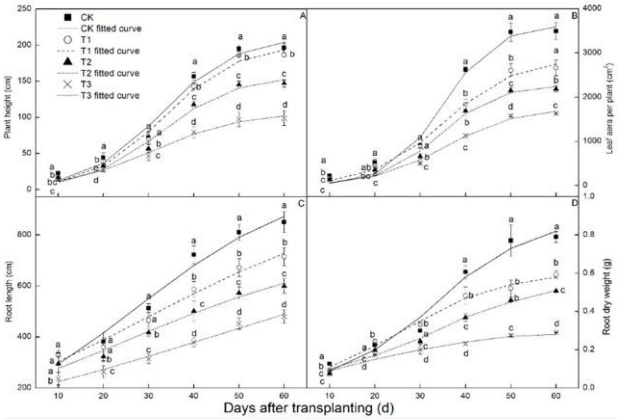

Fig. 3: Comparison of cucumber growth indexes under different water conditions. Error bars represents SD (n = 9). *Small letters* indicate statistically significant differences between treatments at $P<0.05$ by *Duncan*'s test. CK – normal irrigation; T1 – mild water stress; T2 – moderate water stress; T3 – severe water stress

evaporated products were adjusted to 2.8, and then the products were extracted three times by ethyl acetate, and the aqueous phase products were removed to leave the combined ester phase, which were then reduced to 1 mL under reduced pressure at 37°C. The methanol was added to make the solution up to 2 mL. Prior to HPLC analysis, this reconstituted eluate was filtered using a 0.45-μm microfiber filter, and 20 μL filter liquor was injected into an anion exchange column (5 μm, 250×4.6 mm, Agilent HC-C18, Agilent Corporation, USA). The above process was repeated six times for each sample. The samples were eluted using a mobile phase consisting of methanol and 0.075% acetic acid (45:55, v/v), and then measured at 254 nm. The velocity of flow was 0.7 mL min^{-1} and the column thermostat was set at 35°C. The endogenous hormone concentration was calculated by the external standard peak area method. Standard solutions of 0.1 mg mL^{-1} IAA, 0.01 mg mL^{-1} ZT, 1 mg mL^{-1} GA_3 and 0.01 mg mL^{-1} ABA were diluted to create different gradients in order to construct chromatograms and standard curves for the standard samples (Fig. 2) (Table 1). The standard samples were purchased from Sigma Corporation (Ronkonkoma, NY, USA), the methanol was chromatographically pure, and the other reagents were analytically pure. The water used in the experiment was distilled deionised H_2O.

Statistical Analysis

Logistic functions were fitted against DAT for plant height, leaf area per plant, root length and root dry weight:

$$Y = \frac{A}{1 + B \times \exp(-kx)} \tag{1}$$

Where Y is the plant height (cm), or leaf area per plant (cm^2), root length (cm), and root dry weight (g), A is the maximum plant height, or leaf area, root length, and root dry weight, k is a parameter describing the rate of increment of growth rate, and x represents days after transplanting (DAT).

All of the experimental data were analysed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA), and the differences between treatments were calculated by one-way analysis of variance (ANOVA), followed by *Duncan*'s multiple comparison test at $P \leq 0.05$.

Results

Cucumber Growth

Increasing water stress led to significant differences in cucumber growth (Fig. 3). The seasonal variation of plant height, leaf area per plant, root length, and root dry weight could be described by logistic functions (Table 2). The plant height at 60 DAT declined by 5.00%, 25.13%, and 49.75% under T1, T2 and T3, respectively, compared to CK, and the leaf area per plant under CK was more than double of that under T3. Water stress led to the largest reduction in root length at 50 DAT, and the average decreasing amplitude of root length under T1, T2 and T3 were 26.26%, 42.91%, and 66.92%, respectively, compared to CK. Root dry weights were averagely decreased by 13.89%, 30.88%, and 47.52% under T1, T2 and T3, respectively, compared to CK.

Leaf Gas Exchange Parameters

The leaf gas exchange parameters were significantly affected by water stress. Compared to CK, the averaged P_N of cucumber leaves over the whole growth stages decreased by 9.27%, 27.86% and 42.37%, respectively, under T1, T2, and T3. At 50 DAT, P_N had the most significant reduction, and the decreasing extents were 15.06%, 42.04%, and 59.11%, respectively, under T1, T2, and T3 when comparing with CK (Table 3).

The g_s values of cucumber leaves elevated as the

Table 1: Linear relationships and LODs of the endogenous hormone contents. y – concentration of endogenous hormones; x – peak area; LOD – limit of Detection; LOD was calculated according to signal to noise ratio = 3. ZT – zeatin nucleoside; gibberellin – GA_3, auxin – IAA; abscisic acid – ABA

Endogenous hormone	Regression equation	Linear range (μg mL^{-1})	Correlation coefficient	LOD (μg mL^{-1})
ZT	y = 0.0002x-0.5335	0.312-10	0.9995	0.01
GA_3	y = 0.0222x-18.674	0.977-31.25	0.9995	0.08
IAA	y = 0.0004x-0.3348	0.391-12.5	0.9998	0.07
ABA	y = 0.0001x-0.1054	0.078-2.5	0.9996	0.02

Table 2: The corresponding parameters (with standard errors) of growth fitted curves under different water levels

Water stress levels	Growth traits	*A*	*k*	*B*	R^2
CK	Plant height	211.45±17.51	0.12±0.03	142.63±40.92	0.981
T1		200.73±13.56	0.12±0.02	159.47±30.54	0.988
T2		157.54±11.52	0.12±0.02	137.37±20.49	0.985
T3		107.03±6.61	0.10±0.02	53.77±7.83	0.990
CK	Leaf area per plant	3638.73±227.62	0.17±0.04	1594.54±219.36	0.986
T1		2865.84±163.39	0.13±0.02	247.90±30.14	0.993
T2		2279.43±131.79	0.16±0.03	760.28±90.79	0.989
T3		1755.06±118.70	0.13±0.02	254.00±26.69	0.990
CK	Root length	1027.12±176.48	0.05±0.02	6.48±1.87	0.973
T1		954.99±215.88	0.04±0.01	4.28±0.68	0.982
T2		767.79±143.61	0.04±0.01	3.57±0.52	0.983
T3		808.71±65.14	0.03±0.01	4.25±1.71	0.985
CK	Root dry weight	0.95±0.13	0.08±0.02	34.30±17.35	0.984
T1		0.70±0.15	0.06±0.02	13.11±5.39	0.973
T2		0.68±0.18	0.06±0.02	16.30±5.81	0.977
T3		0.40±0.04	0.07±0.01	12.57±3.49	0.989

Table 3: Comparison of net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (*E*) of *Cucumis sativus* L. leaves at different growth stages under different water conditions. CK – normal irrigation; T1 – mild water stress; T2 – moderate water stress; T3 – severe water stress

Days after transplanting (DAT)	Treatments	Gas exchange parameters			
		P_N (μmol m^{-2} s^{-1})	g_s (mol(H_2O) m^{-2} s^{-1})	C_i (μmol(H_2O) mol^{-1})	*E* (mmol(H_2O) m^{-2} s^{-1})
20	CK	17.37 ± 0.42a	0.29 ± 0.01a	285.33 ± 5.13a	8.82 ± 0.07a
	T1	17.13 ± 0.13a	0.28 ± 0.01a	283.67 ± 3.06a	8.57 ± 0.08a
	T2	15.53 ± 0.86b	0.22 ± 0.01b	277.67 ± 3.51a	7.59 ± 0.34b
	T3	14.07 ± 0.25c	0.19 ± 0.01c	261.67 ± 4.51b	7.11 ± 0.29c
30	CK	20.00 ± 1.04a	0.46 ± 0.04a	339.00 ± 5.29a	8.11 ± 0.16a
	T1	17.90 ± 0.53a	0.39 ± 0.02b	332.00 ± 5.00a	7.08 ± 0.42b
	T2	15.30 ± 0.56b	0.35 ± 0.02b	322.33 ± 1.15b	6.05 ± 0.03c
	T3	12.57 ± 0.45c	0.25 ± 0.03c	310.33 ± 2.52c	6.00 ± 0.10c
40	CK	19.53 ± 0.45a	0.64 ± 0.04a	334.19 ± 2.51a	10.90 ± 0.31a
	T1	18.30 ± 0.44b	0.60 ± 0.02b	313.93 ± 4.71b	9.01 ± 0.32b
	T2	14.17 ± 0.29c	0.48 ± 0.01c	307.06 ± 4.77b	8.62 ± 0.17b
	T3	10.17 ± 0.23d	0.36 ± 0.03d	287.42 ± 7.87c	5.67 ± 0.30c
50	CK	23.23 ± 0.72a	0.80 ± 0.04a	301.67 ± 3.06b	11.21 ± 0.43a
	T1	19.73 ± 0.57b	0.61 ± 0.05b	284.67 ± 1.15c	10.46 ± 0.22b
	T2	13.47 ± 0.58c	0.50 ± 0.01c	278.67 ± 8.62c	8.99 ± 0.37c
	T3	9.50 ± 0.35d	0.41 ± 0.03d	316.00 ± 6.00a	4.85 ± 0.36d
60	CK	23.41 ± 1.09a	0.80 ± 0.04a	328.15 ± 1.09d	12.17 ± 0.65a
	T1	20.33 ± 0.19b	0.61 ± 0.05b	336.95 ± 0.51c	10.91 ± 0.58b
	T2	15.05 ± 0.30c	0.50 ± 0.02c	347.25 ± 1.26 b	9.20 ± 0.20c
	T3	12.02 ± 0.49c	0.41 ± 0.03d	350.50 ± 0.92a	5.52 ± 0.33d

Small letters indicate significance of $P \leq 0.05$ by Duncan's test. All results are presented as mean ± SD (n = 3)

growth stages progressed, and the g_s values under CK were almost 1.18, 1.20, and 1.36 times of that under T1, T2, and T3, respectively. From 20 to 40 DAT, water stress considerably reduced the C_i of cucumber leaves as the water stress levels increased, with the degree of descent being 2.90%, 5.24%, and 10.25%, respectively, under T1, T2, and T3. In the last two stages (50 and 60 DAT), the response of C_i to water stress varied between different stress levels. At 50 DAT, C_i for T1 and T2 decreased by 5.64 and 7.63%, respectively, but increased by 4.75% for T3, at 60 DAT, C_i elevated by water stress, from 2.68 to 6.81% in water stressed plants (Table 3). Water stress reduced the *E* of cucumber leaves. The average *E* under CK was 10.23 mmol (H_2O) m^{-2} s^{-1}, while it ranged from 5.83 to 9.22 mmol (H_2O) m^{-2} s^{-1} under T1, T2, and T3 (Table 3). The correlation between endogenous hormones and photosynthetic parameters was analysed. Photosynthetic parameters have negative correlation with ABA contents, at 20, 30, 40, 50, 60 DAT (Table 4). At 20 DAT, P_N, g_s, and *E* had significant positive correlation with ZT, g_s and *E* had significant correlation with GA_3. At 30 DAT, P_N and g_s had highly significant correlation with ZT (Table 4). At 50 DAT, P_N had significant positive correlation with ZT, g_s had significant positive correlation with GA_3. At 60 DAT, P_N and g_s had significant correlation with ZT (Table 4).

Root Activity

Root activity in plants was greatly influenced by water stress, with the average decreasing amplitude being 13.04%, 26.57%, and 34.41% under T1, T2, and T3, respectively, compared to CK (525.58 μg g^{-1} h^{-1}). At 20 DAT, root activity under T1 was 4.11% higher than CK. At 60 DAT, root activity decreased obviously, which decreased by 24.20, 50.57 and 56.37%, for T1, T2 and T3, respectively, compared to CK (Fig. 4).

Endogenous Hormone Contents

The endogenous hormone contents in cucumber leaves varied with progressing growth stages; ZT, GA_3, IAA, and ABA contents increased gradually. The ZT level decreased as the soil water content fell, with the average reduction amplitude being 14.65%, 28.93%, and 38.62% compared to CK, respectively, for T1, T2, and T3 (Fig. 5). At 20 DAT, the GA_3 concentration declined continuously as the stress level rose. However, from 30 to 60 DAT, the plants under T1 contained the highest GA_3 among all treatments. GA_3 levels reached their peaks at 60 DAT for all treatments, with the peak value for T1 rising by 5.73%, and those for T2 and T3 reducing by 21.53% and 36.21%, respectively, compared to CK (Fig. 5). Over the entire stage, the IAA contents in cucumber leaves under T1 and T2 were higher than those under CK, while those under T3 were the lowest among all treatments. At 40 DAT, there were considerable differences between water stress and sufficiently irrigated plants in IAA contents, which they enhanced by 8.89% and 18.53%, respectively, under T1 and T2 compared to CK, but fell by 18.51% under T3. The ABA level rose as the water stress increased over the whole stage. There was a marked difference in ABA level between T1 and CK at 40 DAT, while no significant differences were found at other growth stages. However, significant differences in ABA level were existed between T2, T3 and CK over the whole growing season, with the average increasing range over the whole growing season being 58.45% and 107.66%, respectively, for T2 and T3 when comparing with CK (Fig. 5).

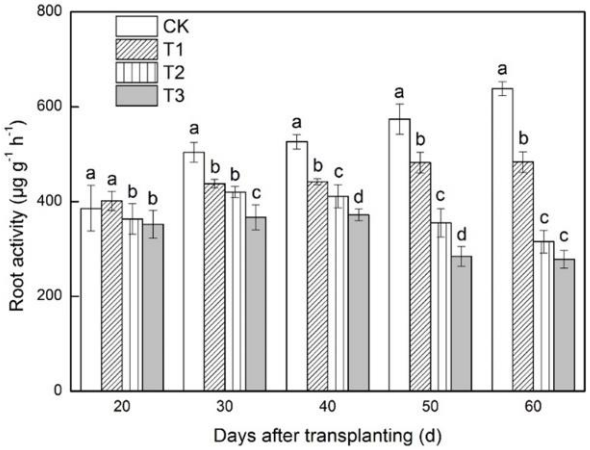

Fig. 4: Comparison of root activity under different water conditions. Error bars represents SD (*n* = 9). *Small letters* indicate statistically significant differences between treatments at *P*<0.05 by *Duncan*'s test. CK – normal irrigation; T1 – mild water stress; T2 – moderate water stress; T3 – severe water stress

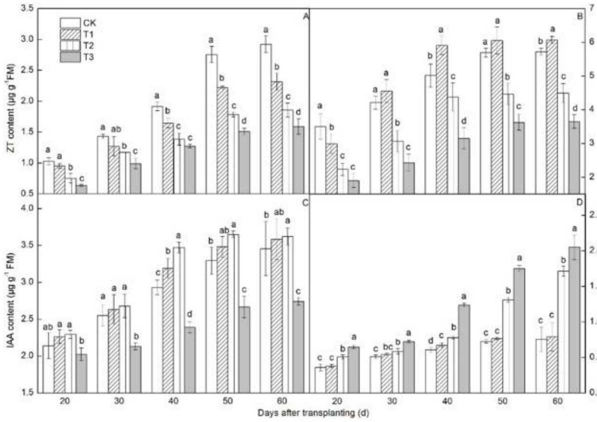

Fig. 5: Comparison of zeatin nucleoside (ZT) (*A*), gibberellin (GA_3) (*B*), auxin (IAA) (*C*) and abscisic acid (ABA) (*D*) concentration in cucumber leaves at different growth stages under different water conditions. Error bars represents SD (*n* = 3). *Small letters* indicate statistically significant differences between treatments at *P*<0.05 by *Duncan*'s test. CK – normal irrigation; T1 – mild water stress; T2 – moderate water stress; T3 – severe water stress

ZT/ABA, GA_3/ABA, IAA/ABA, ZT/IAA, and (GA_3 + ZT + IAA)/ABA Ratios

The ZT/ABA ratios in cucumber leaves were highest under CK in all growth stages, and decreased as the water stress level rose (Fig. 6). At 20 DAT, no significant differences were found in ZT/ABA ratios between T1 and CK, however, the ratios under T2 and T3 declined by 51.57% and 68.35%, respectively, compared to CK. At 60 DAT,

Table 4: Correlation coefficients between photosynthetic parameters, endogenous hormone contents and endogenous hormone ratios of *Cucumis sativus* L. leaves under different water conditions. CK – normal irrigation; T1 – mild water stress; T2 – moderate water stress; T3 – severe water stress

DAT		ZT	GA_3	ABA	ZT/ABA	GA_3/ABA	IAA/ABA	(GA_3 + ZT +IAA)/ABA
20	P_N	0.983^{*}	0.954	-0.999^{**}	0.979^{*}	0.960^{*}	0.999^{**}	0.982^{*}
	g_s	0.997^{**}	0.979^{*}	-0.987^{*}	0.996^{*}	0.984^{*}	0.988^{*}	0.996^{*}
	E	0.998^{**}	0.984^{*}	-0.979^{*}	0.998^{**}	0.989^{*}	0.980^{*}	0.997^{**}
30	P_N	0.993^{**}	0.910	-0.956^{*}	0.994^{**}	0.955^{*}	0.897	0.972^{*}
	g_s	0.994^{**}	0.886	-0.982^{*}	0.998^{*}	0.938	0.941	0.972^{*}
	E	0.924	0.811	-0.789	0.909	0.856	0.682	0.844
40	P_N	0.940	0.903	-0.949^{**}	0.978^{*}	0.981^{*}	0.901	0.986^{*}
	g_s	0.943	0.802	-0.957^{*}	0.988^{*}	0.924	0.912	0.958^{*}
	E	0.905	0.763	-0.961^{*}	0.965^{*}	0.892	0.926	0.939
50	P_N	0.982^{*}	0.932	-0.974^{*}	0.993^{**}	0.966^{*}	0.970^{*}	0.983^{*}
	g_s	0.946	0.959^{*}	-0.994^{**}	0.961^{*}	0.965^{*}	0.990^{**}	0.980^{*}
	E	0.867	0.923	-0.964^{*}	0.871	0.892	0.957^{*}	0.913
60	P_N	0.980^{*}	0.928	-0.974^{*}	0.986^{*}	0.961^{*}	0.977^{*}	0.978^{*}
	g_s	0.997^{**}	0.809	-0.888	0.963^{*}	0.871	0.896	0.905
	E	0.911	0.925	-0.931	0.891	0.887	0.924	0.904

NOTE: *indicate significance of $P \leq 0.05$; **indicate significance of $P \leq 0.01$

the reductions in ZT/ABA ratios reached the highest, which were 25.70%, 72.58%, and 80.03%, under T1, T2, and T3, respectively, compared to CK. At 20 DAT, the GA_3/ABA ratio declined by 20.56%, 55.49%, and 70.32% under T1, T2, and T3, respectively, compared to CK. From 30 to 60 DAT, no significant difference was observed between T1 and CK, and the average GA_3/ABA ratios fell by 47.84% and 69.70% under T2 and T3, respectively, compared to CK (Fig. 6). The IAA/ABA ratios fell as the water stress increased. There was no significant difference in IAA/ABA ratio between T1 and CK over the whole stage. Compared to CK, T2 reduced the IAA/ABA ratios by 25.46, 38.56 and 55.07% at 20, 50 and 60 DAT, respectively, while no significant difference was observed between T2 and CK at 30, 40 DAT, and the average IAA/ABA ratio over the whole stage decreased by 57.37% under T3 compared to CK (Fig. 6). The average ZT/IAA ratios over the whole stage decreased by 19.01, 34.71 and 26.77% under T1, T2, and T3, respectively, compared to CK (Fig. 6). As the water stress levels rose, the (GA_3 + ZT + IAA)/ABA ratios gradually declined. There was no marked difference in (GA_3 + ZT + IAA)/ABA ratios between T1 and CK, but the average reductions through the entire stage under T2 and T3 reached 43.24 and 65.84%, respectively, compared to CK (Fig. 6).

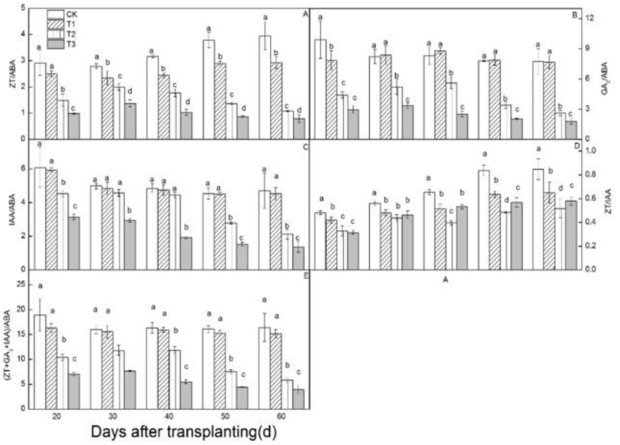

Fig. 6: Comparison of the ratios of ZT/ABA (*A*), GA_3/ABA (*B*), IAA/ABA (*C*), ZT/IAA (*D*)and (GA_3 + ZT + IAA)/ABA (*E*) in cucumber leaves at different growth stages under different water conditions. Error bars represent SD (*n* = 3). *Small letters* indicate statistically significant differences between treatments at $P<0.05$ by *Duncan*'s test. ZT – zeatin nucleoside; gibberellin – GA_3, auxin – IAA; abscisic acid – ABA

Discussion

Due to global warming, water resource shortages have further limited crop growth and development (Katerji *et al.*, 2008), potentially leading to a series of morphological changes in plants. Our study indicated that water stress inhibited cucumber plant growth, which contained above-ground and below-ground parts; this could be proved by the reductions in plant height, leaf area per plant, root length, and root dry weight in water-stressed plants (Fig. 3). It was obvious that root dry weights under T1 were slightly higher than CK at 20, and 30 DAT, with possible increases in root weights contributing to the prevention of mild stress in the early growth stages. In our study, the fitted curves of plant growth traits under different water levels conform to a sigmoid curve, which had high determination coefficients (R^2>0.968); the leaf area per plant presents relatively better fitted effects (R^2>0.986) than other growth traits. In general, some research had similar results to our study with respect to the effects of water stress inhibited plant growth (Jeffrey and David, 1955; Çakir, 2004; Al-Hassan *et al.*, 2015).

Table 5: Correlation coefficients between growth parameters, endogenous hormone contents and endogenous hormone ratios of *Cucumis sativus* L. leaves under different water conditions. CK – normal irrigation; T1 – mild water stress; T2 – moderate water stress; T3 – severe water stress

DAT		ZT	GA_3	ABA	ZT/ABA	GA_3/ABA	IAA/ABA	ZT/IAA	(GA_3 + ZT +IAA)/ABA
20	Ph	0.993**	0.994**	-0.968*	0.993**	0.993**	0.963*	0.974*	0.994**
	LAP	0.997**	0.997**	-0.966*	0.997**	0.997**	0.963*	0.981*	0.998**
	RL	0.972*	0.950	-0.994**	0.967*	0.952*	0.989*	0.905	0.973*
	RW	0.840	0.767	-0.903	0.833	0.785	0.916	0.723	0.834
30	Ph	0.962*	0.967*	-0.960*	0.970*	0.992**	0.913	0.697	0.995**
	LAP	0.949	0.977*	-0.952*	0.958*	0.996**	0.906	0.682	0.994**
	RL	0.988*	0.897	-0.990*	0.994**	0.945	0.955*	0.681	0.980*
	RW	0.830	0.998**	-0.879	0.848	0.983*	0.849	0.528	0.955*
40	Ph	0.930	0.883	-0.971*	0.980*	0.972*	0.931	0.421	0.989*
	LAP	0.956*	0.646	-0.868	0.972*	0.816	0.803	0.592	0.863
	RL	0.980*	0.756	-0.907	0.998**	0.895	0.843	0.585	0.924
	RW	0.978*	0.791	-0.920	0.999**	0.918	0.859	0.567	0.942
50	Ph	0.921	0.966*	-0.996*	0.939	0.959*	0.992**	0.674	0.972*
	LAP	0.994**	0.829	-0.910	0.970*	0.882	0.898	0.863	0.915
	RL	0.992**	0.868	-0.939	0.976*	0.910	0.929	0.836	0.939
	RW	0.975*	0.787	-0.883	0.935	0.833	0.868	0.821	0.874
60	Ph	0.909	0.965*	-0.967*	0.914	0.934	0.961*	0.688	0.943
	LAP	0.994**	0.842	-0.908	0.961*	0.885	0.913	0.879	0.917
	RL	0.989*	0.884	-0.937	0.968*	0.914	0.940	0.853	0.940
	RW	0.965*	0.846	-0.892	0.919	0.853	0.892	0.795	0.885

Note: Ph means plant height, LAP means leaf area per plant, RL means root length, RW means root weight. *indicate significance of $P \leq 0.05$; **indicate significance of $P \leq 0.01$

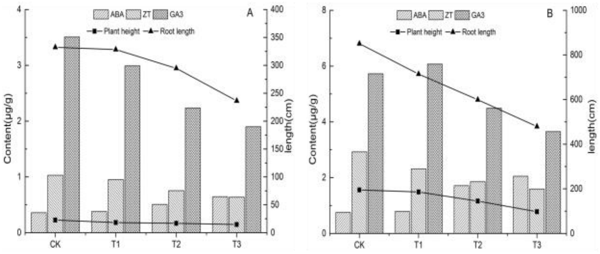

Fig. 7: Correlation between the content of ABA, ZT, GA_3 changes and growth parameters. A:at 20 DAT; B: at 60 DAT

Water stress substantially alters plant metabolism, inhibits photosynthesis, and profoundly affects the ecosystem and agriculture (Cornic and Massacci, 1996). In our study, water stress significantly reduced the P_N, g_s, and E of cucumber leaves, which was consistent with Rahmati *et al.* (2015). Photosynthetic parameters showed close correlations with growth traits (r>0.330, $P < 0.05$). Under water stress, there were two factors influencing photosynthesis: one was stomatal closure, which restricted CO_2 diffusing into the leaf, and the other was non-stomatal responses (Zhou *et al*., 2014). Compared to CK, the P_N, g_s, and E of cucumber leaves declined over the entire growth stage (Table 3), while changing trends of C_i varied in different growth stages. From 20 to 40 DAT, C_i decreased along with the reductions of P_N, and g_s in water stressed plants (Table 3), this suggested that stomatal factor may play key roles in decreasing photosynthesis at the first three stages. However, *Ci* reduced under T1 and T2 but increased under T3 at 50 DAT, *Ci* in water stressed plants were all higher than that in adequately irrigated plants (Table 3). This means that reductions in P_N under mild (T1) and moderate (T2) water stress were correlated with stomatal factors at 50 DAT, but were related to non-stomatal restriction at 60 DAT, and that severe water stress induced CO_2 metabolism congestion in the last two stages (50 and 60 DAT). Xiao *et al.* (2010) reported that non-stomatal restriction is the main inhibitor of *Cucumber* under severe drought stress; the main factor in photosynthesis was chloroplast activity in the leaf. Other researchers have suggested that Rubisco activity, mesophyll conductance to CO_2, and the maximum electron transport rate were involved in non-stomatal responses (Bota *et al*., 2004; Flexas *et al*., 2009). Therefore, in our study, water-stressed plants affected by non-stomatal factors showed a lower ability to fix CO_2, where CO_2 increased by respiration, and stomatal limitation no longer affected photosynthesis.

Generally, when crop is subjected to water stress, it first adjusts its root system shape and distribution, and then responds to water stress through physiological metabolic changes to adapt to water stress. In our study, root activity was measured to evaluate root growth status (Bai *et al*., 1994), which had positive correlations with root length (r=0.711, $P<0.01$) and root dry weight (r=0.721, $P<0.01$). This suggested, cucumber can affect the carbon assimilation product allocation in different organs after feeling stress signal, and finally improve water absorption efficiency by increasing root length and biomass. In our study, root activity decreased, except for the slight increase under mild stress in the early stages (20 DAT). This suggested, cucumber root activity increased under short-term and mild water stress in order to withstand adversity. With aggravated stress and prolonged time, accumulation of ROS in cucumber cells and increase of lipid membrane peroxidation reduce tolerance and decrease root activity of cucumber. In our early study about the impact of cucumber antioxidant enzyme system to water stress, we find that with aggravated stress and prolonged time, the intracellular content of antioxidant enzymes SOD, POD and CAT increased significantly, the cucumber can resist drought by activating key indexes of enzymatic and non-enzymatic system. Similarly, Sun *et al*. (2008) showed that the cucumber root activity increased in mild water stress and decreased with increasing water stress. Other studies have found that the different response mechanism of crops to water stress led to different changes in root activity after crops were subjected to water stress (Yao *et al*., 2009; Yang *et al*., 2012). For example, some drought-resistant alfalfa responds to water stress mainly by changing root characteristics, and its root activity will increase with the increase of stress. After continuous water stress, the stability and osmotic regulation ability of cell membrane system of alfalfa cultivars with weak drought resistance will be easily affected, and the root activity will decrease continuously (Fang and Xiong, 2015; Zhang *et al*., 2017).

Endogenous hormones have important regulatory effects on plant growth. Several researchers have indicated that the adaption of plants to water stress is regulated by ABA-dependent and ABA-independent expression pathways (Lian *et al*., 2006; Rossdeutsch *et al*., 2016). In our study, the ABA content in cucumber leaves rose as the water stress worsened (Fig. 5 and 7). It was inferred that the cucumber adapted to water stress by enhancing ABA levels (ABA-dependent pathway). Research has found that water stress reduced cytokinin content, but increased IAA levels in some plants (Diego *et al*., 2012; Saeedipour, 2013). In our study, the IAA contents under T1 and T2 were higher than under CK, but the lowest IAA content was found under T3 (Fig. 5); meanwhile, IAA levels had positive correlations with growth traits (Non-significant correlation), which suggested that mild and moderate water stress could be beneficial to increase in IAA contents, which was good for growth, but severe water stress inhibited cucumber growth by decreasing IAA concentrations. The IAA and ABA content changes in endogenous hormone levels might affect the growth of cucumber by influencing regulatory enzymes and processes. It was considered that water deficit decreased the GA_3 levels in the leaves and roots of some crops. On the contrary, some researchers reported that water stress increased the GA_3 contents (Yuan *et al*., 2012; Krishnan and Merewitz, 2015). In our study, the GA_3 contents in cucumber leaves had strong correlations with growth traits (Table 5), which were promoted by mild water stress, and reduced by moderate and severe water stress at the middle-late growth stage (Fig. 5 and 7). That meant that mild water stress increased the GA_3 contents in order to promote cucumber growth. The differences between researches may be associated with the differences in drought tolerance and drought adaptation between plant species.

Endogenous hormones act synergistically and antagonistically with each other and are in dynamic balance; the ratio between different endogenous hormones affects the response of plants to water stress (Pinheiro *et al*., 2011). Abscisic acid (ABA) can transmit root signal and control stomatal conductance, so as to control plant growth and regulate transpiration. In our study, the correlation between the ZT/ABA, GA_3/ABA, IAA/ABA, (GA_3 + ZT + IAA)/ABA ratios and photosynthetic parameters was analysed (Table 4). The ZT/ABA, GA_3/ABA, IAA/ABA, (GA_3 + ZT + IAA)/ABA ratios have significant positive correlations with Pn, gs, E (Table 4). Water stress increased the content of ABA and decreased the content of ZT. The ZT/ABA ratios fell with the increasing water stress levels over the entire stage (Fig. 6), which had significant correlations with cucumber plant growth (Table 5). This change in dynamic balance limits photosynthesis, is not conducive to the synthesis of crop photosynthetic products, and affects the growth of crops. GA_3 and ABA mostly antagonize each other and affect crop development. At 20 DAT, the GA_3/ABA ratios declined gradually as water stress aggravated, while the GA_3/ABA ratios were highest under T1 and lowest under T3 from 30 to 60 DAT (Fig. 6). This change in dynamic balance is beneficial for ABA to exert anti-stress effect, but affects the elongation of cucumber stem. Mild and moderate water stress improved the synthesis of IAA and ABA, but the increasing extent of ABA was higher than that of IAA, resulting in the decrease of IAA/ABA ratios under water stress (Fig. 6). At 20 ,50 ,60 DAT , IAA/ABA have significant positive correlations with plant height, which suggested that this dynamic balance is expressed in response to water stress in the early stages, while this may promote the premature senility of cucumber in the later stages. Our study indicated that water stress reduced the ZT/IAA ratios (Fig. 6), and that ZT/IAA ratios had greater correlations with root length at 20 DAT (Table 5). This suggested that this dynamic balance may promote root synthesis to response to water stress in the early stages. In general, the increase in ZT, IAA, and GA_3 contents have significant positive correlations with cucumber growth, while the rise in ABA contents was not conducive to the growth of cucumber, but improved the drought resistance of the cucumber plant. Hence, in our study, we adopted the (GA_3 + ZT + IAA)/ABA ratio as a comprehensive indicator of cucumber growth; the (GA_3 + ZT + IAA)/ABA ratios fell as water stress levels increased (Fig. 6), which had a positive influence on root growth significantly at 20, 30 DAT (Table 5). The results showed that the change of dynamic balance is beneficial for cucumber to respond to adversity by adjusting root morphological characteristics under mild and moderate stress.

Conclusion

The plant height, leaf area per plant, root length and root dry weight of cucumber decreased with increasing water stress levels. Water stress reduced P_N, g_s and E of cucumber leaves. Cucumber can resist adversity by increasing root activity under mild stress in the early stages. ZT and GA_3 have significant positive correlations with photosynthetic parameters and cucumber growth, while ABA is negatively correlated. The ZT in cucumber leaves decreased with the aggravation of soil water stress, while the ABA contents in cucumber leaves rose as the water stress increased over the whole water stress process. Mild stress enhanced the GA_3 concentration except at 20 days after transplanting. Moderate and severe water stress inhibited the synthesis of GA_3 contents, compared to CK. The IAA contents increased under both mild and moderate stress, while severe stress lowered IAA concentrations. Endogenous hormones act synergistically and antagonistically with each other. Water stress reduced the ZT/ABA, IAA/ABA, ZT/IAA, and (GA_3 + ZT + IAA)/ABA ratios in cucumber leaves, which have significant positive correlations with photosynthetic parameters and cucumber growth. Compared with CK, the GA_3/ABA ratio decreased under moderate and severe water stress, while mild stress elevated GA_3/ABA ratios with an exception at 20 DAT.

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