

Assessment of genetic diversity of *Prunus salicina* 'Shazikongxinli' by morphological traits and molecular markers

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Abstract

Prunus salicina 'Shazikongxinli' is one of the most economically valuable and reputable *Prunus salicina* varieties in China. Understanding the genetic diversity and population structure of 'Shazikongxinli' is crucial for excellent germplasm breeding and conservation of genetic resources. In the present study, the genetic variation status of 50 'Shazikongxinli' was assessed using morphological traits, IRAP and ISSR markers, while 18 other plum varieties were selected as outgroups. The results show that the average Shannon-Weaver diversity index (H') of 32 qualitative traits in 50 test 'Shazikongxinli' is 0.557, the average coefficient of variation of 11 quantitative traits is 15.57%, and a total of 232 and 105 polymorphic loci are obtained from 22 IRAP and 15 ISSR primers, respectively. All three marker systems show relatively rich polymorphism, especially the IRAP markers. This may be related to the nature of the retrotransposons in the IRAP markers, which may be more suitable for intraspecific variability detection than ISSR. In addition, all three markers cluster the 68-test germplasm into two groups, with all of 'Shazikongxinli' clustered into one group and most of the other plum varieties as outgroups clustered into another group. This suggests a relatively narrow genetic base within the current 'Shazikongxinli'. These results will be useful in understanding the genetic diversity of the germplasm resources of 'Shazikongxinli' and provide comprehensive information for the breeding of superior germplasm.

Introduction

Prunus salicina 'Shazikongxinli', belonging to the Rosaceae family of the genus *Prunus*, is an endemic plum species cultivated in Yanhe county, Southwest China's Guizhou province. It belongs to an indigenous cultivar of *Prunus salicina*, which has been cultivated in Yanhe for about 160 years and have been awarded the designation of "China's Quality Plum". In addition, it has been listed as a National Geographical Indication Product of China. As of 2021, the area of 'Shazikongxinli' planted in Yanhe County had grown to 30 km², and the annual output was about 20000 tons (Zhang et al. 2020). Under the process of long-term natural evolution and human selection, the 'Shazikongxinli' gradually formed a variety group. At the same time, in recent years, a large area of 'Shazikongxinli' has been planted in Yanhe County, but the production of seedlings is chaotic, and the source of scion is heterogeneous, which further promotes the diversification of 'Shazikongxinli' varieties (Wu et al. 2022). The existence of different strains provides germplasm resources for the excavation of high quality germplasm. But it is not clear whether these differences are simply due to environmental factors or different cultivation techniques, or whether the genetic information has been mutated. Therefore, it is necessary to assess the level of genetic variation in the 'Shazikongxinli' population.

Genetic diversity reflects the genetic background, breeding potential and utilization value of a species (Liu et al. 2007, Mei et al. 2021). Learning the extent of genetic diversity in germplasm collections is an important basis for studying the origin and evolution of a species, and is also a crucial step for optimal conservation and breeding of new germplasm (Tao et al. 2014, Urrestarazu et al. 2018). Genetic diversity could be determined using morphological, physiological, chromosome structure, biochemical and DNA marker analysis (Rana et al. 2015). Morphological characters are an important tool for analyzing crop genetic diversity and could estimate the activity of genotypes in given environmental conditions (Ruchi et al. 2020). For crops such as fruit, morphological markers have tremendous reference value and can visually reflect the merit of the germplasm. Moreover, molecular markers are the main technique used in genetic variability analysis and are considered more reliable. Earlier investigations have been conducted to illustrate the genetic diversity of plums based on morphological variability (Zhang and Zhou, 1998, Yu et al. 2011) and molecular markers such as RAPD (Ben et al. 2015), AFLP (Goulao et al. 2001), SSR (Basilio et al. 2012, Pop et al. 2018, Acuna et al. 2019), ISSR (Liu et al. 2007, Wu et al. 2019) and SNP (Wei et al. 2021). Until now, there have been few studies have been conducted for *Prunus salicina* 'Shazikongxinli' genetic variation. In this study, morphological traits and two molecular markers (ISSR and IRAP) were used to analyze the variation of 'Shazikongxinli' germplasm resources, whose aims to appraise the overall genetic diversity of the currently cultivated 'Shazikongxinli', to provide theoretical guidance for the breeding of excellent 'Shazikongxinli' germplasm.

Materials And Methods

Plant materials

Based on the field survey, 50 healthy adult fruit trees were randomly chosen as experimental germplasm from the *Prunus salicina* 'Shazikongxinli' orchard, Nanzhuang Village, China in September 2020. 18 other plum varieties were also chosen as controls at the plum germplasm conservation facility in Baiyi Town, China (Table 1, Fig. 1). Samples of fresh and healthy young blades were collected, frozen in liquid nitrogen, and stored at -80°C for isolation of genomic DNA.

Table 1 Detailed information of plum germplasm used in this investigation

Group	No.	Cultivar	Latitude	Longitude	Altitude(m)	Collection site	Geographic origin
1	1-31	<i>Prunus salicina</i> 'Shazikongxinli'	28°32'35" N	108°33'55" E	418	Nanzhuang Village Luojia Group	Guizhou, China
1	32-36	<i>Prunus salicina</i> 'Shazikongxinli'	28°33'42" N	108°32'2" E	446	Nanzhuang Village Xiaoshan Group	Guizhou, China
1	37-50	<i>Prunus salicina</i> 'Shazikongxinli'	28°32'40" N	108°32'38" E	552	Shierpan Village	Guizhou, China
2	51	Furong plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Fujian, China
2	52	Qianweihong	26°49'11" N	107°0'12" E	1360	Baiyi Town	North, China
2	53	Xingxing plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Guizhou, China
2	54	Red Heart plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	USA
2	55	Sanyue plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Guangdong, China
2	56	Jiuqian plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Guizhou, China
2	57	Qingcui plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Sichuan, China
2	58	Sanhua plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Guangdong, China
2	59	Nanfanghong plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	South, China
2	60	Dahongpao plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	USA
2	61	<i>Prunus cerasifera</i> 'Atropurpurea'	26°49'11" N	107°0'12" E	1360	Baiyi Town	Southwest Asia
2	62	Queen Rose	26°49'11" N	107°0'12" E	1360	Baiyi Town	USA
2	63	Siyue plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Guizhou, China
2	64	Cuihong plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Sichuan, China
2	65	Black plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	USA
2	66	Banbianhong	26°49'11" N	107°0'12" E	1360	Baiyi Town	Yunnan, China
2	67	Fengtang plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Guizhou, China
2	68	Yuhuang plum	26°49'11" N	107°0'12" E	1360	Baiyi Town	Gansu, China

Morphological analysis1

Since September 2020, 43 phenotypic traits (including trait characteristics of flowers, leaves, fruits, etc.) of 68 germplasm were observed in accordance with the morphological trait testing standard for plum(GBT19557-8 Guide for testing specificity, consistency, and stability of new plant varieties - plum). Qualitative traits were scored according to the measurement standard GBT19557-8, and quantitative traits were directly recorded as measured values(Table 2). To avoid errors, data for each trait was measured by the same person.

Table 2 The characterization and taxonomic values of 32 morphological traits of plum

Codes	Traits	Description standard
1	Calyx pose	1: stick to the petals, 2: separated from petals, 3: curved toward the pedicel
2	Calyx color	1: yellow-green, 2: brownish green, 3: green, 4: yellow-red
3	Petal shape	1: round, 2: obovate, 3: oval, 4: wide oval
4	Corolla size	3: <1.5cm, 5: 1.5-2.0cm, 7: >2.0cm
5	Petal stacking off condition	3: petals separate, 5: petals meet, 7: petals overlapping
6	Petal upper edge shape	1: whole, 2: concave, 3: serrated
7	Stigma position	3: stigma below stamens, 5: stigma as high as stamens, 7: stigma above stamen
8	New shoots color	1: tawny, 2: green-brown, 3: green, 4: reddish brown, 5: fuchsia
9	New shoots length	3: <30cm, 5: 30-70cm, 7: >70cm
10	New shoots internode length	3: <1.5cm, 5: 1.5-2.0cm, 7: >2.0cm
11	Leaf color	1: yellow-green, 2: green, 3: red
12	Leaf shape	1: lanceolate, 2: oblanceolate, 3: broad obovate, 4: oval, 5: round
13	Leaf base shape	1: narrow wedge, 2: wedge, 3: round
14	Leaf edge tooth type	1: blunt tooth, 2: sharp tooth
15	Number of leaf glands	1: 2, 2: >2
16	Leaf gland location	1: leaf base, 2: Leaf base and petiole, 3: petiole
17	Fruit shape	1: oblate, 2: round, 3: pear-shaped, 4: oval, 5: heart-shaped
18	Fruit color	1: green, 2: light yellow, 3: yellow, 4: red, 5: fuchsia, 6: blue, 7: black
19	Fruit symmetry	1: symmetrical, 2: asymmetrical
20	Flesh color	1: light yellow, 2: yellow, 3: orange, 4: green, 5: red
21	Flesh juice	3: few, 5: medium, 7: many
22	Viscosity of seed and flesh	3: adhesion, 5: semi-adhesion, 7: non-adhesion
23	Seed shape	1: oblate, 2: round, 3: melon shape, 4: oval, 5: oblong
24	Seed widest position	3: near base, 5: near middle, 7: near top
25	Leaf length (cm)	average of 20 leaf length
26	Leaf width (cm)	average of 20 leaf width
27	Leaf stalk length (cm)	average of 20 leaf stalk length
28	Weight per Fruit (g)	average of 10 fruits weight
29	Fruit hardness kg/cm ²	average of 10 fruits hardness
30	Soluble solid content (%)	average of 10 fruits soluble solids content
31	Seed weight (g)	average of 10 seed weight
32	Seed to fruit ratio	average of 10 fruits

Genomic DNA extraction

Genomic DNA from each sample was extracted using the Plant Genome DNA Extraction Kit (Tiangen, Beijing). The DNA quality and concentration were detected using a nano spectrophotometer. The integrity of total DNA was detected using 1% agarose gel electrophoresis. In addition, each qualified DNA sample was diluted to approximately 30 ng/μl.

Molecular marker analysis

IRAP: In this work, we developed 65 IRAP primers based on the conserved regions of RT sequences of plum reverse transcription transposons *Ty1-copia* and *Ty3-gypsy*. By screening, 22 IRAP primers with good repeatability and high polymorphism were obtained for profiling (Table 3). The PCR

was performed in a reaction volume of 10 µl containing 1.0 µl DNA (30 ng/µl), 5.0 µL Mix, 1.3 µl primers (10⁻⁵ mol/L), 2.7 µl ddH₂O. PCR amplification using the following conditions: 4 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at the specific annealing temperature and 72 °C extensions for 1 min, with a final extension of 10 min at 72 °C. The amplification products were separated on 1.5% (w/v) agarose gel using the 2000 bp (Tiangen, Beijing) ladder as standard in 1×TAE buffer.

ISSR: A set of 100 primers pairs were screened, out of which 15 primers pairs were found reproducible and selected for profiling (Table 3). The PCR reaction system was the same as for IRAP. Each reaction was repeated at least twice.

Table 3 List of IRAP and ISSR primer sequences. The information includes primer name, annealing sequences 5'-3' and temperature (Tm)

Primer	Primer Sequence 5'-3'	Tm(°C)	Primer	Primer Sequence 5'-3'	Tm(°C)
IRAP :					
Ty1-1	GTGTTCCAAGATGGGATGAT	53.2	Ty1-20	TCAGACAATTCGCAGGAGG	55.3
Ty1-2	TGGCCTGAAACAGTCTC	52.0	Ty1-23	GATTGAAGCAGTCACCCAG	54.0
Ty1-3	CGGAGACTTAGAAGAGGAG	53.0	Ty1-25	ACCAGAAGGGTTTGTTGAA	52.1
Ty1-4	TCAAAGGGAAGGAGGACAT	53.6	Ty3-2	GAATCGGTATCCATTGTCTG	52.5
Ty1-6	CAACCACAAGGGTATGAAG	53.1	Ty3-3	TGGCTATCACCAAGTCTGC	55.3
Ty1-7	AAGGAAGTCCCTGTATGGC	54.7	Ty3-5	CAACGCTCCAACCTCTTTC	52.3
Ty1-8	CGAGGGCATGGTTTCAC	54.1	Ty3-6	CCGCCCTTTCATTGACGACT	58.0
Ty1-9	GGTTTGCTGGTTGAAGAAGT	54.3	Ty3-9	ACCAGCACATCCCTAACCT	55.9
Ty1-15	AGAAGGCACTTTACGGATTG	52.6	Ty3-13	TTCGGGTTATGTAATGCTC	50.8
Ty1-17	ATATGGAACAGCCAGAAGG	52.3	Ty3-14	CGAGGATTGATGACTTGCT	52.0
Ty1-18	AAGGCAGTGGAACAAGAAG	53.2	Ty3-16	CCCATACATCCTTCATCACAG	54.5
ISSR :					
UBC811	(GA) ₈ C	54.8	UBC842	(GA) ₈ YG	55.0
UBC824	(TC) ₈ G	54.0	UBC844	(CT) ₈ RC	55.6
UBC825	(AC) ₈ T	54.5	UBC854	(TC) ₈ RG	55.1
UBC826	(AC) ₈ C	54.3	UBC856	(AC) ₈ YA	56.2
UBC834	(AG) ₈ YT	55.8	UBC857	(AC) ₈ YG	55.0
UBC835	(AG) ₈ YC	57.0	UBC868	(GAA) ₆	50.8
UBC836	(AG) ₈ YA	56.2	UBC888	BDB(CA) ₇	53.8
UBC840	(GA) ₈ YT	54.0			

Data analysis

50 *Prunus salicina* 'Shazikongxinli' germplasm were used as the experimental group and 18 other different plum varieties were used as the control group. Excel 2019 was used to organize the experimental data, and the Shannon-Weaver diversity index (H') of each qualitative trait and the standard deviation and coefficient of variation of quantitative traits were calculated. The Shannon-Weaver diversity index calculation formula was: $H' = -\sum P_i \ln P_i$, where P_i was the ratio of the number of materials in the i th level of a trait to the total number of materials (Yao et al., 2022). Genetic similarity coefficients were calculated using NTSYS-pc version 2.10 (Rohlf, 2000), and cluster analysis based on the unweighted pair-group method with arithmetic means (UPGMA) was carried out.

For molecular markers, the amplification results of each primer in each individual was scored as 0 (absence) and 1 (presence) and obtain a binary matrix. Finally, a binary matrix containing the ISSR and IRAP data together was constructed. Genetic diversity parameters including the number of alleles (N_a), the number of effective alleles (N_e), Nei gene diversity (H) (Nei, 1973) and Shannon information index (I) (Shannon, 1949) were calculated by Popgene32 (Yeh, 1997) software. Clustering analysis of the test germplasms according to the Neighbor-joining method using NTSYS2.10 (Rohlf, 2000) and MEGA11 software. Then beautified the cluster map through iTOL (<https://itol.embl.de/>).

Results

Morphological analysis

The quality traits analysis statistics showed that 28 of the 32 quality traits had variation in the experimental group (Table 4), while 31 traits in the control group had different phenotypes. And the 4 traits: calyx pose, petal upper edge shape, leaf edge tooth type, and seed widest position of the 50 'Shazikongxinli' produced only one phenotype and no other variable traits. In the experimental group, the Shannon-Weaver diversity index (H') varies from 0 to 1.199, with an average value of 0.557. There were 18 traits with an index greater than 0.557. Among them, the index of new shoots color was the largest, resulting in 4 types of variation with greenish-brown predominating (0.46). In the control group, the Shannon-Weaver diversity index ranged from 0 to 1.391, with an average value of 0.811. There were 16 traits with a diversity index greater than 0.811. Highest polymorphism was observed in flesh color varied from light yellow (0.21), yellow (0.43), orange (0.07), green (0.07) to red (0.21). In addition, the Shannon-Weaver diversity index was higher in the experimental group than in the control group for 7 quality traits, these traits including new shoots color, new shoots length, new shoots internode length, leaf gloss, fruit symmetry, flesh Juice and viscosity of seed and flesh.

Quantitative trait analysis statistics showed that the variation coefficients of 11 traits in the experimental group ranged from 9.74% to 22.88%, with an average value of 15.57% (Table 5). and there were 4 traits with a coefficient of variation greater than 15.57%, which included: leaf area, weight per fruit, fruit hardness, seed weight. In the control group, the variation range of the coefficient of variation ranged from 11.50% to 55.39% with an average value of 26.89%, and there were 4 traits with a coefficient of variation greater than 26.89%. These traits include: weight per fruit, fruit hardness, seed weight, seed to fruit ratio. The coefficient of variation of leaf length was the smallest in both test germplasm groups, while single fruit weight was the largest in both groups. In addition, the coefficient of variation was higher in the experimental group than in the control group for two quantitative traits which included: leaf width and leaf area.

Dendrogram of 68 test germplasm was constructed based on UPGMA and grouped germplasm into five clusters (Fig. 2). Cluster 1 included 20 'Shazikongxinli' and 2 other plum varieties, which had significantly larger leaves and were mostly oval in shape. Cluster 2 also included 20 'Shazikongxinli' and 2 other plum varieties, and the fruit weight of this cluster was significantly larger than that of the other 'Shazikongxinli' cluster groups. Cluster 3 included 9 'Shazikongxinli', which have the highest soluble solid content and significantly smaller seed weights. In addition, there was only one 'Shazikongxinli' accession in Cluster 4, and this germplasm has much smaller leaf size and leaf stalk length than that other groups. Taken as a whole, all 50 'Shazikongxinli' plums could be classified into one group, while the 14 plum species in the control group were classified into another group. Cuihong Plum, Red Heart Plum, Fengtang Plum and Xingxing Plum were clustered with 50 'Shazikongxinli' germplasm, indicating that 'Shazikongxinli' and these four plum species are more similar in phenotypic traits and closer in kinship.

Table 4 Statistical results of 32 quality traits of 68 test germplasm

Traits	Experimental group 50								Control group 18							
	Frequency of different phenotypic degree								Frequency of different phenotypic degree							
	1	2	3	4	5	7	9	H'	1	2	3	4	5	7	9	H'
Flower buds of 1 year twig	0.98						0.02	0.098	0.94						0.06	0.227
Calyx pose	1.00							0.000	0.81	0.13	0.06					0.605
Calyx color	0.06		0.94					0.227	0.06		0.81	0.13				0.605
Corolla size			0.02		0.16	0.82		0.534					0.31	0.69		0.619
Petal shape	0.96	0.02	0.02					0.196	0.19		0.69	0.13				0.837
Petal stacking off condition			0.02		0.43	0.55		0.770			0.56		0.25	0.19		0.987
petal upper edge shape	1.00							0.000	0.94		0.06					0.227
Stigma position			0.43		0.57			0.699			0.38		0.25	0.38		1.082
New shoots color	0.08	0.46	0.32	0.14				1.199	0.17	0.67	0.06	0.06	0.06			1.076
New shoots length			0.24		0.36	0.40		1.077			0.06		0.47	0.47		0.879
New shoots internode length			0.22		0.64	0.14		0.894					0.47	0.53		0.691
Leaf shape		0.08	0.78	0.12	0.02			0.729		0.41	0.35	0.24				1.076
Leaf tip shape				0.84	0.16			0.440				0.47	0.53			0.691
Leaf base shape	0.92	0.08						0.279	0.53	0.29	0.18					1.004
Leaf edge tooth type	1.00							0.000	0.82						0.18	0.471
Number of leaf glands	0.98	0.02						0.098	0.50	0.50						0.693
Leaf gland location	0.46	0.54						0.690	0.31	0.31	0.38					1.094
Leaf color		0.18	0.82					0.471		0.12	0.82	0.06				0.586
Leaf gloss			0.26		0.62	0.12		0.901			0.18		0.76	0.06		0.686
Fruit shape	0.70	0.30						0.611	0.15	0.54		0.23	0.08			1.157
Fruit top shape			0.02		0.98			0.098			0.23		0.31	0.31	0.15	1.349
Fruit color	0.10	0.84	0.04	0.02				0.584	0.27		0.07	0.40	0.27			1.260

Fruit symmetry	0.30	0.70				0.611	0.23	0.77					0.539
Flesh color	0.62	0.26	0.12			0.901	0.21	0.43	0.07	0.07	0.21		1.391
Flesh Juice			0.14	0.36	0.50	0.990					0.85	0.15	0.423
Flesh fiber			0.24	0.54	0.22	1.008			0.31		0.23	0.46	1.058
Viscosity of seed and flesh			0.20	0.46	0.34	1.046			0.71		0.21	0.07	0.757
Seed shape		0.62	0.02	0.36		0.742	0.07	0.36		0.43	0.14		1.192
Seed symmetry	0.98	0.02				0.098	0.50	0.50					0.693
Seed widest position				1.00		0.000					1.00		0.000
Seed surface status			0.20	0.52	0.28	1.018			0.21		0.29	0.50	1.033
Seed base width			0.08	0.68	0.24	0.807			0.14		0.57	0.29	0.955
Mean						0.557							0.811

H', Shannon-Weaver diversity index

Table 5 Statistical results of 11 quantitative traits of 68 test germplasm

Traits	Experimental group 50							Control group 18						
	Mean	Min	Max	Range	SD	CV(%)		Mean	Min	Max	Range	SD	CV(%)	
Leaf length (cm)	9.47	5.98	11.86	5.88	0.92	9.74		10.67	8.09	13.60	5.51	1.23	11.50	
Leaf width (cm)	4.09	2.29	5.59	3.30	0.57	14.04		4.75	3.34	5.96	2.62	0.66	13.93	
Leaf area cm ²	39.12	13.69	66.30	52.60	8.49	21.71		51.11	27.02	70.09	43.07	10.82	21.17	
Leaf aspect ratio	2.34	1.94	3.24	1.30	0.23	9.98		2.27	1.93	2.98	1.05	0.27	11.89	
Leaf stalk length (cm)	1.18	0.68	1.46	0.78	0.13	10.75		1.32	0.91	1.96	1.05	0.31	23.47	
Weight per Fruit (g)	16.46	8.13	26.37	18.25	3.77	22.88		49.88	15.01	110.85	95.84	27.63	55.39	
Fruit hardness	4.62	2.97	7.32	4.35	0.86	18.59		6.74	2.38	11.96	9.58	2.66	39.53	
Soluble solid content (%)	13.44	10.25	17.76	7.51	1.65	12.28		11.92	6.23	16.06	9.83	2.58	21.60	
Fruit stalk length cm	1.27	0.77	1.70	0.93	0.20	15.39		1.42	0.80	2.10	1.30	0.33	23.03	
Seed weight (g)	0.66	0.32	0.91	0.59	0.14	20.65		1.30	0.63	1.95	1.32	0.46	35.63	
Seed to fruit ratio %	4.06	2.40	5.53	3.13	0.62	15.27		2.96	1.16	5.40	4.23	1.14	38.65	
Mean					1.60	15.57						4.37	26.89	

SD, Standard deviation, **CV**, Coefficient of variation

IRAP analysis

In the experimental group, the 22 selected IRAP primers generated 249 reproducible amplified loci, giving altogether 232 polymorphic loci(Table 6). Each primer generated 5 to 20 obvious loci, the polymorphism ratio ranged from 66.7% to 100%. There are 10 primers with a polymorphism ratio of 100%, the lowest was Ty1-1 (66.7%), and the average polymorphism ratio was 90.7%. Meanwhile, 22 IRAP primers amplified a total of 261 loci in 19

plum varieties of the control group, with an average of 11.86 loci per primer, and there were 249 polymorphic loci, for an average polymorphic ratio of 93.3%.

Popgene32 software was used to analyze the number of alleles observed (*Na*), number of effective alleles (*Ne*), Nei's gene diversity (*H*) and Shannon information index (*I*) (Table 6). The mean values of *Na* and *Ne* for the 50 'Shazikongxinli' in the test were 1.907 and 1.409, respectively, and the range of Nei's gene diversity (*H*) was 0.139 (Ty3-9) - 0.369 (Ty3-2) with a mean value of 0.255, The Shannon information index (*I*) had a range of 0.244 (Ty3-9) - 0.547 (Ty3-2) and a mean value of 0.397. Meanwhile, the mean values of *Na* and *Ne* for the other 18 plum species in the control group were 1.933 and 1.477, respectively. The *H* value ranged from 0.172 (Ty1-8) to 0.436 (Ty1-9) with a mean value of 0.292. Besides, the range of *I* value was 0.278 (Ty1-8) - 0.626 (Ty1-9), and the mean value was 0.449. As a whole, the genetic indices of the experimental groups were all smaller than those of the control group. This was consistent with the expectation that the diversity level within the same variety was lower than that between varieties.

The genetic relationships of the tested germplasms were unraveled using the Neighbor-joining method based on Jaccard similarity coefficients computed with IRAP markers, and two main clusters, namely, and , were obtained from the dendrogram taking 0.66 as a threshold (Fig. 3). All 50 test 'Shazikongxinli' were clustered together (cluster I), and two other plum varieties were also clustered into cluster I (RedHeart plum and Xingxing plum). This indicated that these two plums were more closely related to 'Shazikongxinli' from IRAP marker results.

Table 6 Polymorphism information of 22 IRAP primers

Primer	Experimental group 50								Control group 18							
	AB	PB	P%	<i>Na</i>	<i>Ne</i>	<i>H</i>	<i>I</i>	AB	PB	P%	<i>Na</i>	<i>Ne</i>	<i>H</i>	<i>I</i>		
Ty1-1	6	4	66.7	1.667	1.545	0.298	0.426	7	6	85.7	1.857	1.306	0.201	0.329		
Ty1-2	9	8	88.9	1.889	1.364	0.224	0.354	8	7	87.5	1.875	1.572	0.330	0.490		
Ty1-3	7	7	100	2.000	1.526	0.334	0.512	6	5	83.3	1.833	1.451	0.273	0.417		
Ty1-4	17	16	94.1	1.941	1.376	0.233	0.368	16	15	93.8	1.938	1.645	0.357	0.521		
Ty1-6	15	15	100	2.000	1.408	0.265	0.419	19	19	100.0	2.000	1.624	0.368	0.548		
Ty1-7	18	18	100	2.000	1.602	0.358	0.536	19	19	100.0	2.000	1.632	0.365	0.542		
Ty1-8	7	5	71.4	1.714	1.226	0.157	0.259	6	4	66.7	1.667	1.251	0.172	0.278		
Ty1-9	15	14	93.3	1.933	1.545	0.320	0.482	16	16	100.0	2.000	1.795	0.436	0.626		
Ty1-15	10	9	90.0	1.900	1.377	0.233	0.367	14	14	100.0	2.000	1.429	0.271	0.428		
Ty1-17	12	11	91.7	1.917	1.339	0.220	0.357	13	12	92.3	1.923	1.464	0.284	0.437		
Ty1-18	5	4	80.0	1.800	1.290	0.182	0.295	9	9	100.0	2.000	1.505	0.312	0.480		
Ty1-20	8	6	75.0	1.750	1.399	0.241	0.364	7	6	85.7	1.857	1.380	0.228	0.349		
Ty1-23	10	8	80.0	1.800	1.267	0.190	0.315	11	9	81.8	1.818	1.409	0.264	0.408		
Ty1-25	9	7	77.8	1.778	1.221	0.151	0.251	13	13	100.0	2.000	1.501	0.320	0.493		
Ty3-2	20	20	100	2.000	1.640	0.369	0.547	19	19	100.0	2.000	1.573	0.342	0.516		
Ty3-3	16	16	100	2.000	1.515	0.312	0.477	16	16	100.0	2.000	1.535	0.322	0.491		
Ty3-5	15	15	100	2.000	1.559	0.331	0.501	15	15	100.0	2.000	1.550	0.334	0.507		
Ty3-6	15	15	100	2.000	1.307	0.210	0.349	12	12	100.0	2.000	1.360	0.246	0.402		
Ty3-9	7	6	85.7	1.857	1.180	0.139	0.244	8	8	100.0	2.000	1.380	0.259	0.419		
Ty3-13	8	8	100	2.000	1.515	0.314	0.479	8	7	87.5	1.875	1.323	0.213	0.344		
Ty3-14	6	6	100	2.000	1.462	0.278	0.432	9	8	88.9	1.889	1.457	0.289	0.445		
Ty3-16	14	14	100	2.000	1.345	0.241	0.398	10	10	100.0	2.000	1.359	0.248	0.406		
Total	249	232						261	249							
Mean	11.32	10.55	90.7	1.907	1.409	0.255	0.397	11.86	11.32	93.3	1.933	1.477	0.292	0.449		

AB, Amplified bands, **PB**, polymorphic bands, **P%**, polymorphism%, ***Na***, Number of alleles observed, ***Ne***, Number of effective alleles, ***H***, Nei's gene diversity, ***I***, Shannon information index

ISSR analysis

Out of 100 ISSR primers, 15 were selected for further analysis based on unambiguous and reproducible loci. The 15 ISSR primers amplified 141 loci in 50 'Shazikongxinli', including 105 polymorphic loci (Table 7). On average, each primer amplified 9.4 loci with 7.0 polymorphic loci. The average polymorphism ratio was 73.5% with a range of 50 to 100%. And there are also 3 primers with a polymorphism ratio of 100%. Moreover, 15 ISSR primers amplified 155 loci, including 136 polymorphic loci in the 18 plum germplasm used as control group. On average, each primer amplified 10.3 loci, of which 9.1 were polymorphic loci. The polymorphism ratios of the 15 ISSR primers ranged from 62.5 to 100%, with an average polymorphism ratio of 86.4%.

In the experimental group, the average number of alleles observed per primer (*Na*) was 1.735, ranging from 1.500 to 2.000, the number of effective alleles (*Ne*) was 1.137 (1.032 to 1.252), the Nei's gene diversity (*H*) was 0.100 (0.031 to 0.158), and the Shannon information index (*I*) was 0.176 (0.073 to 0.252). Meanwhile, the mean values of *Na* and *Ne* for other 18 plum germplasm were 1.864 (1.625 to 2.000) and 1.366 (1.219 to 1.546), and the *H* value was 0.236 (0.159 to 0.328), the *I* value was 0.375 (0.274 to 0.491)(Table 7).

A dendrogram drawn from the genetic distance based on an neighbor-joining method with was constructed using NTSYS-pc2.1 software (Rohlf, 2000). In the analysis of ISSR markers, the total samples were classified into two clusters taking 0.70 as a threshold: cluster 1 was named I, while cluster 2 was named II (Fig. 5). For the ISSR marker, the cluster I consisted of 50 samples, all were 'Shazikongxinli'. while cluster II contained 18 other plum varieties. Nearly all of the 'Shazikongxinli' samples were clustered independently in both the IRAP and ISSR marker systems, showing that the results of the cluster analysis were accurate and trustworthy.

Table 7 Polymorphism information of 22 ISSR primer

Primer	Experimental group 50							Control group 18						
	AB	PB	P%	<i>Na</i>	<i>Ne</i>	<i>H</i>	<i>I</i>	AB	PB	P%	<i>Na</i>	<i>Ne</i>	<i>H</i>	<i>I</i>
UBC811	8	4	50.0	1.500	1.043	0.037	0.077	13	12	92.3	1.923	1.408	0.265	0.419
UBC824	11	11	100	2.000	1.235	0.154	0.216	11	11	100	2.000	1.367	0.253	0.409
UBC825	8	6	75.0	1.750	1.134	0.106	0.192	9	8	88.9	1.889	1.321	0.209	0.340
UBC826	6	3	50.0	1.500	1.042	0.038	0.079	6	4	66.7	1.667	1.258	0.171	0.275
UBC834	9	8	88.9	1.889	1.137	0.115	0.217	10	10	100	2.000	1.226	0.186	0.327
UBC835	11	8	72.7	1.727	1.183	0.133	0.227	9	8	88.9	1.889	1.227	0.159	0.274
UBC836	8	6	75.0	1.750	1.212	0.136	0.234	10	8	80.0	1.800	1.219	0.181	0.323
UBC840	8	4	50.0	1.500	1.076	0.064	0.122	9	7	77.8	1.778	1.360	0.223	0.347
UBC842	14	9	64.3	1.643	1.032	0.031	0.073	16	14	87.5	1.875	1.436	0.267	0.412
UBC844	10	10	100	2.000	1.170	0.124	0.228	10	9	90.0	1.900	1.546	0.328	0.491
UBC854	10	10	100	2.000	1.219	0.138	0.235	11	10	90.9	1.909	1.450	0.270	0.417
UBC856	14	9	64.3	1.643	1.252	0.158	0.252	15	15	100	2.000	1.451	0.290	0.454
UBC857	8	5	62.5	1.625	1.065	0.054	0.108	10	7	70.0	1.700	1.274	0.182	0.293
UBC868	8	6	75.0	1.750	1.114	0.096	0.181	8	8	100	2.000	1.523	0.312	0.476
UBC888	8	6	75.0	1.750	1.140	0.110	0.197	8	5	62.5	1.625	1.424	0.248	0.365
Total	141	105						155	136					
Mean	9.4	7.0	73.5	1.735	1.137	0.100	0.176	10.3	9.1	86.4	1.864	1.366	0.236	0.375

AB, Amplified bands, PB, polymorphic bands, P%, polymorphism%, *Na*, Number of alleles observed, *Ne*, Number of effective alleles, *H*, Nei's gene diversity, *I*, Shannon information index

Discussion

Understanding genetic diversity is necessary for conservation and sustainable management of species (Lynch et al. 1999). Understanding the level of genetic variation and kinship in the germplasm resources of 'Shazikongxinli' can help to efficiently select diverse parental combinations with maximum genetic variability and then breed superior progeny. These were proved useful in crop breeding programs (Liu et al. 2011, Guan et al. 2020). In the current study, morphological markers and two different molecular marker systems (IPAR and ISSR) were utilized for genetic diversity estimation within 68 plum germplasm. The combined use of these 3 datasets allows an accurate assessment of the level of genetic diversity in 'Shazikongxinli'.

The Shannon-Weaver diversity index (H') and coefficient of variation were important indicators of the diversity of germplasm resources for traits (Yao et al. 2022, Hu et al. 2022). In the current study, the mean H' and CV values of 43 traits in 50 'Shazikongxinli' were smaller than the control group, but some traits such as new shoots length, leaf area, weight per fruit, fruit hardness, and seed weight showed higher levels of variation in 50 'Shazikongxinli'. This may be caused by the degradation of the caste of local varieties during long-term cultivation and the mixing of different strains produced during seedling breeding. Leaf and fruit traits were considered reliable in the evaluation of genetic relationships and taxonomic studies (Almajali et al. 2012). In this study, the variation degree of 'Shazikongxinli' in fruit traits was high, which was not conducive to the development of 'Shazikongxinli' to a certain extent. The uneven quality of the fruit tended to damage the brand reputation of 'Shazikongxinli'. On the other hand, it also provided a source of germplasm samples for breeding new varieties of 'Shazikongxinli' with different shapes, colors and hardness (Wang et al. 2015).

The genetic diversity parameters was an important indicator to measure the variation of genetic information (Brenda et al. 2022, Wu et al. 2019). Both molecular markers in this study showed high levels of polymorphism in the test germplasm. By comparing the results of the two molecular markers in the experimental group and the control group, it was found that the polymorphism ratio, N_a , N_e , H and I values of 18 plum germplasms (IRAP and ISSR) were higher than those of 50 'Shazikongxinli' in the experimental group. This was consistent with the expectation that the level of genetic diversity within a single variety is less than that of multiple varieties, and although the number of test materials in the experimental group was much larger than that of the control group, the plum sample in the control group were different varieties formed through long-term mutation or hybridization and thus had richer genetic information (Sehic et al. 2015, Kazija et al. 2014). In this study, several genetic diversity indices of the 50 'Shazikongxinli' tested were also relatively high, especially for the IRAP markers. This indicated that the current genetic information within the 'Shazikongxinli' population had been relatively abundant, and also showed that the IRAP primers developed in this study had high variation detection ability. Moreover, based on the results of this study, it was known that IRAP markers showed a higher level of DNA polymorphism detection compared to ISSR. This could be due to the different nature of the genomic region coverage and loci number of these two techniques, which reveal different characteristics of genetic variation (Karim et al. 2017). The IRAP marker was a marker developed based on the random insertion of retrotransposon to generate polymorphisms (Kalendar et al. 1999). Retrotransposon were prone to be activated under special conditions such as adversity to generate new insertional mutations. Thus, compared with SSR, ISSR and other markers for interspecific genealogy studies, IRAP markers were more suitable for intraspecific variation detection and analysis of genetic variation and kinship at the individual level within a species (Kalendar et al. 2014).

Using phylogenetic analysis, the morphological markers and two molecular markers had similar clustering results and both were able to cluster the 68 test samples into two groups. All 50 'Shazikongxinli' samples were clustered together, while most of the other plum varieties in the control group were clustered into another category, suggesting that these marker systems were effective in studying genetic diversity in the germplasm resources of 'Shazikongxinli'. Furthermore, in the clustering results for both morphological and IRAP markers, both Red Heart plum and Xingxing plum were clustered together with 50 'Shazikongxinli', indicating that these two plum varieties could be more closely related to 'Shazikongxinli'. Moreover, according to the clustering results, the genetic background of 'Shazikongxinli' gourd was inferred narrow in the present study (Ruchi et al. 2020). Hence, it become crucial to broaden the genetic base of the 'Shazikongxinli' gourd to breed superior germplasm. This could be achieved by introgression of genes from other varieties or wild species with enormous variability (Martinez et al. 2003, Wang et al. 2015).

Conclusion

In brief, the investigation revealed a degree of genetic variation within the 'Shazikongxinli' population, which provided a fundamental theoretical basis for the selection and breeding of excellent accessions to the 'Shazikongxinli' germplasm. In addition, morphological markers, ISSR and IRAP markers showed high levels of DNA polymorphism in plum species and could be used to assess the genetic diversity and relationships. The combined use of several marker technologies will be more useful in the integrated evaluation of the genetic diversity of 'Shazikongxinli' from different genetic marker regions. Meanwhile, due to the nature of the markers, IRAP markers were more suitable than ISSR markers for the detection of intra-cultivar or individual level variability.

Declarations

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Competing interests

The authors declare that there are no conflicts of interest. All the authors read and approved the manuscript in its final form.

Author Contributions

Qiao G. conceived and designed the experiments. Wang G. and Li R.R. performed the experiments. Wang G. and Wang L.L. analyzed the data and wrote the paper. Ren F.H. collected samples and managed the materials. Wu M.H. obtained the funding. All authors have read and approved the final manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

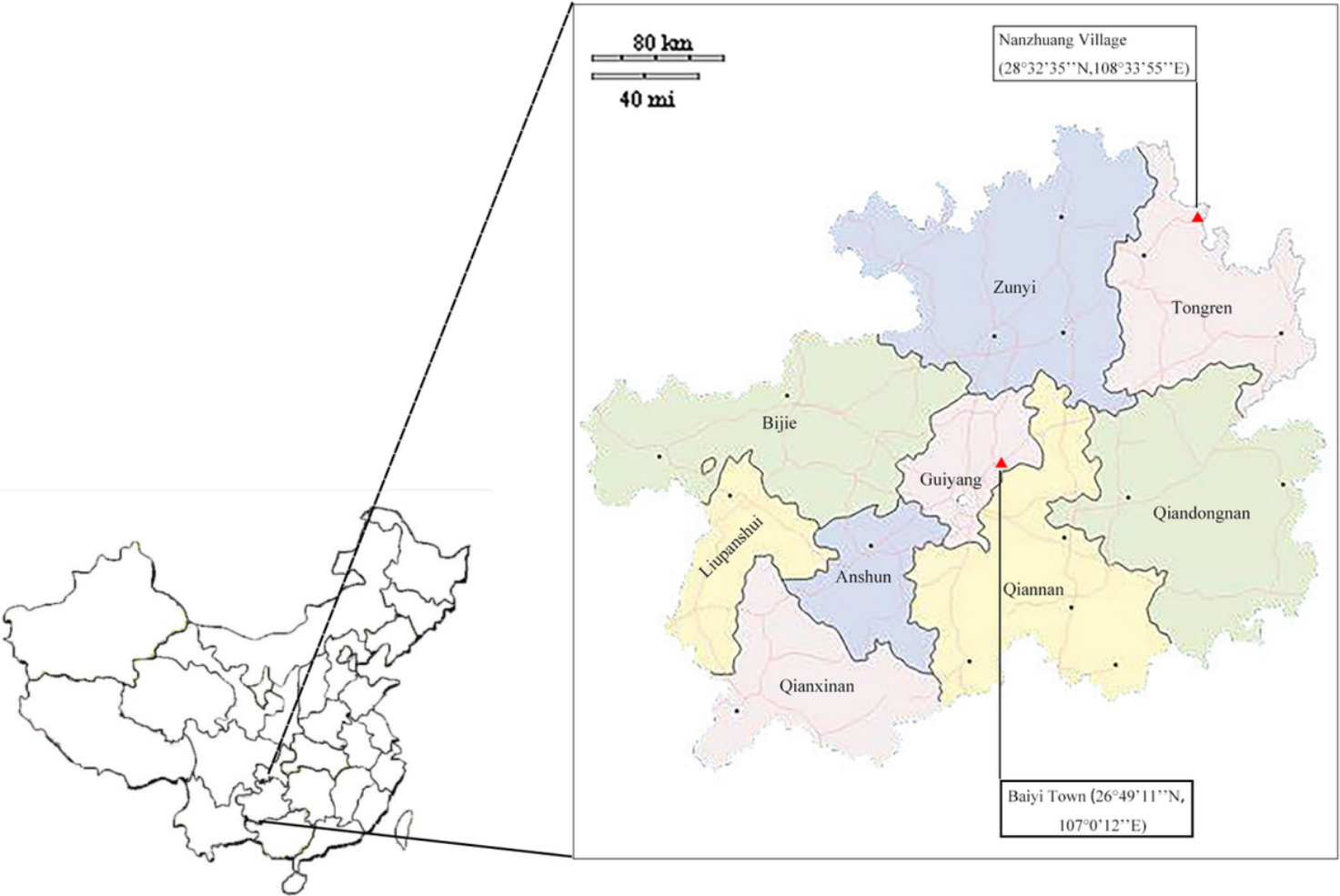


Figure 1

Geographical regions of 68 plum varieties (Table 1) collected from Nanzhuang Village and Baiyi Town in Guizhou Province,China

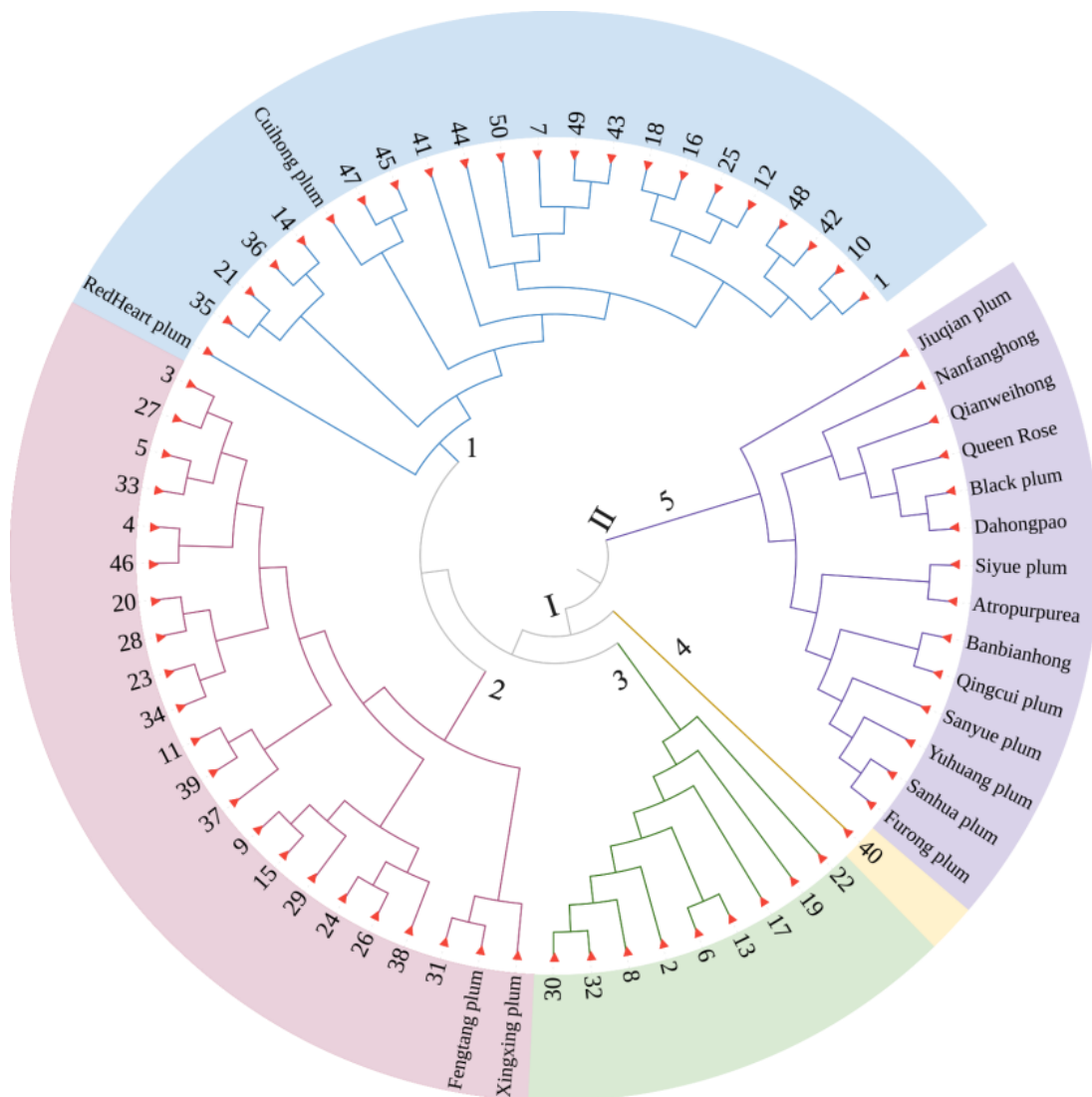


Figure 2
Dendrogram generated of 43 morphological traits based on UPGMA method

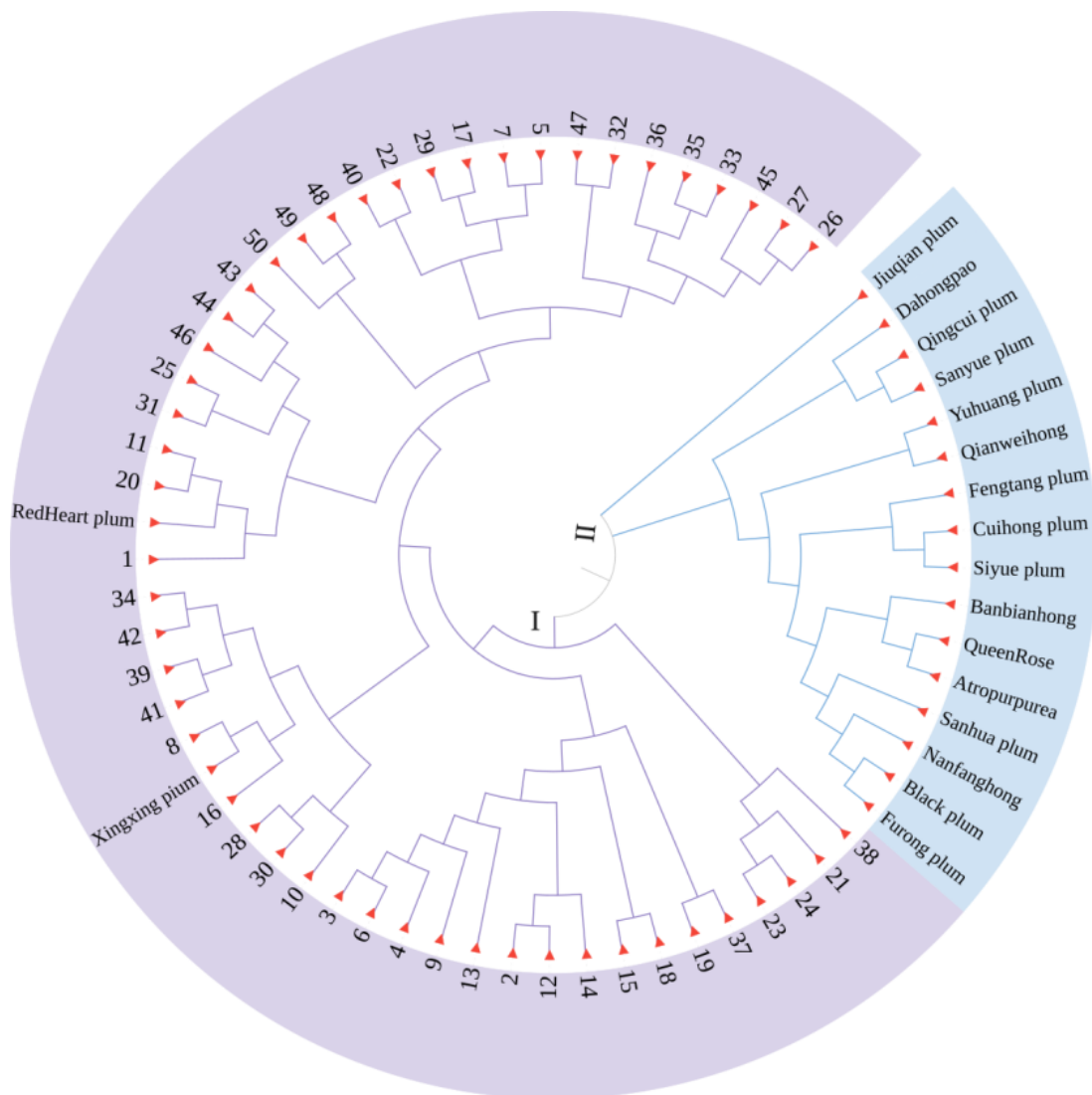


Figure 3

Dendrogram generated of IRAP marker based on Neighbor-joining method

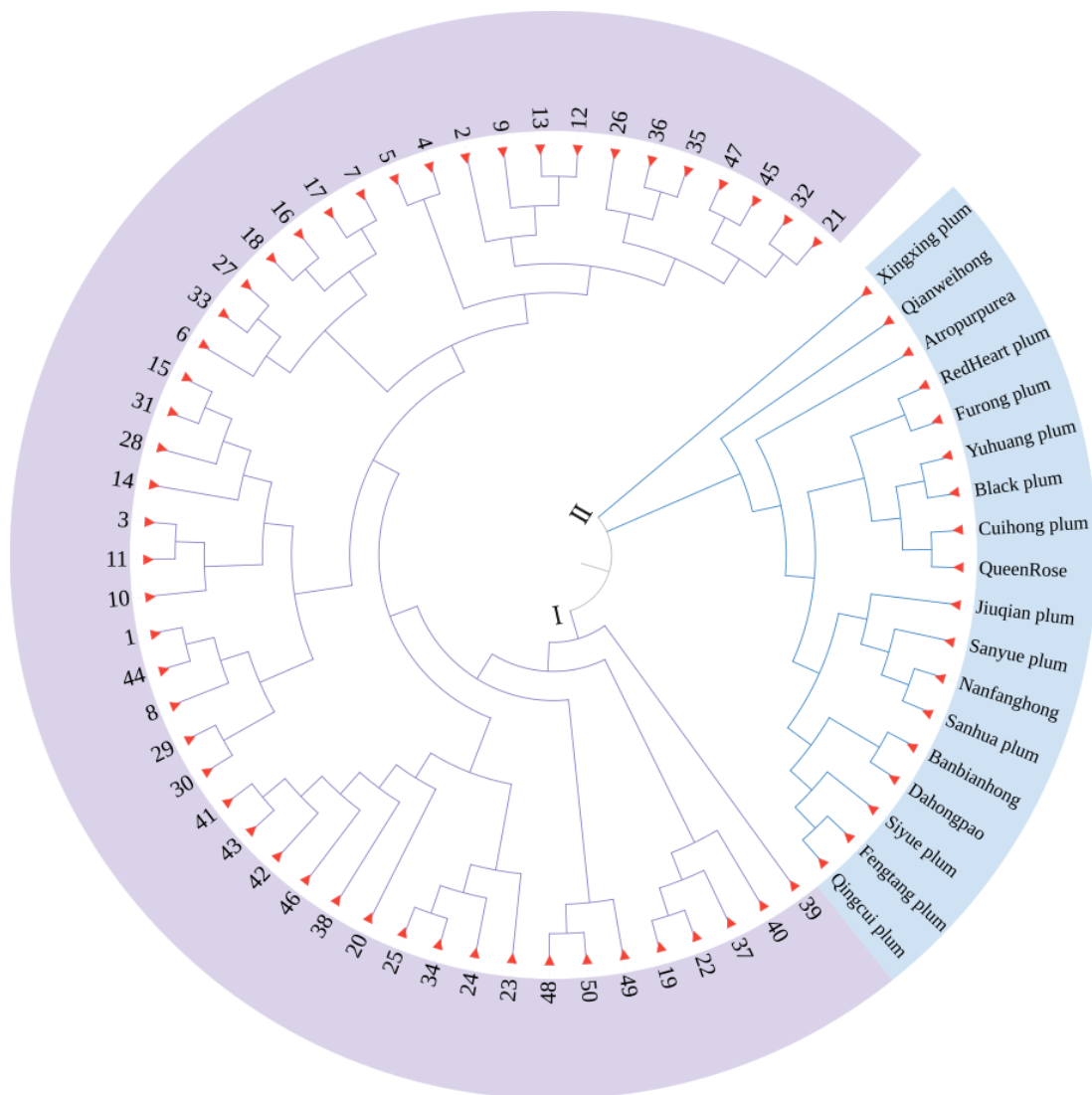


Figure 4

Dendrogram generated of ISSR marker based on Neighbor-joining method