Effects of Alpine Grassland Types and Elevation on Growth and Reproductive Characteristics of the Endangered Tibetan Medicinal Plant Lamiophlomis rotata (Postprint)

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Abstract

To investigate the effects of alpine grassland types and altitude on the growth and reproductive characteristics of Lamiophlomis rotate, random quadrat surveys and sample collections were conducted across three grassland types and three altitude gradients in Maqu, and the growth and reproductive characteristic indices of L. rotate were measured.

The results showed that: (1) Among different grassland types, the aboveground characteristics of L. rotate exhibited the pattern alpine swamp meadow > alpine hillside meadow > alpine shrub meadow, while belowground characteristics showed alpine swamp meadow > alpine shrub meadow > alpine hillside meadow; (2) The growth and reproductive characteristics of L. rotate decreased with increasing altitude; (3) Across different grassland types and altitudes, there was a significant positive correlation between investment in sexual reproductive structures and plant size in L. rotate, whereas no correlation existed between asexual reproductive investment and plant size; (4) Across different grassland types and altitudes, no correlation was observed between the two reproductive modes in L. rotate.

It is concluded that: (1) The resource allocation pattern of L. rotate is influenced by grassland type, representing the outcome of long-term adaptation to the environment; (2) The occurrence of sexual reproduction requires a certain amount of vegetative growth accumulation in the plant, whereas asexual reproductive investment may be an inherent characteristic of the plant, independent of plant size; (3) Rhizome bud asexual reproduction may occur following aboveground damage rather than as an active behavior, and whether the lack of correlation between the two reproductive modes is influenced by factors other than grassland type and altitude requires further investigation.

Full Text

Preamble

Effects of Alpine Grassland Types and Altitude on Growth and Reproduction Traits of the Endangered Tibetan Medicinal Plant *Lamiophlomis rotata*

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Abstract: To investigate the influence of alpine grassland types and altitude on the growth and reproduction traits of *Lamiophlomis rotata*, random quadrat surveys and sample collections were conducted across three grassland types and three altitude gradients in Maqu. Growth and reproduction trait indicators were subsequently measured. The results revealed: (1) Among different grassland types, above-ground traits of *L. rotata* exhibited the pattern: alpine swamp meadow > alpine hillside meadow > alpine shrub meadow, while below-ground traits showed: alpine swamp meadow > alpine shrub meadow > alpine hillside meadow; (2) Growth and reproduction traits decreased with increasing altitude; (3) Across different grassland types and altitudes, a significant positive correlation existed between investment in sexual reproductive structures and plant size, whereas no correlation was found between asexual reproductive investment and plant size; (4) No correlation was observed between the two reproductive modes across different grassland types and altitudes. The conclusions suggest: (1) The resource allocation strategy of *L. rotata* is influenced by grassland type, representing an outcome of long-term environmental adaptation; (2) Sexual reproduction requires a threshold of vegetative growth accumulation, while asexual reproductive investment appears to be an inherent plant characteristic independent of plant size; (3) Rhizome bud-based asexual reproduction may occur following above-ground damage rather than as an active strategy. Whether the lack of correlation between reproductive modes is influenced by factors other than grassland type and altitude requires further investigation.

Keywords: medicinal plant, alpine grassland types, altitude, growth and reproduction traits, rhizome bud

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Lamiophlomis rotata is a perennial herb belonging to the Lamiaceae family. Originally classified under the genus *Phlomis* L., it has since been separated as the sole species of the genus *Lamiophlomis* Kudo. The plant' s inflorescence reaches 2.5–10 cm in height, with a taproot system and 4–6 leaves arranged radially in opposite pairs, growing close to the ground. In China, it is primarily distributed across Tibet, the Yushu and Guoluo regions of Qinghai, Gannan in Gansu, western Sichuan, and sporadically in northwestern Yunnan; internationally, it occurs in Nepal, Bhutan, and Sikkim (Zhu et al., 2018). *L. rotata* represents an important ethnic medicinal resource, documented in classical Tibetan medical texts including *Yuewang Yaozhen*, *Sibu Yidian*, and *Jingzhu Bencao* for over 1,200 years. The medicinal material appears withered yellow or yellow-brown in color, hard and dry in texture, with a fishy odor. It is sweet, bitter, and neutral in nature, entering the liver meridian, and functions to activate blood circulation, stop bleeding, dispel wind, and relieve pain. It is used for traumatic injuries, external bleeding, rheumatic arthralgia, and "yellow water disease" (Chinese Pharmacopoeia, 2015).

Currently, the annual demand for *L. rotata* medicinal materials from Gansu-based pharmaceutical enterprises alone exceeds 1,000 tons. The substantial market demand and limited resource availability led to its classification as a first-class endangered Tibetan medicinal species in 2000 (Li et al., 2002).

Existing literature indicates that *L. rotata* experiences extremely low sexual reproductive success due to constraints in inflorescence structure, style morphology, flower size, and seed dormancy (Jin et al., 2011; Jin et al., 2016). Researchers have attempted to improve sexual reproduction success through breaking seed dormancy (Jin et al., 2011), promoting pollen germination (Jin et al., 2012), and controlling light conditions (Zhang et al., 2007), or to enhance offspring survival through increasing seedling resistance (Cai et al., 2009) and intercropping with other crops (He et al., 2012). However, wild populations remain the primary source of *L. rotata* medicinal materials. The species has not become extinct under long-term natural selection, implying that alternative pathways must compensate for the deficiencies in sexual reproduction.

Although some scholars have argued that *L. rotata* does not exhibit clonal reproduction (Liu, 2006), more recent studies have demonstrated that rhizome buds can develop in subsequent years (Sun et al., 2012; Jin, 2016), confirming that both asexual and sexual reproduction coexist in the species' reproductive system. Existing literature suggests that rhizome bud formation in *L. rotata* is not an obvious phenomenon, raising important questions about the environmental conditions triggering rhizome bud production, their relationship with plant size, and their connection to sexual reproduction. Understanding the species' adaptation to field environments and the relationship between different reproductive modes and environmental factors is crucial for guiding future artificial cultivation efforts.

This study investigated wild *L. rotata* populations through field quadrat surveys and sample analysis to address three specific questions: (1) Do grassland type and altitude influence the growth and reproduction traits of *L. rotata*? (2) How do reproductive traits relate to plant size under different grassland types and altitudes? (3) What is the relationship between the two reproductive modes under different grassland types and altitudes?

1.1 Experimental Site Overview

Alpine meadows on the Qinghai-Tibet Plateau cover extensive areas with significant regional variation in climate and human disturbance. The experimental site was located near Maqu County, Gannan Tibetan Autonomous Prefecture, Gansu Province (102°4 12 E, 33°59 56 N), situated on the northeastern edge of the Qinghai-Tibet Plateau at the first bend of the Yellow River. The county borders Sichuan Province to the southeast and Qinghai Province to the southwest and west. The terrain slopes from high in the west to low in the east, with complex topography and a typical cold, humid plateau climate. The landscape is open and windy, with over 50 days annually experiencing winds exceeding level 8. The mean annual temperature is 1.2 °C, with average temperatures of -10 °C in the coldest month (January) and 11 °C in the warmest month (July). Extreme temperatures range from -29.6 °C to 23.5 °C. Mean annual precipitation is 620 mm. The vegetation type is typical alpine meadow (Wu, 1980).

1.2 Experimental Methods

In July 2019, random 5 m × 5 m quadrat surveys and sample collections were conducted on *L. rotata* populations across different grassland types and altitudes near Maqu County. Each grassland type was surveyed with 1–3 replicates. Altitude was measured using a handheld GPS navigator. Samples from locations numbered 4–6 were purchased from local medicinal material dealers (Table 1). All samples were identified as *L. rotata* by Professor Du Guozhen from Lanzhou University. The number of *L. rotata* individuals and flowering individuals were counted in each quadrat, and complete individuals were randomly excavated and transported to the laboratory. For both field-collected and purchased samples, measurements included plant height, root length, root diameter, horizontal leaf spread, vertical leaf spread, leaf number, inflorescence number, and rhizome bud number. Samples were oven-dried at 80 °C for 24 hours, and above-ground and below-ground biomass were determined using a 1/1,000 electronic balance. Since purchased samples were excavated by herders with incomplete below-ground structures, they could not be used to assess rhizome bud-related indicators. Due to the fragility of roots during excavation, below-ground biomass allocation was expressed as root biomass per unit length ($g \cdot cm^{2}$). Leaf spread area (cm^2) was calculated as horizontal leaf spread × vertical leaf spread to estimate leaf area.

1.3 Statistical Methods

Data analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL). To ensure homogeneity of variance, data were log-transformed prior to analysis, with Levene' s test yielding $P > 0.05$ for all transformed datasets. General linear model (GLM) one-way ANOVA was employed to examine the effects of alpine grassland type and altitude on *L. rotata* growth and reproduction traits. Differences in means among factors were assessed using Tukey' s tests. To examine the influence of grassland type and altitude on relationships between individual size and asexual or sexual structures, analysis of covariance (ANCOVA) was performed with grassland type and altitude as fixed factors and plant size as a covariate. Partial correlation analysis was used to assess the relationship between sexual and clonal reproduction while controlling for plant size.

2.1 Effects of Alpine Grassland Type on *L. rotata* Growth and Reproduction

The survey revealed that *L. rotata* communities commonly comprised species including *Potentilla fruticosa*, *Polygonum viviparum*, *Ranunculus japonicus*, *Nardostachys jatamansi*, *Ligularia virgaurea*, *Anemone rivularis*, *Leontopodium leontopodioides*, *Gentiana macrophylla*, *Xanthopappus subacaulis*, *Potentilla anserina*, and Gramineae species (Table 1).

Alpine grassland type significantly affected plant height ($F_{,}$ = 3.739, P = 0.034), root diameter ($F_{,}$ = 22.731, P < 0.0001), root biomass per unit length ($F_{,}$ = 6.194, P = 0.004), rhizome bud number ($F_{,}$ = 3.403, P = 0.042), leaf area ($F_{,}$ = 3.344, P = 0.044), leaf biomass ($F_{,}$ = 8.417, P = 0.001), inflorescence number ($F_{,}$ = 4.252, P = 0.020), inflorescence biomass ($F_{,}$ = 4.295, P = 0.023), and above-ground biomass ($F_{,}$ = 8.641, P = 0.001), with leaf number being the only non-significant trait ($F_{,}$ = 2.120, P > 0.05). Plant height, leaf area, leaf biomass, inflorescence number, inflorescence biomass, and above-ground biomass all exhibited the pattern: alpine swamp meadow > alpine hillside meadow > alpine shrub meadow. In contrast, root diameter, root biomass per unit length, and rhizome bud number showed the pattern: alpine swamp meadow > alpine shrub meadow > alpine hillside meadow (Table 2).

2.2 Effects of Altitude on *L. rotata* Growth and Reproduction Traits

Altitude significantly influenced plant height ($F_{,}$ = 8.266, P = 0.001), root diameter ($F_{,}$ = 24.085, P < 0.0001), root biomass per unit length ($F_{,}$ = 5.333, P = 0.008), rhizome bud number ($F_{,}$ = 5.284, P = 0.026), leaf area ($F_{,}$ = 12.647, P < 0.0001), leaf biomass ($F_{,}$ = 15.053, P < 0.0001), inflorescence number ($F_{,}$ = 28.914, P < 0.0001), inflorescence biomass ($F_{,}$ = 5.037, P = 0.010), and above-ground biomass ($F_{,}$ = 15.500, P < 0.0001), while leaf number remained non-significant ($F_{,}$ = 1.583, P > 0.05). All measured traits—plant height, root diameter, root biomass per unit length, rhizome bud number, leaf area, leaf biomass, inflorescence number, inflorescence biomass, and above-ground biomass—demonstrated a decreasing trend with increasing altitude (Table 3).

2.3 Relationships Between Plant Size and Sexual Versus Asexual Reproduction

Significant positive correlations between plant size and sexual reproductive structures were observed in alpine hillside meadow (P = 0.005) and alpine swamp meadow (P = 0.017), with a positive trend in alpine shrub meadow (P = 0.429). In contrast, no significant correlations were found between plant size and asexual reproduction in any grassland type (P > 0.05) (Table 4).

Across different altitudes, significant positive correlations between plant size and sexual reproductive structures were detected at all elevation ranges (3,400-3,500 m: $P = 0.017$; 3,500-3,600 m: $P < 0.0001$; 3,600-3,800 m: $P = 0.002$), while no significant correlations were found between plant size and asexual reproduction ($P > 0.05$) (Table 5).

2.4 Relationship Between Sexual and Asexual Reproduction

No significant correlations were found between inflorescence number and rhizome bud number across different alpine grassland types and altitudes ($P > 0.05$), indicating neither competitive nor synergistic relationships between the two reproductive modes in *L. rotata* (Table 6).

3 Discussion

3.1 Adaptive Responses of Growth and Reproduction Traits to Grassland Type and Altitude

The differential expression of *L. rotata* growth and reproduction traits across grassland types indicates multiple resource allocation strategies for environmental adaptation. The plant' s ground-hugging growth habit limits its ability to compete for light and above-ground space in shrub communities, prompting greater resource investment in below-ground tissues and increased asexual reproduction. In shrub-free environments, *L. rotata* tends to allocate more resources to above-ground structures. This strategy of favoring asexual reproduction under harsher conditions aligns with previous research findings (Wang et al., 2005). Swamp meadows, characterized by fewer dominant species and abundant water resources, provide more suitable conditions for *L. rotata* population growth, a conclusion that largely corroborates the field survey results of Sun et al. (2012) on wild *L. rotata* resources.

With increasing altitude, air density, atmospheric pressure, temperature, and saturated vapor pressure decrease while UV radiation increases and daily fluctuations intensify. These unstable abiotic factors enhance plant stress (Ma et al., 2019), thereby limiting *L. rotata* growth and reproduction. Under reduced total resource availability, allocation to individual organs consequently decreases. This conclusion is consistent with studies on *Saussurea dzeurensis* and *Saussurea wellbyi* in western Sichuan and northern Tibet (Wang et al., 2015; Ma et al., 2019).

3.2 Relationship Between Plant Size and Reproductive Investment

The significant positive correlation between sexual reproductive structure investment and plant size, coupled with the absence of correlation between asexual reproductive structure investment and plant size, was consistently observed across different grassland types and altitudes. Rhizome buds in *L. rotata* serve as a compensatory mechanism for sexual reproduction, ensuring population persistence even in harsh environments where seed production fails. This mechanism likely represents an inherent plant characteristic, fundamentally different from sexual reproduction, which requires substantial vegetative accumulation before initiation. Similar results have been reported for *Ligularia virgaurea*, another alpine meadow weed with dual reproductive modes (Xie et al., 2014).

Schmid et al. (1995) proposed that for perennial plants with asexual reproduction, clonal investment constitutes part of vegetative growth and therefore does not require a size threshold if it is merely a component of individual growth processes. In contrast, sexual reproductive investment represents the final plastic structure in a plant' s life cycle, and plants cannot erroneously produce sexual structures before accumulating sufficient resources.

3.3 Relationship Between Sexual and Asexual Reproduction

In perennial plants with dual reproductive modes, relationships between reproduction types may be either competitive (Van Drunen & Dorken, 2012) or synergistic (Xie et al., 2014). However, this study found no correlation between the two reproductive modes in *L. rotata* across different grassland types and altitudes. This phenomenon has also been reported in *Arum italicum* and *Lolium perenne* (Méndez, 1999; Thiele et al., 2008) and may be related to the specific characteristics of *L. rotata* rhizome buds. Rhizome buds in *L. rotata* likely become activated for compensatory growth only after receiving internal signals following complete above-ground withering or harvesting damage, similar to plant responses after mowing (Ma et al., 2010). This differs substantially from plants that actively engage in asexual reproduction. Whether this lack of correlation is influenced by other abiotic factors such as temperature, light, water, and nutrients requires further investigation.

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