Floral Organ Morphological Diversity and Inter-strain Identification in Ningxia Goji Berry (Lycium barbarum L.): A Postprint

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Abstract

This study employed 42 intraspecific strains of Lycium barbarum (L y c i u m b a r b a r u mL.) as experimental materials to investigate the polymorphism of morphological differences through one-way ANOVA among groups, principal component analysis, and cluster analysis, based on scoring and measuring 16 morphological indices of floral organ traits. The results demonstrated that morphological indices of floral organs in the same Lycium barbarum strain exhibited no significant difference across different time points (specifically observed in July, August, and September 2017) ($p<0.05$); concurrently, principal component analysis revealed that the cumulative contribution rate of six floral morphological indices—petal outer margin color, petal front and back venation, petal shape, petal back color, throat color, and position of pistil and stamens—reached 84.791%, constituting the principal components of floral organ differences among different Lycium barbarum strains; cluster analysis, employing a Euclidean distance of 7.5 as the threshold, classified the 42 tested strains into six categories. This study clarified that floral organ morphology of the same Lycium barbarum strain demonstrated certain stability across different time points, serving as one of the diagnostic indicators for distinguishing intraspecific strains of Lycium barbarum; concurrently, six principal components reflecting floral organ morphological differences in Lycium barbarum were identified, and 42 Lycium barbarum samples were classified into six categories, preliminarily establishing a morphological identification method for intraspecific strains of Lycium barbarum, which can provide a basis for morphological research and strain identification of Lycium barbarum.

Full Text

Preamble

Floral Morphological Diversity and Cultivar Identification in *Lycium barbarum* L.

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Abstract

This study investigated 42 cultivars of *Lycium barbarum* L., evaluating 16 morphological traits of floral organs through assignment and measurement. Polymorphism in morphological differences was analyzed using one-way ANOVA, principal component analysis (PCA), and cluster analysis. Results demonstrated that morphological indicators of floral organs within the same cultivar showed no significant differences across different time points (specifically July, August, and September 2017; $p>0.05$). PCA revealed that six floral morphological traits—petal edge color, venation on front and back of petals, petal shape, petal back color, flower throat color, and pistil-stamen position—had a cumulative contribution rate of 84.791%, representing the principal components distinguishing different cultivars. Cluster analysis, using a Euclidean distance threshold of 7.5, classified the 42 cultivars into six groups. This study confirms that floral organ morphology exhibits temporal stability within cultivars, validating its use as a diagnostic criterion for distinguishing *L. barbarum* cultivars. The identification of six principal components reflecting floral morphological differences and the classification of 42 accessions into six groups establishes a preliminary morphological identification method for intraspecific cultivar discrimination in *L. barbarum*, providing a foundation for morphological research and cultivar authentication.

Keywords: *Lycium barbarum* L., floral organ, principal component analysis, cluster analysis, morphological identification

Introduction

The genus *Lycium* Linn. belongs to the Solanaceae family, Solanoideae subfamily, and Lycieae tribe, comprising approximately 80 species worldwide, predominantly distributed in South America with a few species in the Eurasian temperate zone. China hosts seven species of *Lycium* (Lu et al., 2003; Qian et al., 2017; Wang et al., 2011). Previous research from the 1970s to early 2000s has documented morphological distinctions among *Lycium* species based on growth habit, stem, leaf, calyx, corolla, corolla lobes, seeds, and fruit flavor

(Lu et al., 2003; Yuan et al., 2013). These studies indicate substantial morphological differences among the seven species and three varieties of Chinese *Lycium*, enabling discrimination based on calyx, corolla, fruit shape, leaf shape, and stem morphology.

Lycium barbarum L. is widely cultivated throughout northern China as an important economic crop, with fruits valued for tonifying liver and kidney, benefiting essence and vision, and providing anti-aging effects (Dong et al., 2008). Since 2011, cultivation area in Ningxia has exceeded 70,000 hectares, with products exported to over 30 countries and regions (Lian, 2012). Despite its extensive cultivation, no standardized intraspecific identification method currently exists for *L. barbarum*, with cultivar discrimination relying primarily on empirical cultivation experience using ambiguous criteria. This represents both a research gap in cultivar identification and a practical challenge for germplasm collection and purity maintenance during cultivation. As reproductive organs, floral structures exhibit relatively stable morphological characteristics under varying environmental and cultivation conditions, making them valuable for taxonomic classification (Yang, 2002). Based on literature review and field observations, we selected 16 traits for *L. barbarum* floral morphological assessment and employed statistical analysis to develop a preliminary morphological identification method for intraspecific cultivar discrimination.

Materials and Methods

1.1 Experimental Materials and Site

The experiment was conducted at the Lycium Germplasm Resources Nursery of the National Wolfberry Engineering and Technology Center in Yinchuan. Forty-two *L. barbarum* cultivars were evaluated, including 39 accessions from the Lycium Germplasm Resources Nursery in Xixia District, Yinchuan (38°38 N, 106°9 E, elevation 1,114 m) and three from Kongtan Village, Zhongning County (37°48 N, 105°59 E, elevation 1,259 m). Cultivar details are provided in . Observations were conducted from May to September 2017.

During peak flowering periods, five freshly opened, unfaded flowers were collected from vigorous plants of each cultivar between 10:00–11:00 AM. Flowers were placed in disposable plastic petri dishes and stored at 4°C for observation within 12 hours.

1.2 Trait Evaluation and Statistical Analysis

Based on literature review (Liu & Liu, 2012) and practical considerations, 16 floral traits were selected and assigned standardized values (Ouyang, 2010) as detailed in .

1.2.1 Temporal Stability Analysis of Floral Traits Five cultivars (Ningqi 1, Ningqi 3, Baihua 2015, 14-401, and 16-23-8-10) were observed at three time points (July 27, August 22, and September 2, 2017). Measurements were taken directly using vernier calipers (precision 0.01 cm). Data were processed using SPSS software to calculate means and standard deviations, followed by one-way ANOVA among groups.

1.2.2 Morphological Variation Analysis The same methodology was applied to all 42 cultivars, with data subjected to PCA and cluster analysis using SPSS software.

Results

2.1 Temporal Stability of Floral Morphology

Among the 16 traits, 11 descriptive indicators showed consistent expression across time periods, while five quantitative traits exhibited variation (see). One-way ANOVA was performed on these five traits after data standardization (Zhao, 2003). Homogeneity of variance tests () showed significance levels above 0.6 for all morphological indicators across the three collection dates, validating ANOVA assumptions. The ANOVA results () revealed P-values >0.05 for all five traits—flower diameter, corolla tube length, corolla tube width, flower stalk length, and flower throat width—indicating no significant temporal differences. This confirms the stability of floral traits in *L. barbarum* and supports their use for cultivar discrimination.

Note: A-1–A-3: Ningqi 1 on July 27, August 22, September 2; B-1–B-3: Ningqi 3 on July 27, August 22, September 2; C-1–C-3: 14-401 on July 27, August 22, September 2; D-1–D-3: 16-23-8-10 on July 27, August 22, September 2; E-1–E-3: Baihua 2015 on July 27, August 22, September 2.

Plate I. Floral morphology of five cultivars at different time periods.

2.2 Principal Component Analysis for Morphological Identification

PCA of 11 descriptive floral traits yielded two key outputs: total variance decomposition () and component matrix (). The analysis extracted six principal components with contribution rates of 23.265%, 18.945%, 13.599%, 10.827%, 9.874%, and 8.280%, respectively, accounting for a cumulative 84.791% of total variance. This indicates that six principal components adequately represent the 11 original variables.

Traits with absolute component loadings >0.5 were assigned to corresponding principal components. For example, flower color loaded at -0.649 on the first component, exceeding the 0.5 threshold, and was thus assigned to PC1. Following this criterion, traits were grouped into six principal components:

- **PC1** (2 traits): Flower color and petal edge color
- **PC2** (3 traits): Petal rolling pattern, dorsal petal venation, and ventral petal venation

- **PC3** (2 traits): Petal shape and petal fading pattern
- **PC4** (1 trait): Petal back color
- **PC5** (1 trait): Flower throat color
- **PC6** (1 trait): Pistil-stamen position

Only "filamentous degree" showed loading <0.5, indicating weak correlation with all components and was excluded from further analysis.

2.3 Cluster Analysis for Morphological Identification

Cluster analysis using the between-groups linkage method with Euclidean distance was performed on floral traits of 42 cultivars. At a Euclidean distance of 7.5, cultivars were divided into five major groups ([Figure 1: see original paper]).

[Figure 1: see original paper]

Group I comprised 20 cultivars, subdivided into two subgroups: Subgroup 1 (16-1-3-12, NM-03, 16-16-9-3, 16-17-5-11, Baihua 2015, Xin 9, 16-11-5-20, 16-1-1-22, 16-16-9-18, 16-22-8-9, 16-23-8-10, 16-23-7-8, 16-23-13-5) and Subgroup 2 (14-26-4, 16-1-2-16, 14-402, Z44, 14-401, 14-16, 16-1-3-5).

Group II included 12 cultivars: 14-419, Ningqi 3, Ningqi 1, Xiaomaye, 16-23-14-1, Damaye, 14-5-9-1, Ningqi 2, Ningqi 6, 14-6-3-7, NM-02, and Ningqi 5.

Group III contained only Ningqi 7.

Group IV comprised eight cultivars: 14-87, 16-18-7-6, 16-1-3-14, 14-104, 16-16-5-7, 16-19-8-12, NM-01, and 14-2-2-5.

Group V contained only Ningqi 8.

Note: Group I: A (16-22-8-9), B (14-16); Group II: C (14-419), D (14-6-3-7); Group III: E (Ningqi 7); Group IV: F (14-87), G (14-104); Group V: H (Ningqi 8).

Plate II. Floral morphology of five different groups.

Morphological characterization of each group revealed distinct diagnostic features: Group I exhibited purple petal edges, wide flower throats, small pistil-stamen height differences, and frequent bilateral petal reflexing. Group II was characterized by three vascular bundles in petals, involute rolling, fading from outside inward, and narrow flower throats. Group III (Ningqi 7) typically showed a single main vascular bundle, slight petal curling, white petal edges, and pistils exceeding stamens in height. Group IV featured purple petal edges, pronounced pistil-stamen displacement, heart-shaped petals, and narrow throats. Group V (Ningqi 8) had wide corolla tubes, fine venation in addition to three main vascular bundles, apical petal reflexing, and pistils lower than stamens.

Discussion

3.1 Temporal Stability of *L. barbarum* Floral Organs

Under consistent sampling standards and timing, morphological observations of 16 floral traits across five cultivars at three collection dates revealed consistent expression in 11 descriptive traits, with variation only in five quantitative traits. One-way ANOVA of these quantitative traits demonstrated no significant temporal differences ($P>0.05$), confirming the morphological stability of *L. barbarum* floral organs and validating their use for cultivar discrimination. This conclusion regarding temporal stability applies specifically to intraspecific cultivars of *L. barbarum*; further verification is needed to determine whether interspecific stability exists among Chinese *Lycium* species.

3.2 Principal Component Analysis of *L. barbarum* Floral Organs

To comprehensively evaluate floral morphology as a diagnostic criterion, 16 variables reflecting *L. barbarum* floral traits were measured. While these variables exhibited intercorrelations, the large number complicated statistical analysis. PCA addresses this by linearly transforming multiple variables into fewer comprehensive indicators while preserving correlations, thereby simplifying data interpretation. SPSS analysis extracted six principal components from 11 descriptive traits, achieving a cumulative contribution rate of 84.791%.

PC1 comprised flower color (negative correlation) and petal edge color (positive correlation). PC2 included petal rolling pattern, dorsal venation, and ventral venation (all positive correlations). PC3 encompassed petal shape and fading pattern (positive correlations). PC4 comprised petal back color (positive correlation). PC5 included flower throat color (negative correlation). PC6 comprised pistil-stamen position (positive correlation). Based on component coefficients from the component matrix, traits within each principal component can be prioritized for field identification: (1) petal edge color > flower color > flower throat color; (2) ventral venation = dorsal venation > petal rolling pattern; (3) petal shape > fading pattern; (4) petal back color; (5) flower throat color; (6) pistil-stamen position.

While individual researchers may prioritize traits differently during field identification—for example, using flower color as the primary criterion—these results indicate petal edge color has the highest coefficient in PC1. However, regardless of which trait is used first, the final identification outcome remains consistent, suggesting the proposed sequence serves as a reliable reference, though the extracted traits are essential for accurate discrimination.

3.3 Morphological Cluster Analysis of *L. barbarum* Floral Organs

Cluster analysis is a statistical classification method based on the principle that similar objects group together (Ouyang, 2010), maximizing within-group homogeneity and between-group heterogeneity. Using SPSS 23.0, 42 *L. barbarum* cultivars were classified into five groups: Group I exhibited purple petal edges, wide throats, small pistil-stamen height differences, and frequent bilateral reflexing, with spoon-shaped corollas predominating. Group II featured three vascular bundles, involute rolling, small flower diameter but long corolla tubes, outside-inward fading, slight pistil displacement, and narrow throats. Group III (Ningqi 7) typically showed a single main vascular bundle, slight curling, white petal edges, and pistils exceeding stamens. Group IV exhibited purple petal edges, pronounced pistil displacement, heart-shaped petals, and narrow throats. Group V (Ningqi 8) had wide corolla tubes, fine venation in addition to three main bundles, apical reflexing, and pistils lower than stamens. The clustering results align with field observations, confirming the feasibility of software-based morphological identification.

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