Effects of Drought Stress on Physiological Characteristics and Root Growth of Wild Tea Seedlings in Karst Regions (Postprint)

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Abstract

Using clonal seedlings of four wild tea plant species (Sect Thea (L.) Dyer) from the karst region of Guizhou as experimental materials, we employed a pot-culture water control method to investigate their physiological and growth responses to drought stress, conducted a preliminary evaluation of their drought resistance, and verified the results through field simulated continuous drought experiments. The results indicated: (1) With increasing drought stress intensity, the relative water content of leaves in all four wild tea species gradually decreased; plasma membrane permeability, malondialdehyde (MDA) content, and soluble sugar content all exhibited increasing trends; free proline content and superoxide dismutase (SOD) activity initially increased then decreased; peroxidase (POD) activity, except for Camellia gymnogyna Chang which showed an increasing trend with intensifying stress, displayed an initial increase followed by a decrease in the other species. (2) As drought stress intensified, the total biomass dry weight of seedlings of all four tea species gradually decreased, while the root-to-shoot ratio initially increased then decreased; total root length, except for Camellia gymnogyna Chang which showed an initial increase followed by a decrease, gradually declined in the other three species; total root surface area and total root volume gradually decreased in Camellia taliensis and Camellia sinensis, but initially increased then decreased in Camellia tachangensis and Camellia gymnogyna Chang; average root diameter gradually decreased in Camellia gymnogyna Chang, initially increased then decreased in Camellia tachangensis, and gradually increased in Camellia sinensis; root activity gradually decreased in Camellia taliensis, while initially increased then decreased in the other three species; specific leaf area exhibited a decreasing trend in Camellia tachangensis, but initially increased then decreased in the other three species. (3) Drought resistance evaluation based on membership function analysis of growth and physiological indices revealed that MDA content and plasma membrane permeability were closely correlated with drought resistance in wild

tea plants. The drought resistance ranking of the four wild tea seedlings was: Camellia gymnogyna Chang > Camellia tachangensis F. C. Zhang > Camellia Sinensis (L.)-O. Kuntze > Camellia taliensis (W. W. Smish). Field continuous drought experiments validated the accuracy and reliability of the membership function-based drought resistance evaluation method.

Full Text

Preamble

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Effects of Drought Stress on Physiological Characteristics and Root Growth of Wild Tea Plant Seedlings in Karst Regions

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Abstract

Using clone seedlings of four wild tea plant species from the karst region of Guizhou as experimental materials, we investigated their physiological responses to drought stress using a pot water control method to preliminarily evaluate their drought resistance. The results were verified through a two-year field simulation experiment of continuous drought. As drought stress intensity increased, the relative water content of tea leaves gradually decreased, while cell membrane permeability, malondialdehyde (MDA) content, and soluble sugar content all showed upward trends. Free proline content and superoxide dismutase (SOD) activity initially increased then decreased. Peroxidase (POD) activity, except in *Camellia gymnogyna* which continued to increase with stress intensity, showed an initial increase followed by a decrease in the other species. With increasing drought stress, the total dry biomass of seedlings from all four tea species gradually decreased. The root-shoot ratio initially increased then decreased in all species. Root total surface area and volume in *Camellia taliensis* and *C.*

sinensis gradually decreased, while in *C. tachangensis* they initially increased then decreased. Specific leaf area decreased in *C. tachangensis* but showed an initial increase followed by a decrease in the other three species. Root total length in *C. gymnogyna* initially increased then decreased, while in the other three species it gradually decreased. Root activity gradually decreased in *C. taliensis*, but in the other three species it initially increased then decreased. Based on the membership function analysis of growth and physiological indices, the drought resistance order of the four wild tea species was: *Camellia gymnogyna* > *C. tachangensis* > *C. sinensis* > *C. taliensis*. The two-year field continuous drought experiment validated the accuracy and reliability of the membership function-based drought resistance evaluation method.

Keywords: drought stress; wild tea plants; leaf physiological characteristics; root growth

Introduction

Southwest China' s karst region harbors abundant wild tea plant resources [1]. These resources have long been utilized by local people as beverages and are highly valued in southwestern regions including Guizhou. However, in karst mountainous areas, shallow soil and weak water retention capacity, combined with frequent seasonal and temporary droughts [2], have affected the scientific development and utilization of wild tea plant germplasm resources. Under drought stress conditions, the physiological responses of woody plants mainly manifest as decreased leaf water content, changes in osmotic adjustment substances, and alterations in antioxidant and hormone contents [3-5]. Tea plants are sensitive to drought stress, and under drought conditions, stomatal characteristics and leaf antioxidant system components are affected to varying degrees, causing damage to tea plants [6-7]. Studies on the drought resistance of Tieguanyin and Fuding Dabaicha have shown that the production rate of reactive oxygen species in tea leaves accelerates, cell membrane permeability increases, and SOD and POD activities increase under mild and moderate water stress but decrease under severe water stress. The protective enzyme activities and antioxidant contents of Fuding Dabaicha increase under mild water stress but decrease under severe water stress [8], which is consistent with Liang Jianping et al.' s [9] view that physiological indicators are strongly correlated with water treatments. Roots are the primary organs for water absorption in plants and are the first to perceive drought, rapidly generating chemical signals transmitted upward to promote stomatal closure and reduce water loss [10]. Simultaneously, roots can adapt to altered water environments through adjustments in their morphological and physiological characteristics [11]. To better study plant adaptability to drought stress, we used clone seedlings of four wild tea species, establishing different water treatments through pot water control methods. The physiological responses of tea plants to drought stress share certain commonalities with other woody plants. This study is based on the rich wild tea plant resources in Guizhou' s karst region, comparing the physiological and biochemical characteristics, root growth features, and biomass changes of these resources under soil drought stress, and conducting a comprehensive drought resistance evaluation using membership function weighted averages. The results were validated through field simulation experiments of continuous drought to provide theoretical support for the protection, utilization, and drought-resistant breeding of wild tea plant germplasm resources in karst regions.

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1 Materials

Four wild tea plant germplasm resources from Guizhou' s karst region were used. Following the classification method of Chen Liang et al. [12], the four resources were: *Camellia taliensis* (W. W. Smish), *Camellia tachangensis* F. C. Zhang, *Camellia sinensis* (L.) O. Kuntze, and *Camellia gymnogyna* Chang.

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2 Pot Water Control Experiment Methods

Four water stress gradients were established: CK (regular soil water content at 75% ± 2.5% field capacity), T1 (mild drought stress at 55% ± 2.5%), T2 (moderate drought stress at 40% ± 2.5%), and T3 (severe drought stress at 25% ± 2.5%). The experimental soil was collected from the tea experimental garden of Guizhou University, with properties: pH 4.53, organic matter 10.68 g/kg, total nitrogen 0.88 g/kg, available nitrogen 22.63 mg/kg, available phosphorus 0.19 mg/kg, available potassium 12.73 mg/kg, etc.

Uniform two-year-old wild tea cutting seedlings were selected from the Guizhou University Tea Science experimental base and transplanted into black plastic buckets (26 kg soil each). Holes were drilled in the buckets and covered with fine cotton cloth to prevent soil loss during watering. The buckets were placed in a rain shelter, thoroughly watered to reach soil saturation, then allowed to naturally dry to the target relative water content before initiating water stress treatments. Daily weighing method [13] was used to control water content, with other management practices conducted routinely.

Name and origin of tea germplasms

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1 Physiological and Biochemical Indices

Random samples were collected from the upper leaves of tea seedlings. Leaf relative water content, relative conductivity, MDA content, proline content, soluble sugar content, SOD activity, and POD activity were measured immediately. The methods of Chen Wenrong et al. [14] were referenced for MDA and proline content, Liu Lijun et al. [15] for soluble sugar content, Shi Xiaoling et al. [16] for leaf relative water content, relative conductivity, and SOD/POD activities, and Qiman Yunus et al. [13] for root activity.

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2 Growth Indices

Biomass and root indices were measured following the methods of He Yuejun and Zhong Zhangcheng [17]. After treatment, the complete plant was removed by breaking the bucket, and roots were carefully washed clean.

Analysis of Root Total Surface Area

Root total surface area, total volume, average diameter, and total length were analyzed using an Epson Expression 10000XL 1.0 scanner. After scanning, root biomass dry weight and above-ground biomass dry weight were measured, and the root-shoot ratio was calculated.

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4 Drought Resistance Membership Function Analysis

The membership function calculation method was as follows: For parameters positively correlated with stress resistance (leaf relative water content, proline content, root activity, and specific leaf area), the formula was used: Uij = (Xij - Xjmin) / (Xjmax - Xjmin). For parameters negatively correlated with stress resistance (electrical conductivity, MDA content), the formula was: Uij = 1 - (Xij - Xjmin) / (Xjmax - Xjmin). Where Uij represents the drought resistance membership function value for species i and indicator j, Xij is the measured value, Xjmin is the minimum value of indicator j across all species, and Xjmax is the maximum value. Weights were calculated using objective weighting methods based on the ratio of values relative to the control group. The comprehensive evaluation value D = Σ(Uij × Wj), where larger D values indicate stronger drought resistance.

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5 Field Simulation Drought Experiment

The weight of each evaluation indicator was calculated using the formula Ij = (Sj/Cj), where Sj is the average measured value of indicator j under stress treatment, and Cj is the measured value of the control group. Ij is a dimensionless number. Weights were normalized to obtain Wj [20].

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1 Field Simulation Drought Experiment Design

The experiment was conducted at the Guizhou University experimental tea garden. A randomized block design was used with wide-narrow double-row planting. Each plot (1.5 m $\times$ 0.3 m $\times$ 0.3 m) was planted with one-year-old cuttings of the four wild tea species. Normal weeding and fertilization were performed. After root establishment, rain shelters were installed in August. When soil water content fell below 25%-35% of field capacity, water control was implemented. The experiment was repeated over two years.

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2 Soil Water Content Measurement and Supplement Calculation

Soil water content was measured using a TZS-1K soil moisture meter at 0-60 cm depth with 10 cm intervals. Supplement calculation followed Huo Zhiguo et al. [21]. The supplement amount P (mm) = Σ[i $\times$ hi $\times$ (r - Wi/Ti)] $\times$ Ti / 1000, where is soil bulk density, h is soil layer thickness, r is field capacity, W is soil water content, T is time, and n is the number of soil layers.

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2 Results and Analysis

2.1 Effects of Drought Stress on Leaf Physiology of Wild Tea Seedlings

As water stress intensity increased, the relative water content of leaves from all four wild tea species gradually decreased. Under moderate and severe drought stress, leaf relative water content was significantly lower than the control group. Under severe drought stress, differences among species became more pronounced. Cell membrane permeability and MDA content showed significant upward trends. *Camellia taliensis* and *C. sinensis* showed greater membrane system damage, while *C. gymnogyna* and *C. tachangensis* showed smaller increases in cell membrane permeability and MDA content, indicating less membrane damage.

Free proline content showed an initial increase followed by a decrease with increasing drought stress, with values significantly higher than control. Proline content in *C. tachangensis* and *C. gymnogyna* peaked under moderate stress, while in *C. taliensis* it peaked under mild stress. Under severe drought, interspecies differences in proline content reached significant levels.

Soluble sugar content increased with drought stress intensity. Under moderate stress, soluble sugar content in all species was significantly higher than control. Under severe stress, increases were smaller in *C. gymnogyna* compared to other species.

SOD and POD activities showed initial increases followed by decreases. SOD activity peaked under mild stress in *C. taliensis* and *C. sinensis*, and under moderate stress in *C. tachangensis* and *C. gymnogyna*. Under severe stress, only *C. gymnogyna* showed SOD activity significantly higher than control, while other species showed significantly lower activity. POD activity in *C. gymnogyna* increased with stress intensity, while other species showed initial increases followed by decreases.

Effects of drought stress on leaf physiological parameters of wild tea seedlings

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2.2 Effects of Water Stress on Biomass and Root Indices of Wild Tea Seedlings

Under control conditions, *C. taliensis* had significantly higher biomass than other species. Under moderate and severe water stress, its dry matter accumulation decreased by 24.69%, 36.33%, and 51.61% respectively. Total root length and root activity showed significant decreasing trends. Specific leaf area showed no significant difference under mild drought. Root total surface area and volume were significantly lower than control under moderate and severe stress. Root average diameter was significantly higher than control. The root-shoot ratio showed no significant difference from control. Drought stress hindered both above-ground and below-ground growth of *C. taliensis*.

Camellia tachangensis control biomass was significantly lower than other species. Under moderate and severe stress, biomass decreased by 23.75%, 30.40%, and 40.62% respectively. Under mild stress, root-shoot ratio, root average diameter, and root activity were significantly higher than control, indicating strong adaptability to mild drought. Under moderate stress, root average diameter and root activity remained significantly higher, compensating for reduced root absorption area. Under severe stress, all root morphology indices were significantly lower than control.

Camellia sinensis biomass decreased by 18.46%, 44.61%, and 54.77% under mild, moderate, and severe stress respectively. All root indices were significantly lower than control, while root average diameter and root activity were significantly higher. Specific leaf area was significantly higher than control under moderate and severe stress.

Camellia gymnogyna biomass decreased by 1.07%, 4.09%, and 34.54% under mild, moderate, and severe stress respectively, with no significant difference from control under mild and moderate stress. Under mild and moderate stress, root total surface area, volume, length, and activity were significantly higher than control, while root average diameter was significantly lower. Under severe stress, specific leaf area, root volume, and length were significantly higher than control, while root average diameter and activity were significantly lower. This indicates *C. gymnogyna* could maintain high root water absorption capacity through morphological changes under various drought conditions.

Effects of drought stress on biomass and root parameters of wild tea seedlings

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3 Comprehensive Drought Resistance Evaluation of Wild Tea Seedlings

Based on the weighted average membership function values of multiple indicators, a comprehensive evaluation of drought resistance was conducted for the four wild tea germplasm resources. The indicators most closely related to drought resistance were MDA content and cell membrane permeability, with average weights > 0.1. The drought resistance order was: *C. gymnogyna* (membership value 0.936, strong drought resistance) > *C. tachangensis* (0.801, relatively strong) > *C. sinensis* > *C. taliensis* (0.032, poorest drought resistance).

Comprehensive evaluation of drought resistance of wild tea germplasms

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4 Field Continuous Drought Experiment Results

The two-year field continuous drought experiment showed that all four wild tea species suffered varying degrees of damage. *Camellia gymnogyna* showed the least damage and lowest mortality rate (11.43% in 2012, 14.17% in 2014), with significantly higher net plant height increment than other species. *Camellia tachangensis* and *C. sinensis* showed moderate damage, while *C. taliensis* suffered the most severe damage with mortality rates of 59.17% ± 6.09% in 2012 and 55.00% ± 1.53% in 2014, and net height increments less than 4 cm.

Survival rate and growth of four wild tea germplasms under drought stress in field experiments

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3 Discussion and Conclusion

3.1 Effects of Drought Stress on Leaf Physiological Indices

Under drought conditions, stronger leaf water retention capacity and less cell membrane damage indicate stronger drought resistance [22-23]. The relative water content of wild tea seedlings gradually decreased with increasing drought stress intensity. Under moderate and severe drought stress, *C. gymnogyna* and *C. tachangensis* showed less cell membrane damage, while *C. taliensis* and *C. sinensis* showed greater damage. When cell membranes are damaged, excess reactive oxygen species (ROS) are produced. If not promptly cleared, oxidative stress occurs, leading to increased membrane lipid peroxidation products (MDA) and plasma membrane permeability [24]. As leaf relative water content decreases and cell membranes are damaged, plant cells activate protective enzyme systems (SOD, POD) to scavenge ROS and avoid membrane damage [25-26]. Higher enzyme activity indicates stronger drought resistance [11,27]. Plants also adapt through osmotic adjustment by accumulating soluble substances [28]. Soluble sugars and free proline are important osmotic adjustment substances [10,29].

Our results show that with increasing drought stress, cell membrane permeability and MDA content gradually increased, while proline content and SOD/POD activities initially increased then decreased. All four species activated osmotic adjustment mechanisms and protective enzyme systems. However, these systems were gradually inhibited under severe stress. *C. taliensis* and *C. sinensis* showed maximum values under mild stress, indicating sensitivity to drought. *C. gymnogyna* and *C. tachangensis* were less sensitive, with *C. gymnogyna* showing gradually increasing POD activity without inhibition, demonstrating stronger drought adaptation ability.

3.2 Effects of Drought Stress on Root Indices, Specific Leaf Area, and Total Biomass

Roots are the primary organs for water absorption and first perceive drought, adapting through morphological adjustments [30]. Root-shoot relationships represent integrated effects of genetic and environmental factors [31,32]. This study shows drought stress inhibited dry matter accumulation and root growth in wild tea seedlings. *C. taliensis* showed weak drought regulation under mild stress, making it vulnerable to drought damage. *C. tachangensis* adapted to mild stress by increasing root morphology indices and root activity, and to moderate stress by significantly increasing root average diameter and activity to compensate for reduced absorption area. *C. sinensis* alleviated drought damage under mild stress by increasing root average diameter and activity, but showed poor response to moderate and severe stress. *C. gymnogyna* maintained high root water absorption capacity through morphological changes under light and moderate stress, ensuring normal growth. Under severe stress, it maintained certain water absorption capacity through increased specific leaf area, root volume, and length, avoiding excessive above-ground growth inhibition.

3.3 Comprehensive Drought Resistance Evaluation of Wild Tea Seedlings

Currently, there is no unified evaluation method for tree drought resistance, which is influenced by multiple complex factors and achieved through various pathways [33-34]. Most methods focus on yield traits, requiring harvest for evaluation, making them unsuitable for perennial woody plants like tea. Seedling stage evaluation offers advantages of short duration and strong repeatability [10]. Since single-factor evaluation has limitations and indicator trends show inconsistencies, membership function analysis improves accuracy [23,36]. Using objective weighting methods combined with membership functions, our comprehensive evaluation results were consistent with field continuous drought experiments, demonstrating good reliability and application value.

The drought resistance order was: *C. gymnogyna* > *C. tachangensis* > *C. sinensis* > *C. taliensis*. Drought stress reduced total biomass, but drought-resistant *C. gymnogyna* could maintain growth by increasing osmotic adjustment substances, activating protective enzyme systems, enhancing root activity, and maintaining high root water absorption capacity, thereby preserving leaf water content and specific leaf area.

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Note: Figure translations are in progress. See original paper for figures.