

Phenotypic Diversity Analysis in Blue Honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye) Populations in Qilian Mountain area, China

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Abstract: **【Objective】** Blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye), distributed in the Qilian Mountains, is a rare plant with abundant nutrients and medicinal value in high-altitude areas. Studying its phenotypic diversity is of significant importance. **【Method】** To explore the phenotypic diversity of different populations of blue honeysuckle and their variation patterns under different geographical and climatic conditions, we conducted measurements and analyses of 41 phenotypic traits of 40 plants from eight populations in the Qilian Mountains region. **【Result】** The study revealed rich phenotypic variation in blue honeysuckle, with significant differences among populations and close correlations among many traits. Leaf traits exhibited a gradient change pattern primarily influenced by altitude and precipitation, while fruit traits were influenced by a combination of various geographical and climatic factors. Principal component analysis (PCA) identifies leaf size and crown diameter as the main traits influencing the phenotypic diversity of blue honeysuckle. The 40 blue honeysuckle plants clustered into five groups, which did not completely follow geographic distance clustering. **【Conclusion】** This study lays the foundation for further analysis of the genetic mechanism of phenotypic traits in blue honeysuckle and provides reference for the protection of its wild populations and the breeding of improved varieties.

Key words: blue honeysuckle; geographical climate variation; *Lonicera caerulea*; morphometric analysis; natural populations; phenotypic diversity.

Introduction

Phenotypic diversity, embodying the plethora of observable traits within and across plant populations, is a fundamental aspect of plant biology, shaped by the interplay of genetic underpinnings and environmental influences (Raj *et al.*, 2006). Plant growth reflects the adaptation of genotypes to environmental changes, irreversible changes in long-term stress selection, and new phenotypes after stable inheritance, which underlines the critical role of phenotypic diversity (Pigliucci *et al.*, 2006). This diversity is a vital research variable employed to gauge species diversity, further emphasizing the inherent adaptability and evolutionary narratives of plant species (Yang *et al.*, 2014). Therefore, understanding the phenotypic variation of plants across different environments is essential for grasping their genetic variation, fitness, and evolutionary adaptability and the understanding plays a crucial role in germplasm resource conservation and management, substantially aiding in plant introduction, breeding, and the identification of new cultivar varieties (Li *et al.*, 2021). Furthermore, phenotypic diversity serves as a cornerstone in discerning plant responses to ecological shifts, thereby fostering a deeper understanding of plant development and evolution (Donohue *et al.*, 2001). The current landscape of phenotypic diversity research is enriched by methodologic

al advancements, prominently featuring multivariate analyses like correlation analysis, principal component analysis, and cluster analysis, which are adept at handling the large datasets typically generated from plant morphological characterizations(Balkaya *et al.*, 2010). These analytical paradigms have found application across a variety of plant species, facilitating a nuanced exploration of phenotypic traits, which in turn, aid in identifying economically and ecologically valuable germplasm resources for subsequent variety development(Omire *et al.*, 2020). Moreover, the utilization of these analytical techniques has enabled the grouping of diverse germplasm materials into distinct categories, thereby laying a robust foundation for the effective utilization of these resources in breeding new, improved varieties, and furthering our understanding of the genetic diversity landscape(Ebrahimi *et al.*, 2020).

The Qilian Mountains, located at 38.08 N, 98.88 E, straddle the border between Qinghai Province and Gansu Province in the western region of China. They sit on the northeastern edge of the Qinghai-Tibet Plateau, spanning an elevation range from 2000 to 5800 m a.s.l. The landscape is broadly composed of numerous snow-capped peaks extending from northwest to southeast, punctuated by intermountain valleys. The Qilian Mountains are surrounded by the Loess Plateau of central Gansu, the Alxa Desert region, and the Qaidam Basin. To the west lies the uninhabited Lop Nur. Despite the harsh environmental conditions, the high mountain meltwaters of the Qilian Mountains foster the creation of many rivers and lakes. Regulated by these water bodies, the region around the Qilian Mountains flourishes with vast grasslands and oases, showcasing the adaptability and richness of the local ecosystems. The climate of the region is influenced by the interaction of the Siberian high, East Asian monsoon, and westerly jet streams (Chen *et al.*, 2011). Precipitation mainly occurs during the warm months due to the East Asian monsoon, and it decreases from east to west influenced by the topography. The climatic conditions also contribute to the formation and characteristics of glaciers in the Qilian Mountains. There are two types of glaciers: subcontinental (subpolar) and extreme continental (polar)(Tian *et al.*, 2014). In the eastern portion, subcontinental glaciers are prominent, characterized by annual precipitation ranges of 500~1000 mm a⁻¹, an annual average air temperature of -12 to -6°C, a summer mean air temperature of 0~3°C at the ELA, and an ice temperature of -10 to -18°C within the upper 20 m ice layer. The other type, extreme continental (polar) glaciers as per Shi and Liu, 2000, also forms a part of the glacial landscape of the Qilian Mountains. The Qilian Mountains play a critical role in water conservation for three inland river basins in the Hexi Corridor, serving as a significant water supply source for both the Yellow River and Qinghai Lake. This underscores its importance for biodiversity conservation in China(Zongxing *et al.*, 2021), as the region is an essential ecological barrier providing myriad ecosystem services.

Blue honeysuckle (*Lonicera caerulea* L.), also known as honeyberry and haskap, is a perennial deciduous shrub. It is an excellent source of various beneficial substances, and consuming its fruit can help prevent multiple chronic diseases. Scientific research has shown that blue honeysuckle possesses rich nutritional and medicinal values(Kula *et al.*, 2016). Being a pure natural wild edible berry, blue honeysuckle has caught people's attention in recent years due to its excellent characteristics and has been hailed as

the 'King of the Third Generation of Small Berries.' This plant, originating from the cold climates of Europe, North America, and Northern Asia (Thompson *et al.*, 2006), is now widely distributed in the Greater and Lesser Xing'an Ranges, Changbai Mountain in Northeast China, and in Northwestern regions such as Xinjiang, Inner Mongolia, Qinghai, and Gansu. Blue honeysuckle exhibits outstanding cold resistance, requiring no soil burial for overwintering, and can withstand low temperatures down to -50°C , and even during the flowering period, it can tolerate temperatures from -8°C to 0°C (Frier *et al.*, 2016). Moreover, blue honeysuckle has strong soil adaptability, thriving in soil environments with a pH range of 4.5 to 7.5. It also demonstrates strong disease resistance with less susceptibility to pests and diseases. Blue honeysuckle is an emerging small berry species following bayberry, blueberry, black currant, raspberry, and seabuckthorn, serving as an economic tree species with multiple functions. Its branches, leaves, flowers, and fruits have developmental and utilization values. It has been processed into various types of food products like juice, dried fruit, fruit wine, fruit tea, jam, pastry, freeze-dried powder, yogurt, and ice cream (Khattab *et al.*, 2016; Oszmiański *et al.*, 2018).

In the Qilian Mountain area, the variety of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye) is widely distributed at elevations of 2000 to 3000 meters, typically growing under spruce forests, along forest edges, and in ditches, showcasing a strong ability to adapt to harsh environments. It is one of the rare important edible and medicinal precious plants in high-altitude areas. However, due to the lack of basic research and attention, the resources in this area have not been fully developed and utilized. Therefore, the primary goal of this research.

Materials and Methods

Experimental materials

In mid-July to early August 2023, a comprehensive survey was conducted on different forest stands in various regions of Qilian Mountain area. This survey aimed to gather information on the growth conditions of the forest stands. Within the selected regions, five healthy and undamaged intermediate to nearly mature blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye) shrubs were chosen for investigation and sampling. Various growth parameters were measured, including tree height, number of branches, the diameter of the thickest branch, height from the ground to the lowest branch, crown width, branch thickness of both large and small fruiting branches, the number of leaves on fruiting branches per unit length, and the number of fruits on fruiting branches. The initially selected superior individual shrubs were assigned unique identifiers and labeled for tracking purposes. Detailed records of latitude, longitude, altitude, aspect, slope position, and slope gradient were documented, forming comprehensive records of these outstanding trees.

According to the distribution records of blue honeysuckle in the Chinese Virtual Herbarium (<http://www.cvh.ac.cn/>, accessed on 12 May 2023) and iPlant the field investigation, this study selected 8 counties as the research population sites in the areas where blue honeysuckle is distributed in the Qilian Mount

ain area.

The blue honeysuckle in the study area is mostly distributed in the suburbs, roads, or villages, and a small part is distributed in parks or temples. Almost all of these areas, there is soil drought and poor site conditions. In the field investigation, a GPS positioning system was used to obtain the altitude, longitude, and latitude of each group. The climate data were obtained from the WorldClim data website, covering the period from 2001 to 2020, with a spatial resolution of 2.5 minutes. The soil data is sourced from the 2023 edition of the Harmonized World Soil Database version 2.0, with a depth range of 0-20 cm. The data were processed and extracted using ArcGIS software (Table 1 and Fig. 1).

Tab. 1 Geographical locations and main climatic and environment information conditions of eight blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye) populations.

Populations	Sample size	Longitude (°E)	Latitude (°N)	Altitude (m)	Annual Mean Temperature (°C)	Annual precipitation (mm)	Mean precipitation of wettest quarter (mm)	Mean temperature of the wettest quarter (°C)	Mean precipitation of driest quarter (mm)	Mean temperature of the driest quarter (°C)	Soil type
Ledu(LD)	5	102.40	36.70	2745.2	2.37	474.5	284.2	11.65	3.0	-8.25	CMe
Yongdeng(YD)	5	102.71	36.65	2507.1	4.39	419.0	246.8	14.15	2.9	-6.89	CHI
Huzhu(HZ)	5	102.51	36.83	2576.2	2.89	438.0	262.9	12.48	2.9	-8.24	CHI
Tianzhu(TZ)	5	102.67	36.92	2825.0	2.78	415.6	252.4	12.33	3.5	-7.86	CHI
Pingan(PA)	5	101.91	36.34	2728.8	3.78	487.2	288.2	12.98	3.6	-6.93	LPe
Menyuan(MY)	5	102.02	37.15	2779.3	-0.19	524.8	315.4	9.48	4.6	-10.85	CHI
Datong(DT)	5	101.78	37.00	2894.5	2.49	502.7	302.9	11.76	4.7	-8.30	CHI
Tongren(TR)	5	101.90	35.63	2900.3	2.70	560.3	317.2	11.77	5.5	-7.41	CHh

ALT, Altitude; LON, Longitude; LAT, Latitude; AMT, Annual Mean Temperature; AP, Annual precipitation; AMRH, Annual Mean Relative Humidity; WP, mean precipitation of wettest quarter; WT, mean temperature of the wettest quarter; DP, mean precipitation of driest quarter; DT, mean temperature of the driest quarter. ST, Soil type

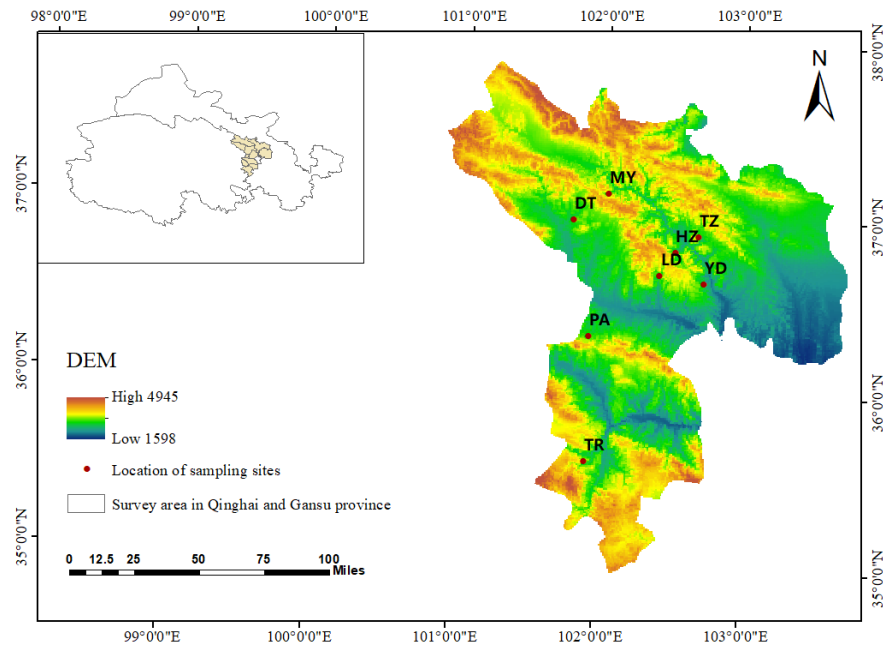


Fig. 1 Geographical distribution of survey sites for various populations of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye). Geo-elevation data were derived from an SRTM DEMUTM 90 M resolution digital elevation in the Geospatial Data Cloud (<http://www.gscloud.cn/>, accessed on 14 December 2023). ArcGIS 10.8 geographic information analysis software was used for data processing to generate distribution maps.

Morphometric Analysis

In each population, five plants with normal growth, no obvious defects and diseases and insect pests, and a plant age > 5a were randomly selected as research samples, and the spacing of samples was more than 15 m. The plant height, diameter at breast height, and branch height of the sample plants were measured, respectively. At the same time, five groups of complete branches, leaves, and fruit were evenly selected from the upper, middle, and lower parts of each sample plant, and the relevant indicators were measured on site.

The phenotypic traits of blue honeysuckle were divided into quantitative traits and quality traits. Quantitative traits, including 28 traits such as tree height, crown diameter, and diameter at breast height, were quantitatively evaluated (Table 2). The qualitative traits included 13 traits such as trunk type, crown roundness, canopy density, and the qualitative evaluation method was used (Table 3).

Tab. 2 Quantitative traits and abbreviations of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye).

No.	Trait	Code	Unit	No.	Trait	Code	Unit
1	Tree height	TH	m	15	Leaf width	LWi	mm
2	Under-branch height	UBH	m	16	Leaf shape index	LSI	-
3	base diameter of the thickest branch	BDTB	mm	17	Leaf thickness	LTh	mm
4	Crown length diameter	CL	m	18	Leaf area	LA	mm ²
5	Crown short diameter	CS	m	19	Leaf weight	LWe	g

6	crown width	CW	m	20	Stipule diameter	StD	mm
Crown ratio of length							
7	diameter to	LS	-	21	Petiole length	PL	mm
short diameter							
8	Branching number	BN	number	22	Petiole diameter	PD	mm
diameter of fruiting twigs							
9		DFT	mm	23	fruit length	FL	mm
diameter of fruiting branches							
10		DFB	mm	24	fruit width	FW	mm
diameter of major fruiting branches							
11		DMFB	mm	25	fruit shape index	FSI	-
number of leaves on fruiting branches							
12		NLFB	number	26	hundred-fruit weight	HFW	g
fruiting density per unit length							
13		FD	number (per50cm)	27	Fruit stalk length	FSL	mm
Leaf length							
14		LL	mm	28	Fruit stalk diameter	FSD	mm

LSI = LL/LW, FSI = FL/FW. The 'Fruiting density per unit length (FD)' is the number of fruits per unit length of 50cm on the branch.

Tab. 3 Grading assignment of quality traits of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye).

No.	Trait	Code	Grading Assignment					
			1	2	3	4	5	6
1	Trunk type	TT	creeping	leaning	upright	-	-	-
Crown roundness								
2		CR	Messy	Moderate	Rounded	-	-	-
Canopy density								
3		CD	Low	Medium	High	-	-	-
Branch color								
4		BC	light Brown	Ash Brown	Dark Taupe	Rusty Red	Dark Brown	Deep Auburn
Leaf shape								
5		LeS	Ovate	Oval	Long Oval	Elliptic	Lanceolate	Narrow Lanceolate
Leaf apex shape								
6		LAS	Caudate	Acuminate	Acute	Obtuse	-	-
Leaf base shape								
7		LBS	Cuneate	Acute	Rounded	-	-	-
Leaf color								
8		LC	Green(a)	Green(b)	Green(c)	Green(d)	Green(e)	-

9	fruit shape	FS	Spherical	Conical	Eggplant-shaped	Oval	Capsicum-shaped	Banana-shaped
10	fruit apex shape	FAS	Depressed	Truncate	Rounded	Acute	Hooked	-
11	fruit base shape	FBS	Depressed	Truncate	Rounded	Acute	-	-
12	fruit color	FC	Medium Lavender Blue	Medium Dark Blue Violet	Dark Blue Violet	Dark Delft Blue	Very Dark Blue Violet	Navy Blue
13	Fruit Taste	FT	Grade A	Grade B	Grade C	Grade D	Grade E	-

* The lowercase letters represent different shades of the same color. The level a is the lightest, and the color is deepened from a to e.

The Trunk and branch indices were determined on-site in the plot, while tree height was measured using a tree-height-measuring instrument. Crown diameter, diameter at breast height, and under-branch height were measured using a tape measure. Ground branching number, fruit count, and leaf count were tallied and recorded. The methodology for assessing leaf and fruit phenotypic characteristics is as follows: For each selected individual shrub, leaves from different orientations (north, south, east, west) and heights (upper, middle, lower) of the fruiting branches, along with all fruits, were collected and segregated into separate bags. These samples were brought back to the laboratory and thoroughly mixed. Subsequently, 50 leaf samples and 100 fruit samples were randomly chosen for leaf and fruit phenotypic diversity assessment. Length measurements were taken using a digital caliper accurate to 0.01mm, and weight measurements were obtained using an electronic balance accurate to 0.0001g. Each measurement was repeated three times. Leaf and fruit length indices were measured using a scale and vernier caliper. Leaf area was determined using a leaf area analyzer. Fruit color, leaf upper surface color, leaf lower surface color, and branch color were compared and recorded using color cards.



Fig. 2 Legend of some traits of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye). (A) TT—Trunk type: (A1) creeping; (A2) leaning; (A3) upright. (B) Three different levels of branch diameter: (B1) DFT—diameter of fruiting twigs; (B2) DFB—diameter of fruiting branches; (B3)DMFB—diameter of major fruiting branches.

Statistical Analysis

Analysis of Variance: Statistically significant differences within and among populations were determined by nested analysis of variance. The linear model comprises the following:

$$\gamma_{ijk} = \mu + \tau_i + \delta_j(i) + \varepsilon_{ijk}$$

γ_{ijk} is the k th observed value of the j th family in the i th population, μ is the total average, τ_i is the effect value among populations, $\mu_j(i)$ is the random effect value of the individual plant (family) in the i th population, and ε_{ijk} is the random error.

Phenotypic Differentiation Coefficient: The phenotypic differentiation within and among populations was reflected by the phenotypic differentiation coefficient (VST). The formula comprises the following:

$$VST = \sigma_{2t/s} / \sigma_{2t/s} + \sigma_{2s}$$

$\sigma_{2t/s}$ is the variance component among populations, and σ_{2s} is the variance component within the population (family)(Ge *et al.*, 1988; Wang *et al.*, 2023).

(3) Descriptive Statistics: Descriptive statistics were performed on the morphometrics of each population using SPSS 20.0 software, including the arithmetic mean ($\bar{X}$), standard deviation (SD), Dun-ca's multiple comparison, and coefficient of variation (CV) to determine the range of variation.

$$CV = SD/\bar{X}$$

(4) Diversity Index: The diversity index of each trait was calculated by EXCEL2010 software. The Shannon-Wiener index (H') is a quantitative expression used to describe the degree of variation of trait diversity. The calculation formula is the following:

$$H' = -\sum (P_i \times \ln P_i)$$

H' is the diversity index and P_i is the effective percentage of the distribution frequency in the i th grade material of a trait. The distribution frequency of quality traits is expressed as the percentage of the number of sample plants at a certain level of traits to the total number of sample plants. The average value and standard deviation of the quantitative traits were calculated, and the obtained quantitative trait data were graded and standardized. According to the total average (X) and standard deviation (SD), they were divided into 10 levels. Among them, level 1 was less than $X - 2SD$, level 10 was greater than $X + 2SD$, and the difference between each level was $0.5SD$ (Maughan *et al.*, 1995; Zeng *et al.*, 2021).

Multivariate Analysis: Multivariate statistical methods were used to further determine the structure and differences in the phenotypic traits of blue honeysuckle. The correlation between each trait and the correlation between traits and geographical climatic factors were obtained by Pearson's correlation analysis.

Principal component analysis (PCA) was used to reduce the dimension of each index to obtain the key indexes affecting the phenotype of blue honeysuckle. Loading plot and biplot analysis were performed according to the obtained PC1, PC2, and PC3. Ward cluster analysis was used to divide the studied samples into different groups, and a circular tree diagram was constructed with the square Euclidean distance as the measurement method (Jain *et al.*, 1975). Before the clustering analysis, the original data were processed by Z standardization (STD) to eliminate the influence of different dimensions on the analysis. The above statistical analysis and graphics were completed by SPSS20.0 and Origin 2022 software.

Results

Phenotypic Variation of Traits

The results of the performed statistical analysis are shown in Table 4. Of all measured traits, the coefficient of variation (CV) ranged from 11.53% to 59.33%, with an average of 29.05%. The highest CV was in Branching number (BN) (59.33%), followed by Leaf weight (LWe) (50.00%), and fruit shape (FS) (42.99%), and the lowest was in fruit width (FW) (11.53%). The average coefficient of variation of traits was greatest in the trunk (32.80%), followed by the branches (30.30%), and leaves (27.09%), and lowest in the fruits (26.92%). The results showed that among blue honeysuckle traits studied, the trunk traits were more phenotypically variable overall than the branch, leaf and fruit traits. In addition, leaf and fruit traits related to its form showed more variability compared to those relating to leaf size.

Diversity Index of Phenotypic Traits

The phenotypic diversity index of 28 quantitative traits ranged from 1.67 to 2.08 (Table 4), with an average of 1.974. Among them, the diversity of the fruit width (FW) was the highest (2.079). The diversity indexes of crown length diameter (CL), diameter of major fruiting branches (DMFB), number of leaves on fruiting branches (NLFB), leaf length (LL), leaf width (LWi), leaf shape index (LSI), stipule diameter (StD), petiole length (PL), petiole diameter (PD), fruit length (FL), fruit width (FW), fruit shape index (FSI), fruit stalk length (FSL), and Fruit stalk diameter (FSD) also exceeded 2. The branching nu

mber (BN) was the lowest (1.668).

On the other hand, there are different degrees of difference in the distribution frequency of 13 quality traits. Among them, the number of sample plants with trunk type (TT), crown roundness (CR), and canopy density (CD) of grade three accounted for 35.0%, 35.1% and 35.0%. Compared to the other two grades, the differences are not significant, indicating a relatively even distribution of blue honeysuckle plant types among different growth patterns. The leaf apex shape (LAS) was mostly acute (44.4%), whereas within the leaf base shape (LBS), acute was the most prevalent (36.0%), and the distribution of branch color (BC), leaf shape (LeS), leaf apex shape (LAS), leaf base shape (LBS), leaf color (LC), fruit shape (FS), fruit apex shape (FAS), fruit base shape (FBS), and fruit color (FC) was small at various levels. According to the Shannon–Wiener diversity indexes of the 13 quality traits, the diversity of the different traits was different (Table S1). The diversity indexes ranged from 0.78 to 1.73, with an average of 1.313. The diversity of Fruit Taste (FT) was the highest (1.732). The diversity of leaf apex shape (LAS) was the lowest (0.781).

In general, 28 quantitative traits exhibited a high phenotypic diversity, surpassing their quality trait diversity indexes significantly. This suggests that the phenotypic quantitative traits of blue honeysuckle are relatively more abundant compared to their quality traits. Overall, both fruit and leaf parts of blue honeysuckle exhibit a high degree of diversity, with fruit characteristics showing slightly higher diversity.

Tab. 4 Summary statistics of the 41 morphological characters studied in the 40 trees of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye) distributed in the 8 natural populations of China.

No.	Trait	Code	Min	Max	Mean	SD	CV(%)	H'
1	Tree height	TH	2.2	6.7	3.49	1.02	29.23	1.79
2	Under-branch height	UBH	0.4	1.81	1.06	0.34	32.08	1.89
3	base diameter of the thickest branch	BDTB	29.83	80.43	47.23	12.63	26.74	1.79
4	Crown length diameter	CL	1.2	5.47	3.21	0.92	28.66	2.04
5	Crown short diameter	CS	0.95	5.32	2.59	0.89	34.36	1.93
6	crown width	CW	1.08	5.4	2.90	0.85	29.31	1.98
7	Crown ratio of length diameter to short diameter	LS	0.27	0.98	0.81	0.15	18.52	1.85
8	Branching number	BN	1	14	4.45	2.64	59.33	1.67
9	Trunk type	TT	1	3	2.15	0.74	34.42	1.05
10	Crown roundness	CR	1	3	2.13	0.72	33.80	1.04
11	Canopy density	CD	1	3	2.15	0.74	34.42	1.05
12	diameter of fruiting twigs	DFT	0.69	2.04	1.14	0.28	24.56	1.96
13	diameter of fruiting branches	DFB	1.23	4.59	2.50	0.69	27.60	2.00
14	diameter of major fruiting	DMFB	2.84	8.99	5.74	1.11	19.34	2.07

	branches							
15	number of leaves on fruit ing branches	NLFB	28	106	61.88	18.65	30.14	2.05
16	fruiting density per unit l ength	FD	23	157	56.86	23.61	41.52	1.88
17	Branch color	BC	1	6	3.08	1.19	38.64	1.55
18	Leaf length	LL	26.60	79.53	46.05	8.92	19.37	2.06
19	Leaf width	LWi	8.50	32.77	15.92	3.52	22.11	2.02
20	Leaf shape index	LSI	1.72	4.63	2.94	0.43	14.63	2.03
21	Leaf thickness	LTh	0.07	0.23	0.14	0.02	14.29	1.99
22	Leaf area	LA	1.48	15.64	4.92	1.96	39.84	1.95
23	Leaf weight	LWe	0.01	0.12	0.04	0.02	50.00	1.95
24	Stipule diameter	StD	2.54	11.93	5.42	1.54	28.41	2.01
25	Petiole length	PL	1.99	7.64	4.11	0.71	17.27	2.06
26	Petiole diameter	PD	0.31	1.66	0.77	0.19	24.68	2.06
27	Leaf shape	LeS	1	6	3.60	1.34	37.22	1.66
28	Leaf apex shape	LAS	1	4	2.28	0.55	24.12	0.78
29	Leaf base shape	LBS	1	3	2.35	0.77	32.77	1.00
30	Leaf color	LC	2	6	3.50	0.96	27.43	1.34
31	fruit length	FL	9.30	24.70	15.83	2.20	13.90	2.06
32	fruit width	FW	4.33	10.16	7.11	0.82	11.53	2.08
33	fruit shape index	FSI	0.24	3.32	2.24	0.31	13.84	2.08
34	hundred-fruit weight	HFW	28.34	75.42	43.36	10.03	23.13	1.91
35	Fruit stalk length	FSL	2.69	12.58	6.24	1.41	22.60	2.06
36	Fruit stalk diameter	FSD	0.36	1.50	0.75	0.16	21.33	2.06
37	fruit shape	FS	1	6	3.28	1.41	42.99	1.69
38	fruit apex shape	FAS	1	5	2.93	1.05	35.84	1.41
39	fruit base shape	FBS	1	4	2.58	0.96	37.21	1.32
40	fruit color	FC	1	5	3.48	1.13	32.47	1.45
41	Fruit Taste	FT	1	6	3.88	1.60	41.24	1.73

Note: Min—minimal value; Max—maximal value; SD—standard deviation; CV—coefficient of variation (%). H' —Diversity index H' . Calculate d using all the data (includes the repeated measurements within a tree).

Phenotypic Differences among and within Populations

The variance analysis of blue honeysuckle quantitative traits showed that 14 traits were significantly different among populations ($p < 0.01$), and all 20 traits were significantly different within populations ($p < 0.01$). It can be observed that significant differences exist among populations for most phenotypic t

raits. However, within the same population, differences among individual plants are even more pronounced. (Table 5).

According to the results of the nested variance analysis, the variance components and phenotypic differentiation coefficients of 20 phenotypic traits of blue honeysuckle among and within populations were calculated, respectively. The phenotypic differentiation coefficients of each trait among populations ranged from 11.96% to 73.45%, with an average of 49.23%. The traits with the largest and smallest phenotypic differentiation coefficient were FSD and LSI. This shows that the Fruit stalk diameter is more differentiated among populations, while the Leaf shape index is less differentiated and relatively stable. The average variance components of phenotypic traits among and within populations accounted for 26.37% and 27.45% of the total variation, respectively. The percentage of variation was slightly higher among the trees within populations compared to that among populations (Table 5 and Fig. 3). However, the error component accounted for the greatest part of the total variation for the majority of measured leaf traits.

Tab. 5 The proportion of variance components, population differentiation coefficients and F values of 20 quantitative traits based on all the data (includes the repeated measurements within a tree) in 8 natural populations of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye) in China.

Traits	F Value		Proportion of Variance Components (%)			Phenotypic Differentiation Coefficients (%)
	Among Populations	Within Populations	Among Populations	Within	Random	
				Populations	Errors	
DFT	3.04 *	16.20 **	28.21	42.37	29.42	39.97
DFB	3.97 **	19.98 **	35.69	41.14	23.17	46.45
DMFB	1.71	12.54 **	16.48	44.02	39.50	27.24
NLFB	7.75 **	2.57 **	46.22	27.26	26.52	62.90
FD	2.20	21.21 **	30.06	62.56	7.37	32.46
LL	8.78 **	6.30 **	33.71	17.54	48.75	65.77
LWi	5.83 **	5.43 **	23.20	18.18	58.62	56.06
LSI	0.62	8.29 **	4.18	30.80	65.01	11.96
LTh	2.91 *	9.98 **	18.87	29.43	51.70	39.06
LA	6.49 **	6.28 **	27.28	19.21	53.51	58.68
LWe	5.20 **	5.98 **	22.36	19.88	57.76	52.94
StD	7.36 **	6.74 **	30.92	19.20	49.87	61.69
PL	2.65 *	3.54 **	8.89	15.33	75.78	36.71
PD	4.17 **	6.60 **	19.99	21.91	58.10	47.70
FL	4.39 **	21.64 **	26.43	27.50	46.07	49.00
FW	5.20 **	11.60 **	21.60	19.01	59.40	53.19
FSI	4.65 **	30.94 **	31.89	31.36	36.75	50.42

HFW	6.56 **	46.69 **	57.66	40.19	2.15	58.93
FSL	6.84 **	3.68 **	20.62	13.78	65.60	59.94
FSD	12.65 **	2.13 **	23.10	8.35	68.55	73.45
Mean			26.368	27.45	46.18	49.23

*, $P < 0.05$; **, $P < 0.01$; phenotypic trait abbreviations are shown in Table 2.

Statistically significant differences among and within populations were confirmed for mostly quantitative studied traits at a significant level $p < 0.01$. The percent of variation was significantly higher among the trees within populations compared to the one among populations (Fig. 3). However, the component of error accounted for the greatest part of total variation for the majority of measured leaf traits. (007)

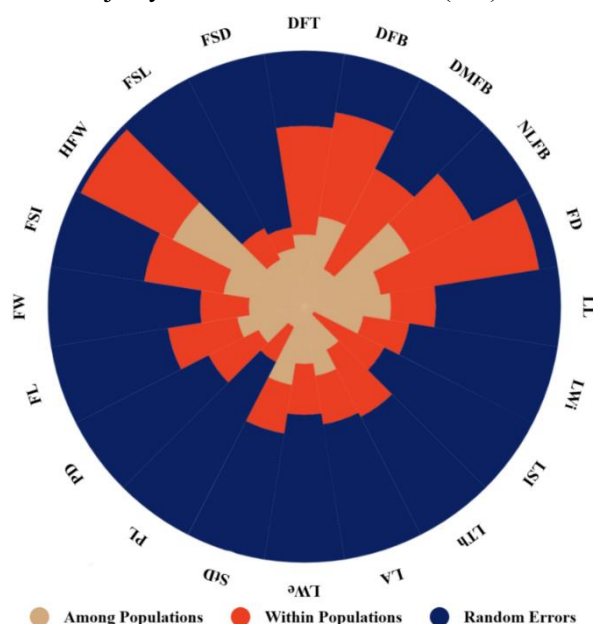


Fig. 3 Partitioning of total variance by hierarchical level for studied phenotypic traits. phenotypic trait abbreviations are shown in Table 2.

Variation Degree of Phenotypic Traits

The variation range and degree of 28 quantitative traits of eight populations are reflected by the mean value, standard deviation, and coefficient of variation(CV). The results show that there was abundant phenotypic variation in the quantitative traits of blue honeysuckle. The variation levels of different populations were different. The average CV of seven in eight populations was more than 15%. The highest CV was found in the Huzhu population(21.10%), and the lowest was in the Datong population(14.48%), but still close to 15%.

There were also significant differences in the variation levels of different traits. The CV of each trait ranged from 11.53% to 59.33%, with an average of 29.05%. Among them, the trait with the highest CV was branch number (BN) (59.33%). At the same time, the CVs of the Leaf weight (LeW)(50.00%), fruit shape(FS)(42.99%), fruiting density per unit length(FD)(41.52%) were also large, with rich polymorphism and variability. The fruit width h(FW)(11.05%), fruit shape index(FSI)(13.84%) and fruit length(FL) (13.90%) are the tree traits with the smallest CVs and relative stability. In general, the CV of traits related to fruits and leaves was small, while the CV of traits related to trunk shape was large. The CV of the same trait was also significantly different among different po

pulations.

Tab. 6 Coefficients of variation and Shannon-Wiener diversity indexes of phenotypic quantitative traits of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye).

Traits	Coefficient of Variation (CV) (%)							
	LD	YD	HZ	TZ	PA	MY	DT	TR
TH	25.20	34.59	47.27	28.98	10.99	29.05	15.81	7.11
UBH	12.54	43.21	15.89	14.50	14.82	50.34	37.29	21.66
BDTB	6.65	23.47	21.40	29.35	21.54	6.42	22.61	21.09
CL	13.39	38.82	35.09	20.29	20.82	21.83	31.83	16.63
CS	8.42	48.21	44.48	28.63	23.96	10.36	33.93	41.93
CW	10.31	41.93	38.96	23.79	21.79	15.18	32.27	23.58
LS	10.19	20.35	15.64	9.59	9.40	19.74	10.67	37.88
BN	73.38	38.03	84.71	35.97	37.42	61.41	42.13	31.04
DFT	20.61	22.55	14.43	19.50	19.45	5.56	15.41	10.11
DFB	21.10	9.62	16.66	29.12	22.10	5.08	16.93	19.34
DMFB	7.21	12.62	21.07	17.97	14.34	13.86	12.24	14.58
NLFB	20.61	15.12	24.32	25.24	18.57	15.78	8.12	12.39
FD	32.69	47.30	29.50	35.66	41.50	26.44	24.56	21.14
LL	8.63	6.30	14.78	11.02	12.55	1.91	4.41	4.16
LWi	16.38	6.03	15.20	7.87	9.30	7.76	5.09	7.37
LSI	10.01	8.57	7.44	12.89	10.98	6.47	1.00	9.86
LTh	7.92	9.88	4.42	6.07	5.73	3.15	3.02	20.77
LA	23.96	8.62	33.28	14.48	16.36	12.59	12.01	5.38
LWe	26.60	12.22	30.92	16.74	14.88	15.13	13.27	9.81
StD	21.30	11.15	16.53	12.19	9.37	19.52	6.00	16.78
PL	5.34	4.52	7.22	6.34	10.59	6.15	7.05	11.92
PD	8.73	7.94	9.00	14.60	17.44	14.87	14.13	15.28
FL	10.56	5.70	4.16	2.75	10.24	12.39	6.39	5.13
FW	6.71	10.20	4.04	4.40	3.64	3.28	1.78	6.00
FSI	9.67	11.21	3.63	5.87	9.38	13.19	5.28	4.59
HFW	22.31	17.14	22.32	7.77	14.04	10.73	13.96	13.34
FSL	11.07	7.02	5.28	6.87	7.71	15.46	5.90	12.45
FSD	6.76	6.48	3.17	3.61	7.27	9.34	2.32	14.18
Mean	16.37	18.89	21.10	16.14	15.58	15.46	14.48	15.55

Phenotypic trait abbreviations are shown in Table 2.

Correlations among Phenotypic Traits

Pearson's correlation coefficient was used to show the correlations between traits (Fig. 4B), and ther

There were different degrees of correlation among 28 phenotypic traits of blue honeysuckle. It was found that 16 pairs of traits were significantly correlated ($p < 0.001$), 19 pairs of traits were significantly correlated ($p < 0.01$) and 49 pairs of traits were significantly correlated ($p < 0.05$), including TH and UBH; CS, CW, LL, LWi, LA, and LWe; PL, PD, BDTB, LL, and LA; FL, FW, and FSI; and DFT, DFB, and FSD. At the same time, the following were also positive correlated with each other: between TH and UBH; between BDTB and DFT; between LS and CS; and between FD, and DFT ($p < 0.01$). This shows that these morphological characteristics are mutually reinforcing and a common variation. At the same time, there were significant negative correlations ($p < 0.05$) between NLFB and LS, BN, DFB; between LSI and LWi, FSD; between LTh and UBH, BDTB; between StD and CL, CW, LL, LWi; between FL and LL, LA, LWe; between FSI and LL, LWi, LA, LWe, FW; and between FSD and CL, LSI.

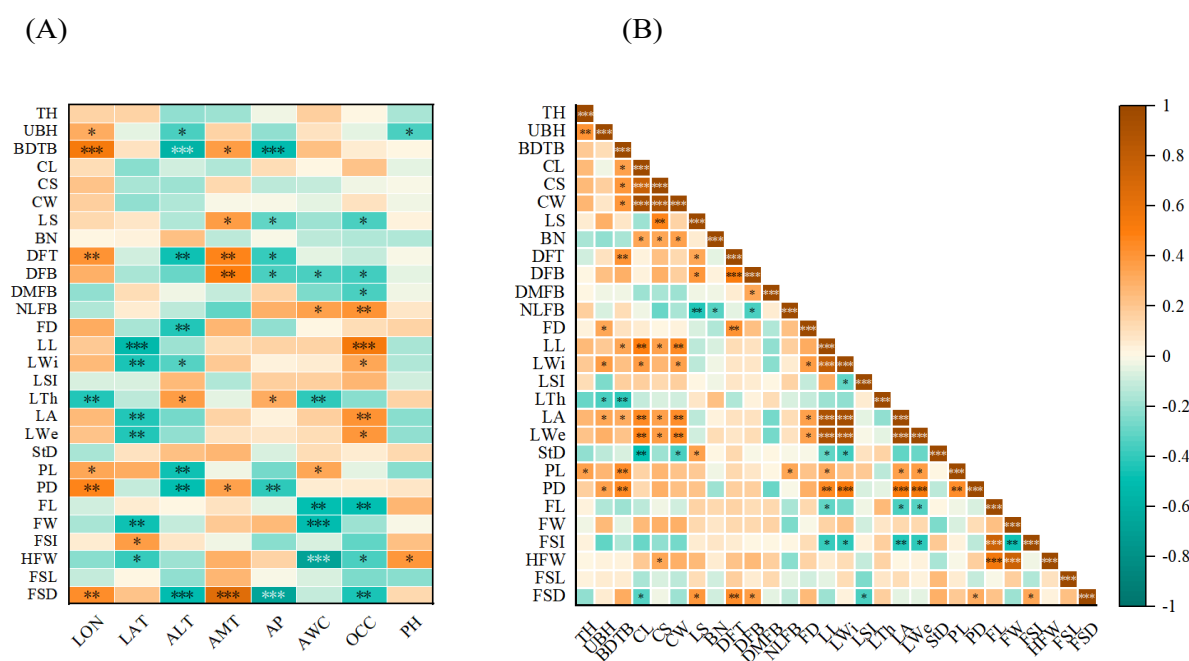


Fig. 4 Correlation heat map of phenotypic traits of blue honeysuckle. (A) Correlations among X phenotypic traits; (B) Correlations between phenotypic traits and geo-climatic factors. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; Black zone: because at least one variable was constant, calculations could not be performed. Geo-climatic factors and phenotypic trait abbreviations are shown in Tables 1–3.

Correlation between Phenotypic Traits and Geo-Climatic Factors

Some phenotypic traits of blue honeysuckle were strongly correlated with geographical and climatic factors (Fig. 4A). Among them, BDTB, DFT, PD, and FSD were positively correlated with longitude and annual mean temperature and negatively correlated with altitude and annual precipitation. UBH and PL was positively correlated with longitude and negatively correlated with altitude. LS and DFB was positively correlated with annual mean temperature and negatively correlated with annual precipitation. On the contrary, between LTh and longitude and AWC for rootable soil depth showed a significant negative correlation, and latitude and annual precipitation showed a significant positive correlation. In addition, there was a significant or extremely significant positive correlation between FSI and latitude, PL, NLFB and AWC for rootable soil depth, NLFB, LL, LWi, LWe, and organic carbon content, and HFW and pH in

water. At the same time, there were significant or extremely significant negative correlations between LL, LWi, LA, LWe, FW, HFW and latitude, FD, LWi, and altitude, DFB, FL, FW, HFW and AWC for rootable soil depth, LS, DFB, DMFB, FL, HFW, FSD, and organic carbon content, UBH and pH in water. The above results show that in the phenotypes of blue honeysuckle, the indexes related to leaf, fruit and branch parts were affected by geographical, climatic and soil factors to a certain extent, among which organic carbon content and altitude had a greater influence on the phenotypic traits of blue honeysuckle than other factors.

Principal Component Analysis of Phenotypic Traits

Principal component analysis was used to reduce the dimension of 41 phenotypic traits of blue honeysuckle, and the top 13 principal components with the largest proportion were extracted. The eigenvalues were all greater than 1, and the cumulative contribution rate was 83.48%, indicating that these 13 principal components could objectively reflect the basic information of phenotypic traits of blue honeysuckle (Fig. 5A). The contribution rate of PC1 was 17.10%, among which LA, LWe, LL, LWi, CW, CL, CS, and PD had higher loading degrees, which mainly reflected the characteristics related to the size of leaf and crown. The contribution rate of PC2 was 10.23%, among which LS, FL, FSD, FS, DFB, HFW, DFT and NLFB had higher loading degrees, which mainly reflected the characteristics of fruit trait and branch size. The contribution rate of PC3 was 9.87%, among which BC, FSD, FD, CS, CL, LeS and CW had higher loading degrees, which mainly reflected the characteristics related to Crown width and fruiting ability. The contribution rate of PC4 was 8.37%, and the loading degrees of LSI, LeS, LAS, BDTB, and TT were higher, which mainly reflected the characteristics of leaf and trunk shape. The resultant nine principal components reflected the characteristics of Tree growth and fruit shape, size and taste, respectively.

The scatter plot constructed according to PC1 and PC2 shows the relationships between samples (Fig. 5B). The geographical distribution of different populations had a certain effect on the phenotypic traits of blue honeysuckle and the effects on leaf size and crown traits (PC1) were greater than those of fruit and branch traits (PC2). The phenotypic traits of Yongdeng and Ledu were relatively less affected by geographical factors, while the phenotypic traits of other populations were greatly affected by geographical factors. The samples of Datong were more concentrated in the scatter plot, while the scatter distribution of samples from Ledu and Yongdeng, as well as Tianzhu and Pingan, exhibited the closest proximity to each other. The samples from Huzhu being the most dispersed.

(A)

(B)

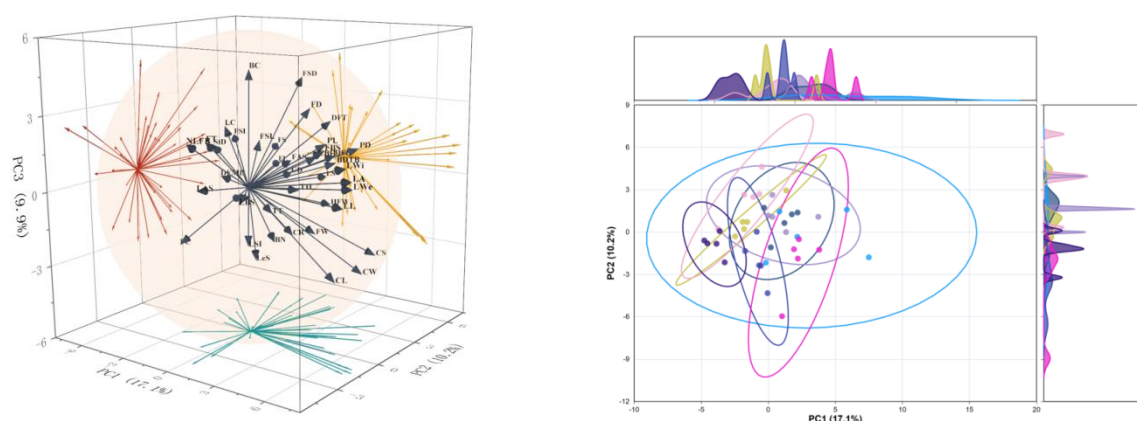


Fig. 5 Principal component analysis results of phenotypic traits of blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye). (A) The load of phenotypic traits and its projections on the first three principal component axes. The length of the line represents the size of the load, (B) Scatter plot of 40 samples based on PC1 and PC2. Phenotypic trait abbreviations are shown in Tables 2 and 3.

Cluster Analysis of Blue Honeysuckle Samples

The Ward method was used for cluster analysis based on the squared Euclidean distance, and 40 samples were clustered into five clusters according to their morphological characteristics (Fig. 6A). Cluster A contains three subclasses, a total of 15 samples, most of which are from Datong, Ledu, and Tianzhu. Cluster A exhibits smaller dimensions across various leaf indicators, with the slenderest petioles and the lowest yield. However, it features rounder crowns and the highest leaf shape index. Cluster B contains eight samples, in two subclasses, half of which originate from Pingan. The features of B cluster encompass larger fruit dimensions, a higher fruit shape index, pointed leaf and fruit tips, thicker foliage, notably increased branch number, and a more erect tree structure. Nevertheless, the plants exhibit shorter stature, slender stem diameter, reduced leaf number, and subpar fruit flavor. Cluster C contains 8 samples, mainly from Menyuan and Tongren. The samples in this cluster display the tallest plant height, the highest leaf count, deep fruit color, and excellent fruit flavor. However, they also exhibit the thinnest fruit-bearing branches, lighter branch coloration, smallest stipule diameter, relatively smaller fruit size, and slender short fruit stalks. Cluster D contains 8 samples in two subclasses, mainly from Huzhu, Tongren, and Yongdeng. The crown width, stem diameter, leaf size, and petiole length of this cluster surpass those of the other clusters. However, its tree form is bent, crown shape irregular, crown density low, leaves thin, leaf color light, fruits short, fruit shape index small, fruit color light, with a relatively favorable taste. Only one plant from Yongdeng was classified into Cluster E. During the survey and sampling process, we encountered a remarkably unique plant, in comparison to other clusters, it had only one branch, with a relatively low tree height but a significant under-branch height. Its crown width was small, yet it boasted a dense crown layer. The fruit-bearing branches were thick, with large stipule diameters. The leaves were deep in color, and both the fruit and leaf stalks were long and thick. This plant yielded an exceptionally high quantity of fruit, characterized by a delightful taste.

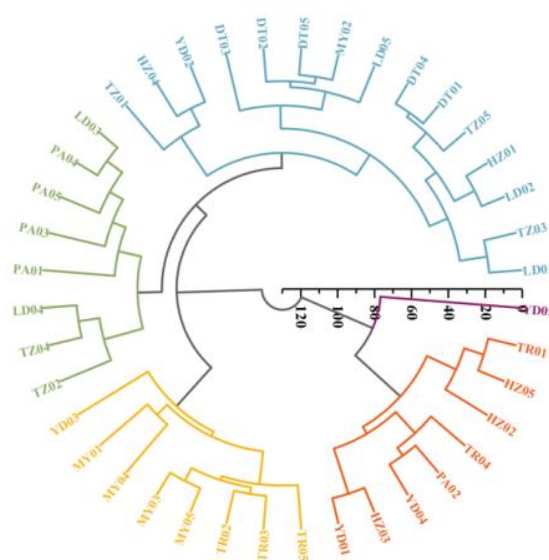


Fig. 6 Ward clustering analysis results based on the squared Euclidean distance for 40 blue honeysuckle (*Lonicera caerulea* L. var. *angustifolia* Bunye) plants.

Discussion

The phenotypic variation of plants is the result of the combined action of genes and the environment over a long period of evolution (Miljković *et al.*, 2019). The abundance of phenotypic variation may reflect, to a certain extent, its genetic diversity. Phenotyping of traits is the most direct method to survey and evaluate the diversity of forest germplasm resources, which is crucial for their reasonable breeding and conservation (Li *et al.*, 2022). Blue honeysuckle is widely distributed in the mountain forests of eastern Qinghai and southern Gansu Province, especially in the mountain valleys of the Qilian Mountains. There are great differences in altitude, temperature, air pressure, annual precipitation, and soil. The changeable natural conditions and strong regional differences make some traits of blue honeysuckle produce genetic differentiation among different populations. In this study, we comprehensively assessed the variation of 41 phenotypic traits in trunk branches leaves and fruits of blue honeysuckle from 8 natural populations. We also investigated the relationships between these traits and geoclimatic factors and the effects of these factors on phenotypic variation.

The research findings indicate that the phenotypic variation of blue honeysuckle was quite rich, the differences in traits both within and among different populations were extremely significant ($p < 0.01$). The phenotypic differentiation coefficient is the percentage of variation among populations in the total variation, reflecting the differentiation of species in traits. The higher the coefficient, the easier the population differentiation. Our study showed that the average phenotypic differentiation coefficient among blue honeysuckle populations was 49.63%, indicating that the genetic differentiation degree of phenotypic traits of blue honeysuckle was relatively high.

gh among populations. This may be due to the large differences in climatic and environmental conditions among the distribution areas of various populations investigated in this study. Among them, the coefficient of variation for fruit traits was the highest (57.49%), followed by leaf traits (49.35%), while branch traits were relatively lower (36.53%). Besides their fundamental biological functions, fruit traits are more influenced by climatic factors such as water and heat. Leaves, on the other hand, are crucial for long-term adaptation, survival, and evolution, which most likely result in their most abundant variation among the traits of interest in natural populations. In addition, blue honeysuckle is mainly distributed in natural secondary forests protected by the government at altitudes of 2000 to 3000 meters, the scattered and discontinuous distribution of habitats, coupled with the limited number of samples, hinders gene flow among populations, resulting in population differentiation and variation in phenotypic traits (Qin *et al.*, 2010).

The average variance component within populations exceeded that among populations, indicating significant variation in phenotypic traits of blue honeysuckle in the Qilian Mountains primarily driven by population-level differences. This suggests that intra-population variability is the main source of phenotypic diversity in blue honeysuckle, with higher diversity levels within populations than among populations. This may be attributed to the environmental heterogeneity within populations, which could subject individuals to diverse selective pressures, thereby resulting in significant phenotypic diversity within the population. Moreover, localized environmental conditions, such as microclimatic variations or soil differences, may lead to distinct phenotypic traits among individuals within the same population. Additionally, genetic drift within small or isolated populations can magnify the effects of random genetic fluctuations, leading to increased intrapopulation variation. This high phenotypic variation endows blue honeysuckle with considerable selection potential and establishes a foundation for subsequent genetic improvements.

The variation levels of eight populations of blue honeysuckle in this study were quite different. The maximum and minimum coefficients of variation were found in the Huzhu population and Datong population, respectively. The reason for the large variation in the Huzhu population may be due to the different distribution environments of the five samples in the population.

The slope, aspect, and elevation of the sampling sites varied significantly, with some samples surrounded by dense growth of large trees such as spruce and birch while others grew in isolation within open forest stands, leading to pronounced phenotypic differences. The relatively small variation in the Datong population may be attributed to the concentrated distribution of blue honeysuckle, which mostly grows on the same side of the road within protected areas, intermixed with other shrubs and herbaceous plants, resulting in similar slope, aspect, and elevation compared to other growth environments. In addition, the coefficient of variation among different phenotypic traits was also quite different. The coefficient of variation of 28 quantitative traits ranged from 11.53% to 59.33%. Among them, the variation in fruiting density per unit length, branching number, crown short diameter, under-branch height, number of leaves on fruiting branch

nches was greater, which may be because compared to other traits, the growth vigor, biomass, and yield of blue honeysuckle plants are more susceptible to geographical, climatic, and environmental factors, as well as tree age. This is similar to the results of the analysis of *Quercus gilva* by Qin et al. (Qin *et al.*, 2010). The traits with a high coefficient of variation have a strong adaptability to their environment and increase their selection opportunities among materials. The traits with low coefficients of variation have a homology and stability among materials (Safdari *et al.*, 2021). In addition, the coefficient of variation of the leaf area and leaf weight of blue honeysuckle was also higher than other traits, which could be explained by the fact that leaf area plays an important role in effective light capture, water balance, and temperature regulation in different habitats (Kidner *et al.*, 2010; Vidakovic *et al.*, 2021).

Within our survey area, the distribution of characteristics such as trunk type, crown roundness, and canopy density of blue honeysuckle appeared relatively uniform. This may be attributed to significant environmental variations within the survey area, resulting in diverse growth conditions for the trees, with various growth states being prevalent. Acute leaf apex and leaf base shapes predominated. This could be due to the relatively dry, cold, and high-light intensity environment of the plateau region, where plants tend to develop sharp-pointed leaf tips and bases to reduce surface area and minimize water loss. Fruits and leaves are newly emerged parts during the year, and due to climatic changes, they exhibit unstable and diverse manifestations, resulting in greater diversity and less distribution at various levels. The Shannon-Wiener diversity index, as an important indicator of plant trait richness, is widely used in genetic resource assessment and phenotypic diversity analysis (Jain *et al.*, 1975; Petruccelli *et al.*, 2013). According to the study of phenotypic diversity of *Prunus Mume* by Wu et al., the Shannon index reaches 1, which is a high degree of diversity (Wu *et al.*, 2011). In this study, the average Shannon index of quality and quantity traits of tested blue honeysuckle populations exceeded 1 for all traits except Leaf apex shape, indicating substantial diversity. Some traits related to branches, leaves, and fruits exhibited higher levels of diversity. In the future, the index could be used as a priority for the analysis of phenotypic diversity of blue honeysuckle. This study also found that the diversity index of quantitative traits was much higher than that of qualitative traits. Li et al. and Szamosi et al. also obtained consistent results for the morphological traits of blueberry and *Cucumis melo* germplasm resources (Szamosi *et al.*, 2010; Li *et al.*, 2020). The reason for this may be that quantitative traits are greatly affected by germplasm resource types, environmental conditions, genotypes, and other factors, while qualitative traits change only slightly and are relatively more stable. In addition, from the overall analysis results of quantitative traits, the variation in the Shannon index and coefficient of variation (CV) is not consistent. For example, the Shannon index of the fruit width is the largest (2.079), while the CV is the smallest (11.53%). The Shannon indexes of the leaf shape index, fruit length, petiole length, leaf length, diameter of major fruiting branches, and fruit shape index are also greater than 2.0, but the CV is

s far lower than 20%. The larger the coefficient of variation, the greater the degree of variation of traits; the larger the diversity index, the richer the trait diversity. However, there is no correlation between the two. This situation is also consistent with the results of He *et al.* and Fu *et al.*'s phenotypic studies on *Dendrobium nobile* and *Salvia splendens* (Fu *et al.*, 2015; He *et al.*, 2021).

The correlation of traits can indirectly achieve the effect of selecting another trait through the selection of one trait, which can improve the selection efficiency of excellent plant varieties and accelerate the process of breeding (Rui *et al.*, 2018). The correlation analysis of phenotypic traits of blue honeysuckle in the Qilian Mountains showed that the correlation between most traits was significant or extremely significant. This is consistent with the previous research results of Wei Li on *Lonicera caerulea* (Li *et al.*, 2020). Correlation analysis has a certain convenience and guidance for the field collection and breeding of blue honeysuckle. Because most traits are closely related, researchers can roughly infer the growth trends of other traits related to one trait, which can reduce the blindness of investigation and research to a certain extent (Norman *et al.*, 2011). However, the linkage mechanism between these traits is still unclear and may need to be explored from biochemical and molecular perspectives. Different species have different geographical variation patterns due to their different adaptability and sensitivity to the environment (Zeng *et al.*, 2018). From this study, it was found that the traits related to the trunk of blue honeysuckle were not significantly affected by environmental factors, the branch traits were mainly affected by temperature, precipitation and organic carbon content, and the leaf traits were synergistically affected by altitude, latitude and organic carbon content, and the fruit traits are significantly affected by AWC, and are also affected by factors such as latitude and organic carbon content. Within suitable ranges, in relatively low altitudes, high longitudes, dry, low latitudes, and warm conditions, blue honeysuckle fruits exhibit high vitality, with thinner leaves, rounder fruits, and higher yields. In areas with low temperatures, high precipitation, low latitudes, and high altitudes, leaves are smaller but thicker, and fruits are longer and larger. This difference may be attributed to the high altitude and relatively low latitude of plateau regions, where temperatures are lower, diurnal temperature variations are greater, and ultraviolet radiation is stronger. The relatively lower altitude and latitude allow blue honeysuckle to fully utilize light and temperature resources, promoting its growth and photosynthesis. In such environments, plants are typically more vigorous, and the warm climate may also help increase fruit yields and promote fruit formation and ripening but could lead to a shorter growing season. Under such pressure, nutrients and water are relatively scarce, leading to smaller and rounder fruits. In high-altitude, low-latitude, and low-temperature regions, with low air density, plant transpiration is restricted. Plants tend to have lower vitality, with leaves experiencing severe wilting and thicker epidermis. Lower temperatures, greater temperature differences, and sufficient moisture facilitate the accumulation of nutrients and water, resulting in larger fruits. At the same time, with a de

crease in altitude, longitude, and precipitation, the size of blue honeysuckle leaves showed an increasing trend. Previous studies have also found that the leaf area of many plants decreases with increasing altitude, while the leaf thickness increases with increasing altitude (Cruz-Maldonado *et al.*, 2021; Huang *et al.*, 2022). This is consistent with our findings. Plants can adapt to environmental changes accompanied by a high altitude by reducing their leaf area and increasing their leaf thickness, mesophyll tissue thickness, and stomatal density (Liu *et al.*, 2020). Li conducted a study on blue honeysuckle in the Changbai Mountain region, within an elevation range of 600-1800 meters. The results revealed a significant increase in leaf characteristics such as TH, CL, LTh, LA, and LSI with increasing altitude. However, contrary to our study's findings, some results showed the opposite trend. This discrepancy may be attributed to differences in the elevation gradients studied. In lower elevation areas, tree height, crown length, and other features tend to increase with altitude due to plants receiving more abundant sunlight and being less affected by negative altitude factors such as excessive ultraviolet radiation and sparse oxygen.

However, in higher elevation areas where most plants struggle to adapt, constraints imposed by altitude-related adverse factors lead to an opposite trend in features such as TH, CL, and LA. This suggests that blue honeysuckle may exhibit its highest adaptability to climatic conditions within a moderate range of altitude. The research findings regarding LA and LSI are consistent with ours, which also aligns with the general pattern of plant leaf characteristics adapting to higher altitudes. Among the 8 populations, Datong and Tianzhu are situated at high elevations, exceeding 2800 meters, and they are also located at high latitudes. In these regions, blue honeysuckle is less distributed, with plants generally being shorter in stature, having lower under-branch height, smaller crown width, narrower and thicker leaves, slender leaf stalks, and the lowest yield. Conversely, Yongdeng and Huzhu have the lowest elevations, below 2600 meters, and in these areas, blue honeysuckle resources are relatively abundant. Crown width, stem thickness, leaf size, and leaf stalk length significantly exceed those in other regions, with thinner leaves and lighter leaf color. This also indicates that the growth of blue honeysuckle is influenced by high altitudes and cold, humid climates.

Principal component analysis can reduce the dimension of a dataset, maintain the characteristics of the largest variance contribution in the dataset, and provide the main information on the comprehensive performance of quantitative traits. In this study, the first 13 principal components were extracted from the phenotypic traits of blue honeysuckle by principal component analysis, and the cumulative contribution rate reached 83.48%. Among the traits related to the plant shape, branch, leaf, and fruit of blue honeysuckle, the primary and key traits affecting the phenotypic diversity are the traits related to leaf size and crown width, which is similar to the phenotypic analysis results of *Elaeagnus angustifolia* in Zhangye City by Zeng *et al.* In future investigations and studies, these traits could be used as key observational traits. Principal component analysis also further showed that the phenotype of blue honeysuckle was greatly affected

by the geographical distribution of different populations. Most of the phenotypic traits of the population in Datong were stable, while the phenotypes of the two populations in Ledu and Yongdeng, as well as Tianzhu and Pingan were closer. The traits of other populations were more abundant and the degree of variation was higher, which may be due to genetic or environmental effects (Karadeniz *et al.*, 2002).

The purpose of clustering analysis is to classify individuals according to their characteristics. Individuals in a cluster are highly similar to each other, and the inhomogeneity and differences among clusters are the largest. According to the clustering results of blue honeysuckle, the geographical distribution of five clusters in 8 populations was obtained (Fig. 6B). The 40 samples were not completely clustered according to geographical distance. The distribution of the five clusters was overlapped, but the distribution of the same cluster had a certain degree of similarity, and the variation in phenotypic traits among populations was discontinuous. This shows that geographical distance does not play a key role in the phenotypic differentiation of the studied blue honeysuckle population (Du *et al.*, 2014). This may be due to the complex and diverse geographical environment and climate differences in the Qilian Mountains. In addition to the specific phenotypic characteristics, the population also has a specific ecological amplitude and distribution range (Cao *et al.*, 2021). The random effects of various specific genotypes or environmental factors often lead to differences in traits between individuals (Fu *et al.*, 2017). Among all clustering types, Type A has the most samples, primarily from Datong, Tianzhu, and Heduo, which are high-altitude and high-latitude regions. They exhibit characteristics more suited to high altitudes and low temperatures, such as smaller and thicker leaves, slender leaf stalks, and lower yields. Samples from Ping'an area mainly belong to Type B, characterized by significantly larger fruit size and the highest fruit shape index. The distribution of Type C blue honeysuckle clusters is the most scattered, indicating that Type C may be a transitional group with less distinct characteristics within the cluster. Type D blue honeysuckle mainly exhibits small fruit size, low fruit shape index, light fruit color, and excellent taste. Yongdeng region has the most diverse types, possibly due to the scattered distribution of sample sites and the greatest environmental differences among the sites, reflecting significant heterogeneity within populations. Of particular note, Type E represents an isolated plant type from Yongdeng, with excellent characteristics in all aspects and significantly higher fruit yield than other plants. In the future, superior blue honeysuckle varieties can be selectively bred based on different purposes.

Conclusions

The phenotypic variation in different populations of blue honeysuckle is rich, and the variation mainly comes from within the population. The phenotypic traits related to plant shape and branches had a large variation, and the phenotypic traits related to fruits and leaves had a small variation. The phenotypic quantitative traits of blue honeysuckle were relatively more abun-

dant, the qualitative traits were more stable, and the diversity of fruits and leaves was higher. Within a certain range, with increasing longitude and temperature, and decreasing altitude, latitude, precipitation, blue honeysuckle exhibits higher vitality, thinner and larger leaves, smaller and rounder fruits, and higher yields. This indicates that excessively high altitude and precipitation within the surveyed area may be limiting factors for the growth of blue honeysuckle. As a topical original study of this species in the Qilian Mountains, the results of our study on the phenotypic trait variation in blue honeysuckle will provide a basis for improving the efficiency of population selection, provide a reference for selecting and establishing gene conservation areas and seed collection areas, and is of great significance for accelerating the selection and breeding. In the future, the corresponding varieties of blue honeysuckle should be selected for different purposes according to their geographical distribution and utilization direction. Using the excellent plant shape and crown characteristics of Cluster A in this study, along with appropriate tree height, under-branch height, and branch number, they could be considered for landscaping decoration. Despite their lower yield, they possess a more compact and rounded appearance. Samples from Cluster B, which exhibit the largest fruit size, attractive fruit shape, and higher production potential, are more suitable for commercial fruit production purposes. Targeted breeding could be applied to gradually cultivate superior varieties. Cluster C, with its dark-colored fruits and excellent fruit taste, is also suitable for high-quality fruit production purposes. Its taller plant height and higher leaf count are also suitable for planting in commercial orchards and gardens. Cluster E demonstrates significant uniqueness compared to other clusters, making it valuable for both landscape decoration and fruit production. However, its value is somewhat limited due to the relatively small sample size. In summary, targeted breeding should be conducted to gradually update and cultivate excellent varieties that combine ornamental and ecological functions, characterized by bright, plump fruits and good tree form. Varieties with high yields, large fruits, and excellent taste should be bred to produce fruits with both high yield and flavor. Additionally, in subsequent research, the nutritional content of the fruits should be determined to more comprehensively develop high-quality varieties of blue honeysuckle.

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References

- Balkaya A, Özbakir M, Kurtar ES. 2010. The phenotypic diversity and fruit characterization of winter squash (*Cucurbita maxima*) populations from the Black Sea Region of Turkey. *African Journal of Biotechnology* 9: 152-162.
- Cao DM, Zhang YH, Cheng XQ, Xiang XD, Zhang L, Hu JJ. 2021. Genetic variation of leaf phenotypic traits in different populations of *Populus*

cathayana. *Scientia Silvae Sinicae* 57: 56–67.

Chen F, Yuan YJ, Wei WS. 2011. Climatic response of *Picea crassifolia* tree-ring parameters and precipitation reconstruction in the western Qilian Mountains, China. *Journal of Arid Environments* 75: 1121–1128.

Cruz-Maldonado N, Weemstra M, Jiménez L, Roumet C, Angeles G, Barois I, Santos M, Morales-Martinez MA, Palestina RA, Rey H et al. 2021. Aboveground-trait variations in 11 (sub) alpine plants along a 1000-m elevation gradient in tropical Mexico. *Alpine Botany* 131: 187–200.

Donohue K, Pyle EH, Messiqua D, Heschel MS, Schmitt J. 2001. Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. *Evolution* 55: 692–702.

Du Q, Xu B, Gong C, Yang X, Pan W, Tian J, Li B, Zhang D. 2014. Variation in growth, leaf, and wood property traits of Chinese white poplar (*Populus tomentosa*), a major industrial tree species in Northern China. *Canadian journal of forest research* 44: 326–339.

Ebrahimi A, Asadi A, Monfared SR, Sahebi M, Rezaee S, Khaledian Y. 2020. Evaluation of phenotypic diversity of the endangered orchid (*Orchis mascula*): Emphasizing on breeding, conservation and development. *South African journal of botany* 132: 304–315.

Frier SD, Somers CM, Sheffield CS. 2016. Comparing the performance of native and managed pollinators of Haskap (*Lonicera caerulea*: Caprifoliaceae), an emerging fruit crop. *Agriculture, Ecosystems & Environment* 219: 42–48.

Fu G, Dai X, Symanzik J, Bushman S. 2017. Quantitative gene–gene and gene–environment mapping for leaf shape variation using tree-based models. *New Phytol* 213: 455–469.

Fu QJ, Li CN, Chen Y, Zhao FK, Sun Y. 2015. Analysis of Phenotypic diversity of major *Salvia splendens* germplasm resources in China. *Plant Genet. Resour* 16: 294–299.

Ge S, Wang MX, Chen YW. 1988. An analysis of population genetic structure of Masson pine by isoenzyme technique. *Scientia Silvae Sinicae* 24: 399–409.

He T, Fan XL, Lu L, Huang TF. 2021. Phenotypic trait diversity of *Dendrobium nobile* from different provenances and their correlations with geographical factors. *Plant Resour. Environ* 30: 1–11.

Huang W, Zhong Y, Zhang C, Ren M, Du Y, Son X. 2022 Leaf traits and water-use characteristics of *Impatiens hainanensis*, a limestone-endemic plant under different altitudes in dry and foggy seasons. *Water* 14:139.

Jain SK, Qualset CO, Bhatt GM, Wu KK. 1975. Geographical patterns of phenotypic diversity in a world collection of durum wheats. *Crop Science* 15: 700–704.

Karadeniz T. 2002. Selection of native “Comelian” cherries grown in Turkey. *Journal of the American Pomological Society* 56: 164.

Khattab R, Ghanem A, Su-Ling Brooks M. 2016. Effect of Juice Extraction Methods on the Physicochemical Characteristics of Haskap Berry (*Lonicera caerulea* L.) Products. *Current Nutrition & Food Science* 12: 220–229.

Kidner C, Umbreen S. 2021. Why Is Leaf Shape so Variable? *International Journal of Plant Developmental Biology* 4: 64–75.

Kula M, Glód D, Krauze-Baranowska M. 2016. Application of on-line and off-line heart-cutting LC in determination of secondary metabolites from the flowers of *Lonicera caerulea* cultivar varieties. *Journal of Pharmaceutical and Biomedical Analysis* 131: 316–326.

Li H, Wang R, Tian Z, Xu J, Sun W, Duan R, Dong L et al. 2022. Phenotypic variation and diversity in fruit, leaf, fatty acid, and their relationships to geoclimatic factors in seven natural populations of *Malania oleifera* Chun et SK Lee. *Forests* 13: 1733.

Li W, Wang P, Qi Q, Zhang Q, Gao X, Lin M, Cui Y. 2020. Phenotypic diversity and variation of *Lonicera caerulea* populations in the Changbai mountain alongside the elevation gradient. *Pol. J. Environ. Stud* 30: 705–716.

Li W, Wang P, Qi QG, Zhang QC, Huang BJ, Xiao ZJ. 2020. Phenotypic diversity analysis of blueberry germplasm resources. *Journal of Beijing Forestry University* 42: 124–134.

Li Y, Li S, Lu X, Wang Q, Han H, Zhang X, Gan X et al. 2021. Leaf phenotypic variation of endangered plant *Tetracentron sinense* Oliv. and influence of geographical and climatic factors. *Journal of Forestry Research* 32: 623–636.

Liu W, Zheng L, Qi D. 2020. Variation in leaf traits at different altitudes reflects the adaptive strategy of plants to environmental changes. *Ecology and Evolution* 10: 8166–8175.

Maughan P J, Maroof MS, Buss GR. 1995. Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. *Genome* 38: 715–723.

Miljković D, Stefanović M, Orlović S, Stanković Nedić M, Kesić L, Stojnić S. 2019. Wild cherry (*Prunus avium* (L.) L.) leaf shape and size variations in natural populations at different elevations. *Alpine Botany* 129: 163–174.

- Norman P, Tongoona P, Shanahan P. 2011. Determination of interrelationships among agr-morphological traits of yams (*Discorea* spp.) using correlation and factor analyses. *J Appl Biosci* 45: 3059–3070.
- Omiere A, Budambula NL, Neondo J, Gituru R, Mweu C. 2020. Phenotypic Diversity of Doum Palm (*Hyphaene compressa*), a Semi-Domesticated Palm in the Arid and Semi-Arid Regions of Kenya. *Scientifica* 2020: 1-13.
- Oszmianański J, Kucharska AZ. 2018. Effect of pre-treatment of blue honeysuckle berries on bioactive iridoid content. *Food Chemistry* 240: 1087-1091.
- Petrucelli R, Ganino T, Ciaccheri L, Maselli F, Mariotti P. 2013. Phenotypic diversity of traditional cherry accessions present in the Tuscan region. *Scientia horticulturae* 150: 334–347.
- Pigliucci M, Murren CJ, Schlichting CD. 2006. Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology* 209: 2362-2367.
- Qin ZK, Liu N, Zhou X, Li ZH, Cao FL, Li H. 2023. Phenotypic diversity of *Quercus gilva* natural populations in middle subtropical China. *Guihaia* 43:1622–1635.
- Raj D, Govindaraju D, Orians C. 2006. Genetic variation among pitch pine *Pinus rigida* families from Walden Woods: Heritability and path analysis of developmental variation of phenotypic traits. *Rhodora* 108: 356-369.
- Rui WJ, Wang XM, Zhang QN, Hu XY, Hu XH, Fu JJ, Gao YM, Li JS. 2018. Genetic diversity analysis of 353 tomato germplasm resources by phenotypic traits. *Acta Horticulturae Sinica* 45: 561–570.
- Safdari L, Khadivi A. 2021. Identification of the promising oleaster (*Elaeagnus angustifolia* L.) genotypes based on fruit quality-related characters. *Food Science & Nutrition* 9: 5712–5721.
- Szamosi C, Solmaz I, Sari N, Bárony C. 2010. Morphological evaluation and comparison of Hungarian and Turkish melon (*Cucumis melo* L.) Germplasm. *Scientia horticulturae* 124: 170–182.
- Thompson MM. 2006. Introducing haskap, Japanese blue honeysuckle. *Journal of the American Pomological Society* 60: 164.
- Tian H, Yang T, Liu Q. 2014. Climate change and glacier area shrinkage in the Qilian mountains, China, from 1956 to 2010. *Annals of Glaciology* 55: 187-197.
- Vidaković A, Liber Z, Šatović Z, Idžojčić M, Volenec I, Zegnal I, Pintar V, Radunić M, Poljak I. 2021. Phenotypic diversity of almond-leaved pear (*Pyrus spinosa* Forssk.) along Eastern Adriatic Coast. *Forests* 12: 1630.
- Wang C, Gong H, Feng M, Tian C. 2023. Phenotypic variation in leaf, fruit and seed traits in natural populations of *Eucommia ulmoides*, a relict Chinese endemic tree. *Forests* 14: 462.
- Wu GS, Sun LD, Hao RJ, Shi WF, Zhang J, Chen JX. 2011. Study on the phenotypic diversity of *P. mume* Sieb. et Zucc. germplasm resources. *Anhui Agric. Sci* 39:12008–12009.
- Yang Q, Fu Y, Wang YQ, Wang Y, Zhang WH, Li XY, Zhang J et al. 2014. Genetic diversity and differentiation in the critically endangered orchid (*Amitostigma hemipilioides*): implications for conservation. *Plant systematics and evolution* 300: 871-879.
- Zeng YF, Zhang JG, Abuduhaiti B, Wang WT, Jia ZQ. 2018. Phylogeographic patterns of the desert poplar in Northwest China shaped by both geology and climatic oscillations. *BMC Evol. Biol* 18: 75.
- Zeng ZH, Li QW, Ti YJ. 2021. Analysis on phenotypic diversity and ornamental of wild *Elaeagnus angustifolia* from Zhangye City of Gansu Province. *Plant Resour. Environ* 30: 12-21.
- Zongxing L, Qi F, Zongjie L, Xufeng W, Juan G, Baijuan Z, Pengfei L et al. 2021. Reversing conflict between humans and the environment-The experience in the Qilian Mountains. *Renewable and Sustainable Energy Reviews* 148: 111333.