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GENETIC DIVERSITY ANALYSIS BASED ON PHENOTYPIC TRAITS AND SSR MARKERS OF *ALLIUM SATIVUM* L. GERMPLASM

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Abstract

By comprehensively utilizing genetic mapping and molecular marker technologies, we emphasize conventional asexual propagation breeding, supplemented by biotechnological breeding methods. This approach integrates traditional phenotype selection with modern genotype selection to provide a basis for variety identification and genetic improvement, aiming to shorten breeding cycles and enhance breeding efficiency. We evaluated 114 garlic germplasm resources for 12 phenotypic traits related to flowering and growth development. We conducted PCR amplification of the genomic DNA from these samples using 10 pairs of simple sequence repeat (SSR) primers. Then, cluster analysis was carried out, and the genetic diversity was assessed based on the polymorphic bands obtained from gel electrophoresis. We performed clustering and principal component analysis using SSR markers on the 114 garlic samples to identify germplasm exhibiting desirable traits. Results showed significant variation in traits related to flowering, while traits associated with growth and development exhibited relatively lesser variation coefficients. Among the 10 pairs of primers used, 6 pairs yielded satisfactory results, detecting a total of 38 allele loci across the 114 samples. Shannon's diversity index (I) ranged from 0.6750 to 1.5319 with a mean of 1.2479, while observed heterozygosity (Ho) and expected heterozygosity (He) ranged from 0.3482 to 0.5526 and 0.4210 to 0.6935, respectively. Cluster analysis classified the population into 4 groups, with close associations observed between grouping and flowering traits.

Key words: *Allium sativum*, SSR markers, Genetic diversity, Genetic mapping, Phenotypic analysis.

Introduction

Garlic (*Allium sativum* L.), a perennial herbaceous plant belonging to the family *Liliaceae* is indigenous to western Asia or Europe. It exhibits two main varieties, namely hardneck garlic and softneck garlic. Throughout history, *A. sativum*, has been cultivated extensively worldwide, showcasing its significant agricultural and cultural importance (Filyushin *et al.,* 2023). *A. sativum*., a medicinal and culinary plant, is celebrated for its delicious flavor and distinctive aroma, making it a popular vegetable and seasoning in cuisines across the globe. Its ubiquitous presence in the dietary cultures of nearly every country highlights the widespread appreciation for garlic's sensory attributes and its versatile culinary applications (Khubber *et al.,* 2020). *A. sativum*., renowned for its nutritional and medicinal properties, exhibits a wide range of effects including antiviral, antioxidant, anti-tumor, and immunomodulatory activities. Its documented efficacy in traditional pharmacopoeias such as "Compendium of Materia Medica" underscores its significant role in promoting health and well-being, as well as its therapeutic potential in various medical applications (El-Saber Batiha *et al.,* 2020). *A. sativum*, a crop propagated through asexual reproduction primarily using bulbs, has historically been identified based on morphological characteristics. However, the prolonged domestication and selective breeding of garlic varieties have led to a significant reduction in genetic diversity. Therefore, the introduction of efficient and accurate molecular marker identification methods is urgently needed to assess and preserve the genetic diversity of *A. sativum.*

Simple sequence repeat (SSR) markers are molecular tools designed based on conserved sequences flanking tandem repeats to analyze the polymorphism of amplified fragment lengths (da Cunha *et al.,* 2014). SSR markers, known for their good repeatability, rich polymorphism, and high universality, have been widely applied in recent years in the fields of plant germplasm identification, genetic diversity analysis, and the construction of DNA fingerprinting (Debbabi *et al.,* 2021). The utilization of SSR molecular markers in garlic was reported relatively late. Initially, Jabbes employed seven sets of ISSR primers to analyze the genetic diversity of 31 garlic varieties from Tunisia and four garlic varieties from France (Jabbes *et al.,* 2011). In recent years, the application of SSR markers in garlic has gradually expanded. Kumar evaluated the genetic diversity of 53 Indian garlic germplasms using SSR markers (Kumar *et al.,* 2019). Li have newly developed 4372 EST-SSR markers to assess the genetic diversity and population structure of 127 garlic germplasms. However, studies simultaneously analyzing garlic diversity using morphological and SSR molecular markers are relatively scarce(Li *et al.*, 2022).

This study utilized 114 germplasms of *A. sativum* to analyze genetic diversity across 12 phenotypic traits. Simultaneously, SSR molecular markers were employed for polymorphic primer screening and genetic diversity analysis. The aim of this study was to investigate the bulb stalk and plant traits of garlic germplasm resources, including scape length, floret length, scape base thickness, scape middle thickness, floret width, and single scape weight, plant height, pseudostem thickness, pseudostem height, plant spread, leaf length, and leaf width.

Materials and Methods

Phenotypic trait determination: All of the 114 germplasm of *A. sativum*. resources selected for the experiment were from Chinese landraces (Table 1). Randomly selected 4-5 garlic plants were measured, and the average values were calculated for statistical analysis. Clustering and principal component analysis were conducted based on SSR markers with the aim of identifying germplasms exhibiting desirable traits. The findings serve as a reference for parent selection and germplasm evaluation, aiding in breeding programs and resource management.

Table 1. 114 Chinese garlic germplasm resources including their accession numbers, names, places of origin, and main commercial types.

Accession	Germplasm name	Place of origin	Main commercial type
9	Kaiyuan Garlic	lishu, Jilin	Solo Garlic
12	Qinchuan Garlic	yangling, Shaanxi	Solo Garlic
14	Xianyang Garlic	xianyang, Shaanxi	Solo Garlic
16	Xinjiang Purple-Skinned Garlic 1	Urumqi, Xinjiang	Solo Garlic
24	Baoding Local Garlic	Baoding, Hebei	Solo Garlic
25	Jinxiang White-Skinned Garlic 1	Jinxiang, Shandong	Solo Garlic
27	Mingshui Garlic	Jinan, Shandong	Solo Garlic
30	Wucheng Garlic	Dezhou, Shandong	Solo Garlic
35	Jiaxiang White-Skinned Garlic	Jiaxiang, Shandong	Solo Garlic
36	Sichuan Soft-Leaf Garlic	Pengzhou, Sichuan	Garlic Scapes
37	Yunnan Fragrant Garlic	Pu'er, Yunnan	Garlic Scapes
38	Tianjin Local Garlic	Tianjin	Garlic Scapes
40	Taizhou Local Garlic	Taizhou, Zhejiang	dual-purpose for scapes and bulbs
44	Kunming Local Garlic	Kunming, Yunnan	Solo Garlic
46	Yantai Local Garlic 1	Yantai, Shandong	Solo Garlic
47	Puning Garlic	Huizhou, Guangdong	
49	Inner Mongolia Local Garlic	Hohhot, Nei Mongol	Solo Garlic
52	Yunnan Solo Garlic	Dali, Yunnan	Garlic Scapes
53	Sichuan Scape Garlic	Pengzhou, Sichuan	Garlic Scapes
55	Dezhou Local Garlic	Dezhou, Shandong	Solo Garlic
56	Yantai Local Garlic 2	Yantai, Shandong	Solo Garlic
58	Chaling Garlic	Zhuzhou, Shandong	Solo Garlic
59	Red Seven-Star 1)	Wenjiang, Sichuan	Garlic Scapes
60	Soft-Leaf Garlic 2	Pengzhou, Sichuan	Garlic Scapes
61	Cangshan Coarse Garlic	Lanling, Shandong	dual-purpose for scapes and bulbs
63	Cangshan Second-Grade Coarse Garlic (Black Soil)	Lanling, Shandong	dual-purpose for scapes and bulbs
64	Cangshan Second-Grade Coarse Garlic (Yellow Soil	Lanling, Shandong	dual-purpose for scapes and bulbs
65	Cangshan Red-Skinned Garlic	Lanling, Shandong	dual-purpose for scapes and bulbs
66	Cangshan PuKe 1	Lanling, Shandong	dual-purpose for scapes and bulbs
70	Pizhou Purple-Skinned Garlic	Pizhou, Jiangsu	Solo Garlic
71	Pizhou White-Skinned Garlic 1	Pizhou, Jiangsu	Solo Garlic
72	Daming Purple-Skinned Garlic	Daming, Hebei	Solo Garlic
76	Qixian Purple-Skinned Garlic	Qixian, Henan	Solo Garlic
77	Qixian Coreless Garlic 1	Qixian, Henan	Solo Garlic
78	Qixian Coreless Garlic 2	Qixian, Henan	Solo Garlic
79	Ji Garlic No. 5	Jining, Shandong	Solo Garlic
80	Qixian Local Garlic	Qixian, Henan	Solo Garlic
81	Qixian Coreless Garlic	Qixian, Henan	Solo Garlic
83	3 Qixian Early-Maturing Garlic 1	Qixian, Henan	Solo Garlic
85	Qixian Early-Maturing Garlic 2	Qixian, Henan	Solo Garlic
88	Yunong Early-Maturing Garlic	Qixian, Henan	Solo Garlic
89	Shucheng Garlic	Shucheng, Anhui	Solo Garlic
91	Changsha Garlic	Changcha, Hunan	Solo Garlic
92	Fuling Garlic	Fulin, Chongqing	Solo Garlic
93	Big Green Leaf Garlic	Liaocheng, Shandong	Solo Garlic
94	Guan County Local Garlic	Liaocheng, Shandong	Solo Garlic
98	Jinxiang White-Skinned Garlic 2	Jinxiang, Shandong	Solo Garlic
102	Jinxiang Purple-Skinned Garlic 1	Jinxiang, Shandong	Solo Garlic
104	Jinxiang Purple-Skinned Garlic 2	Jinxiang, Shandong	Solo Garlic
106	Laiwu Purple-Skinned Garlic	Laiwu, Shandong	Solo Garlic
109	Shuyuan Scape Garlic 1	Qufu, Shandong	Garlic Scapes
111	Feng County Scape Garlic 1	Fengxian, Jiangsu	Garlic Scapes
114	Feng County Scape Garlic 2	Fengxian, Jiangsu	Garlic Scapes
115	Feng County Scape Garlic 3	Fengxian, Jiangsu	Garlic Scapes
119	Shuyuan Scape Garlic 2	Qufu, Shandong	Garlic Scapes
125	Zunyi Purple-Skinned Garlic 1	Zunyi, Guizhou	Solo Garlic
127	Zunyi Purple-Skinned Garlic 2	Zunyi, Guizhou	Solo Garlic

Table 1. (Cont'd.).

Accession	Germplasm name	Place of origin	Main commercial type
129	Shuangfan Garlic	Yuexi, Anhui	Solo Garlic
133	Yunnan Purple-Skinned Garlic	Dali, Yunnan	Garlic Scapes
135	Zhaosu Purple-Skinned Garlic 1	Zhaosu, Xinjiang	Solo Garlic
138	Wanzhou Garlic 1	Wanzhou, Sichuan	Solo Garlic
139	Wanzhou Garlic 2	Wanzhou, Sichuan	Garlic Scapes
140	Qingke Garlic 1	Shanghe, Shandong	Solo Garlic
142	Cheng Purple-Skinned Garlic	Tianshui, Gansu	Solo Garlic
143	Zhaosu Purple-Skinned Garlic 2	Tianshui, Gansu	Solo Garlic
145	Pizhou White-Skinned Garlic 2	Tianshui, Gansu	Solo Garlic
146	Haidaixiang Purple 1	Daihai, Nei Mongol	Solo Garlic
147	Haidaixiang Spicy 2	Daihai, Nei Mongol	Solo Garlic
148	Wudang Mountain Wild Garlic	Wudangshan, Hubei	Solo Garlic
150	Yongnian Garlic	Yongnian, Hebei	Solo Garlic
152	Qinghai Purple-Skinned Garlic	Haixi, Qinghai	Solo Garlic
155	Space No. 2	Jinxiang, Shandong	Solo Garlic
157	Dezhou Red-Skinned Garlic	Dezhou, Shandong	Solo Garlic
158	Qingke Garlic 2	Shanghe, Shandong	Solo Garlic
159	Linqing Garlic	Lingqing, Shandong	Solo Garlic
160	Zoucheng Xiangcheng Garlic	Zoucheng, Shandong	Solo Garlic
162	Majiang Red Garlic	Majiang, Guizhou	Solo Garlic
163	Nanjing Four-Six Clove Garlic	Nanjing, Jiangsu	Solo Garlic
167	Ershui Early Garlic 2	Pengzhou, Sichuan	Garlic Scapes
168	Red Seven-Star 2	Wenajiang, Sichuan	Garlic Scapes
179	Laiwu Red-Skinned Garlic	Laiwu, Shandong	Solo Garlic
180	Laiwu White-Skinned Garlic	Laiwu, Shandong	Solo Garlic
182	Ledu Purple-Skinned Garlic	Ledu, Qinghai	Solo Garlic
183	Xinjiang Purple-Skinned Garlic 2	Urumqi, Xinjiang	Solo Garlic
184	Baodi Garlic	Baodi, Tianjin	Solo Garlic
185	Xingping Garlic	Xingping, Shaanxi	dual-purpose for scapes and bulbs
186	Xindu Garlic	Xindu, Sichuan	Garlic Scapes
187	Acheng Garlic	Acheng, Heilongjiang	Solo Garlic
188	Jimusaer Garlic White-Skinned Garlic	Jimusaer, Xinjiang	Solo Garlic
189	Heishui Garlic	Heishui, Sichuan	Solo Garlic
190	Haicheng Garlic	Hicheng, Liaoning	Solo Garlic
191	Shanghe Garlic (Red-Skinned)	Shanghe,Shandong	Solo Garlic
192	Shanghe Garlic (White-Skinned)	Shanghe, Shandong	dual-purpose for scapes and bulbs
193	Tuanwan Garlic	Qingdao, Shandong	dual-purpose for scapes and bulbs
194	Lianghe Garlic	Anqiu, Shandong	dual-purpose for scapes and bulbs
195	Cangshan PuKe 2	Lanlin, Shandong	dual-purpose for scapes and bulbs
196	Qixian Garlic	Qixian, Henan	Solo Garlic
197	Dali Solo Garlic	Dali, Yunnan	Garlic Scapes
198	Shanggao Purple-Skinned Garlic	Shanggao, Jiangxi	Solo Garlic
199	Linhu Garlic	Shangrao, Jiangxi	dual-purpose for scapes and bulbs
200	Changning Garlic	Changning, Shanxi	Solo Garlic
201	Beidong Garlic	Beidong, Shanxi	Solo Garlic
202	Xiliu Purple-Skinned Garlic	Xiliu, Nei Mongol	Solo Garlic
203	Haidaixiang Garlic	Daihai, Nei Mongol	Solo Garlic
204	Ledu Purple-Skinned Garlic	Ledu, Qinghai	Solo Garlic
205	Gengzhuang Garlic	Hicheng, Liaoning	Solo Garlic
P131	Jiqing Garlic 1	Jining, Shandong	Solo Garlic
JS-1	Ji Garlic 1	Jining, Shandong	Solo Garlic
JS-2	Ji Garlic 2	Jining, Shandong	Solo Garlic
JS-3	Ji Garlic 3	Jining, Shandong	Solo Garlic
Jin-3	Gold Garlic 3	Jinxiang, Shandong	Solo Garlic
Jin-4	Gold Garlic 4	Jinxiang, Shandong	Solo Garlic
jiucong	Leek	Guangxi	Garlic Scapes
rb	Leek Close Relative Species	Guangxi	Garlic Scapes

SSR molecular markers: Genomic DNA from 114 garlic germplasms was extracted using a plant genome DNA extraction kit (Qingke Biotechnology Co., Ltd.). The quality and concentration of the extracted DNA were assessed using 1% agarose gel electrophoresis and a spectrophotometer nucleic acid detection instrument, respectively. The extracted DNA was stored at -20°C for future use. Synthesis of 7 Pairs of Fluorescent-Modified primer for detection, with FAM, HEX, TAMRA, and ROX modifications at the 5' end of the F primer, and synthesis of PAGE primers for the R primer. Subsequently, synthesis of 3 pairs of adapter primers was conducted, with the addition of a 16-base universal Tag sequence at the 5' end of the F primer during synthesis. Finally, a multiplex amplification approach was employed using Tag-modified primers, F primers containing the Tag sequence, and R primers, totaling three primers, to achieve the objective.

PCR amplification program: Pre-denaturation at 98°C for 2 minutes; Denaturation at 98°C for 10 seconds, Annealing at 55°C-58°C for 10 seconds, Extension at 72°C for 10 seconds, for 35 cycles; Final extension at 72°C for 5 minutes; PCR products stored at 4°C. Amplified PCR products were subjected to agarose gel electrophoresis (2 μL sample + 6 μL loading dye), and template concentration was determined through gel electrophoresis. Samples were diluted with water to the required concentration for capillary electrophoresis, followed by analysis using the ABI3730XL DNA Analyzer. The obtained data were analyzed using Genemapper software. Based on the electropherogram, the presence of bands was recorded as "1" while absence of bands was recorded as "0". A matrix of 1s and 0s was established to determine the presence of specific fragment polymorphisms associated with different primers.

Data analysis: The analysis of phenotypic data was conducted using SPSS software. Genetic diversity indices for SSR loci, including observed alleles (Na), effective alleles (Ne), Shannon's information index (I), polymorphic information content (PIC), observed heterozygosity (Ho), and expected heterozygosity (He), were calculated using Popgen32 and GenAlEx version 6.501 software.The Markov Chain Monte Carlo (MCMC) method was utilized to predefine population grouping (K) and compute the optimal K value based on the approach proposed by Evanno (Evanno *et al.,* 2005). The genetic structure of garlic populations was analyzed using STRUCTURE version 2.3.3 software. The unweighted pair group method with arithmetic mean (UPGMA) was employed to construct a clustering tree for individuals. The UPGMA tree was built using populations-1_2_30 software, and visualization and editing of the clustering tree were performed using FigTree version 1.4.2 software.

Results

Diversity analysis of phenotypic traits: A total of 114 garlic germplasm resources were evaluated for 12 phenotypic traits, including traits related to bolting and those associated with growth and development. Statistical analysis was performed to determine the coefficient of variation (CV) for each trait, indicating the degree of variation within the population (Table 2). Significant differences were observed in the phenotypic traits among the 114 garlic germplasm resources. The CV for traits related to garlic bolting ranged from 57.04% to 70.42%, with the variability primarily attributed to the presence or absence of bolting in certain varieties. Conversely, traits associated with garlic growth and development exhibited a lower CV range of 14.43% to 26.85%, indicating less variability compared to bolting-related traits.

The correlation among traits has a significant impact on distinguishing garlic germplasm materials, and this influence can be mitigated through principal component analysis (PCA). A PCA conducted on 114 germplasm materials revealed the extraction of 3 principal components from 12 traits, with a cumulative contribution rate reaching 85.576% (Table 3). The contribution rate of the first principal component was 55.853%, with traits such as scape length, floret length, scape base thickness, scape middle thickness, floret width, and single scape weight ranking prominently. This indicates that the first principal component represents a comprehensive response to these phenotypic traits, summarizable as the "bolting" factor. The second and third principal components, on the other hand, comprised traits such as plant height and leaf length.

Table 2. Coefficient variation index of phenotypic characters of *Allium sativum* L.

Character	Minimum	Maximum	Mean	SD	Variance	CV /%
Scape length	0.000	74.820	31.663	19.427	377.394	61.35%
Floret length	0.000	35.720	16.895	10.682	114.100	63.22%
Scape base thickness	0.000	1.150	0.543	0.310	0.096	57.04%
Scape middle thickness	0.000	1.040	0.484	0.276	0.076	57.11%
Floret width	0.000	1.520	0.711	0.408	0.167	57.39%
Single scape weight	0.000	53.680	12.903	9.087	82.569	70.42%
Plant height	20.300	87.630	60.058	9.606	92.279	15.99%
Pseudostem thickness	0.570	4.100	1.840	0.494	0.244	26.85%
Pseudostem height	4.800	46.000	29.293	6.859	47.039	23.41%
Plant spread	14.530	99.370	55.746	13.218	174.704	23.71%
Leaf length	28.100	71.130	55.227	7.968	63.484	14.43%
Leaf width	0.200	7.770	3.464	0.802	0.644	23.16%

Table 3. Principal components analysis of phenotypic characters of *Allium sativum* L.

Character	Principal component		
	1	2	3
Bulb stalk length	0.203	-0.074	-0.026
Flower bud length	0.17	-0.035	-0.01
Bulb stalk base thickness	0.192	-0.042	-0.019
Bulb stalk middle thickness	0.197	-0.048	-0.026
Flower bud width	0.214	-0.084	-0.04
Single bulb weight	0.168	0.012	-0.07
Plant height	-0.095	0.085	0.443
Pseudostem thickness	-0.094	0.356	0.05
Pseudostem height	-0.032	-0.082	0.487
Plant spread	-0.036	0.379	-0.331
Leaf length	-0.069	0.308	0.032
Leaf width	-0.033	0.264	0.09
Cumulative contribution rat	55.853%	71.718%	85.576%

Analysis of SSR loci and primer polymorphisms: Out of 10 pairs of primers, 6 pairs were selected for their better detection results. These 6 pairs of primers detected a total of 38 allele in 114 samples (Table 4). The minimum number of alleles was 3 (SSR-80) and the maximum number of alleles was 9 (GB-ASM-04 and GB-ASM-05), with an average of 6.3333 alleles per locus. The total number of effective alleles was 16.9821, with a range from 1.7217 (SSR-80) to 3.2301 (SSR-53), and an average of 2.8303 effective alleles per locus. The Shannon's Index (I) ranged from 0.6750 (SSR-80) to 1.5319 (GB-ASM-04), with an average value of 1.2479. The polymorphism information content (PIC) ranged from 0.3478 (SSR-80) to 0.6577 (GB-ASM-04), with an average value of 0.5814, indicating all 6 pairs of primers had high polymorphism information content (PIC>0.25). Observed heterozygosity (Ho) and expected heterozygosity (He) ranged from 0.3482 (GB-ASM-07) to 0.5526 (SSR-80) and 0.4210 (SSR-80) to 0.6935 (SSR-53), respectively, with mean values of 0.4508 and 0.6322. The average inbreeding coefficient was 0.2838, with a range from -0.3184 (SSR-80) to 0.4726 (GB-ASM-07).

Opulation genetic structure analysis: The most suitable K value was determined to be 3, indicating the presence of three genetic clusters within the 114 samples. When K=3, the genetic composition of the 114 samples was primarily derived from genetic clusters 1 and 2 (Fig. 1a-b,Table 5).

Table 5. The sample proportions at K=3 in the STRUCTURE analysis.

K=3	1	2	3
Samples	50.46%	41.67%	7.87%

The genetic similarity matrix of different Chinese garlic germplasm was calculated using the populations-1_2_30 software, followed by UPGMA cluster analysis. The UPGMA dendrogram based on individuals showed a considerable amount of inter-group mixing, indicating substantial genetic variation within the population. Cluster analysis divided the population into four categories. Comparing various fertility traits, the division of three groups showed a close correlation with garlic bolting traits (Fig. 1-c). Specifically, seven germplasms numbered 9, 36, 38, 47, 60, 139, and 186 clustered together, all exhibiting non-bolting traits with completely sterile scapes. These scapes only produce membranous bracts without flowers or bulbils, making them primarily useful for green garlic shoots. Another group, consisting of 57 germplasms including numbers 12, 24, and 25, clustered together as head garlic types, characterized by moderate flower scape growth vigor. Based on the Nei's genetic distance, principal co-ordinates analysis (PCoA) on 114 samples, revealing that the samples could be roughly divided into 4 groups, which was consistent with the clustering analysis results (Fig. 2).

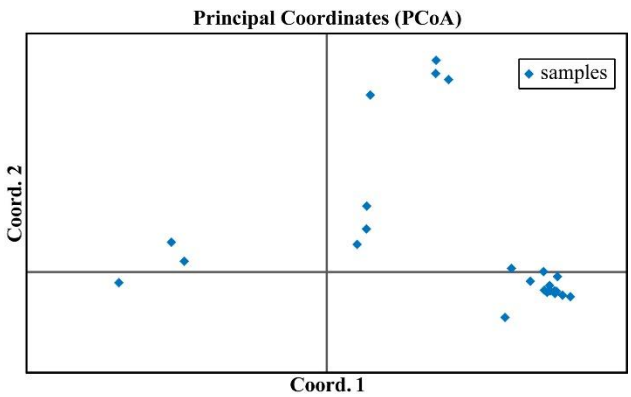

Fig. 2. The PCoA results of the 114 samples. (The 114 samples were roughly divided into 4 groups).

Table 4. 6 pairs of SSR loci and SSR polymorphism primers.

Locus	Primers	Na	Ne	I	PIC	Ho	He	F	PHWE
Asa24	F: TTGTTGTGCCGAGTTCCATA R: CAGCAATTTACCAAAGCCAAG	5	2.8112	1.2147	0.5912	0.4737	0.6471	0.2648	0.000000***
B-ASM-04	F: CACAGCAACATGCACCAT R: TGCCGGAACTCGATATT	9	3.1847	1.5319	0.6577	0.4071	0.689	0.4066	0.000000***
GB-ASM-05	F: CTTGCCGGAACTCGATATT R: CACAGCAACATGCACCAT	9	3.0915	1.4691	0.643	0.4144	0.6796	0.3874	0.000000***
GB-ASM-07	F: CACGCGAATCTTTCTTGG R: TGCAAAGCAATATGGCAG	7	2.9429	1.3206	0.6148	0.3482	0.6632	0.4726	0.000000***
SSR-53	F: ACAAGGTCGACATCGTTTG R: GGGCTTCACCTGAACACA	5	3.2301	1.2763	0.634	0.5089	0.6935	0.2629	0.000000***
SSR-80	F: AATCTCCCTCCAAAGTCCC R: CTGTATTTTGTGTAAAGCATCA	3	1.7217	0.675	0.3478	0.5526	0.421	-0.3184	0.002702**
Mean		**6.3333**	**2.8303**	**1.2479**	**0.5814**	**0.4508**	**0.6322**	**0.2838**	**0.0005**

Note: Na: Number of allele, Ne: Effective number of allele, I: Shannon's index, PIC: Polymorphism information content, Ho: Observed heterozygosity, He: Expected heterozygosity, F: Inbreeding coefficient, PHWE: Hardy-Weinberg equilibrium.
*: p<0.05, **: p<0.01, ***: p<0.001

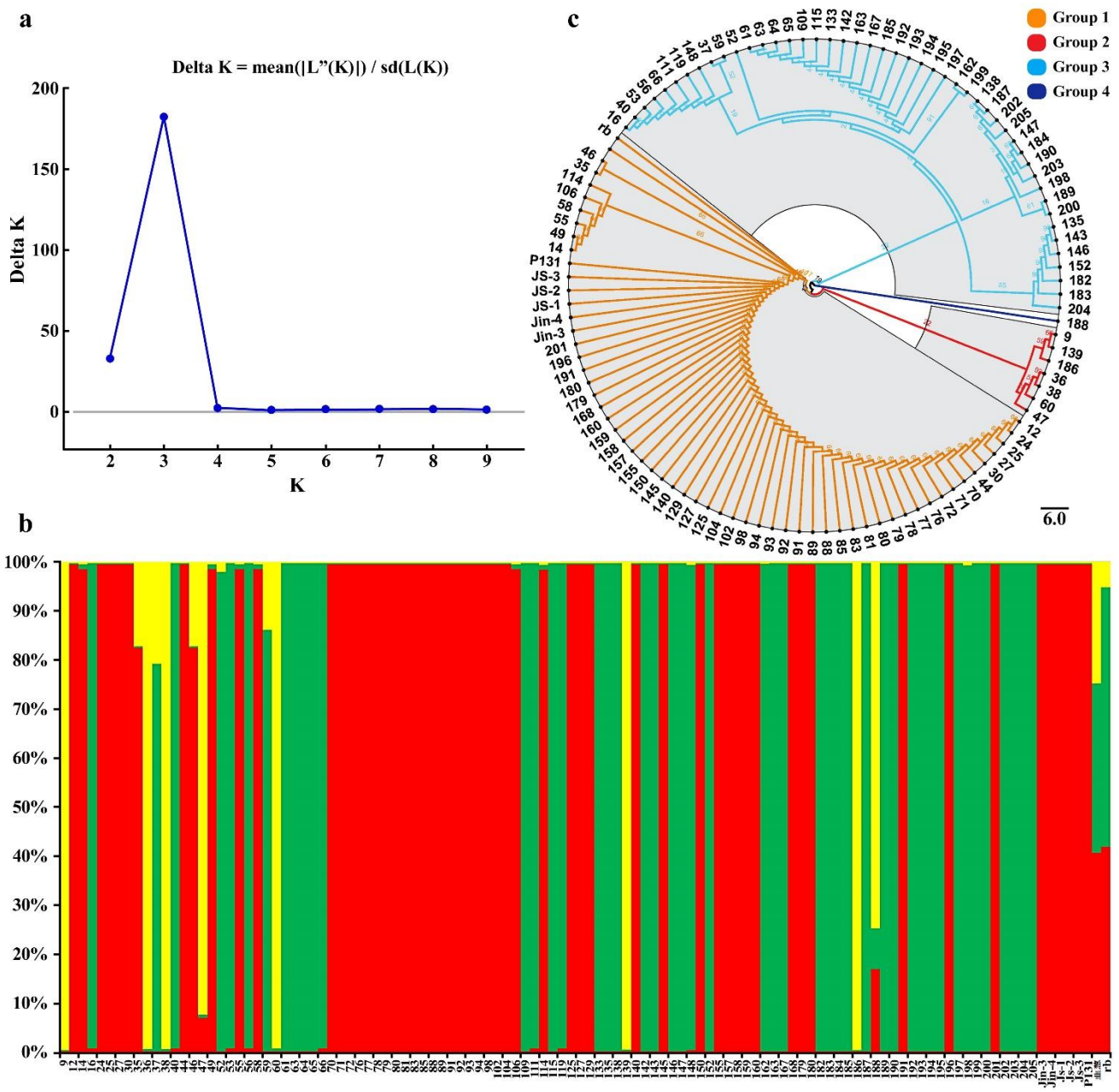

Fig. 1. Opulation genetic structure analysis: a, the plot of K values variation obtained using the ΔK method in the STRUCTURE analysis; b, the STRUCTURE results for 114 samples at K=3; c, The UPGMA results of the 114 samples. (The 114 samples were roughly divided into 4 groups.

Discussion

This study analyzed the genetic diversity of 114 garlic germplasm materials by utilizing phenotypic traits and SSR molecular markers. The results showed that the variation coefficients of 12 phenotypic traits fluctuate significantly, suggesting differences among germplasm materials. However, the average variation coefficient of phenotypic traits and the average Shannon index of 6 SSR primers were relatively low, indicating narrow genetic background among garlic germplasm. Principal component analysis extracted 3 principal components from the 12 phenotypic traits, with a cumulative contribution rate of 85.576%. This analysis was able to highlight important traits, eliminated overlapping and insignificant traits. Based on the model-based grouping method using STRUCTURE(Earl & vonHoldt, 2012), six subpopulations within the garlic germplasm were estimated according to the genome scores. The results of cluster analysis, PCoA, and population structure grouping were consistent to a certain extent, which demonstrated the reliability of the overall analysis.

Germplasm resources form the foundation of crop genetic breeding. The discovery and utilization of excellent traits in plant germplasm resources will further promote breeding work (Chung & Staub, 2003). China has rich germplasm resources of *A. sativum*, but its genetic base is narrow. Garlic *(A. sativum*) reproduces only asexually, yet it exhibits significant morphological variation both within and among varieties (Singh *et al.,* 2012). Therefore, analysis of genetic diversity and relatedness among individuals is important for garlic improvement. SSR markers can effectively reveal the genetic diversity of garlic (Chand *et al.,*

2015). By using two methods, namely phenotypic traits and SSR molecular markers, the genetic diversity of 114 garlic germplasm resources was analyzed. Through the comparison of the two methods, the SSR cluster analysis divided the garlic into 4 groups, showing a close correlation with the bolting traits of *A. sativum*. Among them, 7 germplasms numbered 9, 36, 38, 47, 60, 139, and 186 clustered together, all exhibiting non-bolting traits. Unlike plants that are grown from seeds, clonally propagated species like garlic require regeneration and maintenance every year, which in turn increases the maintenance cost (Etoh & Simon, 2002). Therefore, molecular marker analysis is very useful in identifying potential duplicates and further reducing annual maintenance costs.

In conclusion, to further improve the accuracy of garlic germplasm evaluation in future breeding work, a comprehensive approach that integrates phenotypic and molecular marker studies is recommended. In terms of phenotypic traits, strictly adhering to standardized descriptions, increasing the number of observed traits, and at the molecular level, increasing the number of primers to obtain more genetic loci are essential. This will contribute to a more comprehensive and accurate differentiation of germplasm resources and characterization of genetic diversity.

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