Current Science
CS 5 (6), 5373-5385 (2025)
Received 22 Oct 2025 | **Accepted** 07 November 2025 | **Online Available** 11 November 2025
https://doi.org/10.52845/CS/2025-5-6-8

Current Science
Current Science

OPEN ACCESS
ISSN(O) 2795-8639

Original Article

Comprehensive Assessment of Phenotypic Diversity Analysis and Selenium Enrichment Capacity in 62 Wild *Cardamine Enshiensis* Germplasm Resources from Enshi, Hubei Province

Yarong Li[2], Hongqing Yin[1], Jiajia Ming[1], Wu Xu[1], Zhen Hu[2], Chenghuan Yan[2], Fengling Guo[2*], Jiqian Xiang[1*]

[1]Enshi Tujia and Miao Autonomous Prefecture Academy of Agricultural Sciences, Enshi, Hubei 445000, China

[2]Institute of Industrial Crops, Hubei Academy of Agricultural Sciences / Key Laboratory of Ecological Cultivation of Alpine Vegetables, Ministry of Agriculture and Rural Affairs / Hubei Key Laboratory of Vegetable Germplasm and Genetic Improvement, Wuhan, Hubei 430064, China

***Corresponding Author: Fengling Guo, Jiqian Xiang**

Abstract:
The study systematically analyzed the diversity, correlation, and comprehensive performance of phenotypic traits at both seedling and flowering stages using 62 wild *Cardamine enshiensis* germplasm resources. The results showed that: the phenotypic traits exhibited substantial variation, with coefficients variation (CV) ranging from 10.61% to 30.88%, while seedling-stage traits generally displayed greater variability than flowering-stage traits; plant height, leaf length, and leaf width at the seedling stage were significantly positively correlated with those at the flowering stage (r = 0.77-0.82), indicating that early growth can predict later performance; the selenium content results showed that the leaves of *Cardamine enshiensis* contained selenium levels as high as 3,695.71 μg/kg; cluster analysis grouped the resources into three distinct types, the third group exhibiting exceptionally high biomass potential; principal component analysis (PCA) extracted three principal components, cumulatively explaining 79.12% of the total variation. Based on comprehensive evaluation scores, 10 elite resources with robust growth, high tillering ability, and strong selenium accumulation were identified. This study provides the theoretical foundation and material basis for breeding high-yield, selenium-enrich *C. enshiensis* varieties.

Keywords: ***Cardamine enshiensis*; Phenotypic traits; Cluster analysis; Principal component analysis; Comprehensive evaluation**

1. Introduction

Cardamine enshiensis, commonly known as “wild rapeseed” (*Shi et al.*, 2015), is an annual or perennial herbaceous plant belonging to the family Brassicaceae (*Flora of China*, 1987). It was first discovered in 1981 in Yutangba, Xintang Township, Enshi Prefecture, Hubei Province, within a region characterized by selenium-rich soils. This species represents the first selenium (Se) hyperaccumulator identified and reported in China (*Lei et al.*, 2015) and is endemic to the country. Under natural conditions, the selenium concentration in its dried leaves can reach 1427 mg/kg, while under artificial cultivation with exogenous selenium supplementation, the selenium content in dry matter exceeds 9000 mg/kg, demonstrating an exceptional capacity for selenium accumulation (*Shao et al.*, 2016; *Rao et al.*, 2020).

In addition to its remarkable selenium-enriching ability, *C. enshiensis* is rich in proteins, vitamins, polysaccharides, flavonoids, amino acids, and cellulose (*Xu*, 2023; *Ma et al.*, 2023; *Lin et al.*, 2022). Through years of domestication and cultivation, large-scale artificial planting of *C. enshiensis* has been successfully established. Furthermore, it has been approved by the National Health Commission of China as a leafy vegetable ingredient, allowing its inclusion in the management of edible plant resources (*Technical Regulations for the Production of Cardamine enshiensis*, 2022). Consequently, *C. enshiensis* holds significant potential as a selenium-enriched food source and as a plant-based selenium supplement, contributing to selenium biofortification and functional agriculture. However, systematic evaluation of this species remains limited, which constrains its genetic improvement and large-scale utilization.

Phenotypic characterization serves as the fundamental approach for identifying, classifying, evaluating, and selecting breeding materials from plant germplasm resources. For instance, *Duan et al.* (2023) classified 296 pear germplasms into five categories—excellent, good, moderate, poor, and very poor—based on systematic cluster analysis of fruit lenticel traits, including size, density, and surface morphology. *Peng et al.* (2025) evaluated 148 garlic germplasms for agronomic traits and identified 37 superior genotypes suitable for cultivation in Hubei Province. Similarly, *Leng et al.* (2021) and *Li et al.* (2023) identified 20 elite garlic accessions from Guizhou and 5 superior garlic and green garlic germplasms, respectively, through comparable phenotypic assessments. *Zhi et al.* (2025) analyzed phenotypic characteristics in 96 peony cultivars and identified 10 elite varieties, including ‘Yubanbai’ and ‘Daojin,’ exhibiting superior overall performance. *Zhang et al.* (2025) conducted phenotypic and nutritional evaluations of 235 tillering onion germplasms, selecting 10 outstanding cultivars for commercial promotion. Additionally, phenotypic analyses have been successfully employed to identify superior germplasms in crops such as soybean, barley, chestnut, and *Lolium multiflorum*, offering valuable insights for resource utilization and breeding (*Di et al.*, 2023; *Gao et al.*, 2023; *Guo et al.*, 2022; *Sun et al.*, 2016).

In 2022, *C. enshiensis* was officially approved as a leafy vegetable by the National Health Commission of China, and selenium concentrations in its leaves have been reported to exceed those in the entire plant. Therefore, evaluation of leaf-related phenotypic traits is essential for the identification of superior selenium-rich germplasms. In this study, 62 wild *C. enshiensis* accessions were investigated. Leaf-related traits at the seedling and full-bloom stages were characterized, and leaf selenium content was quantitatively determined. Multiple analytical methods were employed to screen for germplasms with both high yield potential and superior selenium accumulation capacity, providing a theoretical foundation for the breeding of new high-selenium cultivars of *C. enshiensis*.

2. Materials and Methods

2.1 Plant Materials

All *C. enshiensis* germplasm resources used in this study were obtained from the Enshi Academy of Agricultural Sciences (Hubei, China). For the convenience of subsequent analyses, each accession was assigned a unique code consisting of the abbreviation “Ce” (derived from the Latin name) followed by a sequential Arabic numeral. In total, 62 *C. enshiensis* accessions were evaluated and characterized. Detailed information on these germplasm resources is provided in Table 1.

Table 1 ***Cardamine enshiensis*** **germplasm resources**

New ID	Original ID	Geographic Origin	New ID	Original ID	Geographic Origin
Ce02	CYY G25	Shuanghe, Enshi, Hubei Province	Ce33	S20	Shuanghe, Enshi, Hubei Province
Ce03	CYY P13	Shuanghe, Enshi, Hubei Province	Ce34	S21	Shuanghe, Enshi, Hubei Province
Ce04	CYY P15	Shuanghe, Enshi, Hubei Province	Ce35	S23	Shuanghe, Enshi, Hubei Province
Ce05	CYY P16	Shuanghe, Enshi, Hubei Province	Ce36	S25	Shuanghe, Enshi, Hubei Province
Ce06	CYY P24	Shuanghe, Enshi, Hubei Province	Ce37	S27	Shuanghe, Enshi, Hubei Province
Ce07	CYY P3	Shuanghe, Enshi, Hubei Province	Ce38	S28	Shuanghe, Enshi, Hubei Province
Ce08	CYY P6	Shuanghe, Enshi, Hubei Province	Ce39	S29	Shuanghe, Enshi, Hubei Province
Ce09	CYY-1010	Shuanghe, Enshi, Hubei Province	Ce40	S3	Shuanghe, Enshi, Hubei Province
Ce10	CYY-1014	Shuanghe, Enshi, Hubei Province	Ce41	S30	Shuanghe, Enshi, Hubei Province
Ce11	CYY-102	Shuanghe, Enshi, Hubei Province	Ce42	S31	Shuanghe, Enshi, Hubei Province
Ce12	CYY-1022	Shuanghe, Enshi, Hubei Province	Ce43	S32	Shuanghe, Enshi, Hubei Province
Ce13	CYY-1023	Shuanghe, Enshi, Hubei Province	Ce44	S33	Shuanghe, Enshi, Hubei Province
Ce14	CYY-104	Shuanghe, Enshi, Hubei Province	Ce45	S34	Shuanghe, Enshi, Hubei Province
Ce15	CYY-108	Shuanghe, Enshi, Hubei Province	Ce46	S36	Shuanghe, Enshi, Hubei Province
Ce16	CYY-109	Shuanghe, Enshi, Hubei Province	Ce47	S4	Shuanghe, Enshi, Hubei Province
Ce17	CYY-12	Shuanghe, Enshi, Hubei Province	Ce48	S5	Shuanghe, Enshi, Hubei Province
Ce18	S0-1	Shuanghe, Enshi, Hubei Province	Ce49	S6	Shuanghe, Enshi, Hubei Province
Ce19	S0-4	Shuanghe, Enshi, Hubei Province	Ce50	S8	Shuanghe, Enshi, Hubei Province
Ce20	S0-6	Shuanghe, Enshi, Hubei Province	Ce51	S9	Shuanghe, Enshi, Hubei Province
Ce21	S0-7	Shuanghe, Enshi, Hubei Province	Ce52	SH-1	Shuanghe, Enshi, Hubei Province
Ce22	S1-1	Shuanghe, Enshi, Hubei Province	Ce53	SH-2	Shuanghe, Enshi, Hubei Province
Ce23	S1	Shuanghe, Enshi, Hubei Province	Ce54	SH-3	Shuanghe, Enshi, Hubei Province
Ce24	S10	Shuanghe, Enshi, Hubei Province	Ce55	SH-4	Shuanghe, Enshi, Hubei Province
Ce25	S11	Shuanghe, Enshi, Hubei Province	Ce56	SH-5	Shuanghe, Enshi, Hubei Province

Ce26	S12	Shuanghe, Enshi, Hubei Province	Ce57	SH-6	Shuanghe, Enshi, Hubei Province
Ce27	S13	Shuanghe, Enshi, Hubei Province	Ce59	Hong	Shuanghe, Enshi, Hubei Province
Ce28	S14	Shuanghe, Enshi, Hubei Province	Ce60	Hong10.4	Shuanghe, Enshi, Hubei Province
Ce29	S15	Shuanghe, Enshi, Hubei Province	Ce61	Ming Wei	Shuanghe, Enshi, Hubei Province
Ce30	S17	Shuanghe, Enshi, Hubei Province	Ce64	Chang10.4	Shuanghe, Enshi, Hubei Province
Ce31	S18	Shuanghe, Enshi, Hubei Province	Ce65	Chang Bo	Shuanghe, Enshi, Hubei Province
Ce32	S2	Shuanghe, Enshi, Hubei Province	Ce66	ZZPZ	Shuanghe, Enshi, Hubei Province

2.2 Phenotypic Trait Investigation

2.2.1 Agronomic Trait Survey

Phenotypic traits were investigated at both the seedling and full-flowering stages of *C. enshiensis*. Plant height, leaf length, leaf width, and petiole length were measured using a measuring tape, while the number of tillers per plant was recorded during the full-flowering stage. For each accession, six healthy plants of uniform growth were randomly selected for measurements, and the mean values were used for subsequent statistical analyses. Field management, including pest and disease control, irrigation, and fertilization, was conducted following standard agronomic practices.

2.2.2 Determination of Fresh Leaf Selenium Content

In agricultural production, the leaves of *C. enshiensis* at the seedling stage are primarily consumed or processed, and previous studies have shown that selenium concentrations in leaves are higher than those in whole plants. Therefore, fresh leaves collected during the seedling stage were used for selenium content determination. For each accession, leaves from eight plants were combined to form one composite sample. Three parallel measurements were performed, and the mean value was used for further analysis. Selenium concentration was determined using the hydride generation atomic fluorescence spectrometry (HG-AFS) method as specified in the national standard GB 5009.93–2017, following microwave-assisted acid digestion for sample pretreatment.

2.3 Data Processing and Statistical Analysis

Raw data were organized and summarized using Microsoft Excel 2022, and basic descriptive statistics—including minimum, maximum, mean, standard deviation, and coefficient of variation—were calculated for each phenotypic trait. Cluster analysis, correlation analysis, and principal component analysis (PCA) of agronomic traits were conducted using SPSS version 27.0.

Following the method described by *Di et al.* (2023), comprehensive germplasm evaluation was performed based on PCA-derived eigenvectors. Specifically, all phenotypic data were first standardized, and the standardized values of each accession were multiplied by the corresponding eigenvectors of each principal component to obtain principal component scores. Subsequently, weighted comprehensive scores were calculated for each accession by summing the scores across all principal components, using their respective variance contribution rates as weighting coefficients.

3. Results and Analysis

3.1 Phenotypic Diversity of *Cardamine*

enshiensis Germplasm Resources

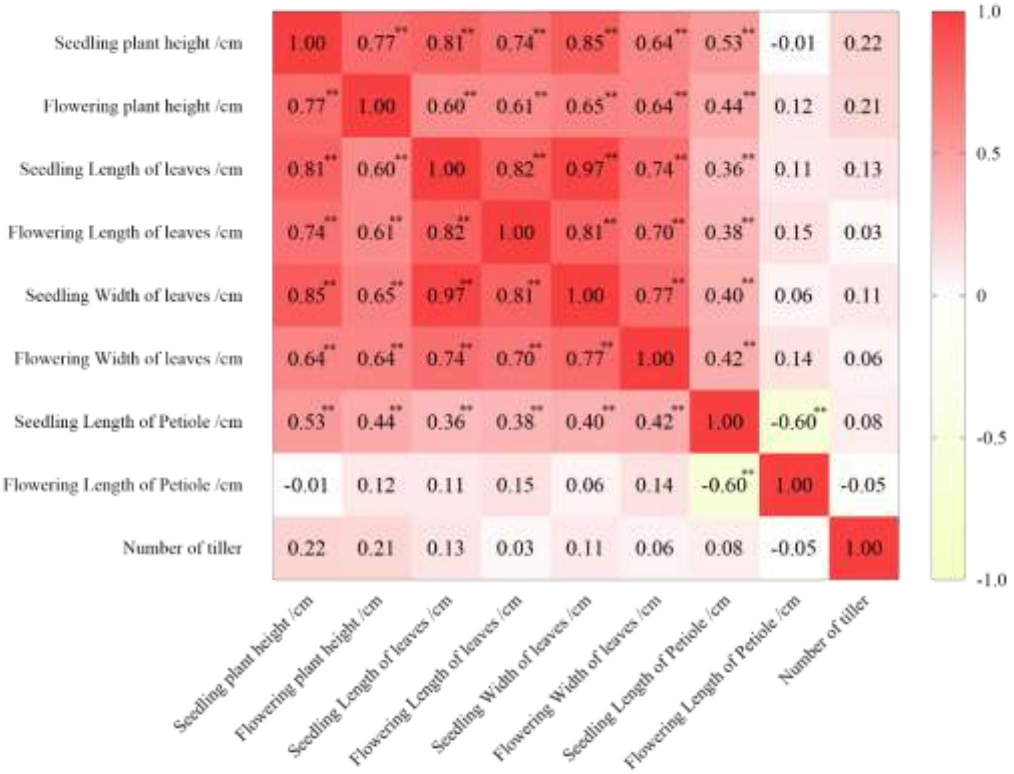
Significant variation was observed among the 62 *C. enshiensis* germplasm resources in terms of phenotypic traits, with coefficients of variation (CVs) ranging from 10.61% to 30.88% (Table 2). The traits were ranked by their CVs in descending order as follows: seedling-stage leaf width > seedling-stage plant height > seedling-stage leaf length > seedling-stage petiole length > flowering-stage leaf length > flowering-stage petiole length > flowering-stage leaf width > flowering-stage plant height > tiller number. Seedling-stage leaf width exhibited the highest variability (CV = 30.88%), with a range of 2.62–8.60 cm and a mean of 5.13 cm. In contrast, tiller number showed the lowest variability (CV = 10.61%), ranging from 5.00 to 8.20 with an average of 6.37. Notably, the CVs of seedling-stage plant height, leaf length, leaf width, and petiole length were all greater than those of the corresponding flowering-stage traits, indicating that phenotypic variation was higher at the seedling stage. Overall, the broad range of phenotypic diversity suggests abundant genetic variability within the *C. enshiensis* germplasm resources.

Table 2 Phenotypic traits diversity analysis of *Cardamine enshiensis* germplasm resources

Trait	Max	Min	Mean	SD	CV (%)
Seedling plant height / cm	11.54	2.68	7.29	2.24	30.73
Flowering plant height / cm	62.04	33.22	47.63	6.23	13.07
Seedling Length of leaves / cm	6.94	2.20	4.02	1.18	29.27
Flowering Length of leaves / cm	8.73	4.02	6.19	1.49	24.14
Seedling Width of leaves / cm	8.60	2.62	5.13	1.58	30.88
Flowering Width of leaves / cm	12.30	6.98	10.36	1.39	13.46
Seedling Length of Petiole / cm	8.09	2.42	5.17	1.38	26.63
Flowering Length of Petiole / cm	11.40	3.74	7.50	1.46	19.48
Number of tillers	8.20	5.00	6.37	0.68	10.61

3.2 Correlation Analysis of Phenotypic Traits

To assess the relationships among the phenotypic traits of *C. enshiensis*, correlation analysis was performed (Fig. 1). Significant and extensive correlations ($P < 0.01$) were detected among most growth indicators. Specifically, seedling-stage plant height was strongly and positively correlated with seedling leaf length, leaf width, and petiole length, as well as with flowering-stage plant height, leaf length, and leaf width ($r = 0.53$–0.85). Flowering-stage plant height was significantly correlated with seedling leaf length, width, and petiole length, and flowering leaf length and width ($r = 0.44$–0.65). Seedling leaf length and width were highly correlated ($r = 0.97$), and flowering leaf length was significantly correlated with seedling leaf width, seedling petiole length, and flowering leaf width ($r = 0.38$–0.81). Furthermore, seedling leaf width showed a significant positive correlation with seedling petiole length and flowering leaf width ($r = 0.40$–0.77), while flowering leaf width was significantly correlated with seedling petiole length ($r = 0.42$). Notably, significant positive correlations were found between seedling and flowering stages for plant height, leaf length, and leaf width, with correlation coefficients of 0.77, 0.82, and 0.77, respectively. These findings suggest that phenotypic performance at the seedling stage can serve as a reliable indicator of plant vigor and morphological traits at later growth stages.



	Seedling plant height /cm	Flowering plant height /cm	Seedling Length of leaves /cm	Flowering Length of leaves /cm	Seedling Width of leaves /cm	Flowering Width of leaves /cm	Seedling Length of Petiole /cm	Flowering Length of Petiole /cm	Number of tiller
Seedling plant height /cm	1.00	0.77**	0.81**	0.74**	0.85**	0.64**	0.53**	-0.01	0.22
Flowering plant height /cm	0.77**	1.00	0.60**	0.61**	0.65**	0.64**	0.44**	0.12	0.21
Seedling Length of leaves /cm	0.81**	0.60**	1.00	0.82**	0.97**	0.74**	0.36**	0.11	0.13
Flowering Length of leaves /cm	0.74**	0.61**	0.82**	1.00	0.81**	0.70**	0.38**	0.15	0.03
Seedling Width of leaves /cm	0.85**	0.65**	0.97**	0.81**	1.00	0.77**	0.40**	0.06	0.11
Flowering Width of leaves /cm	0.64**	0.64**	0.74**	0.70**	0.77**	1.00	0.42**	0.14	0.06
Seedling Length of Petiole /cm	0.53**	0.44**	0.36**	0.38**	0.40**	0.42**	1.00	-0.60**	0.08
Flowering Length of Petiole /cm	-0.01	0.12	0.11	0.15	0.06	0.14	-0.60**	1.00	-0.05
Number of tiller	0.22	0.21	0.13	0.03	0.11	0.06	0.08	-0.05	1.00

Fig 1 Phenotypic traits correlation analysis of *Cardamine enshiensis* germplasm resources

Note： * indicates a significant correlation at the 0.05 ($P < 0.05$) level, and ** indicates a highly significant correlation at the 0.01 ($P < 0.01$) level.

3.3 Cluster Analysis of Phenotypic Traits

Cluster analysis of the nine phenotypic traits was conducted using Ward's method based on squared Euclidean distance (Fig. 2). When the distance threshold was set to 5, the 62 *C. enshiensis* accessions were classified into three distinct groups. Cluster I contained 16 accessions (25.81%), Cluster II included 24 accessions (38.71%), and Cluster III comprised 22 accessions (35.48%).

The three clusters differed markedly in overall biomass and growth vigor. In Cluster I, the mean seedling plant height, leaf length, and leaf width were 4.64 cm, 2.80 cm, and 3.44 cm, respectively; at the flowering stage, the mean plant height, leaf length, and leaf width were 41.42 cm, 4.66 cm, and 8.88 cm. In contrast, Cluster III exhibited the strongest growth, with mean seedling plant height, leaf length, and leaf width of 9.56 cm, 5.26 cm, and 6.81 cm, and flowering-stage plant height, leaf length, and leaf width of 53.50 cm, 7.77 cm, and 11.63 cm, respectively. These results indicate that Cluster III possessed the greatest biomass and most vigorous growth, exhibiting the most favorable comprehensive phenotypic performance among all groups.

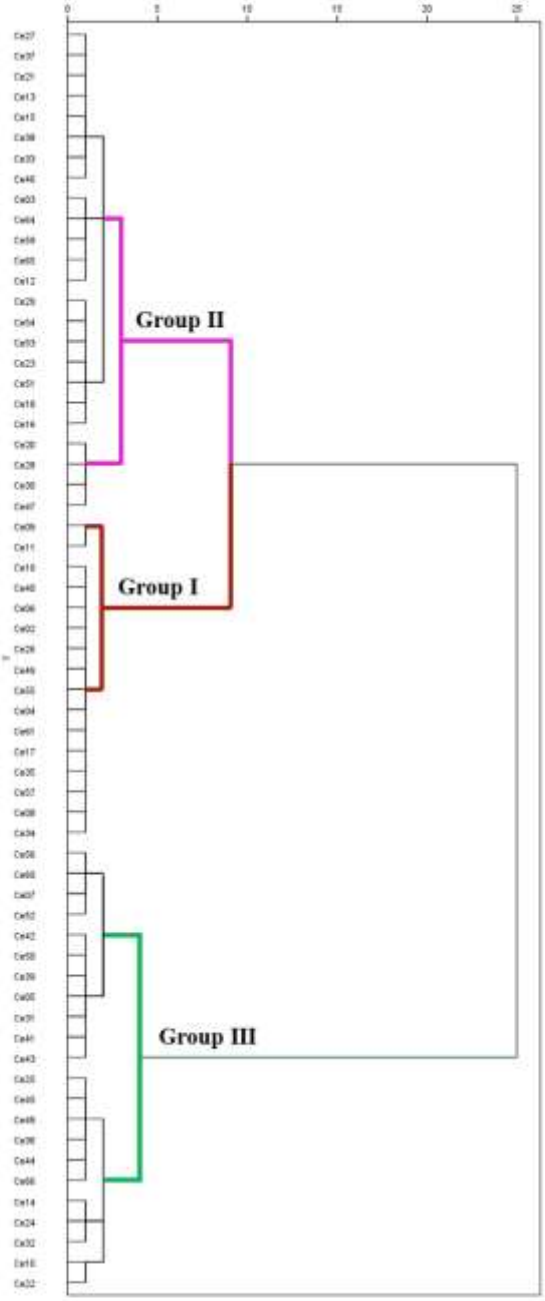


Fig 2 Cluster analysis of phenotypic traits of *Cardamine enshiensis* germplasm resources

3.4 Selenium Accumulation Capacity in *C. enshiensis* Leaves

The selenium concentrations in fresh leaves of *C. enshiensis* varied substantially among germplasms, ranging from 7.18 µg/kg to 3695.71 µg/kg (Fig. 3). Twenty-seven accessions exhibited selenium contents below 100 µg/kg, twenty accessions had selenium contents between 100 µg/kg and 300 µg/kg, and fifteen accessions (24.19%) exceeded 300 µg/kg. Among these, seven accessions contained 300-500 µg/kg, five contained 500-1000 µg/kg, and three exceeded 1000 µg/kg. Notably, the selenium concentrations in the leaves of accessions Ce05 and Ce08 were 1364.91 µg/kg and 1490.45 µg/kg, respectively, while Ce66 exhibited the highest selenium accumulation capacity, reaching 3695.71 µg/kg. These results demonstrate wide variation in selenium enrichment ability among *C. enshiensis* germplasms, indicating considerable potential for selecting high-selenium accessions.

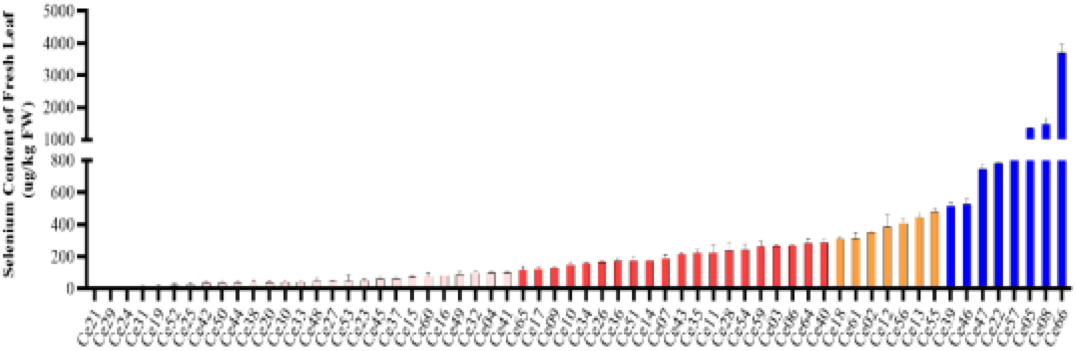


Fig 3 Analysis of Selenium Content in Fresh Leaves of *Cardamine enshiensis* germplasm resources

Note: Pink indicates resources with selenium content below 100 µg/kg. Red indicates resources with selenium content between 100 µg/kg and 300 µg/kg. Yellow indicates resources with selenium content between 300 µg/kg and 500 µg/kg. Blue indicates resources with selenium content above 500 µg/kg.

3.5 Principal Component Analysis of Phenotypic Traits and Selenium Content

Principal component analysis (PCA) was conducted on ten traits of the 62 *C. enshiensis* accessions. The Kaiser-Meyer-Olkin (KMO) value was 0.737, and Bartlett's test of sphericity was significant ($P < 0.01$), confirming the suitability of the dataset for PCA (KMO > 0.5, $P < 0.05$). All communalities were above 0.5, indicating that most variables were well represented by the extracted factors. According to the criterion of eigenvalues > 1, three principal components were extracted, accounting for a cumulative variance contribution of 79.12%. Principal Component 1 (PC1) had an eigenvalue of 5.021 and explained 50.213% of the total variance, mainly representing seedling plant height (0.916), flowering plant height (0.802), seedling leaf length (0.923), flowering leaf length (0.871), seedling leaf width (0.942), flowering leaf width (0.837), and seedling petiole length (0.553). Principal Component 2 (PC2) had an eigenvalue of 1.554 and explained 15.54% of the variance, primarily representing flowering petiole length (0.949). Principal Component 3 (PC3) had an eigenvalue of 1.337 and explained 13.37% of the variance, mainly associated with tiller number (0.677) and leaf selenium content (0.841).

3.6 Comprehensive Evaluation of Phenotypic Traits and Selenium Enrichment Capacity

Using the eigenvector coefficients derived from PCA (Table 4), principal component function expressions were constructed. For instance, the function for PC1 was:

$$F_1 = 0.409X_1 + 0.358X_2 + 0.412X_3 + 0.389X_4 + 0.420X_5 + 0.374X_6 + 0.247X_7 + 0.016X_8 + 0.079X_9 - 0.003X_{10}$$

where X_1–X_{10} represent the standardized values of seedling plant height, flowering plant height, seedling leaf length, flowering leaf length, seedling leaf width, flowering leaf width, seedling petiole length, flowering petiole length, tiller number, and leaf selenium content, respectively. Similarly, PC2 and PC3 were expressed as:

$$F_2 = -0.056X_1 + 0.016X_2 + 0.114X_3 + 0.127X_4 + 0.071X_5 + 0.096X_6 - 0.602X_7 + 0.761X_8 - 0.109X_9 + 0.002X_{10}$$

$$F_3 = 0.059X_1 + 0.246X_2 - 0.117X_3 - 0.075X_4 - 0.118X_5 - 0.095X_6 + 0.013X_7 + 0.144X_8 + 0.585X_9 + 0.727X_{10}$$

A comprehensive evaluation function was then constructed using the variance contribution rates of each component as weights: $F_{composite} = 0.5021F_1 + 0.1554F_2 + 0.1337F_3$.

Table 4 Phenotypic traits principal component analysis of *Cardamine enshiensis* germplasm resources

Traits	Principal components		
	PC1	PC2	PC3
Seedling plant height cm	0.916	-0.07	0.068
Flowering plant height cm	0.802	0.02	0.285
Seedling Length of leaves cm	0.923	0.142	-0.135
Flowering Length of leaves cm	0.871	0.158	-0.087
Seedling Width of leaves cm	0.942	0.088	-0.136
Flowering Width of leaves cm	0.837	0.12	-0.11
Seedling Length of Petiole cm	0.553	-0.75	0.015
Flowering Length of Petiole cm	0.036	0.949	0.167
Number of tillers	0.178	-0.136	0.677
Leaf selenium content μg/Kg	-0.007	0.003	0.841
Eigenvalue	5.021	1.554	1.337
Variance contribution rate %	50.213	15.54	13.368
Cumulative contribution rate %	50.213	65.752	79.12

The comprehensive evaluation index ($F_{composite}$) was calculated for all 62 accessions, with higher scores indicating superior overall performance. The top 10 accessions (Fig. 4) exhibited scores ranging from 1.41 to 2.46. Their mean flowering plant height was 55.20 cm, mean flowering leaf length was 7.82 cm, mean flowering leaf width was 11.63 cm, mean tiller number was 6.78, and mean leaf selenium content was 657.93 μg/kg (Table 5). Among these, accession Ce43 achieved the highest comprehensive score (2.46), followed by Ce41 (2.10). Accessions Ce05, Ce66, and Ce22 also demonstrated strong performance, with scores of 1.67, 1.65, and 1.61, respectively, combining vigorous growth with high selenium accumulation capacity. These elite accessions can serve as valuable breeding materials for developing high-yield, selenium-enriched *C. enshiensis* cultivars, providing a theoretical basis for future breeding programs.

Table 5 Top 10 of *Cardamine enshiensis* germplasm resources with comprehensive evaluation

Accession ID	Seedling plant height /cm	Flowering plant height /cm	Seedling Length of leaves /cm	Flowering Length of leaves/cm	Seedling Width of leaves/cm	Flowering Width of leaves /cm	Seedling Length of Petiole /cm	Flowering Length of Petiole /cm	Number of tiller	Leaf selenium content /μg/Kg	comprehensive score	Rank
Ce43	11.4	59.02	6.42	7.95	8.02	11.92	3.51	11.2	7.2	215.54	2.46	1
Ce41	10.9	54.90	6.94	8.26	8.52	12.11	5.02	8.84	6	99.08	2.10	2
Ce31	11.54	54.55	5.96	8.73	8.6	11.74	4.85	9.16	6	14.95	2.01	3
Ce50	10.02	54.29	6.3	7.90	8	11.56	5.99	8.5	6.8	32.86	1.79	4
Ce05	10.6	50.58	6.16	6.12	7.76	11.33	4.40	8.72	7.2	1364.91	1.67	5

Ce66	9.46	62.04	3.38	7.76	4.3	10.67	7.3	9.14	7	3695.71	1.65	6
Ce22	9.4	58.84	5.52	7.43	7.24	11.84	7.72	5.96	7.2	783.76	1.61	7
Ce10	10.2	52.82	5.96	7.75	7.92	12.30	4.73	4.73	7.6	149.56	1.50	8
Ce07	8.76	53.11	5.5	8.49	6.86	11.34	4.09	10.28	6	190.13	1.44	9
Ce42	10.64	51.85	5.26	7.77	6.74	11.45	5.00	9.02	6.8	32.78	1.41	10

Fig 4 Images of the top 10 *Cardamine enshiensis* seedling-stage based on comprehensive score

4. Discussion

A higher coefficient of variation among germplasm resources indicates a greater degree of phenotypic diversity. The results of this study revealed abundant phenotypic variation among *Cardamine enshiensis* accessions, with coefficients of variation ranging from 10.61% to 30.88%. Notably, the variation coefficients of seedling-stage traits were generally higher than those of flowering-stage traits, suggesting stronger phenotypic plasticity during early growth and providing valuable guidance for directional selection at the seedling stage. The highly significant positive correlations between plant height, leaf length, and leaf width at the seedling and flowering stages ($r > 0.76$) indicate that early growth performance can effectively predict phenotypic expression in later stages.

Numerous studies have confirmed the exceptional selenium (Se) accumulation capacity of *C. enshiensis*. Under natural conditions in Enshi, the selenium concentrations in leaves (>1400 mg/kg) and whole plants (200–816 mg/kg) are remarkably high (Shi et al., 2015; Wu et al., 2023; Zhu et al., 2019). Xiang (2006) further demonstrated differential Se distribution within the plant, with the middle leaves containing significantly more Se than upper or lower leaves. Even in the absence of exogenous selenium supplementation, *Cardamine hirsuta* was able to accumulate substantial Se from the environment, with Se concentrations in roots and leaves reaching (38.5 ± 4.5) mg/kg and (28.1 ± 3.2) mg/kg dry weight, respectively. In the present study, several *C. enshiensis* accessions (e.g., Ce66, Ce05, and Ce08) exhibited strong Se accumulation ability. However, the underlying molecular mechanisms, such as the roles of Se transporters and related gene expression, remain unclear. Future studies integrating transcriptomic and metabolomic analyses are needed to elucidate the molecular basis of Se hyperaccumulation in this species.

Principal component analysis (PCA) and comprehensive scoring approaches have been widely applied in evaluating germplasm resources and selecting elite breeding materials. For example, Zhang et al. (2022) employed PCA and cluster analysis to classify 260 persimmon (*Diospyros kaki*) accessions into several breeding groups (flattened, spherical, oblong, and large-fruited types), meeting different selection goals. Zhu et al. (2025) performed comprehensive phenotypic evaluation of 124 okra (*Abelmoschus esculentus*) accessions, identifying early-maturing and high-yielding elite germplasm for new cultivar development. Lin et al. (2025) assessed seven agronomic traits and yield components of winter wheat in Xinjiang, finding that the number of effective spikes, grains per spike, and spikelets per spike were key yield-determining factors. Similarly, PCA-based comprehensive evaluations have been successfully applied to rice quality traits, cucumber fruit quality, and Chinese kale agronomic characteristics (Jiang et al., 2025; Wang et al., 2025; Cao et al., 2025).

In this study, a PCA-based comprehensive evaluation model was constructed using ten phenotypic traits of *C. enshiensis*. The model integrated three core dimensions—biomass at the seedling and flowering stages, petiole development at flowering, and the coordination between tillering capacity and Se accumulation—to quantitatively assess the comprehensive potential of each accession. High-ranking accessions such as Ce43, Ce41, and Ce66 exhibited superior plant architecture, high tillering capacity, and strong Se enrichment ability, validating the reliability of the model and providing valuable candidates for breeding high-yield and Se-rich cultivars. In particular, accession Ce66 demonstrated both high biomass and extremely high Se accumulation, highlighting its potential as a key parental line in future breeding programs. Considering that phenotypic traits are jointly regulated by genetic and environmental factors, combining molecular markers with phenotypic evaluation in future studies will further clarify the genetic diversity and population structure of *C. enshiensis* germplasm resources.

Overall, this study provides a multidimensional phenotypic assessment that elucidates the diversity patterns and breeding potential of *C. enshiensis*. The established comprehensive selection model and identified elite germplasm offer both theoretical foundations and practical guidance for the development of Se-enriched functional cultivars.

Acknowledgments

Ya-Rong Li analyzed the data and drafted the manuscript. Zhen Hu assisted with the selenium content determination. Cheng-Huan Yan and Feng-Ling Guo supervised the experiment and helped revise the manuscript. Hong-Qing Yin, Jia-Jia Ming, Wu Xu, and Ji-Qian Xiang provided the experimental materials for this study. We confirm that this manuscript is original, has not been published elsewhere. All authors have approved the final version and report no conflicts of interest.

Funding Statement

This work was supported by the Hubei Provincial Seed Industry Development Project (HBZY2023B004) and the Hubei Agricultural Technology Innovation Center Program (2025-620-000-001-007).

References

1. Cao Ping, Huang Kaiyue, Xiong Junhao, Jiang Ping, Zhu Bin, Fang Yong, Cui Tian, Fu Zhenlin, Li Kewen, Xu Xiaodi, Xu Yuying, Zhao Linfeng, Li Qiqiu, Shao Guirong. 2025. Comprehensive evaluation of main agronomic traits in 64 Chinese kale germplasm resources. Fujian Agricultural Science and Technology, 56(4): 31–38.
2. Duan Ruiwei, Zhang Xiangzhan, Li Bo, Wang Mengru, Xie Yarong, Li Pei, Wang Lei, Yang Jian, Xue Huabo. 2023. Comprehensive evaluation of fruit dot traits in 296 pear germplasm resources. Acta Horticulturae

Sinica, 50(11): 2305–2322.

3. Flora Reipublicae Popularis Sinicae (Vol. 33). 1987. Flora Reipublicae Popularis Sinicae Editorial Committee, Chinese Academy of Sciences. Beijing: Science Press. p.202.
4. Gao Zhanning, Yang Yongqian, Wang Shujie, Feng Hui, Xue Zhenggang. 2023. Comprehensive evaluation of 143 barley germplasm resources. Journal of Crop Science, (5): 59–65.
5. Guo Yan, Zhang Shuhang, Li Ying, Zhang Xinfang, Wang Yingjie, Wang Guangpeng. 2022. Leaf phenotypic diversity in chestnut germplasm from Yanshan Mountains. Acta Horticulturae Sinica, 49(8): 1673–1688.
6. Jiang Xiuying, Wang Jia, Li Jianguo, Xie Wenxiao, Pan Zhengyan, Han Yong, Lü Jun. 2025. Comprehensive evaluation of rice quality traits in Liaoning based on PCA and clustering. North Rice, 55(3): 1–7.
7. Lei Hongling, Fang Xiangliang, Zhou Dazhai. 2015. In vivo antioxidant effect of selenoprotein from *Cardamine violifolia*. Journal of Northwest Agriculture and Forestry University (Natural Science Edition), 43(1): 201–205.
8. Leng Jiagui, Li Dewen, Zhou Yali, Yao Zhengzhi, Wang Shaoming, Hou Yinghui, Li Jinhua, Luo Lisi. 2021. Analysis and comprehensive evaluation of agronomic traits in garlic germplasm. Journal of Southern Agriculture, 52(11): 2952–2961.
9. Li Liqiong, Chen Guangping, Ma Jixian, Lü Weiwu, Du Kanghua, Mu Wanfu. 2023. Comprehensive evaluation of agronomic traits in garlic germplasm resources. Acta Agriculturae Jiangxi, 35(12): 84–91.
10. Lin Huailong, Fan Lingyan, Chen Weilin, Yan Kang, Zhang Yueren, Pan Weide, Hou Yachen, Wang Bopeng, Zhang Long. 2025. Agronomic trait comparison and correlation in winter wheat varieties from Xinjiang. China Seed Industry, (6): 114–121.
11. Lin Yue, Li Yue, Cong Xin, Xia Yongmei, Huang Dejian, Chen Shangwei, Zhu Song. 2022. Selenium-enriched peptides isolated from *Cardamine violifolia* are potent in suppressing proliferation and enhancing apoptosis of HepG2 cells. Journal of food science, 87(7): 3235-3247.
12. Ma Yan, Yin Jinjing, Wang Jingyi, Liu Xin, He Jingren, Zhang Rui, Rao Shen, Cong Xin, Xiong Yin, Wu Muci. 2023. Selenium speciation and volatile flavor compound profiles in the edible flowers, stems, and leaves of selenium-hyperaccumulating vegetable *Cardamine violifolia*. Food Chemistry, 427: 136710.
13. Meng Shan, Xu Tingting, Zhu Xiaopin, Di Jiachun, Zhu Yin, Yang Xin, Zou Shuqiong, Yang Xue, Qin Cuihua, Yan Wei. 2023. Phenotypic diversity analysis of soybean landraces from Jiangsu Province. Journal of Plant Genetic Resources, 24(2): 419–436.
14. Peng Dan, Qi Chuandong, Zhao Qingqing, Zhou Jie, Yang Wenjie, Wu Jinping, Guo Fengling. 2025. Comprehensive evaluation and screening of garlic germplasm adaptability. China Vegetables, (5): 59–69.
15. Rao Shen, Yu Tian, Cong Xin, Xu Feng, Lai Xiaozhuo, Zhang Weiwei Liao Yongling, Cheng Shuiyuan. 2020. Integration analysis of PacBio SMRT- and illumina RNA-seq reveals candidate genes and pathway involved in selenium metabolism in hyperaccumulator *Cardamine violifolia*. BMC Plant Biology, 20 (1): 492.
16. Shao Shuxun, Zheng Baoshan, Luo Chong, Su Hongcan, Wang Mingshi, Liu Xiaojing, Pan Ziping. 2006. Discovery of selenium hyperaccumulator plant in Enshi area, Hubei Province. Acta Mineralogica Sinica, 26(2): 130.
17. Shi Aihua, Peng Zuoquan. 2015. Herbal textual research and biological study of *Cardamine violifolia*. *Studies of Trace Elements and Health*, 32(5): 61–64.
18. Sun Ming, Fu Kaixin, Fan Yan, Zhang

Xinquan, Zhang Chenglin, Guo Zhihui, Wang Xia, Ma Xiao. 2016. Phenotypic variation in 15 introduced Lolium multiflorum germplasms. Journal of Plant Genetic Resources, 17(4): 655–662.

19. Technical regulation for production of *Cardamine violifolia*: DB 4228/T 75-2022. 2022. Enshi Tujia-Miao Autonomous Prefecture Market Supervision Administration. Enshi: Enshi Tujia-Miao Autonomous Prefecture Market Supervision Administration.
20. Wang Huiting, Hou Dong, Ma Juanjuan, Tao Haixia, Zhang Dongqin, Xie Jianming, Huang Shuchao, Yue Hongzhong, Li Yali. 2025. Comprehensive evaluation of fruit quality in cucumber germplasm resources. Jiangsu Journal of Agricultural Sciences, 41(5): 943–951.
21. Wu Hongmei, Yang Wei, Cong Xin, Cheng Shuiyuan, Rao Shen. 2023. Research progress on selenium hyperaccumulator *Cardamine violifolia*. Food Science and Technology, 48(11): 26–32.
22. Xiang Tianyong. 2006. Biological characteristics and selenium-containing compounds in leaves of *Cardamine enshiensis* [Ph.D. Dissertation]. Changsha: Hunan Agricultural University.
23. Xu Hui. 2023. Nutritional components and product development of selenium-enriched vegetable *Cardamine violifolia*. Farm Products Processing, (21): 102–104.
24. Zhang Yue, Suo Yujing, Sun Peng, Han Weijuan, Diao Songfeng, Li Huawei, Zhang Jiajia, Fu Jianmin, Li Fangdong. 2022. Analysis of fruit morphological diversity in persimmon germplasm resources. Acta Horticulturae Sinica, 49(7): 1473–1490.
25. Zhang Hui, Hu Yunjie, Jing Hongbo, Huang Yue, Wang Xinyu, Dong Ruihan. 2025. Genetic diversity and trait-quality correlation in tillering onion germplasm. Northern Horticulture, 1–15.
26. Zhi Hui, Yang Jingjing, Luo Jianrang. 2025. Phenotypic diversity and ornamental evaluation of 96 peony cultivars. Acta Horticulturae Sinica, 52(3): 635–645.
27. Zhu Dan, Liu Ziji, Qin Yuling, Yin Xiaomin, Wang Xianli, Liu Weixia. 2025. Phenotypic diversity analysis of 124 Abelmoschus esculentus germplasms. Chinese Journal of Tropical Crops, 46(7): 1627–1637.
28. Zhu Song, Du Chaodong, Yu Tian, Cong Xin, Liu Yuanfa, Chen Shangwei, Li Yue. 2019. Antioxidant activity of selenium-enriched peptides from the protein hydrolysate of Cardamine violifolia. Journal of Food Science, 84(12): 3504-3511.