Effects of Drought Stress on Growth, Gas Exchange, and Chlorophyll Fluorescence Parameters of Two Tung Tree Seedlings (Postprint)

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Abstract

To investigate the changes and responses of photosynthetic physiological characteristics of two tung tree species (three-year tung and thousand-year tung) seedlings under drought stress, a pot experiment was conducted to examine the effects of different water treatments (normal water supply, mild drought, moderate drought, severe drought) on seedling growth, leaf gas exchange, and chlorophyll fluorescence parameters. The results showed that, compared with the control, mild drought stress had no significant effect on growth, gas exchange, or chlorophyll fluorescence parameters in either tung tree species ($P > 0.05$). Moderate and severe drought significantly decreased ($P < 0.05$) chlorophyll SPAD value, growth, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), stomatal limitation value (Ls), maximum net photosynthetic rate (Pnmax), light saturation point (LSP), apparent quantum yield (AQY), dark respiration rate (Rd), maximum photochemical efficiency (FvFm), actual photochemical quantum efficiency (ΦPS), electron transport rate (ETR), and photochemical quenching coefficient (qP) in both species, with rapid declines observed under severe drought stress. Conversely, intercellular CO2 concentration (Ci), water use efficiency (WUE), light compensation point (LCP), initial fluorescence (Fo), and non-photochemical quenching coefficient (NPQ) increased significantly ($P < 0.05$). Under moderate drought stress, the reduction in Pn of tung tree seedlings resulted from both stomatal factors and non-stomatal factors associated with decreased photosynthetic apparatus activity, whereas under severe drought stress, the decline in photosynthesis was primarily caused by non-stomatal factors related to reduced photosynthetic apparatus activity. The photosynthetic apparatus activity and photosynthetic efficiency of three-year tung were higher than those of thousand-year tung, indicating stronger adaptability to drought stress.

Full Text

Influence of Drought Stress on Growth, Leaf Gas Exchange, and Chlorophyll Fluorescence in Two Species of Tung Tree Seedlings

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Abstract

This study investigated the effects of drought stress on seedling growth and leaf photosynthetic physiological characteristics in two species of tung tree (*Vernicia fordii* and *Vernicia montana*). A pot experiment was conducted to examine the impacts of different water treatments (normal irrigation, light drought, moderate drought, and severe drought) on seedling growth, leaf gas exchange, and chlorophyll fluorescence parameters. The results showed that light drought stress had no significant effect ($P > 0.05$) on growth, leaf gas exchange, or chlorophyll fluorescence compared to normal irrigation. Moderate and severe drought significantly decreased ($P < 0.05$) chlorophyll SPAD values, growth parameters, net photosynthetic rate (Pn), stomatal limitation value (Ls), maximum net photosynthetic rate (Pmax), stomatal conductance (Gs), transpiration rate (Tr), light saturation point (LSP), apparent quantum yield (AQY), dark respiration rate (Rd), maximum photochemical efficiency (Fv/Fm), actual photochemical quantum efficiency (ΦPSII), electron transport rate (ETR), and photochemical quenching coefficient (qP). Severe drought stress increased intercellular CO concentration (Ci), water use efficiency (WUE), light compensation point (LCP), initial fluorescence (Fo), and non-photochemical quenching coefficient (NPQ). Under moderate drought stress, the decrease in net photosynthetic rate was primarily caused by both stomatal and non-stomatal factors, whereas under severe drought stress, the decline was mainly due to non-stomatal factors related to reduced photosynthetic apparatus activity. *Vernicia fordii* exhibited higher photosynthetic apparatus activity and efficiency, along with stronger drought adaptability, than *Vernicia montana*.

Keywords: *Vernicia fordii*; *Vernicia montana*; drought stress; photosynthetic characteristics; light saturation point; chlorophyll fluorescence

Introduction

Global climate change has led to increasing duration and severity of drought events. Combined with uneven seasonal and regional precipitation distribution in China, water shortages have become increasingly apparent, with high temperatures and drought occurring during summer in most southern regions, severely affecting plant growth. Drought is one of the primary environmental factors limiting tree growth, distribution, and survival. Both drought and waterlogging affect mineral element absorption and transport in plants, thereby influencing photosynthesis. Lawlor and Cornic demonstrated that drought stress causes stomatal closure and reduces photosynthetic enzyme activity, leading to decreased photosynthetic rates. Ahmed et al. found that drought stress reduces maximum photochemical quantum efficiency and electron transport rate (ETR) in mung bean leaves. Drought stress affects gas exchange, enzyme activity, and chlorophyll fluorescence parameters during photosynthesis. Investigating photosynthetic physiological changes and drought responses in two tung tree species under different soil drought conditions can provide insight into the intrinsic mechanisms of drought damage.

Tung tree is a collective term for species in the Euphorbiaceae family (*Vernicia*), with a cultivation history of over one thousand years in China. Along with tea-oil camellia, walnut, and Chinese tallow tree, it constitutes one of China' s four major woody oil crops. With population growth and rapid economic development, energy crises and ecological construction have gained attention, making tung tree a promising bioenergy crop for alleviating energy shortages. However, the physiological responses and drought resistance of different tung tree species remain unclear, and the effects of drought stress on photosynthetic gas exchange and chlorophyll fluorescence parameters have not been reported. This study used seedlings of *Vernicia fordii* (three-year tung) and *Vernicia montana* (thousand-year tung) to investigate drought stress effects on growth and photosynthetic characteristics, aiming to elucidate the main factors causing photosynthetic changes and understand water stress adaptation mechanisms, providing theoretical basis for cultivation management and drought-resistant variety breeding.

Materials and Methods

Plant Materials

Experimental materials were three-year tung (*Vernicia fordii* Hemsl.) and thousand-year tung (*Vernicia montana* Lour.) seedlings. Mature seeds of both species were collected in early November 2013 from Qingping Town, Yongshun County, Hunan Province (110°29 E, 28°32 N, elevation 530–600 m), which has a mid-subtropical monsoon climate with 1400 mm annual rainfall, 1306 sunshine hours, 16.0°C mean temperature, and 276 frost-free days. The parent rock is limestone with uneven soil depth. Seeds were air-dried indoors and stored in sand until the following March.

Experimental Design

The pot experiment was conducted on the rooftop of the Life Science Building at Central South University of Forestry and Technology in 2014 under a transparent rain shelter. Four water treatments were established: normal irrigation (CK, control), light drought (LS), moderate drought (MS), and severe drought (SS), with soil water contents at 85-90%, 75-70%, 55-50%, and 35-30% of field capacity, respectively. Soil water content was controlled using the weighing method.

Surface soil from tung tree forest was air-dried, sieved, and placed in plastic pots (30 cm inner diameter, 40 cm height) with 8 kg of soil per pot. Field capacity was determined, and pots were weighed after watering to establish baseline weights. Uniform seedlings were transplanted into pots and maintained under normal water management for one month before initiating drought treatments. During the experiment, water was supplemented daily at 18:00 to maintain target soil moisture levels. To reduce evaporation, plastic bags were placed over pot mouths. Photosynthetic and chlorophyll fluorescence parameters were measured three months after initiating treatments.

Growth Indicators and Chlorophyll Content

Plant height and ground diameter were measured using a ruler and vernier caliper, respectively. Relative chlorophyll content was measured using a SPAD-502 chlorophyll meter (Konica Minolta) on three points on both sides of the main vein at the middle of each leaf, with averages calculated.

Gas Exchange Measurements

Diurnal variations in gas exchange were measured using a LI-6400xt portable photosynthesis system (LI-COR, USA). Healthy, mature leaves with consistent position and growth status were selected and maintained at natural angles. Measurements were taken from 7:00 to 19:00 at 2-hour intervals to ensure consistency. Measured parameters included net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO concentration (Ci), and transpiration rate (Tr). Water use efficiency (WUE) was calculated as Pn/Tr, and stomatal limitation value (Ls) was calculated as (Ca - Ci)/Ca $\times$ 100%, where Ca is ambient CO concentration.

Light response curves were measured between 8:00–11:30 using the LI-6400xt. Photosynthetic photon flux density (PPFD) gradients were set at 0, 25, 50, 75, 100, 150, 200, 300, 600, 900, 1200, 1500, 1800, 2100, and 2400 mol·m ^{2}·s ^{1}. CO concentration was maintained at 400 mol·mol ^{1} using a small cylinder. Parameters including maximum net photosynthetic rate (Pmax), light saturation point (LSP), light compensation point (LCP), apparent quantum yield (AQY), and dark respiration rate (Rd) were fitted using the modified rectangular hyperbola model.

Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence parameters were measured using the LI-6400xt. After full dark adaptation, minimal fluorescence (Fo) was measured before dawn using detection light, followed by a saturating pulse at 7200 mol·m ^{2}·s ^{1} for 0.8 s to measure maximal fluorescence (Fm). After 30 minutes of light adaptation, actual photochemical quantum efficiency (ΦPSII), electron transport rate (ETR), photochemical quenching coefficient (qP), and non-photochemical quenching coefficient (NPQ) were measured. Calculations followed Roháček' s methods.

Data Analysis

Data were processed and graphed using Excel 2007. SPSS 17.0 software was used for one-way ANOVA and significance testing ($P < 0.05$).

Results

Effects of Drought Stress on Chlorophyll Content

Under light drought stress, SPAD values of both species decreased slightly but not significantly ($P > 0.05$). Under moderate and severe drought stress, SPAD values decreased rapidly and significantly ($P < 0.05$). Under moderate drought, chlorophyll content decreased by 30.55% and 36.9% in *V. fordii* and *V. montana*, respectively, while under severe drought, decreases were 20.64% and 30.75% ($P < 0.05$). These results indicate that drought stress inhibits chlorophyll synthesis and accelerates chlorophyll degradation, with *V. montana* showing higher chlorophyll content than *V. fordii* under control conditions.

Effects of Drought Stress on Seedling Growth

Under all water treatments, *V. montana* showed greater plant height, aboveground dry weight, and root dry weight than *V. fordii*, indicating faster growth. As drought stress intensified, plant height, aboveground dry weight, and root dry weight gradually decreased in both species. Light drought had no significant effect on these growth parameters ($P > 0.05$). Under moderate drought, *V. fordii* height, aboveground dry weight, and root dry weight decreased by 18.02%, 17.05%, and 22.67% ($P < 0.05$), respectively, while *V. montana* decreased by 18.29%, 14.98%, and 10.75% ($P < 0.05$). Under severe drought, *V. fordii* showed decreases of 35.65%, 38.06%, and 51.94% ($P < 0.05$), while *V. montana* decreased by 43.34%, 42.76%, and 43.82% ($P < 0.05$), demonstrating that severe drought seriously affected normal seedling growth.

Effects of Drought Stress on Diurnal Variation of Gas Exchange Parameters

The diurnal variation of Pn, Gs, Tr, and Ci in both species showed distinct patterns. Under control and light drought conditions, Pn displayed a single-peak curve with peaks at 11:00 for *V. fordii* and 13:00 for *V. montana*. Under moderate drought, both species showed double-peak curves with midday photosynthetic depression, with peaks at 9:00 and 13:00 for *V. fordii* and 9:00 and 11:00 for *V. montana*. Under severe drought, Pn remained consistently low throughout the day. The diurnal variation amplitude of Pn was smaller under light drought, with higher values in the morning than afternoon. Under moderate and severe drought, Pn differed significantly from control ($P < 0.05$).

Drought stress affected Tr more than Pn, as plants reduced transpiration to increase WUE. The diurnal average of Ci showed a decreasing-then-increasing trend, with minimum values occurring at different times across treatments. Under severe drought, Ci remained high, indicating that the decrease in Pn was primarily due to non-stomatal factors related to damaged photosynthetic enzyme activity and reduced metabolism, rather than stomatal limitation.

Effects of Drought Stress on Photosynthetic Light Response Curves

When PPFD 200 $mol \cdot m^{2} \cdot s^{1}$, Pn increased linearly with light intensity. As PPFD increased from 200 to 600 $mol \cdot m^{2} \cdot s^{1}$, the rate of Pn increase was greater under control and light drought than under moderate and severe drought. When PPFD $>$ 600 $mol \cdot m^{2} \cdot s^{1}$, the increase in Pn gradually slowed and plateaued. Under different treatments, *V. fordii* showed higher Pmax, LSP, and AQY but lower LCP than *V. montana*. Light drought had no significant effect on these parameters ($P > 0.05$). Under moderate drought, *V. fordii*' s Pmax, LSP, AQY, and Rd decreased by 46.64%, 25.96%, 33.75%, and 16.56% ($P < 0.05$), respectively, while *V. montana* decreased by 45.45%, 31.19%, 22.67%, and 19.70% ($P < 0.05$). Under severe drought, *V. fordii* showed decreases of 71.53%, 38.36%, 57.50%, and 34.44% ($P < 0.05$), while *V. montana* decreased by 71.60%, 43.03%, 37.33%, and 33.33% ($P < 0.05$). These results demonstrate that both moderate and severe drought significantly inhibited photosynthetic capacity.

Effects of Drought Stress on Chlorophyll Fluorescence Parameters

Light drought had no significant effect on Fo, Fv/Fm, ETR, ΦPSII, or qP in either species ($P > 0.05$). Under moderate drought, *V. fordii*' s Fv/Fm, ETR, ΦPSII, and qP decreased by 3.70%, 13.04%, 21.30%, and 19.15% ($P < 0.05$), respectively, while *V. montana* decreased by 6.17%, 27.27%, 19.79%, and 31.11% ($P < 0.05$). Under severe drought, *V. fordii* showed decreases of 20.75%, 30.43%, 62.04%, and 59.57% ($P < 0.05$), while *V. montana* decreased by 7.41%, 45.45%, 63.54%, and 68.89% ($P < 0.05$). NPQ increased significantly under drought stress, indicating that both species dissipated excess light energy as heat to protect the photosynthetic apparatus. The impact of severe drought on photosynthetic apparatus activity was far greater than that of moderate drought.

Discussion

Effects of Drought Stress on Light Response Parameters

Light is a crucial environmental factor for plant survival and growth. Photosynthetic light response curves reflect how photosynthetic rate changes with light intensity and are important indicators for assessing how environmental changes affect photosynthetic efficiency. Under moderate and severe drought, both species showed significantly lower Pmax than control, while light drought had no significant effect, indicating that moderate and severe drought impaired photosynthetic capacity. This aligns with studies on sweet potato photosynthetic responses. The gradual decrease in AQY with intensifying drought was not significant under light drought but decreased significantly under moderate and severe drought, indicating that drought stress, especially moderate and severe levels, inhibited the ability to utilize weak light and convert solar energy into net energy. This is consistent with findings that drought reduces both weak and strong light utilization capacity. The decrease in Rd under severe drought helps reduce consumption of photosynthetic products. Our measured Rd values for tung tree (0.88–1.51 $\mathrm{mol \cdot m^{2} \cdot s^{1}}$) were similar to those reported for apricot (1.37–2.08 $\mathrm{mol \cdot m^{2} \cdot s^{1}}$) and periwinkle (0.67–1.92 $\mathrm{mol \cdot m^{2} \cdot s^{1}}$), suggesting different species have varying photosynthetic physiological adaptations to weak light environments, likely representing long-term adaptive mechanisms.

Effects of Drought Stress on Leaf Gas Exchange Parameters

Stomatal conductance is primarily controlled by soil moisture and root signals, with root signals regulating ABA concentration in xylem sap based on root water potential. Soil water content significantly affects photosynthesis, and stomata are the main channels for CO entry into mesophyll cells, directly determining photosynthetic rate. Photosynthetic limitation factors can be divided into stomatal and non-stomatal limitations. During dynamic changes in leaf gas exchange, decreased Pn accompanied by decreased Ci indicates stomatal limitation, while decreased Pn with increased Ci indicates non-stomatal limitation due to reduced photosynthetic apparatus activity. Under light drought, the decrease in Pn was mainly caused by stomatal limitation, while under moderate drought, both stomatal and non-stomatal factors contributed. Under severe drought, non-stomatal factors dominated. The diurnal variation of Pn showed single-peak curves under control and light drought without midday depression, indicating light drought had minimal impact. Under moderate drought, double-peak curves with midday depression appeared, suggesting photoinhibition. Under severe drought, consistently low Pn indicated severe damage to photosynthetic apparatus. Water use efficiency (WUE) reflects plant water utilization status and environmental adaptability. Our study found that WUE increased under drought stress, primarily due to reduced transpiration, representing an evolutionary self-protection mechanism. Under severe drought, although Gs was low, Ci remained high because reduced photosynthetic activity prevented CO assimilation, leading to CO accumulation in mesophyll cells.

Effects of Drought Stress on Chlorophyll Fluorescence Parameters

Under drought stress, increased Fo primarily results from photoinhibitory damage to PSII reaction center complexes and partial inactivation of reaction centers. Decreased Fv/Fm reflects reduced maximum photochemical efficiency, indicating increased photorespiration and environmental stress. Our results showed that under light drought, Fv/Fm (0.80–0.83) was not significantly affected, but under moderate and severe drought, Fv/Fm decreased to 0.76 and 0.74, respectively, indicating photoinhibition and reduced photosynthetic apparatus activity. Plants dissipate excess light energy as heat to avoid light system damage. Under severe drought, increased NPQ and decreased qP indicated that reaction centers entered a closed state, obstructing electron transport and increasing heat dissipation, thereby reducing photosynthetic efficiency. These findings are consistent with studies on other species.

Conclusion

Tung tree species have high photosynthetic rates related to their fast growth. *Vernicia fordii* showed higher photosynthetic efficiency and stronger drought adaptability than *Vernicia montana*. Light drought stress had no significant effect on normal growth or physiological indicators, though *V. fordii* had lower chlorophyll content and growth than *V. montana*. Moderate and severe drought significantly reduced chlorophyll content, photosynthetic rate, and various gas exchange and fluorescence parameters. Under moderate drought, the decrease in photosynthetic rate resulted from both stomatal and non-stomatal factors, while under severe drought, non-stomatal factors dominated. Tung tree possesses certain drought resistance, with normal growth unaffected when soil water content exceeds 70% of field capacity, but severe physiological impacts occur when soil water content falls below 50% of field capacity.

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** Effects of drought stress on chlorophyll SPAD values of two tung tree species

**** Effects of drought stress on growth of two tung tree seedlings (mean±SE)

[FIGURE:2] Effects of drought stress on diurnal variation of leaf gas exchange parameters of tung tree

[FIGURE:3] Responses of net photosynthetic rate to photosynthetic active radiation (PAR) under different drought stress

**** Characteristic parameters of net photosynthetic rate responses to photo-

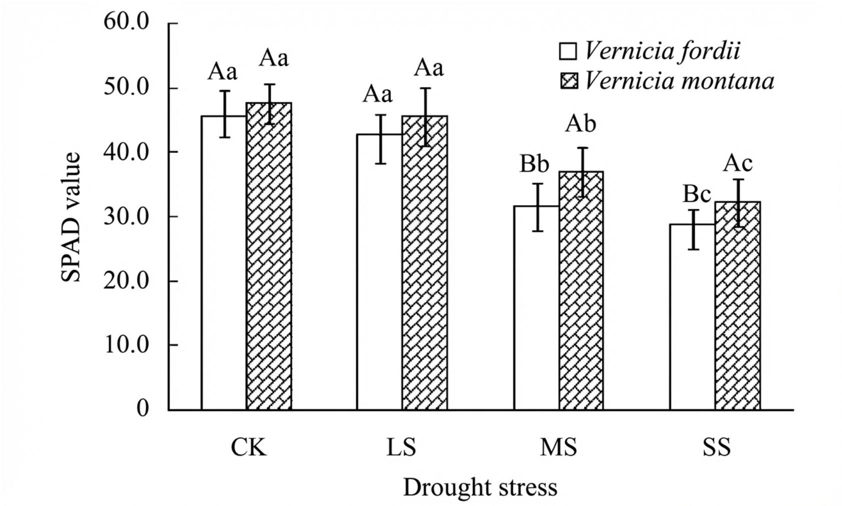

Figure 1: Figure 1

synthetic active radiation under different drought stress (mean±SE)

[**FIGURE:4**] Effects of drought stress on Fo, Fv/Fm, ETR, ΦPSII, qP, and NPQ of two tung tree species

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