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THE IMPACT OF DROUGHT STRESS ON TWO MEDICINAL PLANTS IN KARST AREAS

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Abstract

Karst ecosystems are characterized by shallow soils, rapid water loss, and frequent seasonal drought, posing significant challenges to the growth and sustainability of medicinal plants. To investigate drought-induced morphological adjustment patterns in karst medicinal species, a controlled pot experiment was conducted using two representative plants, *Bulbophyllum odoratissimum* and *Pyrrosia petiolosa*. Five soil moisture gradients were applied to simulate increasing drought intensity. Key morphological traits, stomatal density, and root and aboveground biomass were measured to evaluate plant responses to water deficit. The results showed that increasing drought stress significantly affected all measured morphological indices and biomass parameters in both species ($P < 0.05$). Under extreme drought conditions, aboveground biomass declined by 54.19% in *B. odoratissimum* and 57.40% in *P. petiolosa*, while root biomass decreased by 42.19% and 42.55%, respectively. Leaf length, width, thickness, and area exhibited progressive reductions with increasing drought severity. In contrast, stomatal density displayed a unimodal response, increasing under mild to moderate drought and declining under severe drought stress. Notably, the magnitude of variation in most morphological traits and stomatal density was greater in *P. petiolosa* than in *B. odoratissimum*. These findings indicate species-specific differences in drought sensitivity, with *B. odoratissimum* exhibiting relatively lower morphological sensitivity to drought stress compared to *P. petiolosa*. The study highlights the utility of integrative morphological indicators for assessing drought response strategies in medicinal plants and provides a scientific basis for the selection and management of drought-tolerant species in karst environments.

Key words: Karst; Drought stress; Ecological index; *Pyrrosia petiolosa*; *Bulbophyllum odoratissimum*

Introduction

Karst ecosystems are among the most fragile terrestrial environments, characterized by shallow soils, rapid water drainage, and pronounced seasonal drought. In southwest China, particularly in Guizhou Province, exposed karst landscapes account for more than 60% of the total land area and simultaneously serve as important reservoirs of medicinal plant diversity. However, ongoing rocky desertification and increasing drought frequency severely constrain plant growth, biomass accumulation, and long-term population sustainability in these systems (Lu *et al*., 2022; Liu *et al*., 2023; Rahman *et al*., 2024; 2025).

Drought stress is a major abiotic factor limiting plant productivity and survival. Under water deficit, plants exhibit coordinated morphological adjustments—such as reductions in leaf area, leaf thickness, and biomass allocation—aimed at minimizing water loss and maintaining internal water balance. These morphological responses are closely linked to underlying physiological regulation, including stomatal adjustment, photosynthetic limitation, and altered carbon allocation patterns (Zhang & Wu, 2023; Bistgani *et al*., 2024; Hui *et al*., 2024).

Although physiological traits are often emphasized in drought research, morphological traits provide integrative indicators of plant drought sensitivity and adaptive capacity, particularly in environments where physiological measurements are difficult to obtain at scale.

Medicinal plants are of particular interest in drought studies because water stress not only affects growth but can also influence medicinal quality through changes in biomass distribution and tissue structure. Previous studies have demonstrated that drought can induce adaptive trade-offs between growth and stress tolerance, often reflected in root–shoot allocation and stomatal regulation strategies (Motlagh *et al*., 2024; Luo *et al*., 2024). However, how such morphological adjustment patterns differ among medicinal species inhabiting karst environments remains poorly understood.

Bulbophyllum odoratissimum and *Pyrrosia petiolosa* are two medicinal plant species widely distributed in the karst regions of Guizhou and traditionally used by local ethnic communities. Despite their ecological and medicinal importance, comparative information on their drought response strategies under controlled water-deficit conditions is limited. In particular, it is unclear whether

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differences in drought sensitivity between these species can be explained by contrasting patterns of morphological adjustment and biomass allocation.

These changes can potentially impact both plant growth and medicinal quality. To mitigate such effects, plants employ a crucial regulatory strategy by adjusting stomatal density and aperture, thereby controlling transpiration rates and maintaining leaf water potential—an essential self-protective mechanism under drought stress (Luo *et al*., 2024).

Bulbophyllum odoratissimum and *Pyrrosia petiolosa* are two medicinal plants indigenous to the Guizhou karst region, traditionally used by local Miao, Yao, and Dong communities. The dried rhizomes and pseudobulbs of *B. odoratissimum* are characterized by their effects in nourishing yin, clearing the lungs, resolving phlegm, and relieving cough. They are also known to promote qi circulation and alleviate pain, and are commonly prescribed for tuberculosis-related cough, hemoptysis, chronic bronchitis, chronic pharyngitis, and irregular menstruation (Lu *et al*., 2022). *P. petiolosa* possesses heat-clearing, detoxifying, diuretic, and stasis-eliminating properties (Mehdi *et al*., 2024). Despite this long-standing therapeutic application, the physiological and ecological responses of these two species to drought stress under karst conditions remain largely unexplored.

Therefore, this study hypothesizes that the two species differ in their drought response strategies, with *B. odoratissimum* exhibiting relatively lower morphological sensitivity to increasing drought stress compared to *P. petiolosa*. To test this hypothesis, a controlled pot experiment was conducted using five soil moisture gradients to simulate drought intensities typical of karst environments. By quantifying changes in leaf morphology, stomatal density, and root and aboveground biomass, this study aims to

(i) characterize drought-induced morphological adjustment patterns in both species, and
(ii) evaluate their relative drought sensitivity based on integrative morphological indicators.

The findings provide a scientific basis for selecting medicinal plants suitable for cultivation and conservation in drought-prone karst regions.

Materials and Methods

Study area and plant materials: The experiment was conducted using plant materials collected from the Helang Forest understory in Huishui County, Qiannan Prefecture, Guizhou Province, China (26°15′56″ N, 106°43′29″ E; altitude 1016.2 m). This region represents a typical karst ecosystem characterized by shallow soils, rapid water drainage, and seasonal drought stress.

Healthy and uniformly sized individuals of *B. odoratissimum* and *P. petiolosa* were selected for the experiment. Prior to transplantation, plants were trimmed to standardize root size and aboveground rhizome length to minimize initial variability among treatments.

Experimental design and pot setup: Plants were transplanted into uniform brown plastic pots (25 cm diameter × 16.5 cm height). Each pot contained locally collected karst soil that was air-dried, homogenized, and sieved to remove stones and coarse debris. All treatments used the same soil type to ensure consistency in physical and nutritional properties.

Four plants were planted per pot and evenly spaced. Three pots constituted one biological replicate, and three replicates were established per drought treatment, resulting in a total of nine pots per treatment. The experiment followed a completely randomized design. The temperature of Dapeng is controlled at 22±0.5°C, and the relative humidity is maintained at 50% to 60% RH for only 30 days.

Drought stress gradients: Five soil moisture treatments were applied to simulate increasing drought intensity:

a. Control (100% of field capacity)
b. Mild drought (80%)
c. Moderate drought (60%)
d. Severe drought (40%)
e. Extreme drought (20%)

Due to inherent differences in water-holding capacity and growth characteristics between the two species, the absolute soil water content corresponding to each drought level differed slightly. For *B. odoratissimum*, soil moisture ranged from 37.33% (control) to 17.52% (extreme drought), while for *P. petiolosa*, values ranged from 45.22% to 18.36%. These treatments were designed to represent relative drought intensity within each species rather than identical absolute soil moisture levels, enabling meaningful comparative analysis of drought responses. Drought stress treatments were maintained for a period of 30 days following plant establishment.

Environmental conditions: All pots were maintained under controlled greenhouse conditions. Air temperature was maintained at 22±0.5°C, and relative humidity was controlled between 50% and 60%. Plants were exposed to natural daylight conditions supplemented as required to maintain consistent photoperiod exposure throughout the experimental period.

Measurement of morphological traits and biomass: At the end of the drought treatment period, morphological parameters including leaf length, leaf width, leaf thickness, and leaf area were measured for each plant using digital calipers and standard measurement procedures.

Aboveground and root tissues were harvested separately. Plant samples were gently rinsed with distilled water to remove adhering soil particles and then oven-dried at 80°C until constant weight was achieved. Dry biomass was determined using an electronic analytical balance with a precision of 0.001 g.

Stomatal density measurement: For stomatal density determination, five fully expanded leaves were randomly selected from each plant. The abaxial epidermis was carefully peeled and mounted on glass slides. Stomata were counted under a light microscope at 400× magnification in five randomly selected fields of view per slide.

Stomatal density (number mm^{-2}) was calculated by dividing the average stomatal count by the calibrated microscopic field area. Prior to measurement, the eyepiece micrometer was calibrated, with each small division corresponding to 9.98 μm.

Statistical analysis

All data were expressed as mean ± standard deviation. Statistical analyses were performed using Origin 8.0 software. One-way analysis of variance (ANOVA) was applied to evaluate the effects of drought stress on morphological traits, biomass, and stomatal density. Differences among treatment means were assessed using Duncan's multiple range test at a significance level of $p<0.05$.

Results

Effects of drought stress on biomass accumulation: The effects of drought stress on aboveground and root biomass of *B. odoratissimum* and *P. petiolosa* are summarized in Tables 1 and 2 and illustrated in Figs. 1 and 2. Drought intensity significantly influenced biomass accumulation in both species ($p<0.05$). Compared with the control, mild drought resulted in only minor changes in biomass, whereas moderate to extreme drought caused marked reductions.

Under extreme drought conditions, aboveground biomass decreased by 54.19% in *B. odoratissimum* and 57.40% in *P. petiolosa*. Root biomass declined by 42.19% and 42.55%, respectively. In both species, reductions in aboveground biomass were more pronounced than reductions in root biomass as drought stress intensified.

Responses of leaf morphological traits to drought stress: Leaf morphological responses to drought stress are presented in Tables 1 and 2 and Figs. 3–6. Increasing drought severity resulted in progressive reductions in leaf length, leaf width, leaf thickness, and leaf area in both species. Mild drought caused relatively small changes, whereas severe and extreme drought treatments produced significant reductions compared with the control ($p<0.05$).

Under extreme drought, leaf length decreased by 28.05% in *B. odoratissimum* and 21.55% in *P. petiolosa*. Leaf width declined by 19.87% and 38.85%, leaf thickness by 30.23% and 33.33%, and leaf area by 38.26% and 43.84%, respectively. Across most leaf traits, the magnitude of variation was greater in *P. petiolosa* than in *B. odoratissimum* as drought stress increased.

Variation in stomatal density under drought stress: Changes in stomatal density under different drought treatments are shown in Tables 1 and 2 and Fig. 7. In both species, stomatal density exhibited a non-linear response to increasing drought stress. From the control to moderate drought, stomatal density increased, reaching peak values under moderate drought conditions. Specifically, stomatal density increased by 42.08% in *B. odoratissimum* and 23.25% in *P. petiolosa* relative to the control.

As drought stress progressed from moderate to extreme levels, stomatal density declined in both species. Under extreme drought, stomatal density decreased by 16.67% in *B. odoratissimum* and 23.37% in *P. petiolosa* compared with values under moderate drought conditions. The overall amplitude of change in stomatal density was greater in *P. petiolosa* than in *B. odoratissimum*.

Comparative trends across morphological indicators: Figs. 1–7 collectively illustrate the overall trends of biomass accumulation, leaf morphology, and stomatal density in response to increasing drought stress. For both species, aboveground biomass, root biomass, leaf size parameters, and leaf area consistently declined as drought severity increased, with minimum values observed under extreme drought conditions.

Across most measured parameters, *P. petiolosa* displayed larger relative changes across drought treatments than *B. odoratissimum*. These differences were consistently reflected in biomass parameters (Figs. 1–2), leaf morphological traits (Figs. 3–6), and stomatal density responses (Fig. 7).

Table 1. Effects of different drought treatments on the morphological indices and root biomass indices of *Bulbophyllum odoratissimum* (n=30).

Treatment/Degree of drought	Above-ground biomass / g	Root biomass / g	Leaf length / mm	Leaf width / mm	Leaf thickness/ mm	Leaf area/ mm²	Stomatal density/ piece/mm²
CK	7.75 ± 0.55a	0.64 ± 0.02a	74.23 ± 7.49a	18.52 ± 2.49a	0.86 ± 0.09a	1163.56 ± 150.95a	46.22 ± 4.92bc
Mild drought	7.32 ± 0.77a	0.62 ± 0.09a	69.10 ± 9.30ab	18.77 ± 2.11a	0.78 ± 0.07ab	953.78 ± 150.39b	51.78 ± 9.15b
Moderate drought	5.84 ± 1.14b	0.57 ± 0.07a	65.61 ± 10.61ab	17.74 ± 1.69a	0.72 ± 0.07b	916.78 ± 116.64b	65.67 ± 12.26a
Severe drought	4.32 ± 0.69c	0.45 ± 0.08b	61.52 ± 8.27bc	16.24 ± 1.95bc	0.72 ± 0.07b	901.33 ± 51.15b	41.11 ± 4.31c
Extreme drought	3.55±0.77c	0.37 ± 0.03b	53.41 ± 6.31c	14.84 ± 1.83c	0.60 ± 0.05c	718.44 ± 70.59c	39.44 ± 6.04c

Note: Different lowercase letters in the same column indicate significant ant differences among different treatments in the same column ($p<0.05$), (The same below)

Table 2. Effects of different drought treatments on the morphological indices and root biomass indices of *Pyrrosia petiolosa* (n=30).

Treatment/Degree of drought	Above-ground biomass / g	Root biomass / g	Leaf length / mm	Leaf width / mm	Leaf thickness/ mm	Leaf area/ mm²	Stomatal density/ piece/mm²
CK	3.38 ± 0.40a	0.47 ± 0.04a	44.09 ± 6.04a	32.07 ± 2.26a	1.05 ± 0.08a	1111.78 ± 118.44a	86.56 ± 6.91b
Mild drought	2.69 ± 0.64ab	0.43 ± 0.03ab	40.52 ± 2.81ab	28.89 ± 1.23b	0.98 ± 0.06ab	997.56 ± 122.54ab	95.89 ± 12.64b
Moderate drought	2.37 ± 0.86b	0.40 ± 0.05b	40.70 ± 1.67ab	26.86 ± 0.82c	0.91 ± 0.06bc	909.11 ± 150.03bc	112.78 ± 16.9a
Severe drought	1.99 ± 0.70bc	0.32 ± 0.06c	38.60 ± 1.38bc	25.26 ± 0.70c	0.83 ± 0.06c	753.00 ± 122.63cd	71.22 ± 5.76c
Extreme drought	1.44 ± 0.75c	0.27 ± 0.04c	34.59 ± 2.87c	19.61 ± 1.45d	0.70 ± 0.06d	624.33 ± 154.92d	66.33±5.79c

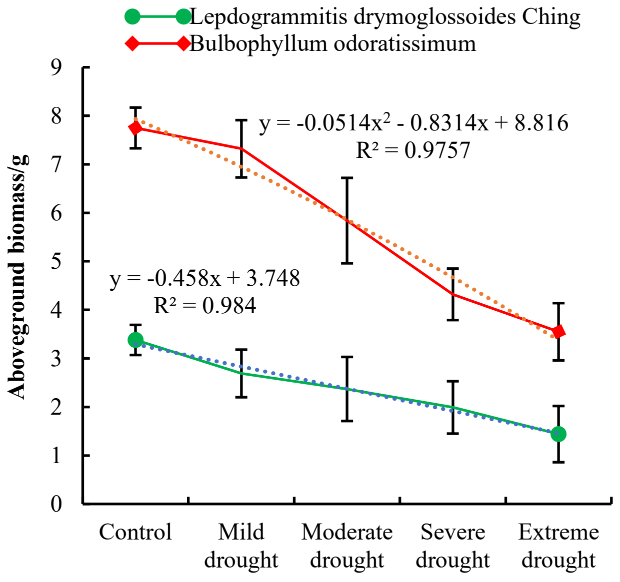

Fig. 1. Biomass of the aboveground.

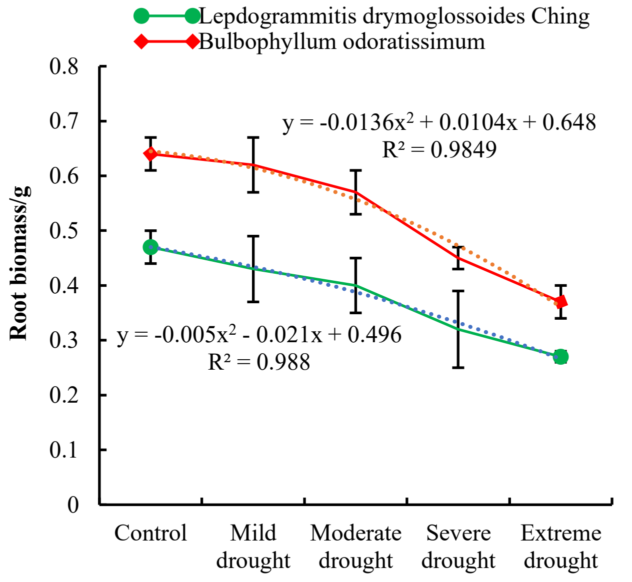

Fig. 2. Root biomass of two plants.

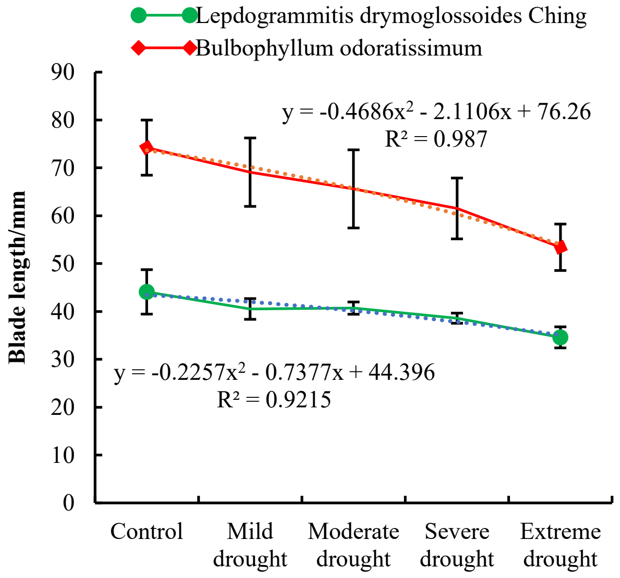

Fig. 3. Leaf length of two plants.

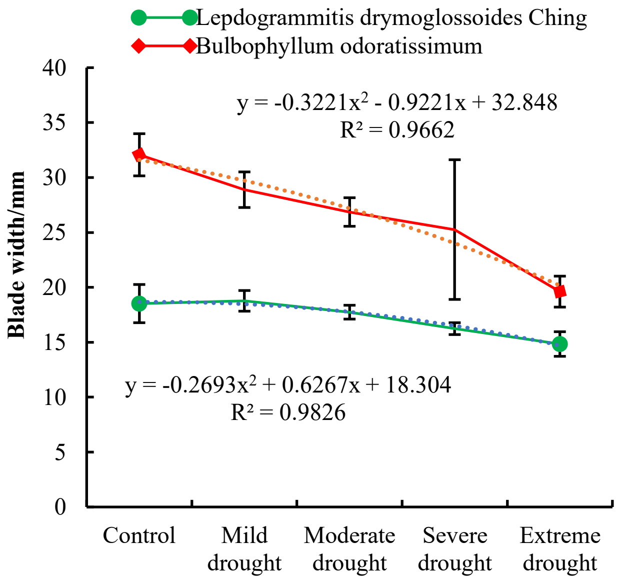

Fig. 4. Leaf width of two plants.

Discussion

This study demonstrates that drought stress exerts a pronounced influence on biomass accumulation, leaf morphology, and stomatal density in *B. odoratissimum* and *P. petiolosa*. Across increasing drought gradients, both species exhibited consistent reductions in aboveground biomass, root biomass, and leaf morphological traits, indicating that water limitation strongly constrains growth under karst conditions. Such responses are characteristic of plants growing in environments with shallow soils and limited water retention, where drought acts as a primary selective pressure shaping plant form and function (Chaves *et al*., 2003; Flexas *et al*., 2014).

Morphological adjustment under drought stress is widely recognized as an integrative strategy through which plants reduce transpiring surface area and metabolic demand, thereby maintaining internal water balance (Poorter *et al*., 2012; Bistgani *et al*., 2024). The progressive decline in leaf length, width, thickness, and area observed in both species reflects a conservative growth strategy aimed at minimizing water loss under prolonged water deficit. Similar drought-induced reductions in leaf size and biomass have been reported across a range of herbaceous and medicinal plants, particularly in water-limited ecosystems (Fraser *et al*., 2009; Liu *et al*., 2023).

The greater reduction in aboveground biomass relative to root biomass observed in both species suggests a shift in biomass allocation under drought stress. Such shifts are commonly interpreted as adaptive responses that prioritize belowground structures involved in water uptake, even when overall growth is constrained (Poorter *et al*., 2012; Yan *et al*., 2024). Although root biomass declined under severe drought, the comparatively smaller reduction relative to shoots indicates a degree of allocation plasticity that may support plant survival under transient drought conditions.

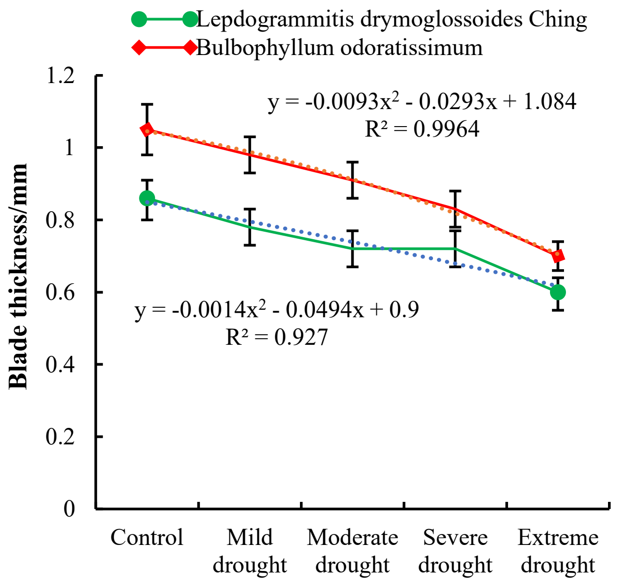

Fig. 5. Leaf thickness of two plants.

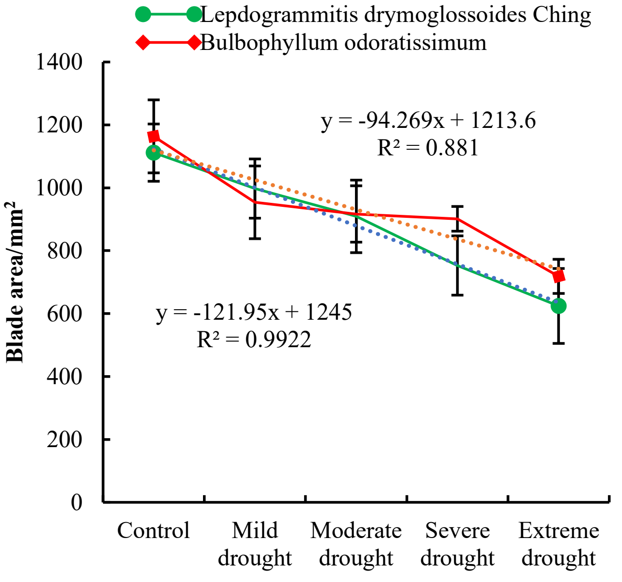

Fig. 6. Leaf area of two plants.

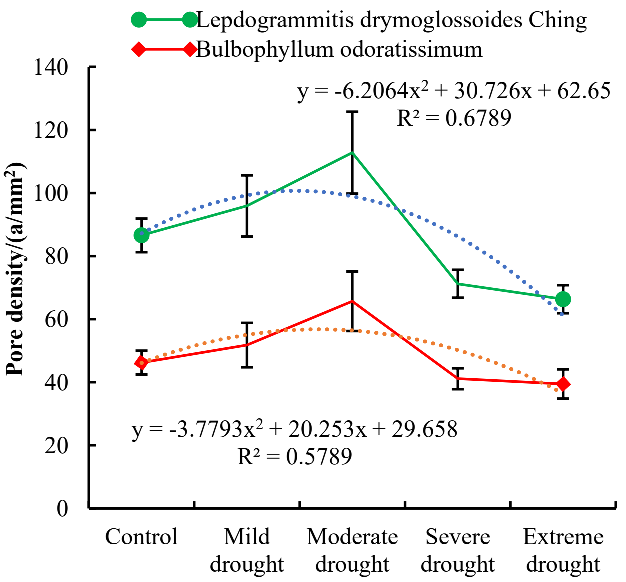

Fig. 7. Stomatal density of two plants.

Comparative analysis revealed that *P. petiolosa* exhibited larger relative changes across most biomass and leaf morphological parameters than *B. odoratissimum*. Greater amplitudes of morphological change under stress are often associated with higher drought sensitivity, reflecting a reduced capacity to maintain structural stability under water limitation (Flexas *et al.*, 2014; Shang *et al.*, 2024). In contrast, the relatively smaller magnitude of change in *B. odoratissimum* suggests a more conservative growth adjustment strategy, which may confer advantages in environments characterized by recurrent drought.

Stomatal density exhibited a unimodal response to increasing drought stress in both species, increasing under mild to moderate drought and declining under severe and extreme drought. This pattern has been widely reported in drought studies and reflects a balance between maximizing carbon assimilation under moderate stress and minimizing water loss under severe water limitation (Fraser *et al.*, 2009; Zhao *et al.*, 2019).

An increase in stomatal density under moderate drought may enhance stomatal control efficiency, allowing plants to regulate gas exchange more precisely when water availability becomes limiting. However, under extreme drought conditions, reduced stomatal density likely reflects structural and developmental constraints imposed by prolonged water deficit, as well as a shift toward water conservation (Chaves *et al.*, 2003; Yang *et al.*, 2020). The larger fluctuation in stomatal density observed in *P. petiolosa* suggests greater sensitivity of stomatal development to drought stress, whereas the more moderated response in *B. odoratissimum* indicates comparatively stable stomatal regulation.

It is important to note that the present study evaluates drought responses primarily through morphological and biomass indicators rather than direct physiological measurements such as photosynthetic rate or water-use efficiency. Therefore, the observed differences between species should be interpreted in terms of relative morphological sensitivity to drought stress, rather than definitive drought resistance. Morphological traits integrate plant responses over time and provide valuable insights into drought response strategies, particularly in long-term or field-relevant contexts (Poorter *et al.*, 2012; Bistgani *et al.*, 2024).

Within this framework, the smaller amplitude of morphological change exhibited by *B. odoratissimum* across drought gradients suggests a lower degree of drought-induced structural disruption compared to *P. petiolosa*. Such stability in morphological traits under stress is commonly associated with enhanced persistence in drought-prone habitats, especially in karst ecosystems where water availability fluctuates sharply (Luo *et al.*, 2024).

The differential drought response strategies observed in the two medicinal plants have important ecological and practical implications. Species exhibiting lower morphological sensitivity to drought may be better suited for cultivation and conservation in karst regions, where seasonal drought and shallow soils impose strong constraints on plant growth. Understanding species-specific drought response patterns can therefore inform the selection of medicinal plants for sustainable utilization and habitat restoration under increasing drought frequency associated with climate change (Ansari *et al.*, 2023; Abdullaev *et al.*, 2025).

Conclusion

In this study, five drought gradients ranging from control to extreme stress were applied to systematically evaluate the responses of two medicinal plants, *B. odoratissimum* and *P. petiolosa*, under conditions representative of karst environments. By examining aboveground biomass, root biomass, leaf morphological traits (length, width, thickness, and area), and stomatal density, the study provides an integrated assessment of drought-induced structural responses in both species.

The results demonstrate that increasing drought stress significantly restricted plant growth, leading to progressive reductions in biomass accumulation and leaf morphological parameters. As drought severity intensified, leaves became smaller and thinner, and overall biomass declined markedly, indicating strong growth limitation under water deficit conditions. Stomatal density exhibited a non-linear response, increasing under mild to moderate drought and declining under severe drought, reflecting a transition from growth regulation to water-conservation strategies as water availability decreased.

Comparative analysis revealed clear differences between the two species. Across most morphological and biomass indicators, *P. petiolosa* exhibited larger relative changes with increasing drought stress, indicating higher morphological sensitivity to water deficit. In contrast, *B. odoratissimum* showed comparatively smaller amplitudes of change across drought gradients, suggesting greater structural stability under drought conditions. These differences reflect contrasting drought response strategies between the two medicinal plants when evaluated through integrative morphological indicators.

It is important to emphasize that the conclusions of this study are based on morphological and biomass responses rather than direct physiological measurements. Therefore, the observed interspecific differences are best interpreted as differences in morphological sensitivity to drought stress, rather than definitive physiological drought resistance. Nonetheless, morphological stability under drought represents an important adaptive feature in karst ecosystems, where shallow soils and rapid water loss impose persistent constraints on plant growth.

Overall, this study enhances understanding of drought response patterns in medicinal plants inhabiting karst regions and provides a scientific basis for species selection, conservation, and cultivation in drought-prone environments. In the context of increasing drought frequency associated with global climate change, these findings contribute valuable insights for the sustainable utilization and management of medicinal plant resources in water-limited ecosystems.

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Conflict of Interest: The authors declare no conflict of interest.

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