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Biomass and Morphology Responses of *Fritillaria unibracteata* to Shading and Warming

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Abstract *Fritillaria unibracteata* Hsiao and Hsia (Liliaceae) (*F. unibracteata*) is a perennial and protected species distributed in the meadow or under the shrub at the eastern Tibetan Plateau. To understand how *F. unibracteata* response to changing environment, OTC (open top chamber) and PSN (Polypropylene shading nets) were used to simulate warming and shading effects. Two years later, dry biomass of each organ (leaf, stem, bulb and root), leaf length (LL), leaf area (LA) and special leaf area (SLA) were measured individually to determine *F. unibracteata* responses to environmental change. The results showed that: (1) total biomass (TB), leaf biomass (LB) and root biomass (RB) increased significantly under warming treatment ($P<0.05$), but no significant change under shading; (2) LB/RB was decreased under warming, while both LB/RB and AB/BeB showed no significant responses to shading; (3) leaf morphological characteristics responded significantly to warming and shading; leaf length (LL) significantly increased under both shading and warming treatments ($P<0.05$); leaf area (LA) increased significantly under warming treatment; special leaf area (SLA) increased significantly under shading treatment. Based on the results above, we get conclusions as below: (1) *F. unibracteata* responded to warming and shading effects differently; (2) For perennial alpine plants such as *F. unibracteata*, morphological traits may be more sensitive to environment variations than other traits.

Keywords: *Fritillaria unibracteata, shading, warming, biomass allocation, leaf morphology*

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1. Introduction

Biomass allocation is one of the most important adaptation mechanisms of plants. Due to different physical separation of resources between above and under-ground, plants may allocate their biomass differently to organs for different functions. From an economical viewpoint, plants would keep a higher root allocation in response to under-ground resource like water and nutrients stress on one hand, while on the other, a higher shoot allocation to increase their capability to acquire limited resources like light. This flexibility in allocation of biomass is important for plants to maximize their growth rate and survive in the environment with variable resource availability [1,2,3].

Despite these earlier works, this notion has been questioned. For example, Wright and Mcconnaughay [4] and Štěpán et al. [5] found that variation of allocation among plants was influenced by both resource variation and ontogenetic drift, plant development would muddy or magnify the biomass allocation observed in experiment. In addition, although allocation variation appears to contribute more to the high diversity in ecological strategies than physiological mechanisms at cellular or molecular levels, there are evidences supporting that morphological characteristics such as leaf area ratio (LAR) and special leaf area (SLA) are more sensitive and important in plants' adjustment to environmental variation [6,7]. Despite these studies, it still remains unclear how some plant species adjust their biomass allocation to varying resource. Although biomass allocation is the best index to characterize plant's response to environmental variation, it is still not fully understood for various reasons.

Fritillaria unibracteata Hsiao and Hsia (Liliaceae) is one of the traditional medicine plants '*Chuanbeimu*' functioning at relieving cough and eliminating phlegm [8]. It is a perennial herb growing at an attitude of 3200~4500 m a.s.l. in a shady and humid habitat. In recent years, because of over-harvest and impacts of climate change, the population of *F. unibracteata* has been threatened. According to the investigations by Xu et al. [9,10], the biomass, biomass allocation and morphological characteristics (eg, leaf area and special leaf area) of *F. Unibracteata* were influenced by environment factors such as altitude, but changes of morphological characteristics were more consisted with environment variation. In order to understand how *F. unibracteata* adapt to environment changes, this research was undertaken to scientifically understand the response of this species to alpine environment in terms of its adaptation of biomass and morphology to warming and shading treatments.

2. Materials and Methods

2.1. Experimental Site

This experiment was carried out in the Chuanbeimu Research Station located in Songpan County, Sichuan Province, China (32°56′18" N, 103°42′45″E) where the elevation is 3,300 m a.s.l. Climate at the experimental site is characterized by cold winter and mild summer. Annual mean temperature is 2.8°C, with -7.6°C minimum monthly mean temperature in January, and 11.7°C maximum monthly mean temperature in July. Water is not a limiting factor for plant growth since the annual mean precipitation is as high as 717.7 mm, and 80% of which falls in the form of rain during the growing season from May to October.

2.2. Material and Treatment

The growth of *F. unibracteata* comprises three main phases (named as phase A, B and C) in line with its morphological development. Phase A (one year old) is marked by needle shaped leaves. Phase B, two or three years old, the plant has only one leaf and no erected stem. Phase C, four or more years old, the plant exhibits erected stem with prominent leaves. In this study, individuals which had grown two years were selected as the subjects.

Plants with similar sizes were carefully transplanted into three experimental fields, each of which had three 1.5×1.5 m^2 plots. In every plot, plants were randomly planted at a distance of 10 cm. Three treatments (including the control, warming and shading) were started respectively in three fields immediately after transplantation. Warming was simulated with open top chambers (OTC) made from Polymethy lmethacrylate plates, 1.5×1.5×1.5 m^3. Shading treatment was simulated with Polypropylene shade nets. The net was supported at 70 cm height above the ground. The light below the net was 40% of full light.

2.3. Harvest and Measurement

After two-years, five living plants were harvested from each plot. The plants under different treatments were carefully dug out and washed. After that, each plant was divided into four parts: root, bulb, stem and leaf. Two leaf morphological characteristics, i.e. leaf lengths and leaf area, were measured by electronic vernier and leaf scanner (Canon, LiDE 220) respectively. Then all the parts of plant were oven-dried at 80°C for 48 h and weighed.

2.4. Statistical Analysis

The analyses was carried out using “Statistical Package for Social Sciences” program (SPSS, 16.0) at *P*=0.05 level. One-way ANOVA was used to analyze the differences of biomass, biomass allocation, leaf length (LL), leaf area (LA) and special leaf area (SLA) among three treatments. Here biomass includes the total biomass (TB), root biomass (RB), bulb biomass (BB), stem biomass (SB) and leaf biomass (LB). Biomass allocation refers to the ratio of leaf biomass to root biomass (LB/RB) and above-ground biomass (leaf and stem biomass) to below-ground biomass (root and bulb biomass) (AB/BeB).

3. Results

3.1. Biomass and Its Allocation

Warming significantly increased *F. unibracteata* root biomass (RB) and leaf biomass (LB), and correspondingly induced a significant increase of total biomass (TB) ($P<0.05$). On the contrast, shading treatment showed no significant influence on biomass (see Figure 1).

Resources variation not only led to the change of *F. unibracteata* biomass, but also shifted its allocation. However, in this experiment, shading did not change LM/RM or AM/BM. Moreover, responses of LB/RB and AB/BeB were different under warming treatment. LB/RB decreased significantly, while AB/BeB showed no obvious change, which suggesting the different significance of LB/RB and AB/BeB on *F. unibracteata* functions.

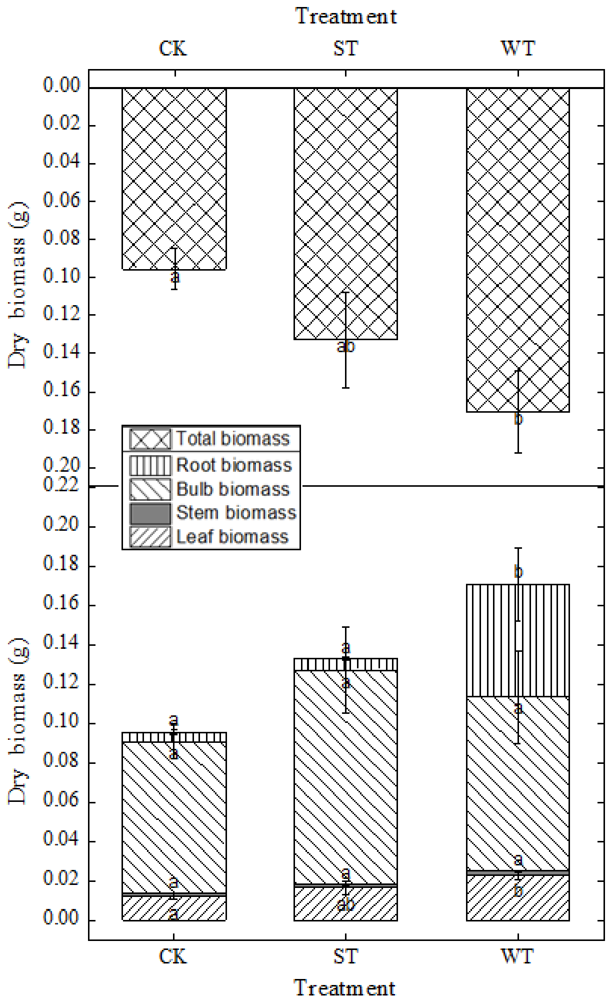

Figure 1. Biomass variations under three treatments: the control (CK), shading treatment (ST) and warming treatment (WT). Vertical bars represent S.E. (n =15)

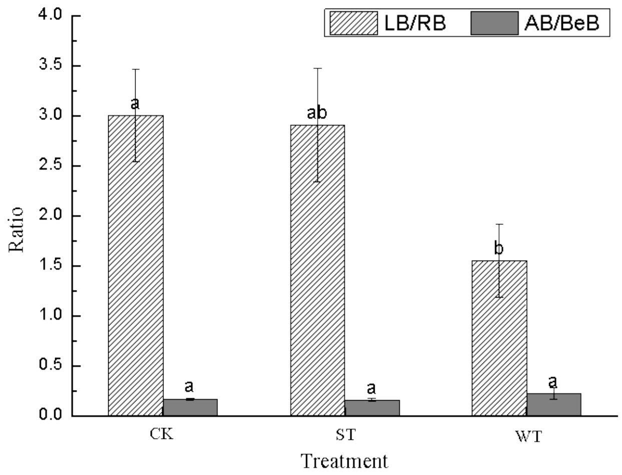

Figure 2. Biomass allocation under three treatments: the control (CK), shading treatment (ST) and warming treatment (WT). Vertical bars represent S.E. (n =15)

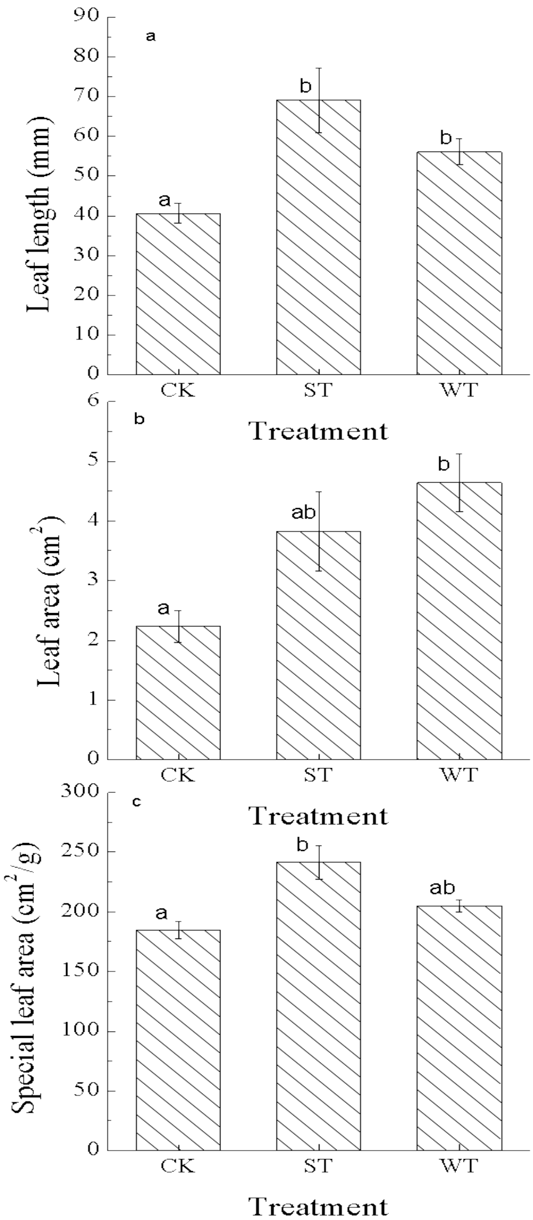

Figure 3. Leaf length (a), leaf area (b) and special leaf area (c) variations under three treatments: the control (CK), shading treatment (ST) and warming treatment (WT). Vertical bars represent S.E. (n =15)

3.2. Variations of Leaf Morphological Characteristics

Shading and warming treatments dramatically influenced *F. unibracteata* leaf morphological characteristics. As shown in Figure 3, leaf length (LL) significantly increased under both shading and warming treatments (P<0.05); leaf area (LA) increased significantly under warming treatment, while leaf special area (LSA) increased significantly under shading treatment.

4. Discussion

4.1. Responses of Biomass and Its Allocation to Warming and Shading Treatments

Total biomass increased significantly under warming treatment compared with the controlled treatment, indicating that the growth of *F. unibracteata* in this area was temperature-limited. Temperature increase in the future may be good for the growth of *F. unibracteata*. On the other hand, total biomass showed an increase under shading, but the response was not statistically significant. It was suggested that shade-tolerant species were capable of maintaining a uniform pattern of growth and metabolism over a wide range of light intensities [11,12]. Although light is limit under shading treatment, for shade-tolerant species, reduced respiration rate and saturate photosynthesis point could trade off the reduction caused by light limitation or even enhance plant production [13,14]. This was confirmed by studying results from Li et al. [15] that shading was beneficial for the growth of *Fritillaria cirrhosa*.

Biomass allocation was one of the most important strategies for plant to adapt to environment change. Significant decrease of LB/RB was induced by warming (P<0.05). Effect of temperature on growth was greater than on resource acquisition ability. Thus, under a higher temperature, nutrients and water become limit, and more biomass need to be allocated to root to meet the demand for their growth [16]. However, shading did not show significant effect on biomass allocation (P<0.05). Geng et al. [17] proposed that since perennial species allocate more to shoots during their growth and development, under above-ground resource limitation, biomass allocation may show no apparent plasticity. So did *F. unibracteata*, which showed no significant responses to light.

4.2. Morphological Responses to Environmental Variations

Consisting with previous studies, morphological characteristics of *F. unibracteata* leaf such as leaf length (LL), leaf area (LA) and special leaf area (SLA) responding dramatically to shading or warming treatments [18,19,20]. A common mechanism of plants is to increase their light interception and photosynthetic rate under shading by increasing LL and SLA [21,22]. Similarly, plants could also enhance its production under the condition of increased temperature by increasing LL and SLA [23].

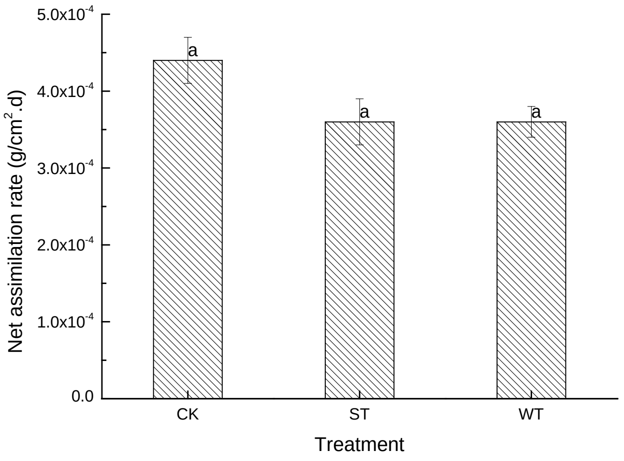

Figure 4. Net assimilation rate (total biomass per unit leaf area and unit time) under three treatments: the control (CK), shading treatment (ST) and warming treatment (WT). Vertical bars represent S.E. (n =15)

Changes of leaf morphology are common for plants' adaption to environmental variation which may be more important than biomass allocation sometimes [7]. Our results suggested that morphological adjustment of *F. unibracteata* leaf was more important than biomass allocation, since the morphological traits of *F. unibracteata* leaf changed greatly under both shading and warming treatments, while biomass and biomass allocation showed significantly change only under warming treatment.

Besides biomass allocation and morphology adjustments, plants also respond to varying environment through physiological modifications. It was suggested that for shading-tolerant species, morphological traits may be more important than physiological traits in response to environmental variation [24-26]. In our study, NAR (net assimilation rate, i.e., total biomass per unit leaf area and unit time) showed no significant difference among three treatments (P>0.05). This is consistent with some other studies, in which it was suggested that plants' responses to environmental variation were due to morphological adjustment other than physiological processes [27,28].

In conclusion, just as some other species, morphology of *F. unibracteata* was most important in its response to environment compared with biomass allocation and physiological adjustments [26,28].

5. Conclusion

In this study, total biomass allocation and the ratio of leaf to root biomass (LB/RB) all changed significantly under warming and showed no significant change under shading treatment; leaf length (LL) increased significantly under both shading and warming treatments; leaf area showed significant change under warming treatment, while special leaf area showed significant change under shading treatment. Even though biomass, biomass allocation and leaf morphology all showed responses to environment variations, for *F. unibracteata* adjustment to environmental variation, morphological traits would be more critical than biomass and its allocation, since leaf morphological traits changed dramatically under both warming and shading treatments. For *F. unibracteata*, morphological traits such as leaf length (LL), leaf area (LA) and specific leaf area (SLA) may be more import for the indication of its adaptation to environment variation.

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