Effects of Drought on Water and Nitrogen Utilization and Assimilate Allocation in Biyu Poplar Seedlings (Postprint)

Authors:

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Abstract

To further elucidate the physiological and ecological response processes of poplar to soil drought and interspecific differences in drought resistance strategies, we investigated the response changes in leaf photosynthetic characteristics, water and nitrogen utilization, and assimilate allocation in potted Populus × euramericana 'Biyu' cutting seedlings under continuous 2-year drought stress. The results showed that: (1) Under drought, leaves of one-year-old Populus × euramericana 'Biyu' seedlings became smaller and thicker, stomatal conductance and transpiration rate showed little change, net photosynthetic rate was constrained by non-stomatal limitations, leading to simultaneous declines in instantaneous water and nitrogen use efficiency. (2) Two-year-old Populus × euramericana 'Biyu' exhibited even smaller and thicker leaves with increased carbon-to-nitrogen ratio, while leaf nitrogen content remained unchanged, which enhanced leaf drought resistance; under drought, net photosynthetic rate increased slightly, instantaneous water and nitrogen use efficiency did not decrease, and moderate drought significantly improved whole-plant nitrogen use efficiency. (3) Continuous 2-year drought treatment substantially reduced the growth of Populus × euramericana 'Biyu', with greater allocation of dry matter and non-structural carbon to stem organs, resulting in decreased root-to-shoot ratio, which may impair root water and nitrogen uptake and whole-plant drought resistance. In summary, although Populus × euramericana 'Biyu' seedlings could reduce transpiration water loss through smaller leaves and maintain relatively high leaf nitrogen content under drought, the reduced photosynthetic area caused substantial decreases in whole-plant growth, and assimilate allocation to roots was diminished, preliminarily indicating that Populus × euramericana 'Biyu' possesses poor drought resistance and is unsuitable for large-scale afforestation in arid and semi-arid regions.

Full Text

Effects of Drought on Water and Nitrogen Utilization and Carbohydrate Allocation in Populus × euramericana 'Biyu' Cuttings

Shang Jiazhou[1], Zhao Yuqi[1], Wang Weifeng[1], Gao Tianhui[1], Zong Yuzheng[2]

[1]College of Forestry, Shanxi Agricultural University, Jinzhong 030801, Shanxi, China
[2]College of Agronomy, Shanxi Agricultural University, Jinzhong 030801, Shanxi, China

Abstract

To clarify the ecophysiological responses of poplar to soil drought and interspecific differences in drought resistance strategies, we investigated changes in leaf photosynthetic characteristics, water and nitrogen utilization, and carbohydrate allocation in potted Populus × euramericana 'Biyu' cuttings under two years of continuous drought stress. The results showed that: (1) Under drought, one-year-old 'Biyu' poplar seedlings developed smaller, thicker leaves with relatively stable stomatal conductance and transpiration rate. However, net photosynthetic rate was limited by non-stomatal factors, causing simultaneous declines in instantaneous water and nitrogen use efficiency. (2) In two-year-old seedlings, leaves became even smaller and thicker with increased carbon-to-nitrogen ratio, while leaf nitrogen content remained unchanged, which enhanced leaf drought resistance. Net photosynthetic rate increased slightly under drought, and instantaneous water and nitrogen use efficiency did not decrease; moreover, moderate drought significantly improved whole-plant nitrogen use efficiency. (3) Drought treatment substantially reduced 'Biyu' poplar growth, with more dry matter and non-structural carbohydrates allocated to stems, leading to decreased root-to-shoot ratio. This allocation pattern may impair root water and nitrogen uptake and whole-plant drought resistance. (4) In summary, although 'Biyu' poplar seedlings reduced transpiration water loss through smaller leaves and maintained high leaf nitrogen content under drought, this strategy simultaneously reduced photosynthetic area, causing substantial growth reduction and lower carbohydrate allocation to roots. These findings indicate that 'Biyu' poplar has relatively poor drought resistance and is unsuitable for large-scale afforestation in arid and semi-arid regions.

Keywords: poplar; drought; leaf gas exchange; water and nitrogen use; carbohydrate allocation

Introduction

Poplar (*Populus* spp.) is a crucial tree species in northern China, valued for its rapid growth and high survival rate in afforestation. For decades, it has been the primary species for ecological shelterbelts in the "Three Norths" region, with extensive plantations of *P. simonii*, *P. popularis*, and other varieties. However, these plantations have contributed to soil desiccation and the formation of dried soil layers, which in turn inhibit vegetation growth and create large areas of stunted "small old trees" [1-3]. Under future climate change, drought frequency and intensity in this region are projected to increase [4], further limiting poplar plantation productivity and potentially causing stand mortality.

Previous studies have documented poplar' s physiological responses to drought. Reduced soil water availability causes stomatal closure, decreased photosynthetic rates, and inhibited individual growth [5-7]. To maintain whole-plant water balance and survival, poplars alter carbon and nitrogen resource allocation patterns among organs [8-10], with response magnitude varying by species, organ function, and growth stage [11]. Most poplar species are sensitive to soil water stress, adjusting root spatial distribution to enhance water uptake [12], reducing xylem vessel diameter to maintain water transport safety [13], and decreasing leaf number and area to reduce water loss [14]. Drought-resistant poplars can improve water use efficiency through sensitive stomatal regulation [15,16].

The new *Populus euramericana* cultivar 'Biyu' has been reported to possess strong cold and drought resistance, growing well in arid regions such as central-western Inner Mongolia and Shandan County, Gansu Province [17,18]. Field observations show that 'Biyu' poplar exhibits obvious rapid growth characteristics under adequate irrigation, but its growth decreases substantially under controlled drought conditions [19]. However, its root-shoot relationships, non-structural carbon accumulation, and other ecophysiological responses to drought remain unclear. Using 'Biyu' poplar cuttings as experimental materials, this study investigated responses of leaf photosynthetic characteristics, water and nitrogen utilization, and inter-organ carbohydrate allocation under continuous soil drought through pot experiments. The results will help clarify drought response mechanisms in poplar and provide theoretical support for efficient cultivation of poplar plantations in arid and semi-arid regions.

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1. Materials and Methods

1.1 Experimental Site The experiment was conducted in a high-light transmission rain-sheltered nursery at the College of Forestry Experimental Farm, Shanxi Agricultural University, Taigu County, Jinzhong City, Shanxi Province (112.57°E, 37.43°N; elevation 796 m). The region has a warm temperate continental climate with mean annual temperature of 10.4 °C, annual sunshine duration of 2527.5 h, annual precipitation of 397.1 mm, and annual evaporation of 1649 mm. The experimental soil was surface soil from the nursery, with loam texture, organic matter content of 26.94 $g \cdot kg^{-1}$, total nitrogen of 1.79 $g \cdot kg^{-1}$, pH of 7.22, and field capacity of 18 kg.

1.2 Experimental Materials and Treatments In early March, healthy, uniform one-year-old 'Biyu' poplar stems were selected and cut into 12 cm long cuttings. Each cutting was planted in a plastic bucket (35 cm height, 30 cm diameter) containing 18 kg of air-dried soil. The bottom of each bucket was layered with pebbles and newspapers to isolate the soil, and a rigid plastic tube inserted to the bottom facilitated uniform water absorption from bottom to top while preventing soil compaction. Each bucket was planted with one cutting and managed under normal pot conditions.

Starting at the end of May, continuous soil drought treatments were imposed using the weighing method: (1) Normal water supply (control): soil water content maintained at 75% ± 5% of field capacity; (2) Moderate drought: 50% ± 5% of field capacity; (3) Severe drought: 25% ± 5% of field capacity. Each treatment had 10 replicates. Buckets were weighed and replenished every evening to maintain target soil water content. Leaf gas exchange, carbon and nitrogen content, and other indicators were measured in early September for both the first and second years.

1.3 Gas Exchange Parameter Measurements For each treatment, three functional leaves per plant were randomly selected between 10:00–11:30 using a Li-6400XT portable photosynthesis system (Li-COR, USA). Measurements included net photosynthetic rate (Pn, $mol \cdot m^{-2} \cdot s^{-1}$), stomatal conductance (Gs, $mol \cdot m^{-2} \cdot s^{-1}$), intercellular CO_2 concentration (Ci, $mol \cdot mol^{-1}$), transpiration rate (Tr, $mmol \cdot m^{-2} \cdot s^{-1}$), with red-blue light intensity set at 1500 $mol \cdot m^{-2} \cdot s^{-1}$, leaf temperature at 25 °C, relative humidity at 60-70%, and ambient CO_2 concentration (Ca) at 410–430 $mol \cdot mol^{-1}$. Stomatal limitation value (Ls) and instantaneous water use efficiency (WUE) were calculated according to Farquhar and Sharkey [23].

1.4 Leaf Carbon and Nitrogen Content Determination Leaves adjacent to those used for gas exchange measurements were harvested. Leaf area was determined with an EPSON DS50000 scanner (Japan). After oven-drying at 105 °C for 30 minutes and then at 75 °C to constant weight, specific leaf weight was calculated. Dried leaf samples were ground and passed through a 0.1 mm sieve. Leaf nitrogen content was determined using the Kjeldahl method with a FoodALYTD5000 OMNILab analyzer following sulfuric acid-catalyst digestion. Leaf carbon content was measured by the potassium dichromate-sulfuric acid oxidation method. Based on these measurements, leaf nitrogen content per unit area, total leaf nitrogen, whole-plant nitrogen use efficiency (NUE), and photosynthetic nitrogen use efficiency (PNUE) were calculated [25].

1.5 Growth Index and Non-structural Carbohydrate Content Determination Plant height and ground diameter were measured with vernier calipers and tape. At harvest, roots were carefully washed and the whole plant was separated into roots, stems, and leaves, which were oven-dried to constant weight. For non-structural carbohydrate (NSC) analysis, dried root, stem, and leaf samples from two-year-old plants were ground and passed through a 0.1 mm sieve. Soluble sugar and starch contents were determined using the phenol-sulfuric acid method [22].

1.6 Data Analysis Raw data were processed in Excel 2013. One-way ANOVA was performed using SPSS 17.0, and Duncan' s multiple range test ($\alpha = 0.05$) was used for post-hoc comparisons.

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2. Results

2.1 Effects of Drought on Gas Exchange Parameters in 'Biyu' Poplar Drought responses of gas exchange parameters differed significantly between years. In the first year, Pn decreased significantly with increasing drought severity ($P < 0.05$), while Gs, Ls, Tr, and WUE also declined. Ci showed no significant difference from the control ($P > 0.05$). In the second year, Pn under moderate and severe drought decreased by 32.15% and 44.81% compared to the control ($P < 0.05$). Gs and Tr showed no significant change, while Ls and WUE increased, showing opposite trends to the first year [Figure 1: see original paper].

2.2 Effects of Drought on Growth and Biomass Allocation in 'Biyu' Poplar Drought significantly affected plant growth. In the first year, single leaf area decreased gradually while specific leaf weight increased with drought severity ($P < 0.05$). In the second year, moderate drought showed no significant effect on leaf area or specific leaf weight ($P > 0.05$), but severe drought reduced leaf area by 39.11% and increased specific leaf weight by 12.39% ($P < 0.05$). Drought significantly reduced biomass accumulation, with organ dry weight and total plant dry weight decreasing significantly ($P < 0.05$). Plant height and root-to-shoot ratio also declined significantly ($P < 0.05$). In the first year, leaf mass fraction decreased while root mass fraction increased under drought. In the second year, root biomass allocation decreased significantly ($P < 0.05$), while stem allocation increased significantly ($P < 0.05$) [FIGURE:2, TABLE:1].

2.3 Effects of Drought on Leaf Carbon/Nitrogen Content and Nitrogen Utilization in 'Biyu' Poplar In the first year, leaf nitrogen content decreased significantly under drought ($P < 0.05$), while carbon content and carbon-to-nitrogen ratio decreased significantly ($P < 0.05$). Nitrogen content per unit leaf area and PNUE showed no significant change, but NUE increased significantly under moderate drought ($P < 0.05$). In the second year, leaf nitrogen content decreased significantly ($P < 0.05$), while carbon content, carbon-to-nitrogen ratio, NUE, and PNUE showed no significant change. With prolonged drought, the relationship between leaf carbon and nitrogen content changed: in the second year, leaf carbon and nitrogen content increased, nitrogen content per unit area increased ($P < 0.05$), and carbon-to-nitrogen ratio increased significantly ($P < 0.05$). Regression analysis revealed a highly significant positive correlation ($P < 0.01$) between leaf WUE and PNUE across treatments [TABLE:2, FIGURE:3].

2.4 Effects of Drought on Non-structural Carbohydrate Content in Organs Non-structural carbohydrate content decreased in the order: leaves > stems > roots. After two years of drought, leaf soluble sugar content decreased significantly ($P < 0.05$), while stem soluble sugar content increased significantly under severe drought ($P < 0.05$), and root content showed no significant change. Starch content in all organs followed the same trend as soluble sugar content ($P < 0.05$). Combining organ biomass data revealed that the allocation proportion of total soluble sugar and total NSC in stems increased gradually with drought severity, while allocation to leaves and roots decreased [FIGURE:4, FIGURE:5].

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3. Discussion

3.1 Effects of Drought on Photosynthesis and Water-Nitrogen Use in 'Biyu' Poplar Drought inhibition of photosynthesis depends on drought intensity, duration, and species, and can be categorized as stomatal or non-stomatal limitation [23]. In this study, first-year 'Biyu' poplar showed continuously decreasing Pn under drought, while Ls and Ci did not differ significantly from the control [Figure 1: see original paper], indicating non-stomatal limitation. This may result from damage to photosynthetic systems in mesophyll tissue and reduced conductance from substomatal cavities to photosynthetic sites [24]. Maintaining high leaf nitrogen content is crucial for photosynthesis [25]. Compared with *P. popularis* [13], 'Biyu' poplar maintained leaf nitrogen levels under drought. However, mesophyll tissue membrane systems in *P. euramericana* are more vulnerable to drought-induced damage [26], likely causing significant reductions in mesophyll conductance. Consequently, 'Biyu' poplar sacrificed leaf area to maintain relatively high Gs and Ci, resulting in high transpiration water loss and reduced WUE. This aligns with studies showing that reduced mesophyll conductance is a key factor causing simultaneous declines in WUE and PNUE [24], though further verification through antioxidant enzyme activity and mesophyll conductance measurements is needed.

In contrast to the first year, second-year drought-treated 'Biyu' poplar showed smaller leaves with increased specific leaf weight, while nitrogen content per unit leaf area remained stable, Gs, Ci, and Pn all decreased significantly. Under moderate and severe drought, although leaf area decreased significantly, Pn increased slightly, and moderate drought significantly improved NUE. These results suggest that two-year-old 'Biyu' poplar seedlings exhibited enhanced leaf drought resistance compared with one-year-old seedlings, possibly related to increased leaf carbon investment to maintain high leaf nitrogen content under prolonged drought. However, this strategy reduced photosynthetic area, leading to substantially decreased whole-plant biomass accumulation.

Drought significantly reduced both biomass and NSC allocation proportions in 'Biyu' poplar roots, which is inconsistent with optimal allocation theory and clearly detrimental to water and nitrogen uptake. In contrast to *P. simonii*, which shows increased root-to-shoot ratio under drought [14], 'Biyu' poplar allocated more dry matter and NSC to stems under moderate and severe drought, while root allocation decreased significantly [FIGURE:2, FIGURE:5]. This pattern differs from most herbaceous plants and some trees like *P. simonii* [14] and *P. tomentosa* [28]. Increased stem carbon investment in drought environments typically results from elevated carbon consumption for xylem construction, such as reduced vessel diameter with thickened walls to lower cavitation risk and enhance xylem water transport safety [29,30]. Soluble sugar accumulation may promote embolism repair through osmotic regulation and maintain water transport [31,32]. Regardless of the mechanism, the consequence is that 'Biyu' poplar reduced leaf area to limit transpiration but simultaneously decreased photosynthetic area, causing substantial growth reduction. With declining leaf water-nitrogen use capacity and reduced carbohydrate allocation to roots, 'Biyu' poplar's overall drought resistance is poor, making it more likely to form stunted "small old tree" stands when planted in arid and semi-arid regions.

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4. Conclusions

1) Under drought, one-year-old 'Biyu' poplar seedlings developed smaller, thicker leaves and maintained high leaf nitrogen content. However, due to non-stomatal limitations, both photosynthetic capacity and instantaneous water-nitrogen use efficiency decreased significantly.

2) Two-year-old 'Biyu' poplar exhibited smaller leaves with increased carbon-nitrogen ratio and specific leaf weight, enhancing drought resistance. Under moderate and severe drought, the species maintained leaf nitrogen content, photosynthetic capacity, and instantaneous water-nitrogen use efficiency by reducing leaf area, and moderate drought significantly improved whole-plant nitrogen use efficiency.

3) Soil drought caused 'Biyu' poplar to allocate more dry matter and non-structural carbohydrates to stems, reducing root-to-shoot ratio. While this may help maintain stem water transport safety, it is detrimental to root water-nitrogen absorption and whole-plant drought resistance.

4) These results indicate that 'Biyu' poplar has poor drought resistance and

is unsuitable for large-scale afforestation in arid and semi-arid regions.

References

[1] Li Yushan. Effects of forest on water circle on the Loess Plateau[J]. Journal of Natural Resources, 2001, 16(5): 427-432.

[2] Tian Junliang. Essential exploration of soil aridization in Loess Plateau[J]. Acta Pedologica Sinica, 2004, 41(1): 1-6.

[3] Lü Wen, Zhang Weidong, Bao Jun. Discussion on developing of poplar and construction of Three Norths Protection Forest Project[J]. Protection Forest Science and Technology, 2000(2): 68-70.

[4] Burkett V R, Suarez A G, Bindi M, et al. Climate Change 2014: Impacts, Adaptation, and Vulnerability[M]. Cambridge: Cambridge University Press, 2015: 151-206.

[5] Yin Chunying, Li Chunyang. Advance in research on drought resistance of *Populus*[J]. Chinese Journal of Appled & Environmental Biology, 2003, 9(6): 662-668.

[6] Meng S, Zhang C, Li S, et al. Nitrogen uptake and metabolism of *Populus simonii* in response to PEG induced drought stress[J]. Environmental & Experimental Botany, 2016, 123: 78-87.

[7] Garavillon Tournayre M, Gousset Dupont A, Gautier F, et al. Integrated drought responses of black poplar: How important is phenotypic plasticity?[J]. *Physiologia Plantarum*, 2018, 163(1): 30-44.

[8] Viger M, Smith H K, Cohen D, et al. Adaptive mechanisms and genomic plasticity for drought tolerance identified in European black poplar (*Populus nigra* L.)[J]. *Tree Physiology*, 2016, 36(7): 909-928.

[9] Zhao Yuqi, Gao Miaoqin, Li Tao, et al. Effects of drought stress on the leaf gas exchanges and organ dry matter distribution of *popularis*' cuttings[J]. *Acta Ecologica Sinica*, 2020, 40(5): 1683-1689.

[10] Gao Tianhui, Shang Jiazhou, Song Liting, et al. Responses of *Populus simonii* leaf photosynthetic and anatomical characteristics in cuttings to drought and re watering[J]. *Science of Soil and Water Conservation*, 2021, 19(6): 18-26.

[11] Sala A, Woodruff D R, Meinzer F C. Carbon dynamics in trees: Feast or famine?[J]. *Tree Physiology*, 2012, 32(6): 764-775.

[12] Klein T, Zeppel M J B, Anderegg W R L, et al. Xylem embolism refilling and resilience against drought induced mortality in woody plants: Processes and trade offs[J]. *Ecological Research*, 2018, 33(5): 839-855.

[13] Li Aiping, Su Xiaohua, Wang Xiaojiang, et al. Introduction trial of new varieties of *Populus euramericanacn* in Inner Mongolian[J]. *Journal of Northeast Forestry University*, 2015, 43(1): 5-8.

[14] Chen Jinlong. Growth observation and seedling management of *Populus* sp. poplar covered with plastic film in arid region[J]. *Forestry Science & Technology*, 2018, 43(4): 9-11.

[15] Parker J. Drought resistance in woody plants[J]. *Botanical Review*, 1956, 22(4): 241-289.

[16] Wiley E, Huepenbecker S, Casper B B, et al. The effects of defoliation on carbon allocation: can carbon limitation reduce growth in favour of storage?[J]. *Tree Physiology*, 2013, 33(11): 1216-1228.

[17] Poorter H, Niklas K J, Reich P B, et al. Biomass allocation to leaves, stems and roots: Meta analyses of interspecific variation and environmental control[J]. *New Phytologist*, 2012, 193(1): 30-50.

[18] Lü Shuang. The Effects of Water and Soil Environment on the Growth of *Populus euphratica* Seedlings[D]. Beijing: Beijing Forestry University, 2015.

[19] Chen Jiacun, Li Yangyang, Zuo Lixiang. The hydraulic acclimation of old and dwarf *Populus simonii* trees growing on sandy soil in northern Shaanxi Province, China[J]. *Acta Ecologica Sinica*, 2014, 34(15): 4193-4200.

[20] Wang Yilin, Wang Weifeng, Zhang Yunxiang, et al. Responses of leaf morphological structure and physiological characteristics of *Populus euramericana* to drought stress[J]. *Forestry Science*, 2019, 55(4): 45-53.

[21] Dong T, Duan B, Zhang S, et al. Growth, biomass allocation and photosynthetic responses are related to intensity of root severance and soil moisture conditions in the plantation tree *Cunninghamia lanceolata*[J]. *Tree Physiology*, 2016, 36(7): 807-817.

[22] Landhäusser S M, Chow P S, Dickman L T, et al. Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates[J]. *Tree Physiology*, 2018, 38(12):1764-1775.

[23] Farquhar G D, Sharkey T D. Stomatal conductance and photosynthesis[J]. *Annual Review of Plant Physiology*, 1982, 33(1): 317-345.

[24] Warren C R, Adams M A. Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis[J]. *Plant, Cell & Environment*, 2006, 29(2): 192-201.

[25] Liang G, Bu J, Zhang S, et al. Effects of drought stress on the photosynthetic physiological parameters of *Populus* × *euramericana*[J]. *Journal of Forestry Research*, 2019, 30(2): 409-416.

[26] Field C, Merino J, Mooney H A. Compromises between water efficiency and nitrogen use efficiency in five species of California evergreens[J]. *Oecologia*, 1983, 60(3): 384-389.

[27] Deng Xiuxiu, Xiao Wenfa, Zeng Lixiong, et al. Transport and distribution characteristics of photosynthates of *Pinus massoniana* seedlings[J]. *Scientia Silvae Sinicae*, 2019, 55(7): 27-34.

[28] Lu Y W, Miao X L, Song Q Y, et al. Morphological and ecophysiological plasticity in dioecious plant *Populus tomentosa* under drought and alkaline stresses[J]. *Photosynthetica*, 2018, 56(4): 1129-1139.

[29] Janssen T A J, Hölttä T, Fleischer K, et al. Wood allocation trade offs between fiber wall, fiber lumen and axial parenchyma drive drought resistance in neotropical trees[J]. *Plant, Cell & Environment*, 2020, 43(4): 965-980.

[30] Hesse B D, Goisser M, Hartmann H, et al. Repeated summer drought delays sugar export from the leaf and impairs phloem transport in mature beech[J]. *Tree Physiology*, 2018, 39: 192-200.

[31] Brodersen C R, Roddy A B, Wason J W, et al. Functional status of xylem through time[J]. *Annual Review of Plant Biology*, 2019, 70(1): 407-433.

[32] Zwieniecki M A, Holbrook N M. Confronting Maxwell' s demon: Biophysics of xylem embolism repair[J]. *Trends in Plant Science*, 2009, 14(10): 530-534.

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