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Physiological and biochemical characteristics of cucumber seedlings under different levels of drought stress (PEG 6000 concentrations)

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Abstract: The effects of different drought stress levels on the plant growth, physiological and biochemical characteristics of cucumber (*Cucumis sativus* L.) seedlings were investigated using different mass fractions of polyethylene glycol (PEG) 6000 (0, 2.5, 5, 10%) to simulate the drought stress. The results showed that the plant height, root length, leaf dry weight (DW), stem DW, root DW, and plant DW all showed no significant difference under mild (2.5% PEG 6000) drought stress, while they exhibited a decreasing trend with an increasing level of PEG. The changing trend in the photosynthetic efficiency, chlorophyll *a,* and carotenoid content was consistent with the growth index under drought stress. Besides, the content of chlorophyll *b* in the cucumber seedlings increased under moderate (5% PEG 6000) drought stress, but decreased under severe (10% PEG 6000) drought stress. However, the antioxidase activities, soluble protein content, malondialdehyde (MDA) content, total phenols, and total flavonoid content in the cucumber seedlings increased gradually with the increase in the mass fraction of PEG. The results indicated that higher the level of drought stress, the higher inhibition on the seedling growth or more severe damage on the cell membrane, and higher contents of total phenols and flavonoids were obtained.

Keywords: *Cucumis sativus* L.; antioxidase; chlorophyll content; flavonoids; phenols; photosynthesis

With global warming and the intensification of human activities, the occurence of droughts is increasing, especially in arid and semi-arid areas. Drought stress is one of the main environmental factors restricting plant growth and production (Qi et al. 2018). Previous research indicated that drought stress has a negative impact not only on the whole plant, but also at the cell level, which was manifested by growth inhibition, cell dehydration, decreased photosynthetic capacity, stoma-

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tal closure, and the production of reactive oxygen species (ROS), and the reduction of osmotic potential (Blum 2017; Sallam et al. 2019; Kuromori et al. 2022); moreover, excessive ROS is potentially harmful to DNA, proteins, and lipids (Jiang et al. 2019), and leads to membrane integrity damage and chlorophyll degradation (Ergo et al. 2018).

Water-saving agriculture has become an inevitable choice for agricultural development. Although drought stress restrains crop growth and productivity at all growth stages, plants respond to water shortages in many ways according to their growth stages, and various physiological and molecular mechanisms are activated to mitigate the effects of drought stress, such as by activating stress-related pathways which involve antioxidant defences, and osmolyte production. Moreover, drought stress can stimulate the production and accumulation of secondary metabolites in plants to allow adaption to severe drought, maintain high antioxidant activities, and rearrange the growth regulators (Li et al. 2018; Burbulis et al. 2022). Certain drought stresses will cause a change in the flavonoid content in plant leaves, and previous research has shown that the total flavonoid content was accumulated after the drought stress (Scott et al. 2019). In addition, other secondary metabolites, phenols, are involved in the defence against salt stress, and remarkably promote the antioxidant activity of plant tissues (Bharti et al. 2013; Sarker, Oba 2018).

The cucumber (*Cucumis sativus* L.) is one of the most grown worldwide horticultural crops, which is very sensitive to drought stress due to its high-water demand and unfavourable soil moisture conditions, which may limit the quality and thus limit the yield parameters (Sun et al. 2016; Li et al. 2018; Cui et al. 2019). Moreover, the cucumber is often subjected to drought stress at the seedling stage, and it affects the growth of seedlings, and ultimately leads to a decline in the yield. Therefore, more and more attention has been paid to study the drought resistance of cucumber seedlings (Du et al. 2022). In order to solve the problems caused by drought, the present research studied the drought resistance of cucumber seedlings, examined the effects of different levels of drought stress on the cucumber seedlings' plant growth, chlorophyll, and malondialdehyde (MDA) content, and certain functional substance contents in different tissues in order to provide a reference for cucumber breeding and selection of drought-resistant varieties in arid and semi-arid areas.

MATERIAL AND METHODS

Plant material and experimental design. Healthy "Jinyan 4" cucumber seeds were used to germinate and culture seedlings. First, the seeds were germinated in moist sand, and grown for ten days (second leaf stage) in a greenhouse at 22 °C/25 °C (day/night), then the seedlings were transferred to 3 L plastic buckets filled up with a basal nutrient solution (BNS) (Janicka-Russak et al. 2012), the pH was 5.6 ± 0.1. Seven averagely spaced holes were bored on the lid of the bucket, and there were two plants per hole, which were fixed with a sponge.

PEG 6000 was dissolved in a nutrient solution to simulate rhizosphere drought stress (Li et al. 2018). Four treatments with three replicates were imposed after seven days of transplanting: control (BNS), mild drought stress (BNS + 2.5% PEG 6000), moderate drought stress (BNS + 5% PEG 6000), and severe drought stress (BNS + 10% PEG 6000). There was a total of 12 plastic buckets, with 3 buckets for each treatment, and the solution was aerated continuously using gas pumps and replaced at five-day intervals.

Plant growth analysis. After ten days of PEG 6000 exposure, three plants were uprooted from each bucket for the plant height and root length measurement using a scale. Then, the cucumber seedlings were separated into their roots, stems, and leaves, and dried at 80 °C until a constant weight was reached for their dry weight (DW) determination.

Determination of the chlorophyll content and photosynthesis parameters. The second fully expanded leaf was used to determine the chlorophyll content and the photosynthesis parameters. Fresh compound leaves (0.1 g) were extracted in the dark at room temperature for 48 hours in 10 mL of acetone/ethanol (1 : 1, v/v), and then the supernatant was used to determine the chlorophyll content using a UV-visible spectrophotometer (UV3600, Shimadzu, Japan) according to Lichtenthaler (1987). The net photosynthetic rate (*Pn*), transpiration rate (*Tr*), intercellular CO_2 concentration (*Ci*), and stomatal conductance (*Gs*) were determined via a fully automatic portable photosynthesis system (LC Pro-SD, England).

Enzyme activity assay. The fresh compound leaves, stems and roots of each plant were collected for the enzyme determination after treatment. All the samples were washed three times using distilled water, then 0.5 g of the leaf, stem, or root was homogenised in 8 mL of 50 mM sodium phosphate buffer (PBS, pH 7.8) using a prechilled mortar and pestle, then centrifuged at 10 000 × g for 15 min at 4 °C. The supernatant was designated as a crude enzyme extract, which was stored at 4 °C for the assays of various antioxidant enzyme activities, MDA, and protein. The superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) activity determination were performed spectrophotometrically as described by Zhang et al. (2021). The SOD activity was assayed by the nitro blue tetrazolium (NBT) method by measuring the photoreduction of NBT at 560 nm, one unit of SOD was defined as the quantity of enzyme that produced 50% inhibition of the NBT reduction under the experimental conditions. For the analysis of the APX, ascorbate (AsA) was used as the substrate and the decrease in the ascorbate concentration followed as a decline in the optical density at 290 nm, and the activity was calculated using the extinction coefficient (e) 2.8 (mM/cm) for ascorbate. The reaction mixture for POD consisted of 100 μL of enzyme extract, 100 μL of guaiacol (1.5%, v/v), 100 μL of H_2O_2 (300 mM), and 2.7 mL of 25 mM PBS with 2 mM of Ethylenediaminetetraacetic acid (EDTA) (pH 7.0). The increases in the absorbance were measured spectrophotometrically at 470 nm (e = 26.6 mM/cm).

Determination of the lipid peroxidation, soluble protein content, total phenols, and flavonoids content. The level of lipid peroxidation was expressed as malondialdehyde (MDA) content, which was determined by the thiobarbituric acid (TBA) reaction on the basis of Wu et al. (2003), the amount of the MDA-TBA complex (red pigment) was calculated from the extinction coefficient of 155 mM/cm. The content of soluble protein was analysed according to Bradford (1976) using bovine serum albumin (BSA) as standard. Total phenols content was determined using the Folin-Ciocalteu reagent reduction (Singleton et al. 1999), the absorbance was recorded at 765 nm, and the result was expressed as mg gallic acid (GAE) per g fresh weight (FW); while the flavonoid content was determined colourimetrically according to Zhishen et al. (1999), and the absorbance was determined at 510 nm, the result was expressed as mg rutin equivalent (RE) per g FW. The abovementioned parameters were assayed using a UV-visible spectrophotometer (UV3600, Shimadzu, Japan).

Statistical analyses. The data are presented as the averages of at least three independent replicates. All the data were analysed using SPSS version 22.0. The statistical analyses were performed with a one-way analysis of variance (ANOVA) based on Duncan's multiple-range test to evaluate the significance of the effects of the different treatments. P values ≤ 0.05 were determined to indicate significance, different letters were statistically different at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Effects of the different levels of drought stress on the growth characteristics of the cucumber seedlings. Figure 1 displays the growth characteristics of the cucumber seedlings under different degrees of drought treatment. The results showed that after 10 days of the PEG 6000 treatment, the mild (2.5% PEG 6000) drought stress had no significant effect on the plant growth. When the drought stress level reached 5%, the stem, root and plant DW were significantly affected; however, the plant height, root length, and leaf DW of the seedlings under the moderate (5% PEG 6000) drought stress were basically the same as the control, and there was no significant difference with the control (Figure 1A–C). Moreover, when the drought stress level increased to 10%, all the growth parameters of the seedlings were significantly lower than those under the control conditions. The root DW had the largest decrease in percentage compared to the other growth traits in the trial, with a decrease percentage of 46% compared with the control (Figure 1E).

Drought stress has a direct and indirect effect on plant growth. Under drought stress, the plant root system was first affected (Kim et al. 2020). In this study, it was found that the root system of cucumber seedlings was more severely damaged with the intensification of drought stress. When the plant is subjected to drought stress, the most direct expression is the change in the plant dry matter. The present research showed that the stem/root DW and plant DW evidently decreased with the increased level of drought intensity, and the decreased percentage of root DW under severe (10% PEG 6000) drought stress was the largest (Figure 1). These results were consistent with previous research findings, moderate and severe drought stress significantly decreased the morphological parameters, such as the plant height, stem diameter and leaf area of cucumber seedlings (Cui et al. 2019). Simultaneously, the plant

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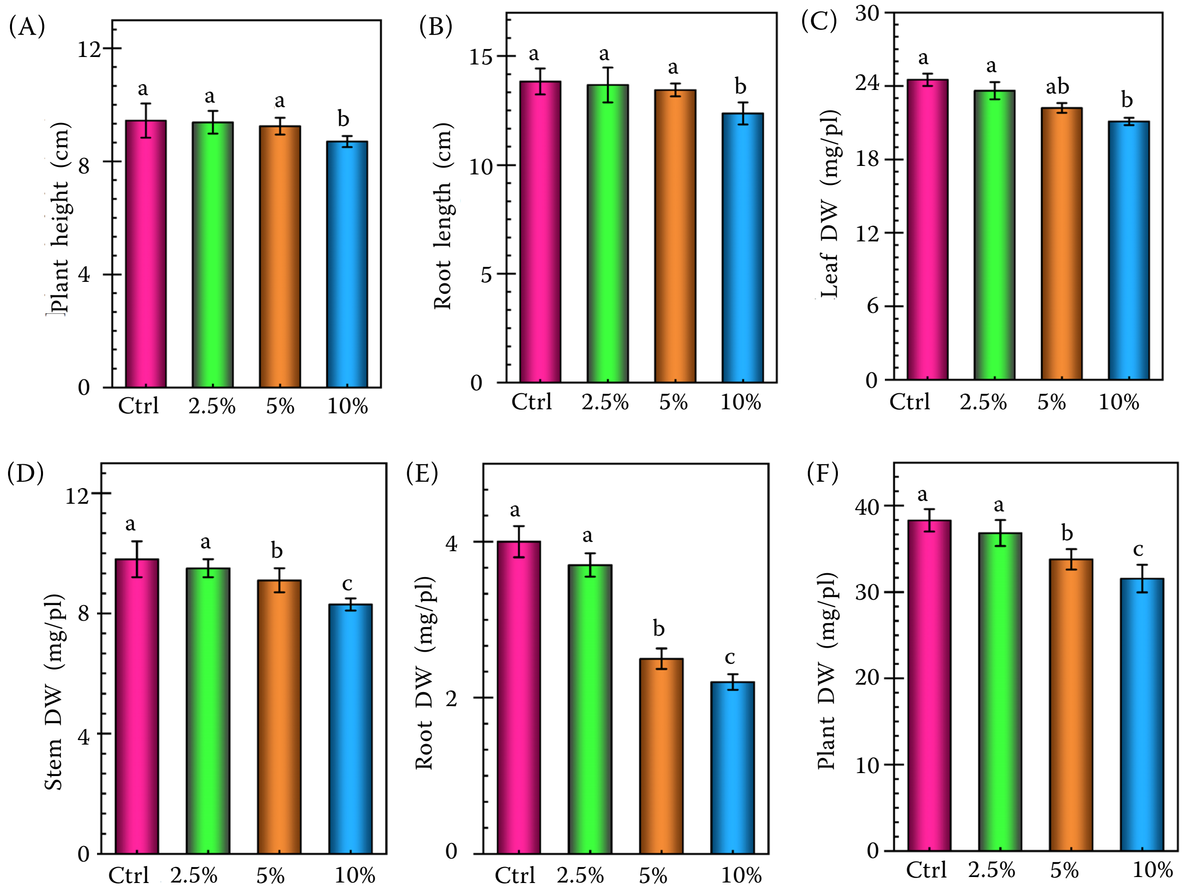

Figure 1. Effect of the different levels of drought stress on the plant growth in the cucumber seedlings. Ctrl, 2.5%, 5%, and 10% represent the control, mild (2.5% PEG 6000), moderate (5% PEG 6000), and severe (10% PEG 6000) drought stress, respectively

DW – dry weight

height, DW, and FW of the cucumber plants decreased with the increase in the drought stress level (Najarian et al. 2018). In addition, under the mild (2.5% PEG 6000) drought stress, all the examined plant growth indices showed no significant difference with the controls, which showed that growth was inhibited when the plants were subjected to the moderate and severe drought stress, while the mild drought stress did little damage to the cucumber seedlings.

Effects of the different levels of drought stress on the pigment content and photosynthetic parameters of the cucumber seedlings. Under the different drought treatments, the chlorophyll content of the seedlings changed differently. Under the mild (2.5% PEG 6000) drought stress, there was no notable difference in the chlorophyll content compared with those of control (Figure 2). However, when the drought stress level reached 5% and 10%, the chlorophyll *a* and carotenoid content increased significantly compared with the control; but the chlorophyll *b* content decreased under the severe (10% PEG 6000) drought stress, while increased under moderate (5% PEG 6000) stress compared with the control (Figure 2B).

A negative effect of the drought stress on the photosynthesis was found in cucumber seedlings, although only the Gs was significantly inhibited after the mild (2.5% PEG 6000) drought stress. Under the moderate (5% PEG 6000) and severe (10% PEG 6000) drought stress, the *Pn*, *Tr*, and *Gs* were evidently reduced, while, the *Ci* was markedly increased, in comparison with the control. Moreover, the degree of the negative effect increased when the level of drought stress intensified (Table 1). Li et al. (2018) also reported that moderate and severe drought stress significantly decreased the *Pn*, *Tr*, and *Gs* of cucumber seedlings, but significantly increased the *Ci*.

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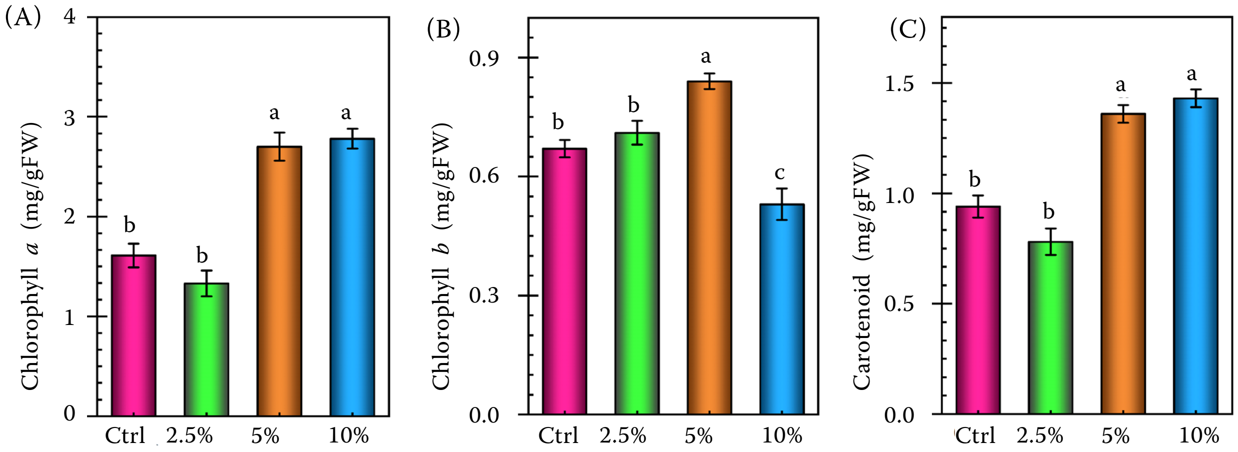

Figure 2. Effect of the different levels of drought stress on the chlorophyll content in the cucumber seedlings
FW – fresh weight

Chlorophyll plays an important role in the assimilation, transmission, and conversion of light energy in the process of photosynthesis (Chen et al. 2021). The chlorophyll *b* content of the cucumbers increased and then decreased under the moderate (5% PEG 6000) and severe (10% PEG 6000) drought stress, indicating that the cucumbers adjusted their energy capture during the early stage of drought, due to the relatively stable electron transfer, normal photosynthesis was maintained, and when the PEG concentration reached 10%, the severe drought stress may damage the chloroplasts and eventually destroy them, resulting in a chlorophyll loss and photosynthetic efficiency reduction (Zahedi et al. 2020). In addition, both chlorophyll *a* and the carotenoid content were significantly increased under the moderate and severe drought stress compared with controls. However, under the mild (2.5% PEG 6000) drought, chlorophyll *a*, *b*, and carotenoid content had no obvious difference with the controls (Figure 2). It suggested that maybe the chlorophyll was not seriously damaged after 10 days of drought stress. Moreover, it was reported that as the stress intensifies during prolonged drought, the chlorophyll degradation accelerates and electron transfer through PSII slows (Chen et al. 2019).

Effects of the different levels of drought stress on the MDA content and certain antioxidase activities of the cucumber seedlings. Different levels of drought stress at the seedling stage increased the MDA content in the leaves, stems, and roots, and with the increase in the PEG concentration, the MDA content gradually increased. Moreover, the change in the MDA content under the mild stress (2.5% PEG 6000) was not significantly different from that under the control (Figure 3). The MDA content under the severe stress was significantly higher than that under the control and mild stress. Under the severe drought stress (10% PEG 6000), the increasing percentage was 24.3%, 40.1%, and 26.1% in the leaves, stems, and roots, respectively, compared with the control plants.

With an increase in the drought stress, the SOD activity evidently increased in all the cucumber seedlings, except for the activity under the mild stress (2.5% PEG 6000). The highest SOD activity in all the drought stress levels was detected in the leaves under the severe drought stress (10% PEG 6000). With an increase in the PEG level to 10%, the SOD activity increased by 52.4%, 74.9%, and 79.1%, in leaves, stems, and roots respectively, compared to the

Table 1. Effect of the different levels of drought stress on the photosynthesis parameters of the cucumber seedlings

Treatment	*Pn* (μmol $CO_2/m^2/s$)	*Tr* (mmol $H_2O/m^2/s$)	*Gs* (mmol $H_2O/m^2/s$)	*Ci* (μmol CO_2/mol)
Control	14.3 ± 1.5^a	2.5 ± 0.2^a	106.4 ± 0.8^a	329 ± 5.1^c
2.5% PEG	13.0 ± 1.2^a	2.3 ± 0.2^a	90.4 ± 0.7^b	335 ± 3.8^c
5% PEG	11.6 ± 0.9^b	2.0 ± 0.1^b	84.5 ± 1.5^c	368 ± 2.4^b
10% PEG	9.3 ± 0.4^c	1.8 ± 0.1^c	78.6 ± 1.8^d	412 ± 3.6^a

Pn – net photosynthetic rate; *Tr* – transpiration rate; *Gs* – stomatal conductance; *Ci* – intercellular CO_2 concentration

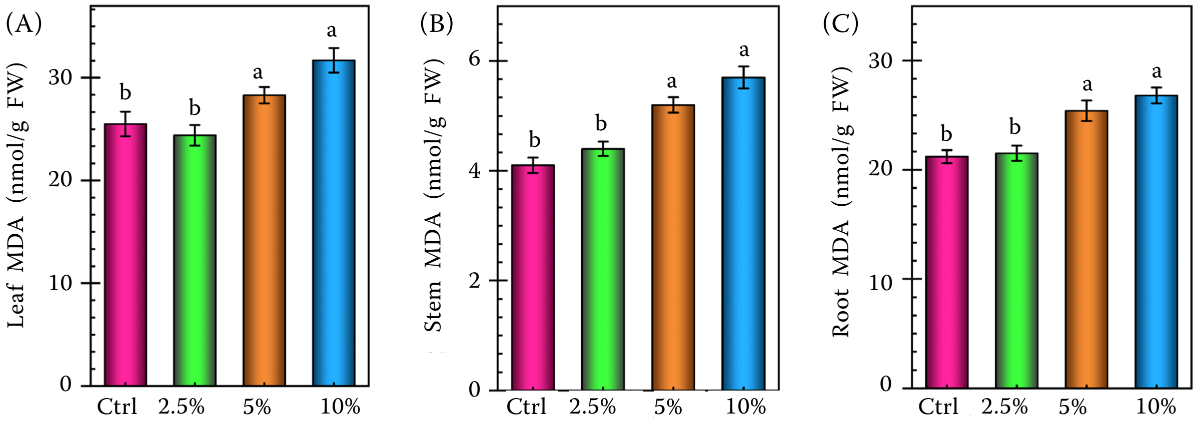

Figure 3. Effect of the different levels of drought stress on the MDA content in the cucumber seedlings
FW – fresh weight

control conditions. The changing trend of the APX activity after the drought stress was similar to that of the SOD activity (Table 2). Compared with the control, an increase in the PEG level increased the POD activity in the cucumber seedlings, except for the leaf POD activity under the 2.5% PEG treatment. However, under the 10% PEG stress, the root POD activity was almost unchanged compared with that of the 5% PEG stress.

MDA is the product of membrane lipid peroxidation when plants are subjected to drought stress, and the MDA content reflects the level of damage to the plant cell membrane. Moreover, the production of ROS is directly related to the overproduction of MDA (Sachdev et al. 2021). The present research indicated that the MDA content in the cucumbers increased with the increase in the PEG concentration, demonstrating that, in cucumber seedlings, drought stress will lead to oxidative damage, and this was consistent with previous studies, e.g., Cui et al. (2019) reported that a gradual increase in the MDA content was detected in cucumber leaves as drought stress became more severe. However, there was no significant difference between the mild (2.5% PEG 6000) drought stress and the control groups. It indicated that when the PEG concentration was less than 2.5%, the damage to the membrane systems of the cucumber seedlings was not significant in all the cucumber tissues, and the membrane system was severely damaged after the PEG concentration was greater than 5% (Figure 3). The increase in the plant antioxidant enzyme activity is a defence mechanism against drought stress, scavenging free radicals, and reduc-

Table 2. Effect of the different levels of drought stress on certain antioxidase activities of the cucumber seedlings

Antioxidase	Tissues	Control	2.5% PEG	5% PEG	10% PEG
SOD (U/g FW)	Leaves	5.94 ± 0.3^{c}	6.41 ± 0.9^{bc}	7.82 ± 0.7^{b}	9.05 ± 0.4^{a}
	Stems	4.15 ± 0.5^{c}	4.24 ± 0.3^{c}	6.31 ± 0.5^{b}	7.26 ± 0.1^{a}
	Roots	2.63 ± 0.2^{c}	2.97 ± 0.4^{c}	3.24 ± 0.6^{b}	4.71 ± 0.4^{a}
POD (µmol/min/g FW)	Leaves	2.21 ± 1.1^{c}	2.62 ± 0.2^{c}	4.25 ± 0.5^{b}	5.93 ± 0.3^{a}
	Stems	1.43 ± 0.4^{d}	2.08 ± 0.3^{c}	4.02 ± 0.4^{b}	6.25 ± 0.4^{a}
	Roots	10.5 ± 0.7^{c}	12.3 ± 0.4^{b}	16.0 ± 0.8^{a}	17.16 ± 1.1^{a}
APX (nmol/min/g FW)	Leaves	3.26 ± 0.16^{a}	3.59 ± 0.21^{a}	2.12 ± 0.10^{b}	1.81 ± 0.12^{b}
	Stems	3.11 ± 0.14^{a}	3.50 ± 0.24^{a}	2.01 ± 0.14^{b}	1.69 ± 0.15^{c}
	Roots	4.35 ± 0.22^{a}	4.55 ± 0.25^{a}	3.14 ± 0.23^{b}	2.32 ± 0.19^{c}

The data are the means of three independent replicates

$^{a-d}$The different letters in each line indicate the significant differences ($P < 0.05$) among the four treatments; FW – fresh weight

SOD – superoxide dismutase; POD – peroxidase; APX – ascorbate peroxidase ing oxidative stress (Hajizadeh et al. 2023). In the present study, the SOD, POD and APX activities were probably increased due to the high levels of drought stress, and the mentioned enzyme activities could be used as factors to measure the response of the plant to drought stress. Meanwhile, Cui et al. (2019) also reported that the PEG treatment increased the activities of APX, POD, and CAT under moderate and severe drought stress.

Effects of the different levels of drought stress on the soluble protein content of the cucumber seedlings. The content of soluble protein in the cucumber seedlings increased with the increase in the drought intensity. Under the mild (2.5% PEG 6000) drought stress, the soluble protein content, only in the leaves, was significantly increased compared with the control, and there was no remarkable difference in the stems and roots (Figure 4). Furthermore, with an increasing supply of PEG, the soluble protein content showed a clear positive linear response. After 10 days of the severe (10% PEG 6000) drought treatment, the soluble protein content reached the maximum. Moreover, the content in the leaves under the severe drought stress was distinctly higher than those under the moderate (5% PEG 6000) drought stress (Figure 4A).

Osmotic regulation is an important strategy for plants to adapt to drought stress, which is related to the accumulation of various osmolites, including protein and carbohydrates. Increasing the synthesis of soluble proteins is one of the important ways of osmotic regulation (Close 1996). The proline, soluble protein and soluble sugar content were increased as drought stress became more severe (Cui et al. 2019). In this study, the soluble protein content increased with the increase in the PEG concentration, but the difference in the roots between 5% and 10% was not significant (Figure 4). This indicated that under the present drought stress, the cucumber seedlings maintained their normal physiological functions through the metabolism of osmotic regulators, which is a positive response mechanism.

Effects of the different levels of drought stress on the total phenols and flavonoid content of the cucumber seedlings. It was observed that a PEG 6000-dose-response relationship was found in the total phenols content, the value increased as the concentration of the PEG increased, which was in the range of 31.3–440.2 mg/g of fresh weight. Furthermore, there were significant differences in total phenols content among the three different levels of drought stress in the different tissues, except for the root total phenols content between the severe (10% PEG 6000) and moderate (5% PEG 6000) drought stress (Figure 5). The highest total phenols value can be found in the leaves of the cucumber seedlings under the severe (10% PEG 6000) drought stress, with an increasing percentage of 158.7% compared with those in the control. Moreover, under the severe (10% PEG 6000) and moderate (5% PEG 6000) drought stress, the total flavonoids content in all the tissues was significantly higher than those of the controls; and there was also a significant difference between these two drought stresses (Figure 6). However, under the mild (2.5% PEG 6000) drought stress, only the leaf/root total flavonoid content was markedly increased compared with the control, and the stem values were similar to the control.

Phenolic compounds are natural compounds in fruits and vegetables, which are attracting more

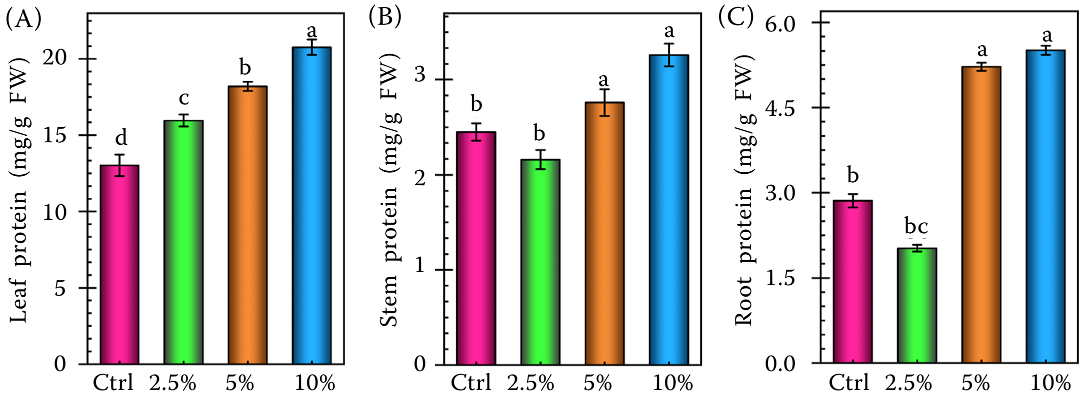

Figure 4. Effect of the different levels of drought stress on the soluble protein content in the cucumber seedlings
FW – fresh weight

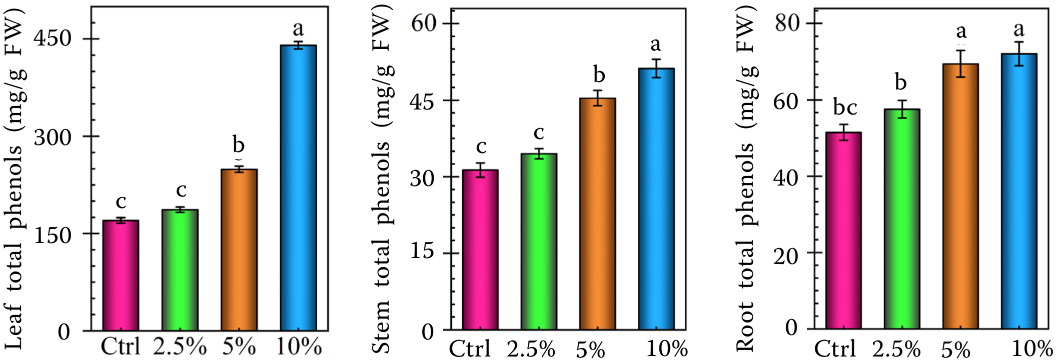

Figure 5. Effect of the different levels of drought stress on the total phenols content in the cucumber seedlings
FW – fresh weight

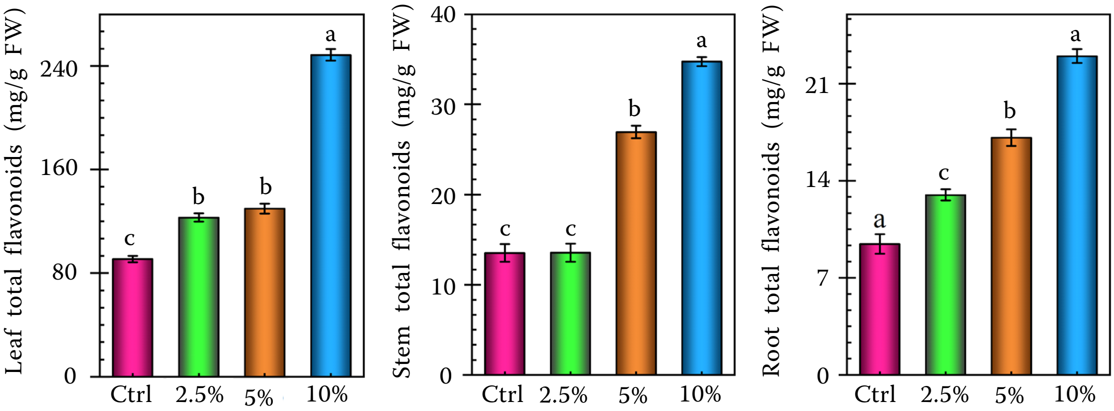

Figure 6. Effect of the different levels of drought stress on the total flavonoid content in the cucumber seedlings
FW – fresh weight

and more attention due to their disease-prevention effects and potential technical applications (Muñoz-González et al. 2021; de Araújo et al. 2021). Drought stress can promote the accumulation of antioxidant substances such as total phenols and flavonoids (Sarker and Oba 2018). In the present study, the drought treatment significantly increased the total phenols and flavonoid content compared to the control plant, and the contents were enhanced with the increasing PEG percentage (Figures 5 and 6). These findings were similar with previous results, where the drought stress enhanced the synthesis of the total phenols and flavonoid content in cucumber seedlings (Ghani et al. 2022; Li et al. 2022). The increase in the total flavonoid contents in response to drought stress may be owing to the upregulation of the flavonol synthesis-related proteins under drought stress (Gu et al. 2020). It suggests that, under certain levels of drought stress, the antioxidant system of cucumber seedlings is stabilised and strengthened by increasing the content of phenols and flavonoids. Recent studies have also revealed that plants can improve their drought, salt, and other abiotic resistance by increasing the accumulation of flavonoids (Li et al. 2019). In addition, both phenols and flavonoids can eliminate ROS, prevent lipid peroxidation, and maintain membrane fluidity and function (Oteiza et al. 2005).

CONCLUSION

It was concluded, from our study, that the plant growth status under the mild (2.5% PEG 6000) drought stress showed no significant difference with the control, while the plants exhibited a decreasing trend with an increased PEG level, and the plant height, root length, leaf dry weight (DW), stem DW, root DW, and plant DW all showed prominent inhibition under the severe (10% PEG 6000) drought stress. Moreover, the changing trend in the photosynthetic efficiency, chlorophyll *a* and carotenoid content was consistent with the growth index under different levels of drought stress. Besides, the content of chlorophyll *b* in the cucumber seedlings increased under the moderate (5% PEG 6000) drought stress, but decreased under the severe (10% PEG 6000) drought stress. However, certain antioxidase activities, soluble protein content, malondialdehyde (MDA) content, total phenols, and total flavonoid content in the cucumber seedlings increased gradually with the increase in the mass fraction of PEG. The results indicated that the higher the level of the drought stress, the higher inhibition on the seedling growth or more severe damage on the cell membranes and the higher contents of total phenols and flavonoids.

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