

Postprint: Study on the Physiological Response Process of *Ulmus glaucescens* Saplings to Soil Drying and Rehydration

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Abstract

The physiological response process of young trees to soil moisture after afforestation in arid regions is an important prerequisite for water management and survival determination. Using young *Ulmus glaucescens* trees as experimental material, and employing potted natural water loss and rewatering methods, this study investigated the response processes of morphological, physiological, and photosynthetic characteristics of young *Ulmus glaucescens* trees to different degrees of soil water loss and rewatering, aiming to provide a theoretical basis for water management in cultivated *Ulmus glaucescens*. Results showed: (1) When soil water loss was minor (soil water content of 15%), leaf relative water content, chlorophyll content, and water use efficiency of *Ulmus glaucescens* showed slight increases, rising by 14.8%, 1.2%, and 43.2% respectively compared with pre-experiment values; net photosynthetic rate, stomatal conductance, and transpiration rate of leaves showed significant decreasing trends, declining by 26.5%, 27.1%, and 48.7% respectively compared with pre-experiment values; (2) When soil water loss was severe (soil water content of 0%), leaf relative water content, chlorophyll content, and water use efficiency of *Ulmus glaucescens* decreased substantially, declining by 78.4%, 65.4%, and 47.3% respectively compared with pre-experiment values; net photosynthetic rate, stomatal conductance, and transpiration rate of leaves all showed gradual decreases, declining by 89.9%, 83.3%, and 79.9% respectively compared with pre-experiment values; one of the main reasons for the decrease in photosynthetic rate was the change in stomatal conductance. (3) Rewatering could alleviate the adverse effects of soil water loss on *Ulmus glaucescens*. Therefore, in an environment of continuous soil water loss, *Ulmus glaucescens* adapts to decreasing soil water content through a series of changes in morphological, physiological, and photosynthetic characteristics, demonstrating strong drought tolerance; if severe soil water loss occurs after planting of *Ulmus glaucescens*, rewatering can alleviate the damage caused.

Full Text

Physiological Response Process of Young Gansu Elm (*Ulmus glaucescens*) to Soil Water Loss and Rehydration

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Abstract

The physiological response of young trees to soil moisture following afforestation is a critical prerequisite for water management and survival in arid regions. This study investigated the morphological, physiological, and photosynthetic responses of young Gansu elm (*Ulmus glaucescens*) to varying degrees of soil water loss and rehydration using potted plants under simulated natural water loss and rewatering conditions, aiming to provide a theoretical basis for water management in Gansu elm cultivation. The results demonstrated that under mild soil water loss (soil water content of 15%), leaf relative water content, chlorophyll content, and water use efficiency of Gansu elm increased slightly by 14.8%, 1.2%, and 43.2%, respectively, compared to pre-experiment levels. In contrast, net photosynthetic rate, stomatal conductance, and transpiration rate decreased significantly by 26.5%, 27.1%, and 48.7%, respectively. Under severe soil water loss (soil water content of 0%), leaf relative water content, chlorophyll content, and water use efficiency declined substantially by 78.4%, 65.4%, and 47.3%, respectively. Net photosynthetic rate, stomatal conductance, and transpiration rate showed gradual decreases of 89.9%, 83.3%, and 79.9% compared to pre-experiment values. Changes in stomatal conductance represented one of the primary causes of photosynthetic rate decline. Rehydration effectively alleviated the adverse effects of soil water loss on Gansu elm. Therefore, in environments with continuous soil water loss, Gansu elm exhibited strong drought tolerance through a series of morphological, physiological, and photosynthetic adjustments. In cases of severe soil water loss after planting, rehydration can mitigate damage to Gansu elm trees.

Keywords: *Ulmus glaucescens*; young trees; soil water loss; morphological and physiological characteristics; photosynthetic characteristics

Introduction

Gansu elm is widely distributed in arid regions of northwestern China and plays an important role in fast-growing timber forests, economic forests, and urban-rural landscape forests. In Gansu elm cultivation management, the survival

and growth of this species are affected by environmental factors such as soil water deficiency due to natural conditions in northern China's arid and semi-arid regions. Therefore, rational water management during the cultivation of young Gansu elm trees is a critical limiting factor for ensuring survival and improving afforestation efficiency. Currently, domestic research on Gansu elm has primarily focused on softwood cutting techniques, introduction and seedling cultivation, population structure, and comparative analysis of drought resistance among several Ulmaceae species through metabolic physiology. During Gansu elm cultivation, the photosynthetic characteristics and growth and physiological indicators of young trees represent important manifestations of their response to different soil water contents. Previous studies have shown that severe soil water loss or drought stress significantly affects plant growth, physiology, and photosynthesis, manifested as restricted growth in basal diameter, plant height, and leaf area, dehydration of leaf mesophyll cells, weakened chlorophyll synthesis, and inhibited photosynthesis. Research on sour jujube (*Ziziphus jujuba*) has demonstrated that severe soil water loss inhibits plant growth and chlorophyll synthesis, reducing leaf relative water content and chlorophyll content. In purple false indigo (*Amorpha fruticosa*) and dryland willow (*Salix matsudana*), decreasing soil moisture leads to varying degrees of decline in net photosynthetic rate, stomatal conductance, transpiration rate, and chlorophyll content. Plants typically employ various strategies to avoid these stresses or adapt to adverse environments through self-modification under soil water deficiency conditions. For instance, when encountering scarce precipitation during the growing season and extremely low soil moisture, *Potaninia mongolica* sheds all its leaves, flowers, and fruits, entering dormancy through a "false death" state. When autumn precipitation replenishes soil moisture, *Potaninia mongolica* rapidly absorbs water and undergoes secondary growth and reproduction. This study aims to simulate the stress conditions resulting from soil water deficiency without normal replenishment after successful afforestation, as well as the growth process following water replenishment from natural rainfall, to investigate the response mechanisms of young Gansu elm trees to soil water loss. This research provides theoretical foundations and practical guidance for optimized cultivation and conservation management of Gansu elm. Therefore, we selected two-year-old Gansu elm seedlings as experimental materials and measured their morphological, physiological, and photosynthetic indicators under natural soil water loss and rehydration conditions to analyze the variation processes of these indicators under different soil water contents.

1 Materials and Methods

1.1 Experimental Materials

Two-year-old Gansu elm seedlings purchased in autumn 2017 from Urad Middle Banner, Bayannur City, were used for pot experiments. Plastic pots with upper and lower diameters of 33 cm and 18 cm, respectively, and a height of 22 cm, along with sandy loam soil, served as the potting medium. The seedlings were

cultivated for six months in a solar greenhouse at Inner Mongolia Agricultural University with sufficient soil water to ensure it would not become a limiting factor for seedling growth. In May 2018, 36 plants with consistent growth and good condition were selected for the experiment. During the experimental period, the average temperature in the greenhouse was 25°C, and the relative humidity was 45%.

1.2 Experimental Design

The experiment commenced on May 20, 2018, after thorough watering. Drought stress was induced through natural soil water loss. Both control and drought treatments had six replicates, with six pots per replicate. During the experiment, the control treatment (CK) received daily water supplementation using the weighing method to maintain soil water content at $36.99\% \pm 6.4\%$. The drought treatment ceased watering on the first day of the experiment. Drought treatment samples were collected on days 6, 12, 24, and 30 after watering cessation; the drought treatment was rehydrated on day 30, and samples were collected at 18:00 on days 33 and 36. The experiment lasted 36 days. At each sampling time, leaves were collected from both control and drought treatments, with three mature leaves collected per replicate. These leaves were immediately placed in liquid nitrogen tanks, transported to the laboratory, and stored in a -80°C refrigerator for later analysis. At each measurement time, soil volumetric water content in the pots was determined using a TDR300 soil moisture meter (12 cm depth), and pot evapotranspiration was measured using the weighing method. Plant growth and physiological indicators were subsequently measured.

1.3 Measurement Indicators

1.3.1 Morphological Indicators Plant height was measured using a tape measure for each plant. Height increment was calculated as the difference between plant height at the end and beginning of drought stress. Basal stem diameter was measured at a marked position on each pot using a vernier caliper at each measurement. Basal diameter increment was calculated as the difference between basal diameter at the end and beginning of drought stress. Leaf area was determined by marking three leaves per plant and scanning them with a leaf area meter to calculate projected area. Leaf area increment was calculated by subtracting the scanned leaf area at the beginning of drought stress from that at the end.

1.3.2 Physiological Indicators Leaf relative water content was measured using the saturated water method. Approximately 500 mg of fresh leaves were randomly collected from each plant, quickly transported to the laboratory, and weighed for fresh weight (m_f). The leaves were then soaked in deionized water for 24 hours, after which saturated weight (m_s) was measured. Finally, the leaves were oven-dried to constant weight (m_d). Leaf relative water content was calculated using the following formula: Leaf relative water content (%) =

$(m_f - m_d) / (m_s - m_d) \times 100\%$. Leaf electrolyte leakage was measured using the conductivity ratio method. Different leaf sections were randomly selected, and initial leaf conductivity was measured using a conductivity meter. The leaves were then heated in boiling water for 30 minutes, and maximum conductivity was measured. Leaf electrolyte leakage was calculated as: Leaf electrolyte leakage (%) = (Conductivity before heating / Conductivity after heating) $\times 100\%$. For total chlorophyll content measurement, collected leaves were immersed in dimethyl sulfoxide, and absorbance was measured at 645 nm and 663 nm using a spectrophotometer. Chlorophyll content was calculated using the formula: Chlorophyll content = $(0.0202 \times A_{\{645\}} + 0.00802 \times A_{\{663\}}) \times V / W$, where V is the volume of dimethyl sulfoxide added, W is leaf dry weight, and A is absorbance.

1.3.3 Photosynthetic Indicators Between 9:00 and 11:00 AM during each sampling, net photosynthetic rate (Pn, $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), intercellular CO_2 concentration (Ci, $\text{mol} \cdot \text{mol}^{-1}$), transpiration rate (Tr, $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), stomatal conductance (Gs, $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and atmospheric CO_2 concentration (Ca, $\text{mol} \cdot \text{mol}^{-1}$) of marked leaves were measured using a GFS3000 portable photosynthesis system. Water use efficiency (WUE) and stomatal limitation value (Ls) were calculated as: $\text{WUE} = \text{Pn} / \text{Tr}$ and $\text{Ls} = 1 - \text{Ci} / \text{Ca}$.

1.4 Data Processing and Analysis

Data were organized using Microsoft Excel 2019. SPSS Statistics 25 was used for one-way ANOVA of each indicator. Indicator values are presented as mean \pm standard deviation. Origin 2018 was used for plotting.

2 Results

2.1 Dynamic Changes of Soil Water Content and Evapotranspiration in Pots

As shown in [Figure 1: see original paper], with prolonged soil water loss, soil water content in Gansu elm pots gradually decreased from 36.99% to 0.28% ($P < 0.01$). Specifically, soil water content showed no significant difference from the control during days 0-6, but decreased significantly by 62.5% and 92.5% on days 12 and 24, respectively, compared to day 0. When water loss continued to day 30, soil water content dropped to 0.28%, representing a significant decrease of 99.7% compared to day 0 ($P < 0.01$). After soil water content fell below 3%, it remained stable at 0.28%. The control treatment maintained soil water content at $36.99\% \pm 6.4\%$ throughout the experiment. Pot evapotranspiration gradually decreased with declining soil water content, decreasing from $18.9 \text{ kg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ during days 0-30 to $11.9 \text{ kg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ on day 6. Evapotranspiration rate was fastest on day 6, reaching $11.9 \text{ kg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. After day 24, evapotranspiration tended to stabilize. When water loss continued to day 30 with soil water content approaching 0%, evapotranspiration reached its minimum at $0.08 \text{ mm} \cdot \text{d}^{-1}$.

2.2 Morphological Responses of Young Gansu Elm to Soil Water Loss and Rehydration

As shown in [Figure 2: see original paper], under natural soil water loss conditions, the increments of plant height, basal diameter, and leaf area of young Gansu elm gradually decreased. Basal diameter increment showed no significant difference during days 0-12, but decreased significantly by 37.5% and 35.9% on days 24 and 30, respectively, compared to day 0 ($P < 0.01$). After rehydration, basal diameter continued to decrease, while the control treatment showed a stable growth trend with an average increment of $0.6 \text{ mm} \cdot \text{d}^{-1}$, significantly higher than the drought treatment ($P < 0.01$). Plant height increment showed no significant difference during days 0-12, but decreased significantly by 33.5% and 33.5% on days 24 and 30, respectively, compared to day 0 ($P < 0.01$). After rehydration, plant height in the treatment group continued to decrease. Leaf area increment showed no significant difference during days 0-12, but decreased significantly by 82.5% and 81.2% on days 24 and 30, respectively, compared to day 0 ($P < 0.01$). After rehydration, leaf area in the treatment group continued to decrease. Visual observations from photographs showed that on day 30, Gansu elm saplings clearly exhibited stem wilting, leaf dehydration and curling, and leaf abscission. These results indicate that soil water content below 3% significantly inhibits leaf and stem growth in young Gansu elm, with no recovery after rehydration, demonstrating that excessively low soil water content suppresses growth.

2.3 Physiological Responses of Young Gansu Elm to Soil Water Loss and Rehydration

As shown in [Figure 3: see original paper], with increasing natural soil water loss, leaf water content and total chlorophyll content showed declining trends. Leaf relative water content decreased from 82.5% to 15.5% during days 0-30, with no significant difference during days 0-6, but significant decreases of 24.4%, 56.2%, and 81.2% on days 12, 24, and 30, respectively, compared to day 0 ($P < 0.01$). After rehydration, leaf relative water content did not increase. Chlorophyll content decreased from $3.36 \text{ mg} \cdot \text{g}^{-1}$ to $0.3 \text{ mg} \cdot \text{g}^{-1}$ during days 0-30, with no significant difference during days 0-12, but significant decreases of 36.5%, 64.5%, and 82.1% on days 24 and 30 compared to day 0 ($P < 0.01$). No significant change occurred after rehydration. Leaf electrolyte leakage showed an increasing trend with water loss, rising from 15.5% to 64.5% during days 0-30, with no significant difference during days 0-12, but significant increases of 55.9% and 63.3% on days 24 and 30, respectively, compared to day 0 ($P < 0.01$). After rehydration, electrolyte leakage decreased but remained significantly higher than the control ($P < 0.01$). These results indicate that prolonged soil water loss intensifies drought stress. When soil water content remains above 15%, it has minimal impact on young Gansu elm. However, soil water content below 3% significantly inhibits leaf tissue relative water content and total chlorophyll content.

2.4 Photosynthetic Responses of Young Gansu Elm to Soil Water Loss and Rehydration

As shown in [Figure 4: see original paper], with increasing soil water loss days, leaf net photosynthetic rate, transpiration rate, and stomatal conductance showed continuous decreasing trends. Net photosynthetic rate decreased from $8.2 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.1 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ during days 0–30, with no significant decrease during days 0–6, but significant reductions of 82.7%, 88.4%, and 95.7% on days 12, 24, and 30, respectively, compared to day 0 ($P < 0.01$). After rehydration, the treatment group showed a continued decrease. Transpiration rate decreased from $2.4 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.1 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ during days 0–30, with no significant difference during days 0–6, but significant decreases of 58.3%, 85.4%, and 87.1% on days 12, 24, and 30, respectively, compared to day 0 ($P < 0.01$). Stomatal conductance decreased from $59.8 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $5.9 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ during days 0–30, with no significant difference during days 0–6, but significant decreases of 89.9%, 84.3%, and 92.8% on days 12, 24, and 30, respectively, compared to day 0 ($P < 0.01$). Water use efficiency (WUE) of Gansu elm leaves showed a trend of first increasing, then decreasing, and then increasing again with soil water loss time. WUE increased from $5 \text{ mol} \cdot \text{mmol}^{-1}$ to $5.9 \text{ mol} \cdot \text{mmol}^{-1}$ during days 0–6, decreased to $1.8 \text{ mol} \cdot \text{mmol}^{-1}$ during days 6–24, and increased again to $3.4 \text{ mol} \cdot \text{mmol}^{-1}$ during days 24–30, with no significant difference compared to day 0. After rehydration, WUE in the treatment group continued to increase, while the control treatment showed a decreasing trend. Stomatal limitation value (Ls) showed a trend of first increasing and then decreasing with water loss time, increasing from 0.12 to 0.57 during days 0–12 and decreasing to 0.08 during days 12–30, with no significant difference before rehydration compared to day 0. After rehydration, the treatment group showed a significant decrease compared to day 0. Intercellular CO_2 concentration (Ci) showed a decreasing then increasing trend with water loss time, decreasing from $287 \text{ mol} \cdot \text{mol}^{-1}$ to $144.6 \text{ mol} \cdot \text{mol}^{-1}$ during days 0–12 and increasing to $199.5 \text{ mol} \cdot \text{mol}^{-1}$ during days 12–30, with no significant difference compared to day 0. These results indicate that soil water content above 15% has minimal impact on Gansu elm photosynthesis, while soil water content below 3% significantly inhibits photosynthesis.

3 Discussion

Soil moisture is a crucial factor affecting normal forest growth. The drought experiment on young Gansu elm demonstrated that excessively low soil water content significantly inhibits growth and physiology. Previous research has shown that drought stress distinctly suppresses photosynthesis and growth in plant seedlings, with inhibition intensifying as drought stress severity and duration increase. This study simulated scenarios of continuous soil water loss and rehydration during Gansu elm cultivation, comparing growth and physiological indicators of Gansu elm under different drought durations and soil water contents with a normally watered control. The results revealed that soil water con-

tent below 3% in the middle and late experimental stages significantly inhibited plant height, basal diameter, and leaf area growth in young Gansu elm. When soil water content fell below 15%, it significantly suppressed leaf tissue relative water content and total chlorophyll content. Visual morphological observations showed clear stem wilting, leaf chlorosis, dehydration, curling, drooping, and abscission on day 30, with no recovery after rehydration. This indicates that excessively low soil water content disrupts the growth mechanisms of Gansu elm, causing growth inhibition, tissue cell dehydration, and decreased turgor pressure. Some studies suggest that plants may enter dormancy due to drought stress between complete leaf senescence and plant death. In this study, when soil water content dropped below 3% for 30 days, Gansu elm leaves dehydrated, wilted, and even abscised. After experiencing soil water loss stress and subsequent rehydration, some Gansu elm plants resprouted, suggesting an adaptive capacity to extreme soil water loss. This indicates that complete leaf abscission in young Gansu elm does not necessarily represent whole-plant death. These findings establish a theoretical foundation for water management in Gansu elm afforestation in arid and semi-arid regions. Therefore, when such morphological changes occur during Gansu elm cultivation, they signal that the trees are suffering from soil water loss or drought impacts, and forest managers should implement timely irrigation to prevent more severe stress.

When plants experience soil water stress, they develop resistance at the physiological level. Prolonged soil water loss alters leaf cell membrane permeability and photosynthetic apparatus in Gansu elm saplings, consistent with findings from Sang Ziyang et al. and Wang Jingying et al. Photosynthesis is one of the most vital life activities in plants, and continuously decreasing soil water content severely affects photosynthetic capacity. Zhou Yufei et al. reported that mild drought stress had minor effects on sorghum (*Sorghum bicolor*), which could regulate itself to maintain unaffected photosynthetic rates. However, when stress intensity exceeded the plant's regulatory capacity, photosynthetic rates decreased substantially. This study found that during continuous soil water loss, soil water content above 15% had minimal impact on Gansu elm, while soil water content below 3% significantly reduced leaf transpiration rate, net photosynthetic rate, and stomatal conductance. Research indicates that drought stress affects plant photosynthetic rates through stomatal and non-stomatal limitations. This study found that the photosynthetic rate decline during days 0-12 may have been caused by stomatal limitation. After day 12, when soil water content dropped below 3%, excessively low soil water content likely damaged photosynthetic organs, directly weakening the light reaction process in chloroplasts, leading to decreased photosynthetic rates and increased intercellular CO₂ concentration. At this stage, photosynthetic rate reduction was primarily non-stomatal limitation, similar to conclusions drawn by Zhang Yanhong et al. Additionally, differences in intercellular CO₂ concentration and stomatal limitation value between control treatments were not significant, showing trends of first decreasing then increasing or first increasing then decreasing, possibly related to unstable atmospheric CO₂ concentrations. Rehydration may reverse these

adverse effects on Gansu elm photosynthesis. For example, during Gansu elm cultivation, timely rainfall or artificial irrigation after extreme soil drought can play a crucial role in ensuring the survival of stress-affected young Gansu elm trees.

4 Conclusion

- 1) Young Gansu elm responds to continuously decreasing soil water content morphologically by reducing basal diameter, plant height, and leaf area to resist adversity caused by soil water loss.
- 2) Young Gansu elm responds to continuous soil water loss at the physiological level by decreasing leaf relative water content and chlorophyll content while increasing leaf electrolyte leakage.
- 3) The net photosynthetic rate, stomatal conductance, and transpiration rate of young Gansu elm all decrease due to continuously reducing soil water content.
- 4) The weakening of photosynthesis in young Gansu elm manifests as stomatal limitation during early drought stress and non-stomatal limitation during middle and late drought stress stages.
- 5) Soil water content below 3% significantly inhibits growth and photosynthesis in young Gansu elm. Gansu elm saplings possess certain drought tolerance to extremely low soil water content, and rehydration promotes recovery of various indicators. In natural afforestation environments, soil water content should be monitored timely for artificial supplementation to avoid damaging Gansu elm growth.

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