

EFFECTS OF DROUGHT STRESS ON GROWTH, PHOTOSYNTHESIS AND ULTRASTRUCTURE OF MESOPHYLL CELL OF *ULMUS SZECHUANICA* FANG SEEDLINGS

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Abstract

Effects of drought stress on growth, photosynthesis and ultrastructure of mesophyll cells of *Ulmus szechuanica* Fang seedlings were studied. Results showed that under drought stress both leaf water content (LWC) and relative water content (RWC) decreased significantly and height growth (GH) and base diameter growth (GD) declined. P_n , T_r , G_s , C_i and L_s were significantly affected by water deficit. With the continuing of drought, P_n decreased significantly, the change of G_s , C_i , T_r was same. There existed significant effects of drought on stomatal density (SD), stomatal length (SL), stomatal width (SW), individual stomatal area (A_s) and percentage of individual stomatal area (A_i) too. Under drought condition, change of structure and chloroplast morphology of the leaves was evident, the number of chloroplasts and orophil granules increased, their proportion in cells amplified. The number of starch granules became smaller, the arrangement of thylakoids among chloroplasts swollen and tend to be disordered.

Introduction

Drought is one of the major constrains on plant growth and survival (Ghafari *et al.* 2020, Khyrollah *et al.* 2021). Drought stress causes a wide range of responses in plants, such as fallen leaves, withered branches, damaged chloroplasts, reduced photosynthesis, and limited metabolic reactions (Hu *et al.* 2014, Ma *et al.* 2015, Wang *et al.* 2019). To cope with drought stress, plants have also evolved various strategies, such as generation of larger and deeper root system to absorb water, reduction of stomatal aperture to limit water loss, as well as increase of some antioxidants to decrease cell oxidative damage (Alessandra *et al.* 2016, Mansoor *et al.* 2019, Chun *et al.* 2021). Drought damages plants by inhibiting and disrupting photosynthesis, which is the main mechanism of plants growth and yield, so most studies on drought in plants have mainly focused on growth and physiological response during young stage. Drought conditions at the plants seedling or vegetative growth stages showed significant decrease in root length, branch, leaf number and growth of height, usually leading a reduction in quality and yields (Zakariyya *et al.* 2018). Therefore, it is of important significance to conduct research the effects of drought stress on growth and physiology of plants seedlings, especially some rare and endangered tree species, which will help to reveal the drought resistance of these species and explore management measures in improving the competitiveness of seedlings and the self-renewal of populations.

Ulmus szechuanica Fang is an endemic species, mainly distributed in southern China (Editorial board of Flora of china 1998). Due to environmental degradation and human disturbances, and its poor ability to generate naturally, this species survives in unstable habitats by stream banks, on steep slopes, or in narrow valleys, and is on the verge of extinction in recent years (Li *et al.* 2019). So far, reports on the drought tolerance of *U. szechuanica* at the early vegetative stages were not available. Therefore, the soil drought stress with different water gradient was carried out with pot experiment and water control, and the leaf water contents

(LWC), photosynthesis parameters, and leaf micro structures were studied. Thus the present study was aimed to reveal the adaptive mechanism of *U. szechuanica* seedlings to drought stress, and provide some theoretical reference for seedlings breeding and forest restoration.

Materials and Methods

Experiments were performed at horticultural experimental station in Jinling Institute of Science and Technology, Nanjing, China (31° 07' 31.77" N, 118° 48' 20.49" E). Seeds of *U. szechuanica* were collected from Tianmu Mountain National Nature Reserve, Zhejiang, China (30°18'36" N, 119°24'18" E), and cultured in a greenhouse at 24 °C under 40–65% relative humidity and a 16 hr light / 8 hr dark photoperiod. After one year cultivation, seedlings of *U. szechuanica* with good growth potential, uniform specification and no pests were selected, and grown into larger plastic pots (15 cm high and 20 cm diameter) containing a mixture of loess and peat 1/1 (v/v) for next experiments.

For drought stress treatments, seven water gradients (D₀, D₂, D₄, D₆, D₈, D₁₀, D₁₂) were prepared. D₂ represents that seedlings were irrigated after two days drying. Single factor test design, 2 d as a treatment period, that was every 2 d, one treatment stopped watering, Other normal watering. To 25 June, 7 water gradients were formed: D₀ (CK), D₂, D₄, D₆, D₈, D₁₀, D₁₂, which represent a drought treatment for 0, 2, 4, 6, 8, 10 and 12 d, respectively. There were 30 replicates per treatment (Fig. 1).



Fig. 1. *Ulmus szechuanica* seedlings under different drought conditions.

From ten pots per treatment, change of soil water content (SWC) was determined by hydra probe soil moisture temperature and salt sensor (Hydra Probe II, USA). Thirty seedlings per treatment were selected and leaf water content (LWC) and relative water content (RWC) were detected by drying method (Li 2000). Single leaf dry mass (SLDW) were determined by same method.

Leaf length and width were measured by using a ruler and vernier caliper (EDHG-150-IP54, China), and growth of height (GH) and growth of diameter (GD) were recorded with the same method.

Relevant photosynthesis indexes were determined with Li-cor 6400 photosynthetic apparatus. Three well-developed seedlings were selected, the 3 well-developed leaves, 4th from top, were selected as experiment sample. Since the leaf photosynthetic rate usually present remarkable daily variation, in order to minimize the error caused by the change of light condition, all the measurement conducted within 1 hr (10: 00 -11: 00 AM) on a clear day, and continuously measured for 3 days. Air temperature was 34.09-34.42°C, CO₂ concentration 396.88-410.50 μmol·mol⁻¹. The artificial light source, standard leaf chamber (2 cm ×3 cm) were used. The leaves

were light-induced 30 min in advance, and photo-synthetically active radiation (PAR) was controlled at $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, stopped 40–60s per leaf, and each repeated 3 times. Measured indexes included: Net photosynthesis rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), air CO_2 concentration (C_a), transpiration rate (T_r), water use efficiency (WUE), stomatal limitation value (L_s). In addition, $L_s = 1 - C_i / C_a$, $\text{WUE} = P_n / T_r$.

The middle part of the leaves (without veins) was removed by nail polish film to observe stomatal structure by the biological microscope (OLYMPUS-BX6, China). The stomatal density (SD), stomatal device length (SL), stomatal device width (SW) and the area (A_s) of a single were measured by Image-Pro Plus 6.0 program (Media Cybernetics, Inc. Cai *et al.* 2016). To complete ANOVA 30 visual field every treatment were taken.

SD = the number of stomata /visual field area.

$A_s = \pi \times \text{SL} \times \text{SW} / 4$, $\pi = 3.14$; $A_t (\%) = A_s \times \text{SD} \times 100$.

A_s reported by Fu *et al.* (2004), the leaf sample was fixed in 4% glutaraldehyde solution for more than 24 hr (4°C), then rinsed with 0.1 mol/l phosphate buffer (PH 7.5) and fixed in 1% acid at 4°C . Subsequently, samples were dehydrated by acetone series, buried by SPI-Pon™ 812, sliced by 1 kb ultrathin slicer, and double stained by dioxane with lead citrate. Lastly, Hitachi transmission electron microscope (H-7650, Japan) was used to observe and photograph.

The ANOVA was conducted with SPSS17.0 software, treatment means were compared by using LSD test at 99 and 95%, confidence interval to estimate their significance under different treatments. Results were represented by Means \pm Standard deviation.

Results and Discussion

From Table 1, it is apparent that, SWC decreased significantly under drought stress. SWC under normal water supply (D_0) was about 40.00%. After 2 d of water stoppage, SWC decreased to 37.33%, that under D_6 decreased to 27.00%. With the extension of water stoppage, soil moisture showed obvious water gradient change, which reflected the process of soil moisture loss in natural state. LWC showed significant descending under drought conditions. LWC under D_2 , D_4 , D_6 were 58.67, 56.41, 58.76%, respectively, and there was no significant difference ($p > 0.05$) compared with CK, that under D_0 was 59.51%. But, LWC under D_8 , D_{10} , D_{12} declined obviously, and that under D_{12} was the lowest (43.38%). Compared with CK, these differences were significant ($p < 0.05$). RWC showed similar change, but in the early days of the drought the decline was less, and there was no significant difference with CK ($p > 0.05$). From the change of dry mass of single leaf (SLDW), there was no significant difference between different treatments, so the short-term water stoppage had little effect on leaf development.

Both GH and GD decreased with drought continuing. ANOVA showed that, both GH and GD under drought stress were significantly affected ($p < 0.05$). GH of D_0 was 15.31 cm, 1.19, 1.48, 1.82, 1.80, 2.10 and 2.10 times D_2 , D_4 , D_6 , D_8 , D_{10} , D_{12} , respectively. Accordingly GD of D_0 was 1.78 mm, that was 1.26, 1.13, 1.16, 1.35, 2.07, 2.37 times of D_2 , D_4 , D_6 , D_8 , D_{10} , D_{12} . Therefore, drought, especially severe drought stress (such as D_8 , D_{10} , D_{12}) would hindered seedlings growth and development.

Changes of photosynthesis parameters under different treatments presented in Table 2, showed that under drought stress P_n showed a marked decline, the difference of P_n was significant between different treatments ($p < 0.05$). P_n under D_2 was $11.32 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the difference of which and D_0 was not significant ($p > 0.05$). However, P_n under other treatments was significantly smaller than CK ($p < 0.05$), and showed an obvious decreasing trend.

Table 1. Changes of SWC, LWC and growth of *U. szechuanica* seedlings under different drought conditions.

Treatment	Soil water content SWC / %	Leaf water content LWC / %	Relative water content RWC / %	Single leaf dry weight SLDW / g	Growth of height GH / cm	Growth of diameter GD / mm
D ₀	40.00 ± 1.00a	59.51 ± 1.38a	93.21 ± 6.62a	0.08 ± 0.01a	15.31 ± 8.81a	1.78 ± 0.26a
D ₂	37.33 ± 2.31b	58.67 ± 3.72a	89.28 ± 3.12a	0.08 ± 0.03a	12.86 ± 3.60b	1.42 ± 0.48b
D ₄	34.67 ± 4.16b	56.41 ± 7.26a	81.61 ± 7.32a	0.12 ± 0.06a	10.29 ± 3.59c	1.57 ± 0.60b
D ₆	27.00 ± 3.46c	58.76 ± 4.25a	87.81 ± 5.68a	0.12 ± 0.06a	8.40 ± 2.70d	1.53 ± 0.66b
D ₈	22.33 ± 3.51d	49.13 ± 5.87b	53.61 ± 1.67b	0.08 ± 0.04a	8.49 ± 2.59d	1.32 ± 0.57c
D ₁₀	11.33 ± 1.53e	44.68 ± 6.20b	32.91 ± 2.60c	0.09 ± 0.01a	7.26 ± 5.32e	0.86 ± 0.58d
D ₁₂	10.67 ± 0.58e	43.38 ± 6.41b	23.51 ± 1.42d	0.12 ± 0.02a	7.28 ± 3.20e	0.75 ± 0.38e

Different lowercase letters indicate significant differences between two treatments at 0.05 level. The same in Table 2.

Table 2. Photosynthesis parameters of *U. szechuanica* seedlings under different drought conditions.

Treatment	Net photo-synthesis rate $P_n / (\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1})$	Stomatal conductance $G_s / (\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1})$	Intercellular CO ₂ concentration $C_i / (\mu\text{mol} \cdot \text{mol}^{-1})$	Transpiration rate $T_r /$ $(\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1})$	Water use efficiency WUE $(\mu\text{mol}/\text{mmol})$	Stomatal limitation value $L_s / \%$
D ₀	13.37 ± 0.22a	0.29 ± 0.01a	306.01 ± 2.94a	6.20 ± 0.26a	2.158 ± 0.02c	0.229 ± 0.01f
D ₂	11.32 ± 1.35a	0.24 ± 0.07b	299.86 ± 16.58a	5.17 ± 1.00a	2.18 ± 0.01c	0.247 ± 0.04e
D ₄	10.08 ± 1.91b	0.20 ± 0.09b	297.96 ± 29.96a	4.57 ± 1.55b	2.20 ± 0.03c	0.266 ± 0.07d
D ₆	8.24 ± 1.73c	0.14 ± 0.05c	291.18 ± 15.32b	3.62 ± 1.05c	2.27 ± 0.02c	0.284 ± 0.04c
D ₈	8.05 ± 0.76c	0.09 ± 0.02d	251.97 ± 28.84c	2.78 ± 0.56d	2.88 ± 0.01b	0.384 ± 0.07b
D ₁₀	7.15 ± 0.84d	0.04 ± 0.00e	136.04 ± 41.46d	1.44 ± 0.07e	4.95 ± 0.03a	0.669 ± 0.10a
D ₁₂	3.13 ± 0.02e	0.05 ± 0.00e	277.12 ± 0.82c	1.01 ± 0.01f	4.10 ± 0.02a	0.290 ± 0.00c

Generally, G_s reflects the ability of the atmospheric CO₂ and water vapor into plant leaves (Gao *et al.* 2016). According to research, G_s is very sensitive to the change of environmental factors, and all kinds of factors affecting plant photosynthesis and leaves water condition might affect G_s (Wang *et al.* 2001). As shown in Table 2, the change trend of G_s under different drought conditions was basically consistent with P_n , correlation analysis which showed that there was a very significant positive correlation between G_s and P_n ($p < 0.01$, Table 3). The change of C_i and T_r was similar to P_n and G_s , basically showing a slow fall with drought stress intensifying (Table 1). The order of C_i and T_r under different treatments was $D_0 > D_2 > D_4 > D_6 > D_8 > D_{10} > D_{12}$ (Table 2). The ANOVA showed that the difference of C_i and T_r under different treatments was significant ($p < 0.05$).

WUE could be estimated with both P_n and T_r , and the results are presented in Table 2. WUE under D₁₀ and D₁₂ remained higher level (4.95, 4.10 $\mu\text{mol}/\text{mmol/l}$), due to low soil moisture and very weak T_r , despite very low P_n , which were significantly higher than that under D₀, D₂, D₄, D₆ (2.18, 2.20, 2.27 $\mu\text{mol}/\text{mmol/l}$) ($p < 0.05$).

From the comparison of L_s under different treatments, with the intensification of drought stress, it showed an increasing trend. L_s under D₀ was minimal (0.229%), and that under D₂, D₄, D₆, D₈, D₁₀ was 0.247, 0.266, 0.284, 0.384, 0.669% in turn, but L_s under D₁₂ decreased to 0.290%

(Table 2). The ANOVA showed that there was significant difference between different treatments ($p < 0.05$).

The correlation between photosynthesis parameters and LWC and growth index was shown in Table 3. Results showed there existed very significant positive correlation between P_n and G_s , T_r , LWC, GH, GD ($p < 0.01$), and the correlation between LWC and GD was so. This observation was in agreement with previous reports in other species, such as *Cinnamomum camphora* (Hu *et al.* 2014) and *Phoebe zhennan* (Wang *et al.* 2019). Therefore, drought stress affected LWC and P_n , and then impacted the growth of seedlings.

Table 3. Relationship matrix of the photosynthesis parameters with LWC and growth of *U. szechuanica* seedlings under different drought conditions.

	P_n	G_s	C_i	T_r	L_s	LWC	GH	GD
P_n	1	0.934**	0.664**	0.950**	-0.672**	0.698**	0.560**	0.726**
G_s		1	0.616**	0.990**	-0.625**	0.669**	0.541*	0.718**
C_i			1	0.688**	-0.961**	0.520*	0.309	0.613**
T_r				1	0.697**	0.690**	0.533*	0.739**
L_s					1	0.526*	0.316	-0.619**
LWC						1	0.471*	0.628**
GH							1	0.397
GD								1

* and ** indicate significant correlation at 0.05 and 0.01 level, respectively.

Stomata, regulate the flow of gases in and out of leaves and thus plants as a whole (Hetherington and Woodward 2003), so SD and A_s directly determine the transpiration and photosynthesis efficiency of plants. The observation showed that there was no stomata in the upper epidermis, but obvious stomata in the lower epidermis (Fig. 2). The microscopic parameters of stomata were shown in Table 4. The results showed that drought stress had significant effects on SD, SL, SW, A_s , A_t ($p < 0.01$). SD under CK was the largest (139.39 mm^{-2}), and significantly higher than under drought stress ($p < 0.05$), but there was no significant difference between D_2 and D_4 ($p > 0.05$). Similar results were also reported on *Typha domingensis* (Cruz *et al.* 2019), almond species (*Prunus L. spp.*) (Rajabpoor *et al.* 2014), and so on.

Table 4. Leaves-stomatal characteristics of *U. szechuanica* seedlings under different drought conditions.

Treatment	Stomata density SD / mm^{-2}	Stomata length SL / μm	Stomata width SW / μm	Single stomata area A_s / μm^2	Percent of stomata area A_t / %
D_0	$139.39 \pm 5.25a$	$27.36 \pm 0.62a$	$14.20 \pm 2.62a$	$305.81 \pm 63.48a$	$4.25 \pm 0.84a$
D_2	$109.09 \pm 9.09b$	$23.18 \pm 1.03b$	$12.52 \pm 0.80a$	$227.58 \pm 11.19b$	$2.48 \pm 0.21b$
D_4	$106.06 \pm 5.25b$	$24.87 \pm 1.46b$	$12.76 \pm 1.51a$	$248.51 \pm 24.14a$	$2.63 \pm 0.17b$
D_6	$92.42 \pm 2.62c$	$25.10 \pm 1.54b$	$10.51 \pm 0.93b$	$206.39 \pm 11.11b$	$1.90 \pm 0.13b$
D_8	$74.24 \pm 18.92d$	$25.44 \pm 1.95a$	$12.19 \pm 1.81a$	$245.19 \pm 53.71a$	$1.85 \pm 0.81bc$
D_{10}	$65.15 \pm 11.44e$	$27.80 \pm 3.40a$	$10.22 \pm 0.42b$	$223.80 \pm 35.52b$	$1.45 \pm 0.35c$
D_{12}	$65.15 \pm 14.61e$	$28.99 \pm 3.21a$	$11.19 \pm 0.88b$	$253.18 \pm 8.78a$	$1.65 \pm 0.38c$

The changes of SL, SW and A_s were not obvious, so short term drought had little effect on stomatal morphology. A_t under CK was the highest (4.25%), and significantly higher than other treatments ($p < 0.05$), indicating that drought stress affected distribution of stomata, resulting in the change of A_t adaptively.

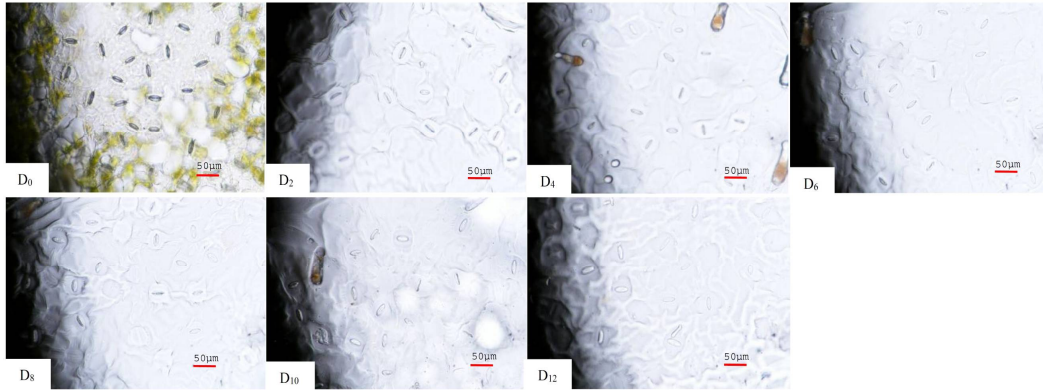


Fig. 2. Stomatal distribution on leaves of *U. szechuanica* seedlings under different drought conditions.

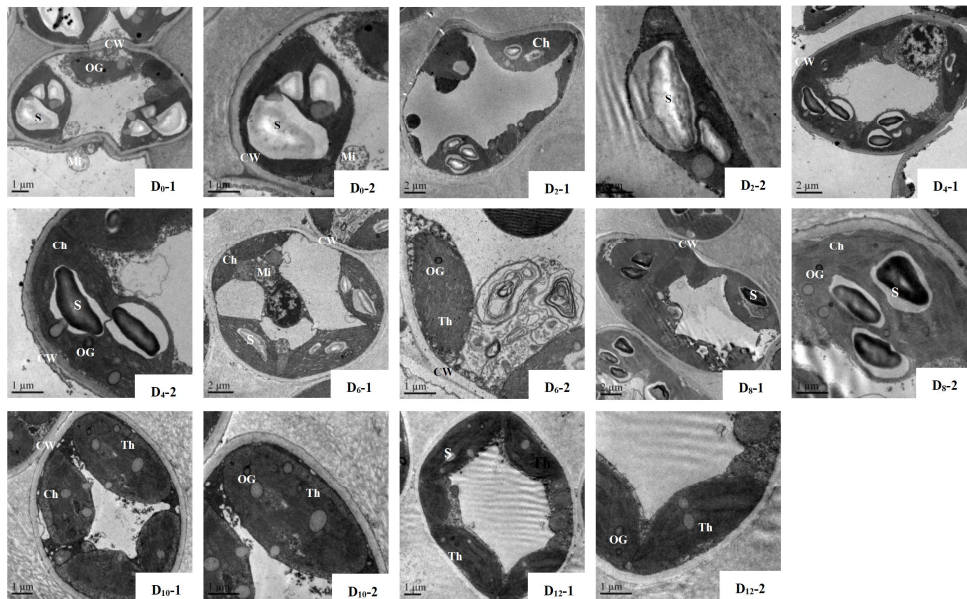


Fig. 3. Ultrastructure of mesophyll cells of *U. szechuanica* leaves under different drought conditions. D₀₋₁, D₂₋₁, D₄₋₁, D₆₋₁, D₈₋₁, D₁₀₋₁, D₁₂₋₁: Whole mesophyll cell; D₀₋₂, D₂₋₂, D₄₋₂, D₆₋₂, D₈₋₂, D₁₀₋₂, D₁₂₋₂: magnified view of chloroplasts and thylakoid membranes in mesophyll cells. CW-Cell wall, Ch- Chloroplast, Th- Thylakoids, OG-Osmiophilic globule, S-Starch grains, Mi-Mitochondrion.

Chloroplast is the site of photosynthesis and the most sensitive organelle in cells (Zhao *et al.* 2020). Therefore, the structure analysis of chloroplasts can explain the physiological responses of plants under adverse drought conditions. Figure 3 revealed the traits of ultrastructure of

mesophyll cells of *U. szechuanica* leaves under normal and stress conditions. Under drought stress, the intracellular structure and chloroplast morphology changed obviously, the number of chloroplasts increased, the proportion of area in cells increased, the number of eosinophils and volume was so. But the shape of chloroplasts gradually becomes globular, the cell wall around the ring becomes smaller, the number of starch grains decreased and the volume became smaller. Moreover, the thylakoid arrangement in chloroplast showed swelling, tended to disorder, resulted in photosynthetic ability of leaves became weak.

In summary, drought being an important environmental factor weakens photosynthesis, decreased seedlings growth, restricted the healthy and physiological development of plants. To meet the needs of growth and development of *U. szechuanica* seedlings in cultivation, the soil water content should not be less than 60%.

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