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Variation of Leaf Functional Traits of Rhododendron Aganniphum Var. Schizopeplum along Altitude Gradient

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Abstract: Rhododendron aganniphum var. Schizopeplum is one of the typical community types in Sejila Mountain, which is of great significance in maintaining biodiversity and exerting ecological service functions. In this experiment, Rhododendron aganniphum with five different altitudes in Sejila Mountain was studied. Through single factor analysis, nine leaf functional traits, such as Pearson correlation analysis, the distribution characteristics of leaf area (LA), dry matter content (LDMC) and N, P, N: P of Rhododendron aganniphum var. Schizopeplum and their relationships were studied to reveal the ecological adaptation strategy of Rhododendron aganniphum to the environment. The results showed that: (1) The functional traits of Rhododendron aganniphum leaves at five altitude gradients in Sejila Mountain are significantly different, where the coefficient of variation of specific leaf area (SLA) is the largest (57.78%), and the coefficient of variation of P is the smallest (4.60%), and the coefficient of variation from small to large is: P<N<N: P<leaf thickness (LT) <LDMC<LA<leaf dry weight (DW) <leaf tissue density (LD) <SLA. (2) The leaf functional traits show a certain trend along the altitude gradient. LA, DW, LDMC and SLA of Rhododendron aganniphum leaves shows a downward trend with the increase of altitude gradient, while N, N: P, LT, P and LD shows an increasing trend with the increase of altitude gradient.(3) Correlation analysis shows that LA is significantly negatively correlated with N and N:P (P<0.05). There is a significant negative correlation between DW and N (P<0.05), and there is a significant positive correlation between N and P, N: P (P<0.01). (4) LDMC, DW and LA can be used as the main indexes of leaf functional traits of Rhododendron aganniphum. It is believed that with the increase of altitude, Rhododendron aganniphum in Sejila Mountain changed from a “pioneering” survival strategy to a “conservative”' survival strategy to gain competitive advantage.

1. Introduction

Plant functional traits are the bridge and link between plants and the external environment, which reflect the adaptation mechanism of plants to living environment and can affect the ecosystem [1]. At the same time, it can not only reflect the ability of plants to acquire and utilize resources, but also closely link individuals with the environment. Therefore, it is still a frontier issue in global ecology research to explore the changes of its under different conditions (such as altitude gradient, soil nutrients) in the context of global warming.

Leas are the organ with the largest contact area with the outside world, which can provide important information of plant growth. Leaf traits directly reflect the survival strategy of plants to the external environment. Therefore, leaf functional traits have become an important bridge for scholars to explore the variation between plants and the environment. At present, the study on the relationship between traits and altitude gradient is mainly carried out on species level. For example, Liu Siwen et al. studied the variation of leaf traits of Pinus from Beijing Songshan National Nature Reserve along the altitudinal gradient [2]. However, due to great differences in longitude, latitude and altitude in the study area, the variation patterns of plant functional traits and environment with altitude are inconsistent. It is still necessary to study the relationship between small-scale spatial environment dominated by different altitude gradients and leaf functional traits.

Rhododendron aganniphum, which is native to Mangkang County of Changdu City, Chayu County and Milin County of Linzhi City in Tibet. The fruit of Rhododendron aganniphum var. Schizopeplum is short and erect or slightly curved, and the corolla is pink to white. At present, the studies on Rhododendron aganniphum var. Schizopeplum mainly focus on leaf anatomical structure and environmental adaptability, seed setting and germination characteristics, population distribution[3] and other aspects. However, the studies on the characteristics of leaf traits of Rhododendron aganniphum with altitude distribution have not been reported.

2. Overview of Research Area and Research Methods

2.1. Overview of Research Area

Located in Linzhi City, the Sejila Mountain belongs to the southern extension of the Nyainqentanglha Mountains. The region average temperature is -0.73 ℃, the average temperature in the warmest month is 9.23 ℃, the average temperature in the coldest month is -14 ℃. The annual average precipitation is 1134 mm, and the annual average evaporation is 544 mm. June to September is the rainy season, accounting for about 80 % of the annual precipitation. [4]. The vegetation of the nature reserve mainly includes Sabina saltuaria and the like. The vegetation types of Sejila Mountain have obvious distribution regularity along with the increase of altitude. It’s a natural laboratory of between vegetation and external environment in this area.

2.2. Sample Plot Setting and Sample Collection

On the basis of the previous field investigation, at the end of July 2021, the altitude of 4003 m – 4385 m was selected as the research area, and the representative Rhododendron aganniphum var. Schizopeplum community was selected at a vertical altitude interval of about 100 m. A horizontal fixed sample line of 130 m was set up on each altitude gradient, with a total of 5 fixed sample lines, and a fixed sample of 10 m × 10 m was set up at every 20 m interval on each sample line, with a total of 25. Three plants with good growth and no pests and diseases (adult individuals with similar tree age and DBH) were randomly selected in each quadrat. A branch with good growth without shelter was collected in four directions (east, west, south and north) in the middle of the canopy by means of high branch scissors and tree climbing. Ten intact mature leaves without diseases were selected on these branches. A total of 120 leaves were selected for each quadrat, and a total of 600 leaves were selected for each transect. They were placed in the middle of the pre-soaked filter paper, put into a self-sealing bag and sealed, brought back to the laboratory to measure each trait index, and recorded the information of each transect (Table 1).

Table 1: Environmental Data of Line Transect

Number	Altitude	Longitude	Latitude	Slope	aspect
1	4385m	E94°42′26.06″	N29°39′25.31″	25 °	119 °
2	4293m	E94°42′31.39″	N29°39′19.93″	24 °	131 °
3	4182m	E94°42′37.01″	N29°39′15.55″	32 °	124 °
4	4097m	E94°42′40.61″	N29°39′12.64″	21 °	127 °
5	4003m	E94°42′45.73″	N29°39′07.62″	20 °	123 °

2.3. Determination of Leaf Functional Traits

The collected Rhododendron aganniphum leaves were taken back to the Tibet Ecological Security Laboratory. The leaves were dried with filter paper, and the leaf area (LA) was measured by leaf area meter (AM-300). The accuracy of 0.01 mm electronic vernier caliperwas used to avoid the main vein and randomly select three points to measure its thickness, and the average value was taken as the leaf thickness (LT)[5]. Then the leaves were put into a cowhide envelope and placed in an oven at 105 ℃ for 20 min, and then dried to constant weight at 80 ℃ to measure the dry weight (DW) of the leaves. Finally, the leaves were crushed through a 100-mesh sieve to determine the contents of leaf nitrogen (N) and leaf phosphorus (P). The nitrogen content of leaves was determined by H2SO4-H2O2 digestion, and the phosphorus content of leaves was determined by H2SO4-H2O2 digestion vanadium molybdenum yellow colorimetric method [6]. Leaf dry matter content (LDMC), specific leaf area (SLA) and leaf tissue density (LD) was calculated, and the calculation formula was as follows:

$$LDMC=DW/FW;\ SLA=LA/DW;\ LD=DW/(LA\times LT) \quad (1)$$

2.4. Data Analysis and Processing

Excel 2010 and SPSS 22.0 were used to analyze the data of traits. Single factor and Duncan method were used for variance analysis and multiple comparisons. Pearson method was used to analyze the correlation of 9 leaf traits. Coefficient of variation (CV)=standard deviation/mean value, weak variation when 20%≥CV, moderate variation when 20%<CV≤50%, and strong variation when 50%<CV[7].

3. Results and Analysis

3.1. Leaf traits of Rhododendron Aganniphum at Different Altitudes

According to Table 2, the average values of LA, LT, DW, LDMC, SLA, LD, N, P and N: P of Rhododendron aganniphum var. Schizopeplum leaves at five altitude gradients in Sejila Mountain were 28.52 cm2, 0.54 mm, 0.41 g, 0.30 g/g, 82.26 cm2/g, 0.30 g/cm3, 16.37 g/kg, 0.99 g/kg and 16.45 respectively. The nine leaf traits of Rhododendron aganniphum had different degrees of variation. The coefficient of variation (CV) from small to large was P, N, N: P, LT, LDMC, LA, DW, LD, SLA, and the CV ranged from 4.60% to 57.78%. The coefficients of variation of P, N, N/P and LT were 4.60%, 15.14%, 15.68% and 16.64%, respectively, which belonged to weak variation, while LDMC, LA and DW belonged to moderate variation, and the coefficients of variation of LD and SLA were 56.12% and 57.78% respectively, which belonged to strong variation, indicating that SLA of Rhododendron aganniphum var. Schizopeplum leaves was the most sensitive to the change of altitude gradient in Sejila Mountain.

Table 2: Variation characteristics of leaf traits in rhododendron aganniphum

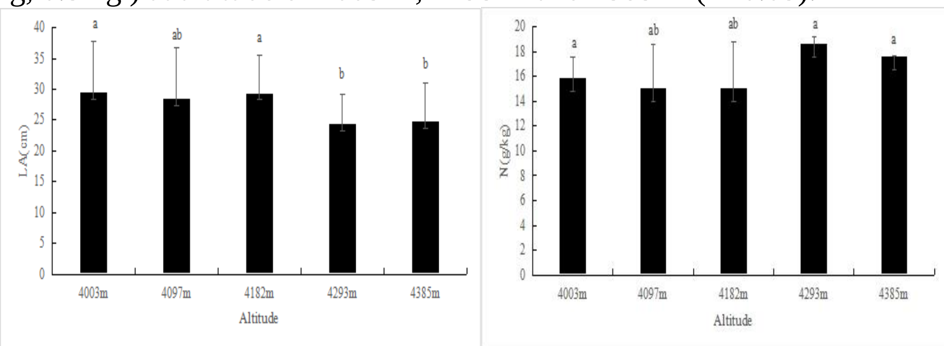
	Maximum	Minimum	Median	Mean ±standard deviation	coefficient of variation
LA(cm^2)	64.45	15.27	27.03	28.52±9.96	34.64%
LT(mm)	0.91	0.37	0.53	0.54±0.09	16.64%
DW(g)	0.90	0.15	0.38	0.41±0.16	38.56%
LDMC(g/g)	0.48	0.15	0.27	0.30±0.08	28.34%
SLA(cm^2/g)	283.82	20.54	71.92	82.26±47.90	57.78%
LD(g/cm^3)	0.99	0.05	0.25	0.30±0.17	56.12%
N(g/kg)	19.34	10.61	17.48	16.37±2.57	15.14%
P(g/kg)	1.06	0.88	1.00	0.99±0.05	4.60%
N:P	20.15	10.01	17.27	16.45±2.61	15.68%

3.2. Changes of Leaf Traits of Rhododendron Aganniphum along Altitude Gradient

The differences of nine leaf functional traits of Rhododendron aganniphum in Sejila Mountain are shown in Figure 1. With the increase of altitude gradient, LA, LDMC and the like of its leaves showed a downward trend, while N, N: P, and LD showed an upward trend.

The LA of leaves showed a maximum of 29.37 cm2 at 4003 m and a minimum of 24.24 cm2 at 4293 m, and the difference between the two was significant ($P<0.05$). The contents of N and P in leaves reached the maximum at 4293 m, which were 18.54 g/kg and 1.02 g/kg, respectively. The SLA of leaves showed a maximum value of 78.98 cm2/g at 4003 m, which was significantly different from other altitudes ($P<0.05$). The LD of leaves reflected the accumulation of plant biomass, and the maximum value was 0.32 g/cm3 at 4097 m, while the minimum value appeared at 4182 m.

The N: P content of leaves was not significantly different at 4293 m and 4385 m, but it was significantly different 4003~4097 m ($P<0.05$), and the lowest at 4182 m (14.68). The LT of leaves reached a maximum of 0.57 mm at an altitude of 4385 m, which was significantly different from that at 4003-4097 m and 4292 m ($P<0.05$). The LT showed a minimum of 0.48 mm at an altitude of 4003 m. The LDMC of leaves reached a maximum of 0.37 g/g at an altitude of 4097 m, which was significantly different from other altitude gradients ($P<0.05$). The DW of leaves reached a maximum of 0.46 g at an altitude of 4097 m, which was significantly different from the DW content (0.38 g, 0.35 g, 0.37 g) at altitude of 4003 m, 4293 m and 4385 m ($P<0.05$).



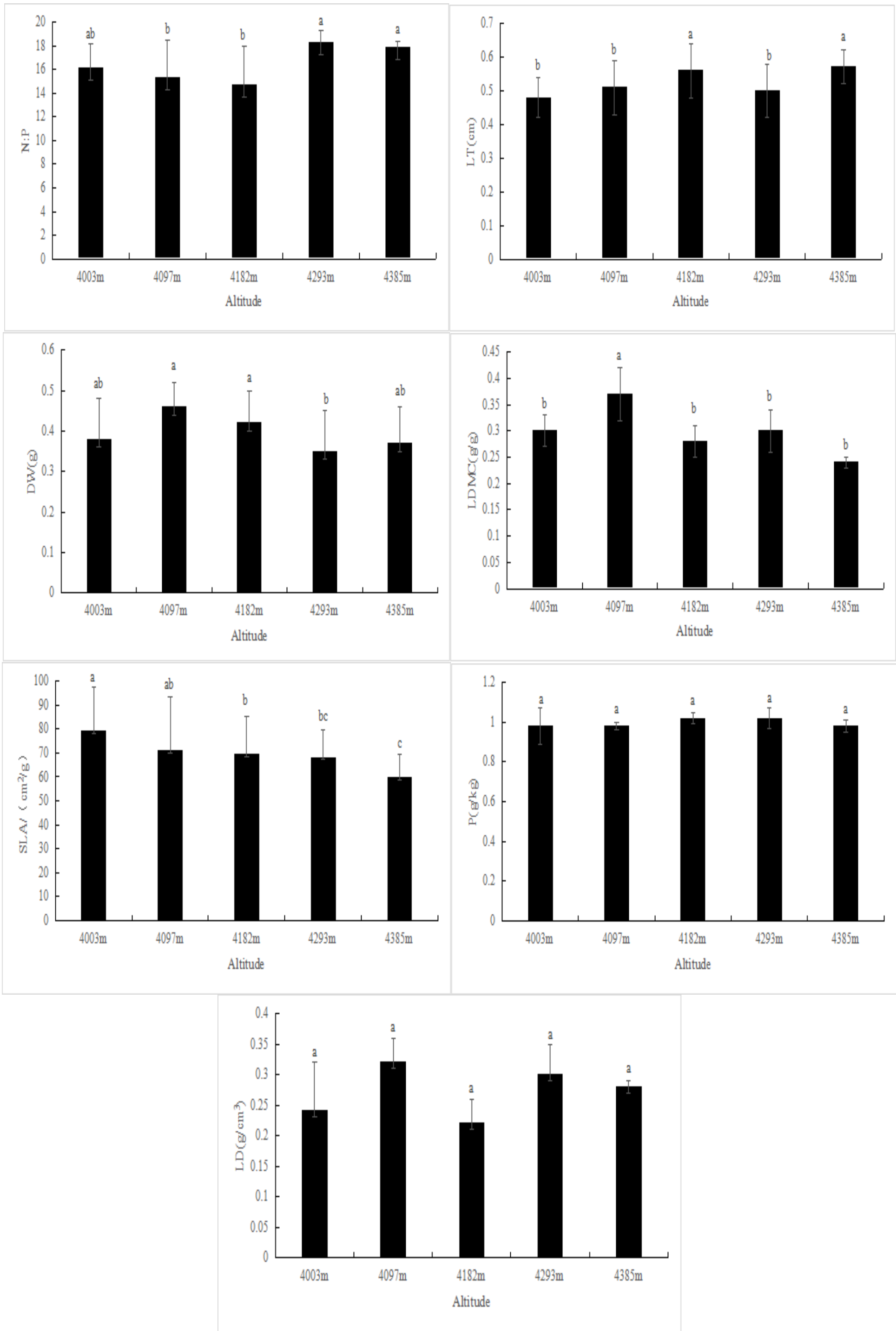


Figure 1: Leaf functional traits of Rhododendron aganniphum at different altitudes

3.3. Relationships among Leaf Functional Traits of Rhododendron Aganniphum

The results of correlation analysis showed that there was a certain relationship between the functional traits of leaves (Table 3). There was a significant negative correlation between LA and N, N: P ($P<0.05$). There was a significant negative correlation between DW and N ($P<0.05$). There was a significant positive correlation between N and P, N: P ($P<0.01$). And there was no significant correlation between the other traits ($P>0.05$).

Table 3: Correlation coefficient between leaf functional traits

	LA	LT	DW	LDMC	SLA	LD	N	P	N:P
LA	1								
LT	-0.203	1							
DW	0.700	0.091	1						
LDMC	0.381	-0.605	0.630	1					
SLA	0.73	-0.786	0.226	0.500	1				
LD	-0.496	-0.248	0.098	0.561	-0.289	1			
N	-0.933*	-0.023	-0.892*	-0.429	-0.481	0.316	1		
P	-0.023	0.182	-0.114	-0.085	-0.028	-0.301	0.927*	1	
N:P	-0.921*	-0.062	-0.877	-0.427	-0.467	0.359	0.975**	-0.115	1

Note: ** indicates significant correlation at 0.01 level, and * indicates significant correlation at 0.05 level.

3.4. Principal Component Analysis of Leaf Functional Traits of Rhododendron Aganniphum

Table 4: Initial Factor Load Matrix and Principal Component Contribution Rate

Functional Traits	Principal Components1	Principal Components 2	Principal Components 3	Comprehenesive Score	Comprehenesive Ranking
LA	0.958	-0.186	-0.186	0.438	3
LT	-0.267	-0.806	0.529	-0.261	7
DW	0.837	-0.035	0.542	0.549	2
LDMC	0.613	0.692	0.221	0.560	1
SLA	0.698	0.328	-0.635	0.349	4
LD	-0.238	0.765	0.577	0.183	5
N	-0.95	0.227	-0.178	-0.491	9
P	-0.047	-0.389	-0.27	-0.180	6
N:P	-0.94	0.299	-0.137	-0.459	8
eigenvalue	4.398	2.149	1.516		
contribution rates/%	48.87	23.87	16.85		
cumulative contribution rate/%	48.87	72.74	89.59		

Based on the principle of the eigenvalue is greater than 1, three principal components were extracted (Table 4). The eigenvalues were 4.398, 2.149 and 1.516 respectively. The contribution rates of the principalcomponents were 48.87%, 23.87% and 16.85%, respectively. The cumulative contribution rate reached 89.59 %, indicating that these three principal components were the main factors affecting the leaf functional traits of this species. By ranking the comprehensive scores of the principal components of leaf functional traits of R. aganniphum, LDMC, DW and LA of leaves can be used as the main indicators of the changes of functional traits of this species.

4. Results and Discussion

The change of altitude often affects the gradient changes of various ecological factors such as water, light, temperature, and forms redistribution of water, heat, steam and the like within a small range of changes. Plants will form different functional traits to adapt to the complex changes of the external environment. This creates good conditions for exploring the changes of leaf functional traits of plants under different altitude gradients. In this paper, the leaf functional traits of Rhododendron aganniphum at five different altitude gradients in Sejila Mountain were studied. It was found that the leaves of this species showed certain intraspecific variation to adapt to different habitats, and the coefficient of variation was between 4.60% and 57.78%. Although the interspecific variation of leaf functional traits is greater than the intraspecific variation, the intraspecific variation is the most direct station of plant adaptation to the external environment, thus, it cannot be ignored. The average intraspecific variation of leaf functional traits of Rhododendron aganniphum was 29.72 %, which was significantly higher than that of Lycium ruthenicum and Ammopiptanthus [8], indicating that Rhododendron aganniphum still has a wide ecological range in the alpine habitat of southeastern Tibet. Among the nine traits of Rhododendron aganniphum, the coefficient of variation of SLA was the largest (57.78%), indicating that the SLA of Rhododendron aganniphum was more sensitive to the change of altitude gradient.

In the high altitude area of southeast Tibet, temperature is the main ecological factor limiting plant growth. In the process of long-term evolution, plants will eventually form a set of optimal functional trait combinations through the coordination of various traits in order to continuously adapt to the changes of the external environment. The leaf functional traits of plants are the best embodiment of the adaptability to the external environment. LDMC reflects the storage capacity and production capacity of plant nutrients. LT is related to resource acquisition and preservation, and LD is related to nutrient storage of leaves, reflecting the tolerance and defense of plant leaves [9]. LA reflects the ability of plants to receive and intercept light energy. In the extreme cold environment, the available resources of plants are relatively limited. When plants invest too much in one of the functional traits, they will inevitably reduce the investment in the remaining traits. Especially under unfavorable factors, the plants will maintain their own survival and reproduction by sacrificing other traits. This study found that the LA, DW, LDMC and SLA of R. aganniphum showed a downward trend with the increase of altitude gradient, while LT and LD showed an upward trend with the increase of altitude gradient. The complementarity of this leaf trait is related to the self-adaptation and self-protection of the plant in the long-term living environment. With the increase of altitude in the study area, the external environment temperature of plants decreased, resulting in the decrease of free water content in the body, inhibiting the increase of leaf area and cell division, thickening the cuticle and palisade tissue of leaves. In order to reduce the loss of water and enhance the resistance to low temperature, plants usually reduce leaf area and stomata area, accumulate photosynthetic products, enhance the maintenance of water in the body and improve the cold tolerance of leaves, resulting in the decrease of SLA, LA and LDMC and the increase of LT and LD in R. aganniphum. In high altitude areas, SLA, LA, LDMC of R. aganniphum leaves are smaller, and the LT and LD are larger, which makes the species grow slowly and the resource acquisition ability is low, that is, the "defensive" investment is more, which belongs to the "conservative" survival strategy ; in low altitude areas, the SLA, LA, LDMC of leaves are larger, and LT and LD are smaller, which makes the species grow faster and have higher resource acquisition ability, that is, the "defensive" investment is less, which belongs to the "pioneering" survival strategy. In summary, Rhododendron aganniphum obtained higher growth rate and resource acquisition ability by adopting a ' pioneering ' strategy in low altitude areas. In high altitude areas, conservative strategies are used to increase their own nutrient storage to gain competitive advantage.

The results of relevant research showed that there was a significant positive correlation between N and P in leaves [10]. This result was consistent with the results of this study. Although there were some differences in the correlation between nitrogen and phosphorus at different research scales, for a stable community plant, the limiting elements of its growth were basically stable, indicating that Rhododendron aganniphum can not only adapt to the soil conditions in southeastern Tibet, but also has the ability to maintain the relative stability of N and P, and it is easy to show the same change rule under extreme environment. Relevant research has shown that there is a negative correlation between LDMC and SLA. This study is inconsistent with its research results. This may be due to the influence of environmental gradients and soil element content. Plants achieve self-regulation to adapt to changes in the environment. Different from the general distribution strategy, a survival strategy corresponding to the environment is formed, which makes LDMC and SLA show a positive correlation. In this experiment, DW, LDMC and LA can be used as the main indicators of the changes of leaf functional traits of Rhododendron aganniphum, which comprehensively reflects that R. aganniphum adapts to the harsh external environment changes in the high altitude areas of southeastern Tibet through its own relevant regulation. The results of this study are inconsistent with the results of Wang Fei et al. On the one hand, this may be caused by different species, on the other hand, the habitat of the study area is different, and the adaptation of leaf functional traits to habitat is different.

5. Conclusion

The variation of altitude will lead to a certain altitude pattern of plant functional traits. In Sejila Mountain, Rhododendron aganniphum has a rapid growth rate and high resource acquisition ability through the "pioneering" strategy in low altitude areas, while in high altitude areas, the species increases "defensive" investment through the "conservative" strategy to gain competitive advantage. It indicated that Rhododendron aganniphum var. Schizopeplum formed an ecological strategy to adapt to the environment through the trade-off mechanism between leaf functional traits.

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