

# Phedimus Aizoon L. Responds to Drought Stress: Growth, Physiological Changes and Mechanism of Drought Resistance

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## Research Article

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# Abstract

A pot experiment was conducted to investigate the growth, physiological changes and mechanism of drought resistance of *Phedimus aizoon* L. under different levels of water content .CK: 75% ~ 80% of the MWHC (maximum water holding capacity), Mild drought: 55% ~ 60%, Moderate drought: 40% ~ 45%, Severe drought: 20% ~ 25%. We observed that the plants grew normally in the first two treatments, even the mild drought promoted the growth of the roots. In the last two treatments, drought stress had a significant negative effect on plant growth, at the same time, *Phedimus aizoon* L. also made positive physiological response to cope with the drought: The aboveground part of the plant (leaf, plant height, stem diameter) was smaller, the waxy layer of the leaves was thickened, the stomata of the leaves were closed during the day, and only a few stomata were opened at night, which proved that the dark reaction cycle metabolism mode of the plant was transformed from C3 cycle to CAM pathway. The activity of antioxidant enzymes (SOD, POD and CAT) was continuously increased to alleviate the damage caused by drought. To ensure the relative stability of osmotic potential, the contents of osmoregulation substances such as proline, soluble sugar, soluble protein and trehalose increased correspondingly. But plants have limited regulatory power, with aggravation of drought stress degree and extension of stress time, the MDA content and electrolyte leakage of leaves increased continuously. Observed under electron microscope, the morphology of chloroplast and mitochondria changed and the membrane structure was destroyed. The plant's photosynthetic and respiratory mechanisms are destroyed and the plant gradually die.

## 1 Introduction

As a worldwide problem, drought is one of the most important stress factors limiting plant growth and yield in arid and semi-arid regions (Anjum et al.<sup>[1]</sup>; Gray and Brady<sup>[2]</sup>). Today, the global arid and semi-arid regions account for one-third of the total land area. Drought is considered a multidimensional stress that restricts plant growth and development, and causes a series of changes in physiological, morphological, biochemical and molecular characteristics of plants (Wu et al.<sup>[3]</sup>; Noman et al.<sup>[4]</sup>). The effects of drought stress predominantly vary with the plant species and genotype, developmental stages as well as its duration, and stress severity (Gall et al.<sup>[5]</sup>; Bhargava and Sawant<sup>[6]</sup>). Generally, plant responses to drought stress are mostly reflected in loss of turgor, drooping, wilting and yellowing of leaves, decline of plant height, shoot dry weight, leaf area index and even damage to blade structure, but proliferation of root length (Jaleel et al.<sup>[7]</sup>; Davis et al.<sup>[8]</sup>; Hufstetler, et al.<sup>[9]</sup>). In addition, drought stress causes oxidative stress in plants through the production of reactive oxygen species (ROS), which leads to metabolic disorders and disruption of ion absorption and translocation (Anjum, et al.<sup>[10]</sup>; Cruz de Carvalho<sup>[11]</sup>).

Studies have shown that the plant drought resistance has certain correlation with the anatomical structure (Wang et al.<sup>[12]</sup>). Besides, the drought resistance of plants is mainly manifested by the combination of osmoregulation substances, changes in membrane lipid components, free radical scavenging, and protein induces the formation of hormone regulation (Smirnoff<sup>[13]</sup>). Drought stress causes imbalance between the generation and scavenging system of active oxygen in plants, meanwhile,

the accumulation of biological free radicals causes membrane damage and membrane lipid peroxidation. Antioxidant defense system consisting of a series of antioxidant enzymes and osmotic regulators (such as soluble sugar, soluble protein, free proline and trehalose in plants) not only removes these reactive oxygen species, but also helps maintain turgor and protects the macromolecular structure of cells (Farooq et al.<sup>[14][15]</sup>). It is widely used for road and roof greening because of its short stature, long flowering and green periods, and strong resistance to stress and barrenness.

*Phedimus aizoon* L. is perennial herb of Crassulaceae, and widely planted in north and south of China. It is widely used for road and roof greening because of its dwarfish height, long flowering and green periods, and strong resistance to stress and infertility (Stephenson,<sup>[16]</sup>). In addition to its strong ornamental value, it also has good edible and medicinal value. *Phedimus aizoon* L. is a wild vegetable selected from traditional breeding and rich in triterpene, sterol, flavonoids and other active substances (Li et al.<sup>[17]</sup>). It is used in foods primarily for its health benefits such as protecting cardiovascular and cerebrovascular, promoting blood circulation and enhancing immunity (Lin et al.<sup>[18]</sup>). It has become green and healthy wild vegetable with high economic value. Now, the research on *Phedimus aizoon* L. mainly focuses on its biochemical component identification and cultivation methods, and the study on stress resistance, especially drought resistance, has not been reported. Therefore, this research studied the effects of different degrees of drought stress on the growth, physiological changes of *Phedimus aizoon* L. by controlling soil moisture of potted plants, which aiming to clarify its drought tolerance mechanism, and provide a theoretical basis for water-saving and high-yield cultivation of *Phedimus aizoon* L. in the arid regions.

## 2 Materials And Methods

### 2.1 Materials

The plants of *Phedimus aizoon* L. for experiment were obtained from cutting propagation for 20 days in mid-May, and they were provided by the college of horticulture, Sichuan Agricultural University (30°42' N, 103° 51' E) .

### 2.2 Experiment design

The experiment was conducted from May 2019 to October 2019 at Chengdu Campus of Sichuan Agricultural University. Before the experiment, the nutrient soil consisted of humus soil: perlite: vermiculite = 4:1:1 (V:V:V) was dried to constant weight, then the plastic pots (14 cm in diameter and height) were filled with above soil, and recorded the quality of dried soil and soil saturated with water, repeated five times and calculated the maximum water holding capacity (MWHC). Then, the seedlings of *Phedimus aizoon* L. with the same growth were selected and transplanted into pots filled with the same quality of dried nutrient soil. Drought stress was simulated by the method of weighing soil. Four drought stress levels were set in the test, which were non-stress (75%-80% of MWHC, Control), mild drought stress (55%-60% of MWHC, T1), moderate drought stress (40–45% of MWHC, T2) and severe drought stress

(20–25% of MWHC, T3). The water was replenished every morning and evening by weighing soil, and a soil three-parameter instrument (WET-2, Zealquest Scientific Technology Co. Ltd, China) was used to monitor soil moisture. Each treatment was replicated for three times with a completely randomized design with 10 cm spacing. The pots were kept under ambient environmental conditions with natural sunlight and temperature in a plastic house. Air temperature ranged between  $28 \pm 2.5$  °C (day) and  $20 \pm 2.5$  °C (night). Ten plants of each treatment were randomly selected on 10d, 20d, and 30d at 9:00 in the morning (3th to 5th leaves from top to bottom) for determination of various physiological indicators. Three plants were randomly selected on 30d at 9:00 am to observe the anatomical structure of the leaves.

## **2.3 Experiment methods**

### **2.3.1 Determination of growth parameters**

The whole plants were gently uprooted and divided into shoots and roots, then rinsed with tap water and washed again with deionized water repeatedly. Plant height and root length were measured with a millimeter scale ruler, stem diameter was measured with vernier calipers.

### **2.3.2 Determination of lipid peroxidation and osmolyte**

Malondialdehyde (MDA) was measured to determine the level of lipid peroxidation following the method used by Kumar and Knowles<sup>[19]</sup>. Electrolyte leakage was measured following the method used by Dionisio-Sese and Tobita<sup>[20]</sup>. Soluble sugar content was measured by the method of anthranone-ethyl acetate (Gill et al.<sup>[21]</sup>). Proline content was assayed by the method of sulfo-salicylic acid (Bates et al.<sup>[22]</sup>). Soluble protein content was measured by the method of coomassie brilliant blue (Bradford<sup>[23]</sup>).

The accumulation of trehalose was determined as per the methods of Kumar et al.<sup>[24]</sup> and Li et al.<sup>[25]</sup> with modifications. Fresh leaves (0.2 g) were ground with 1 mL of 0.5M trichloroacetic acid solution in ice-water baths, then filled the volume of solution to 5 mL, and shocked for 2 hours at 0°C. The solution was centrifuged at 10,000 rpm for 10 min, 0.2 ml of supernatants was mixed with 0.2 N H<sub>2</sub>SO<sub>4</sub> (0.2 ml) and boiled at 100 °C for 10 min, after cooling, 4 ml of anthrone reagent (0.2 g anthrone in 100 ml cold 95% sulfuric acid) was added to above mixture and boiled at 100 °C for 10 min, then chilled again. The absorbance of the above solution were made at 630 nm with spectrophotometer, the concentration was worked out from a standard curve of trehalose.

### **2.3.3 Determination of antioxidant enzyme activities**

Superoxide dismutase (SOD) activity was determined following the method of Beauchamp and Fridovich<sup>[26]</sup> with one unit of activity as the amount of protein required to inhibit 50% initial reduction of nitroblue tetrazolium (NBT) under light. Peroxidase (POD) activity was assayed as described by Omran<sup>[27]</sup>. The activity of catalase (CAT) was assayed as described by Aebi<sup>[28]</sup> and one unit was defined as the amount of enzyme required to decompose 1  $\mu\text{mol min}^{-1}$  of hydrogen peroxide.

### **2.3.4 Observation of leaf structure**

The paraffin method was according to the method of Li and Zhang<sup>[29]</sup>. On the 30th day of drought treatment, the mature leaves of each treatment were randomly selected, small pieces (1 cm × 3 mm) were cut from the part between main-vein to margin of the leaf with a double-sided blade. Then the sample segments were fixed in FAA stationary liquid containing 5% formalin, 5% acetic acid, and 90% ethanol at 4°C. The fixed segments were dehydrated in a graded series of ethanol solutions, and further treated for being transparent, paraffining, embedding, production, dyeing and sealing. The slice thickness was 8 μm. Then the segments were double stained with safraninO-fast green. The cell structure of the samples was observed and photographed with optical microscope (DS-U3, Nikon, Japan), and the microstructure of the leaves was observed and photographed by transmission electron microscopy (H-600IV, Hitachi, Japan) The thickness of leaves, epidermis, and cuticle were measured by microscope graticules, and the values were the average of 40 measurements.

## 2.4 Statistical analyses

All data were analyzed using SPSS 20.0 statistical software (IBM Corporation, Armonk, NY, USA). Data were analyzed by one-way analysis of variance with least significant difference (LSD) at the 5% confidence level.

## 3 Results

### 3.1 Growth

Mild and moderate drought stress had no significant effect on plant height, which was 13.17% ( $P < 0.05$ ) lower than that of the control only at 30 days of moderate stress. Severe drought stress significantly inhibited plant height, which was significantly lower than the control throughout the whole period, and the reduction increased with the increase of stress days (Table 1). The lateral growth of plants was inhibited by drought stress, but there was no discernible rule. At the initial stage of stress (10d), the stem diameter of each treatment was significantly lower than that of the control; at the 20th day, the stem diameter of T3 alone was significantly lower than that of the control, at the 30th day, both T2 and T3 were significantly lower than that of the control (Table 1). The effect of drought stress on root growth was different from that of shoot, which showed a certain promoting effect, but with the extension of stress time, the promotion effect would gradually weaken. In the early stage of stress (10d), the root lengths of T1 and T2 treatments were 38.92% ( $P < 0.05$ ) and 42.89% ( $P < 0.05$ ) higher than that of control, respectively, although the root length of T3 treatment was lower than T1 and T2, it was still significantly higher than that of control. In the middle stage of stress (20d), the root length of T1 treatment was the largest, which was significantly higher than other treatments, while the root length of T3 treatment was not distinct different from the control. In the late stage of stress (30d), mild and moderate drought stress promoted root growth, but there was no significant difference with control, while severe drought stress showed no significant inhibition on root growth (Table 1).

Table 1  
Effects drought stress on plant height, root length and stem diameter in *Phedimus aizoon* L.

	Treatment	10d	20d	30d
	Control	14.27 ± 0.36a	15.82 ± 1.32a	18.75 ± 0.93a
Plant height	T1	13.51 ± 0.74a	16.21 ± 0.54a	18.39 ± 0.48a
(cm)	T2	12.95 ± 1.11a	14.87 ± 0.25a	16.28 ± 0.25b
	T3	11.09 ± 0.69b	11.92 ± 0.65b	12.24 ± 0.64c
	Control	0.482 ± 0.009a	0.454 ± 0.049a	0.486 ± 0.032a
Stem diameter	T1	0.432 ± 0.008b	0.495 ± 0.014a	0.441 ± 0.025ab
(cm)	T2	0.420 ± 0.018b	0.488 ± 0.031a	0.410 ± 0.034b
	T3	0.421 ± 0.020b	0.385 ± 0.006b	0.388 ± 0.012b
	Control	9.84 ± 0.82c	15.32 ± 0.78c	16.45 ± 1.39ab
Root length	T1	13.67 ± 1.07a	18.09 ± 0.63a	17.71 ± 0.48a
(cm)	T2	14.06 ± 0.16a	16.68 ± 0.20b	17.01 ± 0.46a
	T3	12.21 ± 0.49b	15.05 ± 0.72c	15.26 ± 0.99b
<p>Note: Data are means ± SD of three replications. Mean values carrying different letters within each parameter differ significantly (<math>p \leq 0.05</math>) based on Duncan's multiple range test. Control: 75%~80% of MWHC, T1: 55%~60% of MWHC, 40%~45% of MWHC, 20%~25% of MWHC.</p>				

### 3.2 Leaf anatomical structure

The mesophyll cells of leaves in the control were loose and well-hydrated, and the single cell was in large volume.

Because of too much water in cells, the effect of dehydration fixation was poor when the slices were made. However, as the drought stress deepened, the arrangement of mesophyll cells gradually changed from loose to tight, and the mesophyll cells gradually shrunk and lose water. Especially under severe drought stress, the spaces between mesophyll cells the volume of cells were reduced, the leaf fixation after water loss was better, and the leaf slices were more complete. At the same time, the chloroplasts in the mesophyll cells uniformly distributed along the cell wall under suitable moisture conditions, but which were gradually gathered to the middle of cells with the deepening of drought stress. Under severe stress, the chloroplast envelope dissociated, and the chloroplasts partially merged, and there was no complete chloroplast in the cell (Fig. 1).

The statistical results of leaf anatomical structure analysis showed that under mild drought stress, leaf thickness was 3.55 ( $P < 0.05$ ) higher than the control, while the leaf thickness decreased under moderate and severe drought stress and was significantly lower than the control, especially under severe stress,

which was decreased by 25.34% than the control. In terms of upper epidermal thickness, it was significantly lower than that of control only under severe drought stress. Under mild drought stress, there was no obvious change in the thickness of lower epidermis, but under moderate and severe stress, it was 40.87% ( $P < 0.05$ ) and 42.59% ( $P < 0.05$ ) lower than that of control, respectively. Secondly, the difference in cuticle thickness among the treatments was not significant, but there was a tendency of thickening with the deepening of stress (Table 2).

Table 2  
Statistical analysis of leaf anatomical structure of *Sedum aizoon* L. under drought stress

	Control	T1	T2	T3
Leaf thickness ( $\mu\text{m}$ )	740.56 $\pm$ 5.33b	766.81 $\pm$ 4.28a	718.59 $\pm$ 3.34c	552.84 $\pm$ 10.79d
Thickness of upper epidermis ( $\mu\text{m}$ )	38.41 $\pm$ 7.25a	32.45 $\pm$ 3.18a	36.56 $\pm$ 1.99a	28.77 $\pm$ 2.26b
Thickness of lower epidermis ( $\mu\text{m}$ )	24.56 $\pm$ 7.73a	23.15 $\pm$ 3.24a	14.52 $\pm$ 3.10b	14.10 $\pm$ 0.49b
Cuticle thickness ( $\mu\text{m}$ )	3.72 $\pm$ 0.51a	3.96 $\pm$ 0.69a	4.24 $\pm$ 0.87a	4.33 $\pm$ 0.74a

## 3.2 Lipid peroxidation

At the initial stage of stress (10d), there was no significant difference in MDA content among all treatments. At 20d, T2 and T3 treatments were significantly higher than the control. The change of MDA content at 30d was the same as that at 20d, and the T2 and T3 treatments increased by 6.43% ( $P < 0.05$ ) and 15.24% ( $P < 0.05$ ) compared with control, respectively (Fig. 2A). At the drought stress for 10 days, the electrolyte leakage rate was only significantly higher in T3 treatment than control. At 20d, there was no significant difference among the treatments. At the later stage of stress (30d), although there was no significant difference between T1 and control, both T2 and T3 were significantly higher than control, with an increase of 34.92% and 53.89% respectively compared with control (Fig. 2B).

## 3.3 Osmoregulation substances

The changes of soluble sugar content, proline content and trehalose content were basically the same throughout the drought treatment stage, which were increased with the aggravation of drought stress. The soluble sugar of T1 showed no significant difference with the control during the whole stress process, T2 and T3 were significantly higher than the control at 20d and 30d, especially at 30d, which were 99.40% ( $P < 0.05$ ) and 173.28% ( $P < 0.05$ ) higher than control, respectively (Fig. 3A). The content of proline in all treatment was significantly higher than that in the control throughout the period, and the higher the stress intensity, the higher the content and the greater the increase. In each period, the content of proline in T3 was the highest, at 10d, 20d and 30d of stress, which were 107.52% ( $P < 0.05$ ), 197.41% ( $P < 0.05$ ) and 232.27% ( $P < 0.05$ ) higher than that of control respectively (Fig. 3B). Consistent with the

change of proline content, the trehalose content in the drought treatment at all stages was significantly higher than that in the control. At 30d of the drought stress, the difference among treatments were significant, especially in the T3, the trehalose content was the highest, which was 199.37% ( $P < 0.05$ ) higher than that in the control (Fig. 3C). The change of soluble protein content in drought treatment was small in different periods, and there was no obvious change rule, but significantly higher than the control (Fig. 3D).

### 3.4 Antioxidant enzyme activity

With the increase of drought intensity and time, the activity of antioxidant enzymes gradually increased. During the whole period of drought stress, the increase amplitude of SOD activity was small, except for T3 treatment, there was no significant difference between other treatments (Fig. 4A). The changes of POD enzyme activity was coincident with that of CAT, when treated for 10 days, although there were significant differences between different treatments, the change range was small. At 20 days, although there was no significant difference between T1 and control, the differences among T2, T3 and control were all significant. At 30 days of drought stress, the activities of POD and CAT were significantly different among all treatments, both SOD and CAT activity increased with the aggravation of drought stress. In particular, T3 treatment was significantly higher than other treatments, SOD and CAT activity were respectively 169.81% and 230.47% higher than the control at 30d (Fig. 4B, C).

### 3.5 Photosynthetic parameter

On the 30th day of drought treatment, the photosynthetic parameters of *Phedimus aizoon* L. changed significantly. Net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular carbon dioxide concentration (Ci) were negatively correlated with stress intensity, while stomatal limit value (Ls) was positively correlated with stress intensity. Especially under severe drought, Pn, Gs, Tr, Ci of plants decreased by 71.15%, 57.98%, 76.83% and 60.24%, while Ls increased by 127.66%. Under moderate drought, the water use efficiency (WUE) of plants was significantly higher than that of other treatments (Fig. 5).

### 3.6 Stomatal aperture

We observed the stomata of *Phedimus aizoon* L. at day and night (11:00 am. and 11:00 pm.) under scanning electron microscope, It can be seen in (Fig. 6) and (Fig. 7), in CK and T1, stomatal were open in day and night, and there was no significant difference in surface wax layer. However, under T2 treatment, the number of stomatal openings decreased during the day, almost in a closed state, stomatal opening decreased, and the guard cells were slightly deformed and wrinkled. At night, the condition is similar to CK and T1, but the waxy layer thickness increases and the number of waxy crystals increases. Under T3 treatment, we observed that the waxy layer on the leaf surface increased and thickened, and the guard cells of stomata shrank and stomata became smaller (Fig. 8). Stomata are closed during the day and less open at night.

### 3.7 Chloroplast and mitochondrial microstructure

As shown in the (Fig. 9)&(Fig. 10), chloroplasts of plants were evenly distributed along the cell membrane under CK, in a spindle shape, and thylakoid membranes were neatly and closely arranged, with starch grains embedded in them. Mitochondria are spherical, round and clear boundaries. With the deepening of stress formation, chloroplasts were gradually shrivelled, boundaries were rough, thylakoid thin films were loose, lamellae were broken, the number of starch grains decreased and the volume decreased. Mitochondria dissolved inside, swelling white; Osmiophilic granules gradually increased. This indicates that the membrane system was irreversibly damaged by drought.

## 4 Discussion

The growth is a comprehensive response of plants to drought stress and a reliable criterion for evaluating drought stress degree and drought resistance of plants. The decrease of plant height and stem diameter is a common phenomenon in drought stress (Wang et al.<sup>[30]</sup>). The result of this study showed that under drought stress, the shoot growth of *Phedimus aizoon* L. was inhibited, and the stronger the degree of stress, the longer the time, the more significantly inhibited (Table 1), the main reason was that the lack of water led to clogging of vascular tissue and reduced cell elongation (Abdalla and El-Khoshiban<sup>[31]</sup>). Roots are the main organs of plants that absorb water and closely related to the drought resistance, the root system can adapt to drought stress by regulating its own growth and development, water absorption and transportation. The root length proliferation of *Phedimus aizoon* L. was promoted under mild and moderate drought stress (Table 1), which was beneficial for the root system to absorb the deep water of the soil and improve the utilization rate, thus improving the drought tolerance of the plant (Hufstetler et al.<sup>[9]</sup>).

The leaves are sensitive organs to drought during the evolution process. Because of their plasticity, the changes of leaf morphological structure will inevitably lead to the change of physiological and biochemical characteristics of plants. Therefore, the changes of leaf morphological traits can reflect the adaptability of plants to drought stress. (Mahajan and Tuteja<sup>[32]</sup>). The thicker the plant leaves, the greater the tightness of the tissue, the stronger the water storage capacity and the drought tolerance of the leaves (Pu<sup>[33]</sup>). *Phedimus* is a typical phedimus plant with strong drought tolerance, and the leaves belong to special fleshy, which is closely related to its drought tolerance (Gravatt and Martin<sup>[34]</sup>). Through the observation of leaf anatomical structure, it was found that in the absence of drought stress, the leaves of *Phedimus aizoon* L. were thicker, the mesophyll cells were large and irregular, arranged loosely, with large intercellular spaces and strong water storage capacity. Under moderate and severe stress, the mesophyll cells gradually aggregated and smaller, the chloroplast gradually aggregated into the middle of the cell, and the upper epidermis thickness became thinner (Fig. 1, Table 2). The purpose of this change is to reduce the direct contact of plants with radiation and transpiration of water, to preserve the limited water and to make full use of it (Xue et al.<sup>[35]</sup>). Especially under severe stress, the leaf thickness and upper epidermis thickness were significantly lower than that of control, and the tissue arranged from loose to tightly. As the drought deepened, water between the mesophyll cells was consumed, thus the intercellular space was shrunked, the cells were tightly arranged after being squeezed, and the cuticle was thicken

gradually (Fig. 1, Table 2), which indicated that the leaf tissue of *Phedimus aizoon* L. showed the strongest resistance to drought stress under such soil moisture.

The plasma membrane is the original site where plants are firstly damaged in stress. Under drought stress, the plasma membrane is damaged, which is characterized by increased plasma membrane permeability and partial electrolyte leakage (Tan and Blake<sup>[36]</sup>). At the same time, a large amount of reactive oxide species are produced in plants, which induces membrane lipid peroxidation and produces malondialdehyde, thus causes damage to plant cell membrane systems (Yao et al.<sup>[37]</sup>). The level of MDA content and changes of plasma membrane permeability are important indicators reflecting the degree of plasma membrane damage. This study showed that although the MDA content and electrolyte leakage increased with the increase of the time and degree of drought stress, throughout the growth phase, only the electrolyte leakage fluctuated significantly at 30d, and there were no significantly difference between mild drought stress and control from beginning to end (Fig. 2), indicating that *Phedimus aizoon* L. could endure a certain degree and time of water deficit.

The accumulation of osmotic regulatory substances (proline, soluble sugar and soluble protein) is one of the basic characteristics for plants to adapt to drought stress. Under drought stress, plants actively accumulate osmotic regulatory substances to increase the concentration of cell fluid, the main function of which is to maintain cell turgor, balance the infiltration of protoplasm and the external environment, and enable various physiological processes of cells to proceed normally. Moreover, it is generally believed that the drought resistance of plants is positively correlated with its content (Zhang et al.<sup>[38]</sup>). The content of soluble sugar, proline and trehalose in leaves of *Phedimus aizoon* L. was significantly increased under drought stress, especially at 30 days of stress (Fig. 3A, B, C). The result of this study was similar to the study of Guo<sup>[39]</sup>, which also showed that under drought conditions, as the stress time increased, the content of osmotic regulatory substances such as proline and soluble sugar increased sharply. The increase of these osmotic regulatory substances reduced the cell osmotic potential and ensured that *Phedimus aizoon* L. could continue to absorb water from soil. However, the change of soluble protein content was slightly different from that of other osmotic regulatory substances. The possible reason is that as the drought time prolongs, the degree of stress is aggravated, and the anabolism in plants is inhibited, resulting in the inability of soluble protein to continue to rise, and even decreased.

Oxidative stress is usually accompanied by drought stress, and antioxidant defense system is one of the mechanisms of drought response, which provides aerobic metabolism of energy for plant growth and development. The generation and clearance of intracellular reactive oxygen species (ROS) are in a state of dynamic equilibrium in normal state, but when plants are subjected to drought stress, the dynamic balance is destroyed (Cruz de Carvalho<sup>[11]</sup>). Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are effective in scavenging reactive oxygen species and preventing excessive ROS accumulation, and protect plants from hurt (Harb et al.<sup>[40]</sup>). This study showed that in order to remove excess ROS and reduce oxidative damage, *Phedimus aizoon* L. could maintain high antioxidant enzyme activity under different degrees of drought stress. Especially under severe drought

stress and in later period of stress, the activities of SOD, POD and CAT increased sharply (Fig. 4), the main reason is that the production of ROS in the plant increased sharply under this condition, although which caused serious damage to normal metabolism *Phedimus aizoon* L., the plant could still remove these ROS by increasing antioxidant enzymes accordingly, showing its strong resistance to drought stress.

The life activities of plants depend on stomata for gas exchange, and stomata is also a channel of transpiration. The size, opening degree and density of stomata directly affect the transpiration rate of plants (Dong J and Bergmann D C.<sup>[41]</sup>). In this study, stomata closed during the day and slightly opened at night under severe stress, which reduced the evaporation of water and hindered the absorption of CO<sub>2</sub>, which may be one of the factors causing the reduction of intercellular CO<sub>2</sub> concentration (Ci) (Fig. 5). At this time, stomatal conductance (Gs) and transpiration rate (Tr) of leaves significantly decreased, and stomatal limit value Ls significantly increased, leading to a decline in photosynthetic capacity, indicating that stomatal status directly affected the photosynthetic capacity and dark reaction metabolism of plants. For every gram of carbohydrate produced by the CAM pathway, crassulaceae plant consume one-tenth as much water as the C3 cycle. CAM pathway not only reduces water loss through stomata, but also promotes water absorption by plants (Winter K et al.<sup>[42]</sup>). Under drought stress, *Phedimus aizoon* L. can reduce water loss by regulating stomatal state and dark reaction metabolism, which is another important reason for the improvement of drought resistance.

Chloroplasts and mitochondria are the two most important sites for photosynthesis and respiration. Drought stress can change the microstructure of plants. In this study, chloroplasts gradually converge to the middle of cells under moderate and severe stress, and the chloroplasts shrink under severe stress, resulting in fragmentation of the granule lamella, internal dissolution, and destruction of membrane structure, which are consistent with the rise of the MDA content and electrolyte leakage of leaves. The increase of osmiophilic granule, the fragmentation and dissolution of starch granules increased the osmotic potential of cells and contributed to water absorption. The inner lamella of the mitochondria dissolves and become swollen and faded. In this case, the life activities of plants are seriously affected.

## 5 Conclusions

In summary (Fig. 11), when subjected to drought stress, the structure and physiology of *Phedimus aizoon* L. have changed accordingly to adapt to drought. Mild drought stress had little effect on the structure and physiological metabolism of *Phedimus aizoon* L. But under moderate stress, the plant took the initiative to increase the secretion of osmotic regulatory substances, and improved the osmotic regulation ability and reduced the osmotic potential inside and outside plant cells, so as to improve the water absorption and water retention ability of plants. Although plants were grievously damaged under severe drought stress, they could maintain their life activities and reduce water loss by changing the leaf structure, improving the osmotic regulatory substances and antioxidant enzyme activities. Plants have a limited capacity for regulation, under severe drought, plant stomata are almost completely closed, chloroplasts and mitochondrial structures were destroyed, the anabolism and catabolism of plants are seriously affected,

so the plants gradually die. Although *Phedimus aizoon* L. has a strong drought resistance, but when cultivated, the soil moisture content should not be less than 40% of the field water capacity.

## Declarations

### Author Contributions Statement

Y. L. designed the experiments, wrote the manuscript. Z. H. designed the experiments. Y. X. processed the data, reviewed the manuscript. L. S., R. Z., H. W., C. L. and S. L. collected samples reviewed the manuscript and supervised the study.

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### Competing interests

The authors declare no competing interests.

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