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Response of Growth and Other Physiological Characteristics of *Sophora Japonica* L. Saplings to Drought Stress

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Abstract. Potting and continuous drought was applied to study the effect of drought stress (2 days for a treatment interval and 0-14 days of continuous drought) on the growth and photosynthetic characteristics of *Sophora japonica* L. saplings. The results were received as followed. (1) The ground diameter and height increment of *Sophora japonica* L. saplings were inhibited under drought stress. There was no significant difference under mild and moderate drought treatments (drought time 2-8 days) compared with the control group in leaf water content and leaf relative water content; and serious drought treatment (drought time > 10 days) was significantly lower than that of control group ($P < 0.01$). (2) Under drought stress, the content of every photosynthetic pigment of the leaf of *Sophora japonica* L. saplings was first increased and then decreased, reaching the highest point on the eighth day of drought. After drought stress, the net photosynthetic rate, transpiration rate, stomatal conductance and other aspects of the leaf of *Sophora japonica* L. saplings presented various degrees of being inhibited. (3) Under the drought stress, several physiological indexes of the leaf of *Sophora japonica* L. saplings decreased significantly, including the apparent quantum yield (AQY), carboxylation efficiency (CE) of RuBP, light compensation point (LCP), light saturation point (LSP) and CO₂ compensation point (CCP), which shows that drought stress can reduce the utilizing and adaptive capacity of *Sophora japonica* L. saplings to CO₂ in the environment. (4) Under drought stress, the content of soluble sugar and free proline in the leaf of *Sophora japonica* L. saplings increased first and then decreased. They reached the highest in the eighth day of drought but were still higher than that of the control group at the end of the drought stress. (5) Under drought stress, the content of antioxidant enzymes like POD and SOD in the leaf of *Sophora japonica* L. saplings increased first and then decreased; they reached the highest on the eighth day of drought but were still higher than that of the control group at the end of the drought stress.

Key words: *Sophora japonica* L. saplings; drought stress; physiological characteristics; morphological growth.

1. Introduction

The growth of plants is not only controlled by their own genetic materials, but also influenced by many environmental factors, such as light, temperature, water and soil nutrients. Among the many environmental factors, water is one of the most common factors limiting plant growth[1]. Under drought stress, the stomatal density of plant leaves increases, the stomatal conductance decreases, the



absorption rate of CO₂ and release rate of O₂ decreases [2-3], and the plant photosynthetic rate decreases [4]. Through quantitative experiment on physiological indexes of the growth and other physiological characteristics of *Sophora japonica* L. saplings, this research analysed their response mechanism to drought stress. Therefore, the research has certain practical significance to the cultivation and afforestation of *Sophora japonica* L., and also has certain promotion significance to the formulation of drought countermeasures for other greening tree species in seedling stage.

Sophora japonica L. belongs to the Leguminosae Papilionoidea *sophora*. It is a kind of deciduous trees with pinnate compound leaves, conical inflorescence and fleshy pods. Its fruit does not fall down in winter. *Sophora japonica* L. has a developed root system, huge crown and dense foliage. It enjoys light and has shade tolerance and strong wind resistance. In addition, it has strong adaptability and resistance to many kinds of harmful substances, and it therefore is one of the tree species suitable for garden cultivation and urban and rural greening.

2. Materials and methods

2.1. Material processing and experimental design

Sophora japonica L. seeds were collected in the campus of Huainan Normal University in Anhui, and these seeds were dried and stored at low temperature. In April 2016, the seedlings were transplanted into the cultivation containers (white polypropylene plastic basins with 30cm in upper diameter, 25cm in bottom diameter and 25cm in height); the soil in the left-side wood of the campus was used as the ground mass for planting, and the soil characteristics are shown in Table 1. Every basin was loaded with 8kg soil and 3 seedlings were transplanted into each of them. During the growth of the seedlings, daily watering was ensured to keep the water content of the soil more than 20%. When the length of seedlings was about 120cm, one strong sapling was preserved in each culture container and drought stress test began to be carried out. The group with normal water supply was taken as the control group, and 7 experimental treatment group set up at the same time. Each group was treated with interval of 2 days, and the continuous drought period respectively were 2 days (D₂), 4 days (D₄), 6 days (D₆), 8 days (D₈), 10 days (D₁₀), 12 days (D₁₂) and 14 days (D₁₄). The control group was recorded as D₀, and each treatment group had 3 repetitions. In order to facilitate the analysis of the results, these 8 groups were divided into four treatment group according to the soil water content (SWC): the control group (d₀: SWC>20%), mild drought group (D₂ and d₄: SWC 15%-20%), moderate drought group (D₆ and d₈: SWC 10%-15%) and severe drought group (D₁₀, D₁₂ and d₁₄: SWC<10%). These basins were weighed at 18:00 daily and the water lost on the day was supplemented. After drought stress, the SWC and photosynthetic parameters of saplings in control group and treatment groups were immediately determined and relative water content (LRWC) and chlorophyll content of leaves were also determined. At the beginning and end of the drought stress, the physiological indexes of the saplings in each treatment group were measured in time.

Table 1. Physical and chemical properties of cultivating soil

PH	EC value / (Ms/cm)	Organic matter/(g/kg)	N / (g/kg)	P ₂ O ₅ / (g/kg)	K ₂ O / (g/kg)
7.56	3.20	56.31	11.27	5.68	8.36

2.2. Determination items and methods.

2.2.1. The PR2-6 soil profile moisture detector and HH2 handheld reader were employed to determine the soil moisture content (SWC) in the cultivated containers of each treatment group.

2.2.2. The Saturation weighing method [5] was used to determine the water status of the leaves.

2.2.3. Determination of growth indexes. Electronic ruler and Vernier caliper were used to measure the height and diameter of *Sophora japonica* L. saplings, repeated 5 times: the height of a sapling at the

end of drought stress - the height of the sapling at the beginning of drought stress = the tree height increment of the *Sophora japonica* L. sapling; the ground diameter of a sapling at the end of drought stress - the ground diameter of the sapling at the beginning of drought stress = the tree ground diameter increment of the *Sophora japonica* L. sapling;

2.2.4. Determination of physiological parameters of photosynthesis. The Li-6400 portable photosynthesis system was used to measure the net the photosynthetic rate (Pn), intercellular CO₂ concentration (Ci), stomatal conductance (Gs) and transpiration rate (Tr) of *Sophora japonica* L. saplings in every treatment group. Each determination was repeated 3 times. Because leaves of these *Sophora japonica* L. saplings are too small, measured leaves were picked after being measuring; Li-3000 leaf Area Measurement Instrument was used to measure area of leaves which were then input the Photosynthesis System to the data of photosynthesis [6].

2.2.5. Determination of photosynthesis-photo response and photosynthesis-CO₂ response [6] Li-6400XT Photosynthesis System was adopted in this part. The original program of this instrumentation was used to determine the photosynthesis-photo response (Pn-PAR) and photosynthesis-CO₂ response (Pn-CO₂), and the measurement was conducted was at 9:00-11:00.

2.2.6. Determination of chlorophyll content: spectrophotometric method [7].

2.2.7. Determination of soluble sugar content: a throne colorimetric method; determination of free proline content: colorimetric method of sulfonyl salicylic acid extracting ninhydrin [7].

2.2.8. Determination of protective enzyme activity. Determination of SOD activity: Nitro blue tetrazolium (NBT) method; determination of POD activity: guaiacol colorimetric method [7].

2.2.9. Data processing. The experimental data was analysed with Excel and the analysis of variance was processed by SPSS.

3. Results and analysis

3.1. Changes of soil water content under drought stress and the influence on the growth and leaf water content of *Sophora japonica* L. saplings.

Table 2. The influence of soil water content, leaf relative water content, leaf water content and growth of *Sophora japonica* L. saplings under drought stress

Treatment	Soil water content (SWC, φ %)	Leaf relative water content (LRWC, φ %)	Leaf water content (LWC, φ %)	Growth of height (l/mm)	Growth of diameter (l/mm)
d ₀	26.50±0.58a	86.58±6.15b	59.22±0.97a	18.31±3.10a	2.87±0.73a
d ₂	17.88±0.74b	97.89±0.27a	59.89±0.62a	17.02±2.27ab	1.84±0.76b
d ₄	15.08±1.12c	94.14±5.32ab	60.01±1.59a	16.15±2.38b	1.83±0.58b
d ₆	12.85±0.34d	90.62±0.21ab	62.91±0.21a	14.35±1.54c	1.15±0.64c
d ₈	10.28±0.63e	88.27±4.89b	60.24±3.19a	10.77±1.21c	0.87±0.59c
d ₁₀	9.39±0.57e	57.75±5.43c	49.02±3.86b	9.43±2.24cd	0.58±0.31d
d ₁₂	7.32±1.36e	54.64±0.79d	43.75±0.75b	8.96±2.01d	0.56±0.42d
d ₁₄	5.59±0.71e	39.65±4.09e	28.11±3.74c	5.82±1.12de	-0.16±0.91e

As can be seen from Table 2, *Sophora japonica* L. saplings present a downward trend in the increment of height and ground diameter due to drought stress; the severe stress treatments have the biggest impact on the increment of ground diameter --the value is negative.

With the aggravation of drought stress, the leaf water content of *Sophora japonica* L. saplings also decreased as the soil water content (SWC) decreased significantly ($P < 0.05$). Specifically, the value under mild and moderate stress had no significant difference with that of the control group ($P > 0.05$) but had significant difference under severe stress ($P < 0.05$). The leaf relative water content of *Sophora japonica* L. Saplings decreased significantly after significantly rising. Under mild stress, leaf relative water content increased and then decreased gradually; under moderate stress, there was no significant difference in leaf relative water content compared with that of the control group ($P > 0.05$); and relative water content in leaves decreased significantly under severe stress ($P < 0.05$). This change in the relative leaf water content and leaf water content showed that the leaf of *Sophora japonica* L. has strong capacity of water retention; by slowing down the rate of the leaf relative water content, *Sophora japonica* L. can increase water use efficiency and reduce water loss to maintain the normal operation of physiological and biochemical activities, so as to resist drought stress and grow in adversity.

3.2. Effects of drought stress on the photosynthetic pigment content in leaves of *Sophora japonica* L. saplings.

It can be seen from Table 3 that, drought stress has significant effects on various content indexes of the leaf of *Sophora japonica* L. saplings, including Chla, Chl, etc. With the aggravation of stress, these index values show a trend of increasing first and then descending. The content of Chl under mild and moderate stress increases significantly compared with that in the control group ($P < 0.05$). The peak value appears in the D8 treatment group under moderate stress, and the content shows a significant downward trend with the aggravation of drought stress ($P < 0.05$). The Chlb and Car contents present the same changing trend but the difference was not significant. Under different drought treatments, Cha/Chb and Car/Chl in the leaf of *Sophora japonica* L. saplings also have no significant difference.

Table 3. The effect of photosynthesis pigment content and percentage of *Sophora japonica* L. saplings under drought stress

Treatment	Chl/mg·g ⁻¹ DW	Chla/mg·g ⁻¹ DW	Chlb/mg·g ⁻¹ DW	Car/mg·g ⁻¹ DW	Cha/Chb	Car/Chl
d ₀	6.87±0.32a	5.25±0.38a	1.28±0.15a	0.92±0.27a	3.92±0.16a	0.154±0.033a
d ₂	6.83±0.47b	6.13±0.32b	1.45±0.13a	1.05±0.14a	3.97±0.26a	0.161±0.026a
d ₄	6.89±0.57b	6.39±0.40b	1.39±0.14a	1.14±0.12a	4.08±0.38a	0.163±0.023a
d ₆	6.90±0.51b	6.50±0.41ab	1.51±0.11a	1.29±0.10a	4.12±0.09a	0.168±0.017a
d ₈	7.48±0.49b	6.77±0.43ab	1.71±0.08a	1.56±0.14a	4.49±0.05a	0.176±0.014a
d ₁₀	7.12±0.32ab	5.92±0.24a	1.49±0.15a	1.31±0.03a	4.16±0.11a	0.159±0.012a
d ₁₂	6.34±0.31ab	5.49±0.26a	1.05±0.18a	1.19±0.08a	3.78±0.27a	0.147±0.010a
d ₁₄	5.37±0.26ab	4.49±0.1a	0.495±0.15a	0.69±0.13a	3.04±0.28a	0.129±0.025a

3.3. Effects of drought stress on net photosynthetic rate and other parameters of *Sophora japonica* L. saplings.

As can be seen from Table 4, with the aggravation of drought stress, net photosynthetic rate and transpiration rate of *Sophora japonica* L. saplings all show a significant downward trend. Different degree of stress results in different degree of decline. Under mild stress, the transpiration rate presents greater decrease than the net photosynthetic rate and stomatal conductance, which may indicate that the transpiration rate of *Sophora japonica* L. saplings is more sensitive to mild stress compared with

the other two. As the degree of stress increases, photosynthetic rate and transpiration rate decrease, but the decrease degree of the former is less than that of the latter. Therefore, the effect of water use efficiency on drought stress first increases significantly and then decreases significantly. It reaches the maximum in the D10 group, showed that drought stress significantly inhibits the net photosynthetic rate, stomatal conductance and transpiration rate of *Sophora japonica* L. saplings but increases the water use efficiency. With the increasing of stress, the further closure of a large number of stoma leads to the significant decrease of stomatal conductance of *Sophora japonica* L. saplings and transpiration rate also decreases, while the intercellular CO₂ concentration decreases first and then increases.

Table 4. The effects of continuous drought stress on the variation of Pn and diurnal change of gas exchange parameters of *Sophora japonica* L.

Treatment	P _n (CO ₂)/μmol m ⁻² s ⁻¹	T _r (H ₂ O)/mmol m ⁻² s ⁻¹	G _s (H ₂ O)/mol m ⁻² s ⁻¹	C _i (CO ₂)/mmol mol ⁻¹	r _{WUE} (CO ₂ /H ₂ O)/mmol mol ⁻¹
d ₀	6.25±0.28a	3.68±0.02a	0.10±0.01a	281±15.9b	1.97±0.01e
d ₂	5.23±0.30b	2.91±0.03b	0.07±0.01b	273±5.4c	1.99±0.01e
d ₄	2.78±0.02c	0.91±0.01c	0.03±0.01c	225±24e	3.89±0.1d
d ₆	2.58±0.01c	0.51±0.01cd	0.03±0.01d	204±16.1f	5.02±0.2c
d ₈	2.10±0.02c	0.48±0.01d	0.02±0.01d	173±2.6g	5.79±0.3b
d ₁₀	2.01±0.01d	0.47±0.01d	0.01±0.01e	232±17.3d	6.05±0.1a
d ₁₂	1.89±0.02d	0.45±0.01d	0.01±0.01e	278±17.5c	5.87±0.27b
d ₁₄	0.96±0.03e	0.40±0.01d	0.01±0.01e	276±22c	3.21±0.1d

3.4. Effects of drought stress on the response of the leaf of *Sophora japonica* L. saplings to light and CO₂ intensity.

Table 5. Photosynthetic rate parameters in response to light and CO₂ intensity of *Sophora japonica* L. saplings leaves under drought stress

Treatment	P _n -PAR			P _n -CO ₂		
	AQY/mol mol ⁻¹	LSP/μmol m ⁻² s ⁻¹	LCP /μmol m ⁻² s ⁻¹	CO ₂ , CE/μmol m ⁻² s ⁻¹	CSP/μmol m ⁻² s ⁻¹	CCP/μmol m ⁻² s ⁻¹
d ₀	0.0489a	1863.75a	15.89a	0.0398a	1024.18c	66.98f
d ₂	0.0448b	1836.32ab	14.97ab	0.0302b	1309.49b	118.04e
d ₄	0.0349c	1756.48b	13.75b	0.0234c	1519.71a	148.68d
d ₆	0.0302c	1238.59c	8.77c	0.0206c	905.04d	150.02d
d ₈	0.0245d	868.52d	6.81d	0.0198c	885.38e	165.03c
d ₁₀	0.0232e	739.65e	5.78e	0.0166d	836.54f	172.52b
d ₁₂	0.0119f	703.87f 6.21ef 0.0188d 827.98g 192.96a	5.59ef 0.0188d 827.98g 192.96a	0.0137d	807.88g	182.69a
d ₁₄	0.0078g	612.01g	5.98f	0.0101e	809.69g	133.43e

As showed in Table 5, the apparent quantum yield (AQY), light saturation point (LSP) and light compensation point (LCP) decrease with the severity of drought stress; but they show significant differences under mild, moderate and severe drought stress. It can also be seen from Table 5 that, the CO₂ saturation point and compensation point of *Sophora japonica* L. sapling leaves increase first and then decrease with the increase of drought stress; the carboxylation rate (CE) of RuBP gradually decreases with the increase of drought stress, and there were significant differences between different stress levels. This also shows that the photosynthetic capacity of *Sophora japonica* L. saplings is greatly inhibited under serious drought.

3.5. Changes of soluble sugar and proline content in leaves of *Sophora japonica* L. saplings under drought stress.

Table 6. The change of soluble sugar and free proline content of *Sophora japonica* L. leaf under drought stress

	d ₀	d ₂	d ₄	d ₆	d ₈	d ₁₀	d ₁₂	d ₁₄
soluble sugar (%)	0.82±0.041	1.23±0.031	1.51±0.033	2.09±0.032	2.51±0.038	2.48±0.026	2.32±0.029	2.18±0.029
free proline (μg·g ⁻¹)	41.64±8.26	47.48±9.86	65.58±6.75	88.79±6.57	96.37±7.54	85.82±7.13	80.21±5.89	76.96±8.09

As can be seen from Table 6, the content of soluble sugar is very low in leaves of *Sophora japonica* L. saplings in the control group, about 0.82%. Under mild and moderate drought stress, the soluble sugar content in leaves of *Sophora japonica* L. saplings increases gradually, reaching the peak in the D₈ group, and then decreases; it is still higher than that of the control group at the end of the drought stress. The Table 6 also shows that, leaves of *Sophora japonica* L. saplings in the control group have less free proline content. The content in treatment groups increases gradually under mild and moderate drought stress, reaching the peak in the D₈ group, and then continues to decline; it is still higher than that of the control group at the end the drought stress.

3.6. The changes of antioxidant enzymes in leaves of *Sophora japonica* L. saplings under drought stress.

Table 7. The change of POD and SOD content of *Sophora japonica* L. leaf under drought stress

	d ₀	d ₂	d ₄	d ₆	d ₈	d ₁₀	d ₁₂	d ₁₄
POD (U·g ⁻¹ ·min ⁻¹)	53.82 ±7.24	65.23 ±8.31	75.51 ±6.33	90.09 ±5.32	95.51±6.38	87.48 ±5.26	83.32 ±6.29	76.18 ±5.29
SOD (U·g ⁻¹ ·min ⁻¹)	141.64±10.21	147.48±9.86	152.58±8.75	188.79±9.57	215.37±7.54	205.82±7.13	198.21±5.89	176.96±6.09

As can be seen from Table 7, POD and SOD in leaves of *Sophora japonica* L. saplings decrease first and then decrease under drought stress; there is little change in the control group and under mild stress; the values rise obviously in the D₆ treatment group and reach the peak value in the D₈ treatment group. They decrease gradually under severe drought stress, but they are still higher than that of the control group at the end of drought stress. Thus, *Sophora japonica* L. saplings can produce reactive oxygen under drought stress to achieve the biological membrane lipid peroxidation. But in order to maintain the stability of the biological membrane in the sapling, the active oxygen scavengers like antioxidant enzymes are formed to clear the reactive oxygen species.

4. Conclusion and discussion

Drought stress is the main adversity factor [8] that restricts plant growth. The two most important problems in the study of the relationship between plant growth and water are the absorption mechanism of water and the utilization efficiency of water [9]. This study showed that different degree

of drought stress has important effects on the leaf relative water content, photosynthesis pigment content and other physiological indexes of *Sophora japonica* L. saplings and significantly inhibits the growth of saplings.

Under drought stress, the change of relative water content of leaves can reflect the anti-dehydration ability of plant leaf tissue [10]. In this study, the relative leaf water content of each treatment group did not change significantly and remained stable compared with the control group under mild and moderate drought stress. However, under severe drought stress, it decreased rapidly and the leaves wilted and shrank.

The photosynthetic pigments of higher plants are mainly composed of Ch la, Ch lb and Car. In this study, with the increase of water stress, both Chl and Car increased first and then decreased. They reached the peak values under moderate drought stress and decreased under severe drought stress. The overall performance of leaf Ch la/b was decreased with the increase of drought stress. It indicated that *Sophora japonica* L. saplings, to a certain extent, can adapt to changes in the environment by regulating the relative contents of Ch la and Ch lb as well as the content of Car to strengthen the stress resistance.

It is found in this study that, with the aggravation of drought stress, *Sophora japonica* L. saplings decreased their net photosynthetic rate, transpiration rate and stomatal conductance; at the same time, the concentration of intercellular CO₂ decreased under mild and moderate drought stress but increased under severe drought stress. This may indicate that, with the increase of drought stress degree, the reduction of photosynthetic capacity of *Sophora japonica* L. saplings is greatly related to the change of stomatal limitation, which also shows that they have certain adaptability to drought stress.

It is also observed in this study that the apparent quantum yield and RuBP Carboxylation rate of *Sophora japonica* L. saplings decreased significantly under drought stress, which indicates that the drought will reduce their ability to utilize light and CO₂; The significant decrease of light compensation point, light saturation point, CO₂ saturation point and Rubp carboxylase activity lead to the decrease of light and CO₂ utilization and adaptability, resulting in the decline of photosynthetic rate.

The study results showed that, with the aggravation of drought stress, the content of soluble sugar and free proline in *Sophora japonica* L. saplings increased first and then decreased, but it was higher than the control group until the end of the drought stress. This indicates that the changes in soluble sugar and free proline in plants play an important role in the osmotic regulation of plants.

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