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Zhenjie Zhang

Fujian Agriculture and Forestry University

Jiaqi Zhang

Fujian Agriculture and Forestry University

Yulan Zhang

Fujian Agriculture and Forestry University

Yunqi Xu

Fujian Agriculture and Forestry University

Qingqing Zhu

Fujian Agriculture and Forestry University

Sihao Wu

Fujian Institute of Tropical Crops

Zeyao Lin

Fujian Agriculture and Forestry University

Chenxuan Huang

Fujian Agriculture and Forestry University

Lu Xie

Fujian Agriculture and Forestry University

Qintong You

Fujian Agriculture and Forestry University

Xianqian Niu

nxq828@126.com

Fujian Institute of Tropical Crops

Yongyu Li

Fujian Agriculture and Forestry University

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Genetic Diversity Analysis of Passion Fruit (*Passiflora* spp.) Germplasm

Resources Based on Optimized SRAP Markers

Zhenjie Zhang¹² · Jiaqi Zhang¹³ · Yulan Zhang¹³ · Yunqi Xu¹³ · Qingqing Zhu¹³ · Sihao Wu² · Zeyao Lin¹³ · Chenxuan Huang¹³ · Lu Xie¹³ · Qintong You¹³ · Xianqian Niu^{2*} · Yongyu Li^{13*}

¹ Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, China; 15075009417@163.com (Z.Z.); z13363025274@163.com (J.Z.); 18984727116@163.com (Y.Z.); xyqqyx2025@163.com (Y.X.); lo152843@163.com (Q.Z.); 13273675758@163.com (Z.L.); h2201673654@163.com (C.H.); 1207652145@qq.com (L.X.); 15860202261@163.com (Q.Y.)

² Fujian Institute of Tropical Crops, Zhangzhou 363001, China; wusihao777@163.com (S.W.)

³ Institute of Natural Products of Horticultural Plants, Fujian Agriculture and Forestry University, Fuzhou 350002, China

* Corresponding author Email: nxq828@126.com (X.N.); yongyu.li@fafu.edu.cn (Y.L.)

Abstract To clarify the genetic diversity level and genetic relationship of passion fruit germplasm resources, this study took 140 passion fruit accessions as the test materials. Through optimizing the PCR reaction system and screening primers, a stable and efficient SRAP reaction system was established. On this basis, genetic diversity analysis and cluster analysis were conducted. The results indicate that the optimized SRAP system identified 21 primer pairs exhibiting clear bands and high polymorphism. A total of 345 clear bands were amplified, among which 343 were polymorphic bands, with a polymorphism ratio of 99.41%. The Nei's genetic identity ranged from 0.4870 to 0.9275, and the genetic distance ranged from 0.0752 to 0.7196. Cluster analysis classified the 140 tested passion fruit accessions into five groups, revealing clear differences in genetic relationships among materials from different origins. The SRAP molecular markers employed in this study effectively distinguished different passion fruit germplasms. The findings provide a molecular basis and technical support for the identification of passion fruit germplasm resources, the assessment of genetic diversity, and the selection of superior parental lines.

Keywords *Passiflora edulis* Sims; SRAP-PCR; Single-factor experiment; Optimization

Introduction

Passiflora edulis Sims a perennial vine belonging to the Passifloraceae family, has become one of the most important economic fruit trees in tropical and subtropical regions worldwide due to its rich nutritional content, unique flavor, and high medicinal value (Betancur-Ancona et al. 2025; Arshad et al. 2025). In recent years, with the increasing demand for healthy beverages from consumers, the scale of the passion fruit industry has continued to expand. The selection and promotion of superior varieties have become the core driving force for the high-quality development of the industry (Faleiro et al. 2019; Rosado et al. 2019; de Jesus et al. 2022). However, passion fruit exhibits abundant natural variation, with overlapping morphological characteristics among different cultivars. Traditional germplasm resource identification methods based on phenotypic traits are susceptible to environmental interference, making it difficult to accurately distinguish closely related varieties. This leads to ambiguous germplasm classification and frequent disputes over cultivar rights, severely hindering the efficient advancement of passion fruit breeding and the rational utilization of germplasm resources (Viana et al. 2010; Su et al. 2024).

Genetic resources are the material basis for crop genetic improvement. Accurate identification and genetic diversity analysis are the prerequisites for variety breeding, resource conservation and sustainable industrial development (Rajan et al. 2023). Molecular marker technology, by directly revealing genetic differences at the genomic level, offers significant advantages such as high stability, abundant polymorphism, and immunity to environmental influences. It has been widely applied in the study of fruit tree germplasm resources (Grover and Sharma 2016; Agarwal et al. 2008; Li and Quiros 2001). Among these, sequence-related amplified polymorphism (SRAP) molecular marker technology, because its primers are designed based on open reading frames (ORFs), can effectively amplify gene-coding regions (Robarts and Wolfe 2014). Combines ease of operation, low cost, good reproducibility, and high detection rates for polymorphisms (El-Shahed et al. 2017).

Currently, research on molecular markers in passion fruit primarily focuses on techniques such as RAPD and SSR (Carneiro et al. 2002; Cerqueira-Silva et al. 2012; Maciel et al. 2019; Wei et al. 2022). RAPD is simple, convenient, and low-cost, but its low stability and poor reproducibility limit its utility (Roodt et al. 2002). SSR markers are stable, abundant, co-dominant, highly polymorphic, and reproducible; however, primer development for each species requires sequencing, making the method both time-consuming and expensive (Wei et al. 2022). Systematic research on the identification of passion fruit germplasm resources using SRAP markers remains limited. Existing research indicates that SRAP markers demonstrate significant advantages in crop genetic

diversity analysis and the identification of closely related varieties. They have been successfully applied to variety identification and genetic analysis in fruit trees such as pineapple (Mwaniki et al. 2025), kiwifruit (Ma et al. 2024), and kumquat (Liu et al. 2023). Recently, SCOT markers have been employed to assess the genetic relationships among a collection of 140 passion fruit accessions (Zhang et al. 2026). However, given that different molecular marker systems target distinct genomic regions, the use of multiple marker types can provide complementary perspectives on genetic diversity. In this study, we applied SRAP markers to the same germplasm set to further characterize its genetic variation and population structure, thereby contributing additional molecular data to the ongoing characterization of this collection.

In this study, passion fruit germplasm resources were used to optimize the SRAP molecular marker reaction system. SRAP primer combinations with high polymorphism were screened to analyze the genetic similarity and genetic differences among various passion fruit germplasm resources. The findings aim to provide a scientific basis for the precise identification, rational conservation, and innovative utilization of passion fruit germplasm, thereby promoting the healthy and sustainable development of the passion fruit industry.

Materials and methods

Plant material

A total of 140 passion fruit accessions were collected from the Fujian Institute of Tropical Crops (24.63°N, 117.52°E) and Zhangzhou Wanyi Agricultural Development Co., Ltd. (24.58°N, 117.60°E) regions. These accessions are identical to the germplasm set previously characterized using SCoT markers by Zhang et al. (2026). Detailed information on all 140 accessions, including their species, origin, and source, is provided in Table S1 (Supplementary Material). Unless otherwise specified, the accession numbers are denoted by "No.".

DNA extraction

Total genomic DNA was extracted from the leaves of 140 passion fruit accessions using the YALEPIC® Plant Genomic DNA isolation Kit (Polysaccharides& Polyphenolics-rich, RNase A). The concentration and quality of DNA were measured using the Nano-400A instrument, and DNA integrity was assessed by gel electrophoresis. The obtained DNA was uniformly diluted to 50 ng·μL⁻¹ and finally stored in a -20°C freezer for subsequent use.

Single-factor optimization of SRAP-PCR

SRAP primers were designed based on previously published sequences (Budak et al. 2004; Li and Quiros 2001). A total of seven forward and seven reverse primers (Table 1) were selected and randomly combined to generate 49 primer pairs, all of which were synthesized by Fuzhou Sunya Biotechnology Co., Ltd.

Genomic DNA from one passion fruit cultivar was selected as the template. Original PCR system (20 μ L) contained 9 μ L of 2 $\times$ Taq Plus Master Mix II, 0.8 μ mol $\cdot$ L⁻¹ primer, and 20 ng template DNA, adjusted to volume with ddH₂O. Original PCR program: 95°C for 3 min (pre-denaturation); 35 cycles of 95°C for 15 s, annealing for 20 s, 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C. Annealing temperature followed Table 2. Select the primer with the richest and clearest bands as the initial primer.

A single-factor optimization experiment was conducted for three parameters: DNA template amount, primer concentration, and 2 $\times$ Taq Plus Master Mix II volume. Each parameter was tested across six gradient levels (Table 2), with the other two held constant at their initial reaction system values.

Table 1 SRAP primer sequence

Primer	Forward primer (5'-3')	Tm (°C)	Primer	Reverse primer (5'-3')	Tm (°C)
mE1	TGAGTCCAAACCGGAGC	57.4	Em1	GACTGCGTACGAATTAAT	51.2
mE2	TGAGTCCAAACCGGAAT	52.6	Em2	GACTGCGTACGAATTTGC	55.8
mE3	TGAGTCCAAACCGGACC	57.4	Em3	GACTGCGTACGAATTGAC	55.8
mE4	TGAGTCCAAACCGGACT	55.0	Em4	GACTGCGTACGAATTGCA	55.8
mE5	TGAGTCCAAACCGGAAA	52.6	Em5	GACTGCGTACGAATTCAA	53.5
mE6	TGAGTCCAAACCGGAAC	55.0	Em6	GACTGCGTACGAATTCAC	55.8
mE7	TGAGTCCAAACCGGAGA	55.0	Em7	GACTGCGTACGAATTCAG	55.8

Table 2 SRAP-PCR single factor level

Level	DNA quantity (ng)	Primer concentration (μ mol/L)	2 $\times$ Taq Plus Master Mix (μ L)
1	5	0.4	7
2	10	0.6	8
3	15	0.8	9
4	20	1	10
5	25	1.2	11
6	30	1.4	12

Orthogonal optimization of SRAP-PCR

An $L_{16}(4^3)$ orthogonal design was used, with three factors each at four levels, yielding 16 experimental runs. PCR amplification followed the original PCR program; products were resolved on 1% agarose gels (120 V, 35 min), and gel images were documented using a gel imaging system. Bands were scored for specificity, clarity, and intensity on a 0-16 scale (0 = no band; 16=sharp, specific, and intense bands), with three technical replicates per run and the mean score used for analysis (Wu et al. 2013).

Primer screening and data analysis

Template DNA was a mixture from five phenotypically distinct passion fruit varieties. The optimized system was employed to screen primer combinations, followed by PCR amplification of 140 passion fruit accessions. For data analysis, Quantity One software was used to read the bands and construct a (0/1) matrix. Genetic diversity parameters, including the number of observed alleles (N_a), number of effective alleles (N_e), Nei's gene diversity index (H), Shannon's information index (I), Nei's (1972) genetic identity (GI) and Nei's (1978) genetic distance (GD) were computed using PopGen32. Neighbor-Joining (NJ) cluster analysis was performed using MEGA11. The phylogenetic tree was visualized and refined using the iTOL v7.5 online platform (Letunic and Bork 2024).

Results

DNA Extraction Results

The quality of the extracted total DNA is summarized in Table 3. The A_{260}/A_{280} ratios ranged from 1.70 to 2.02, and DNA concentrations varied between 50 and 434 $\text{ng} \cdot \mu\text{L}^{-1}$. Agarose gel electrophoresis of representative samples (Fig. 1) revealed clear, bright bands with no smearing, indicating high purity and integrity of the DNA, which was suitable for subsequent experiments.

Table 3 DNA Purity and Concentration Data

No.	A260/280	ng/ μ L	No.	A260/280	ng/ μ L	No.	A260/280	ng/ μ L	No.	A260/280	ng/ μ L
1	1.89	54	36	1.8	142	71	1.93	123	106	1.79	92
2	1.7	99	37	1.76	146	72	1.86	82	107	1.71	114
3	1.87	70	38	1.81	207	73	1.82	57	108	1.7	50
4	1.9	56	39	1.7	90	74	1.83	147	109	1.72	136
5	1.81	113	40	1.7	55	75	1.88	209	110	1.74	85
6	1.81	75	41	1.84	107	76	1.7	164	111	1.73	148
7	1.63	50	42	1.87	149	77	1.71	111	112	1.75	82
8	1.94	50	43	1.93	199	78	1.7	99	113	1.84	83
9	1.7	56	44	1.94	312	79	1.74	74	114	2.05	482
10	1.8	140	45	1.95	328	80	1.74	94	115	1.82	243
11	1.72	82	46	1.93	80	81	1.75	74	116	1.70	132
12	1.79	76	47	1.76	151	82	1.76	114	117	1.78	147
13	1.76	130	48	2.05	149	83	1.76	71	118	1.73	53
14	1.71	133	49	1.8	59	84	1.71	70	119	1.87	65
15	1.7	50	50	1.75	183	85	1.73	184	120	1.73	142
16	1.87	169	51	1.76	109	86	1.84	78	121	1.84	154
17	1.89	70	52	1.78	138	87	1.8	62	122	1.78	146
18	1.93	104	53	1.7	68	88	1.74	88	123	1.81	128
19	1.83	54	54	1.73	128	89	1.71	102	124	1.83	60
20	1.72	113	55	2.01	57	90	2.02	434	125	1.88	146
21	1.86	71	56	1.83	104	91	1.76	97	126	1.74	137
22	1.88	84	57	1.96	309	92	1.82	104	127	1.79	192
23	2	402	58	1.8	78	93	1.74	131	128	1.71	79
24	1.7	51	59	1.98	92	94	1.79	111	129	1.70	154
25	1.85	132	60	1.9	96	95	1.78	151	130	1.76	120
26	1.72	72	61	1.87	76	96	1.96	313	131	1.79	105
27	1.79	138	62	1.7	93	97	1.82	127	132	1.72	140
28	1.77	92	63	1.91	126	98	1.73	198	133	2.08	161
29	1.74	92	64	1.8	114	99	1.88	173	134	1.84	72
30	1.79	83	65	1.7	69	100	1.92	211	135	1.73	79
31	1.7	53	66	1.77	80	101	1.71	153	136	1.79	173
32	1.75	179	67	1.75	64	102	1.70	58	137	1.71	129
33	1.73	262	68	1.99	145	103	1.91	66	138	1.71	95
34	1.83	100	69	1.7	52	104	1.85	187	139	1.72	67
35	1.78	172	70	2.03	260	105	1.76	93	140	1.74	144

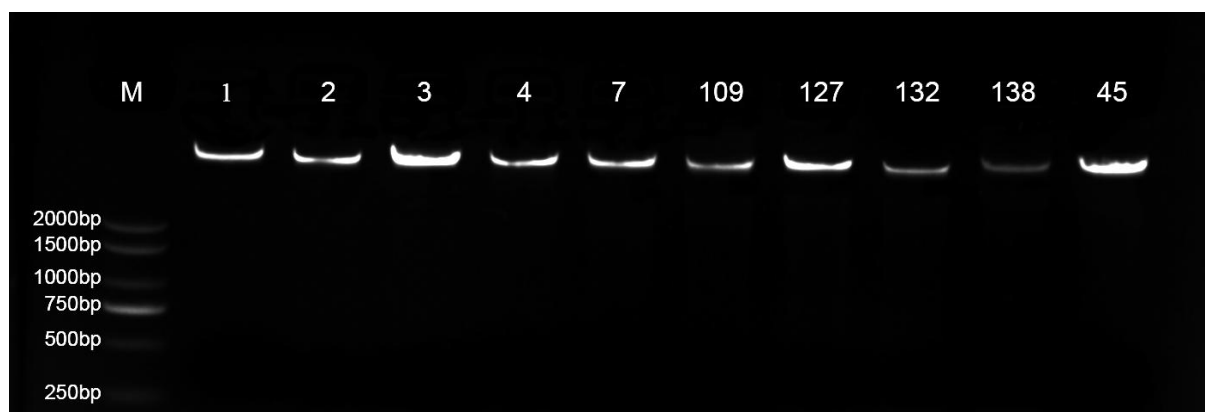


Fig.1 Partial Sample DNA Electrophoresis Results

M: DL2000 Plus DNA Marker; Samples shown are No. 1, 2, 3, 4, 7, 109, 127, 132, 138, 45

Effect of primer concentration on SRAP-PCR of passion fruit

Fig. 2 shows the amplification results under different primer concentrations: at 0.4 $\mu\text{mol/L}$, bands were faintest and least numerous; from 0.6 to 1.0 $\mu\text{mol/L}$, band brightness and number of identifiable bands increased; from 1.0 to 1.4 $\mu\text{mol/L}$, band integrity and clarity stabilized. Considering both amplification efficiency and band quality, the optimal primer concentration was determined to be 1.0–1.4 $\mu\text{mol/L}$.

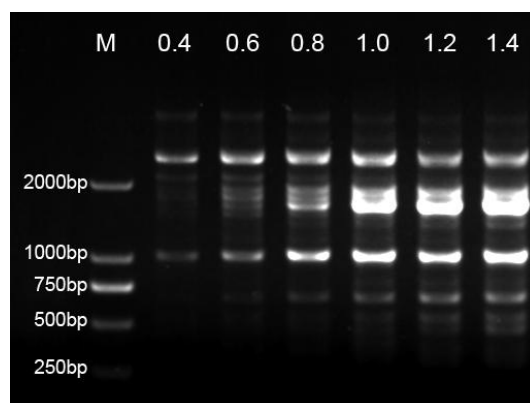


Fig.2 PCR amplification effect of primer concentration gradient

M: *Trans2K*[®] DNA Marker; 0.4–1.4 were different primer concentrations: 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 $\mu\text{mol/L}$

Effect of 2 × Taq Plus Master Mix II on SRAP-PCR of passion fruit

Fig. 3 shows the effect of 2 × Taq Plus Master Mix II volume on PCR amplification: no bands at 7 μL; clear, uniformly bright bands without smearing or nonspecific products at 8–10 μL; blurred bands and reduced brightness stability at 11–12 μL. The optimal volume is 8–10 μL, balancing amplification performance and practicality.

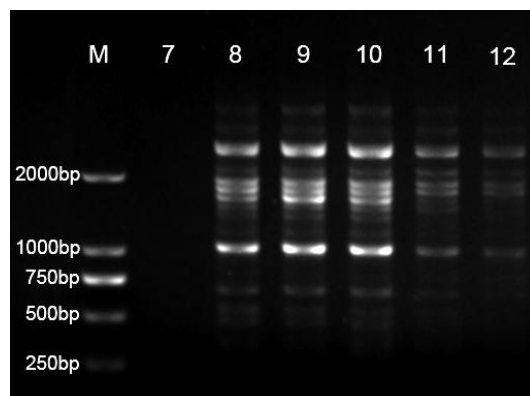


Fig.3 PCR amplification efficiency at different doses of 2 × Taq Plus Master Mix II

M: *Trans2K[®]* DNA Marker; 7–12 denotes different 2 × Taq Plus Master Mix II: 7, 8, 9, 10, 11, 12 μL

Effect of DNA template amount on SRAP-PCR in passion fruit

Fig. 4 shows the effect of template DNA amount on PCR amplification: clear, consistent banding patterns with no dropout, smearing, or ambiguity across 5–30 ng; indicating robust tolerance within this range. The optimal amount is 5–20 ng, balancing amplification efficiency and cost-effectiveness.

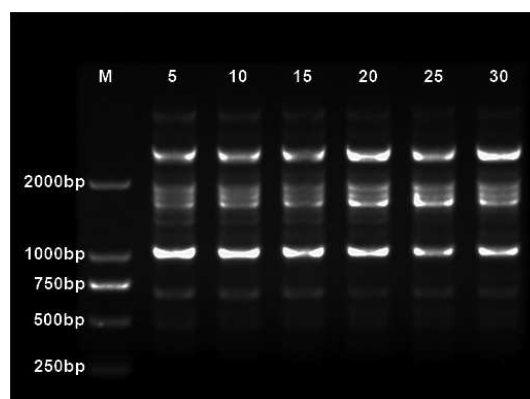


Fig.4 PCR amplification efficiency at different doses of template DNA

M: *Trans2K[®]* DNA Marker; 5–30 denotes different DNA quantities: 5, 10, 15, 20, 25, 30 ng

Results of orthogonal design

Orthogonal experiment results (Table 4, Fig. 5) show significant variation in band number, clarity, and intensity across the 16 condition combinations: combinations 3, 6, 11, and 16 yield abundant, clear, bright bands, indicating optimal SRAP primer compatibility; combinations 5, 9, and 13 produce blurred, smeared, or missing bands, indicating poor specificity and amplification efficiency. Visual scoring of electrophoretograms (mean of three replicates, Table 4) reveals the factor influence ranking: primer concentration > 2 × Taq Plus Master Mix II volume > template DNA amount (R-value analysis). The optimal combination (highest K value) is 15 ng template DNA, 1.2 μmol/L primer, 9 μL 2× Taq Plus Master Mix II, ddH₂O to 20 μL.

Table 4 L₁₆ (4³) orthogonal test score

Comb	DNA (ng)	Primers (μmol/L)	2×Taq Plus Master Mix (μL)	Mean Rating
1	10.00	0.60	9.00	8.67
2	10.00	0.80	9.50	12.33
3	10.00	1.00	10.00	13.33
4	10.00	1.20	10.50	12.67
5	15.00	0.60	9.50	8.33
6	15.00	0.80	9.00	12.33
7	15.00	1.00	10.50	13.33
8	15.00	1.20	10.00	14.00
9	20.00	0.60	10.00	8.00
10	20.00	0.80	10.50	7.67
11	20.00	1.00	9.00	14.33
12	20.00	1.20	9.50	14.00
13	25.00	0.60	10.50	7.67
14	25.00	0.80	10.00	11.00
15	25.00	1.00	9.50	12.67
16	25.00	1.20	9.00	14.67
K1	11.75	8.17	12.50	-
K2	12.00	10.83	11.83	-
K3	11.00	13.42	11.58	-
K4	11.50	13.83	10.33	-
R	1.00	5.67	2.17	-

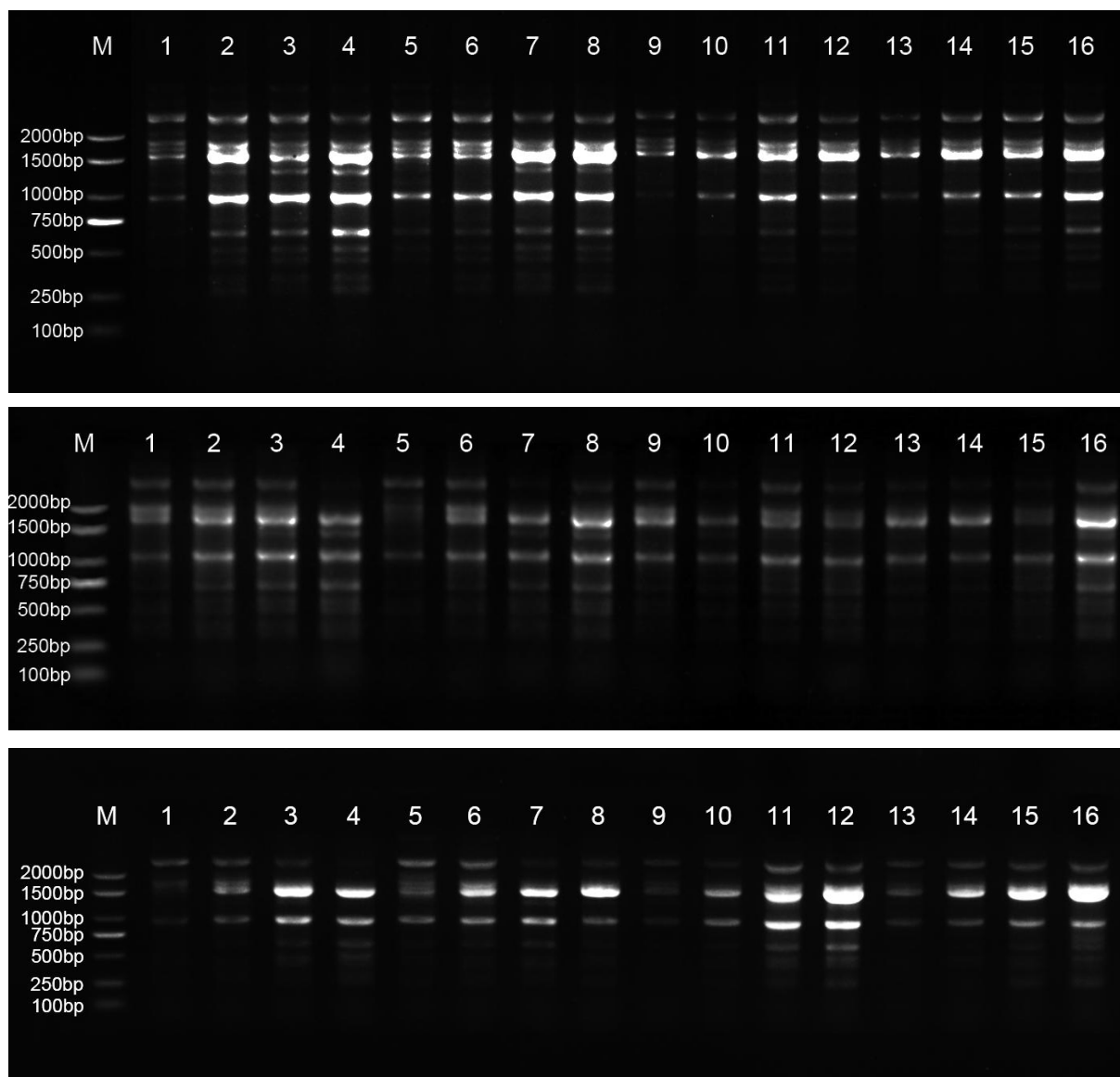


Fig.5 Results of three independent orthogonal experiments

M: DL2000 Plus DNA Marker; 1–16: Sixteen orthogonal treatment combinations

Primer screening

Using a DNA mixture of five varieties (No.1, 36, 60, 102, and 122) as the template, primers were screened under the initial PCR program and optimized reaction system. From 49 randomly combined primer pairs, 21 SRAP primer combinations with clear amplification and high polymorphism were selected (Table 5). Representative results are shown in Fig. 6.

Table 5 Selected Passion Fruit SRAP Primer Combination

No.	Primer combination	No.	Primer combination	No.	Primer combination
1	mE2-Em1	8	mE3-Em4	15	mE4-Em6
2	mE2-Em2	9	mE3-Em5	16	mE4-Em7
3	mE2-Em3	10	mE3-Em6	17	mE5-Em3
4	mE2-Em4	11	mE3-Em7	18	mE5-Em4
5	mE2-Em6	12	mE4-Em2	19	mE5-Em6
6	mE3-Em2	13	mE4-Em3	20	mE6-Em4
7	mE3-Em3	14	mE4-Em4	21	mE7-Em4

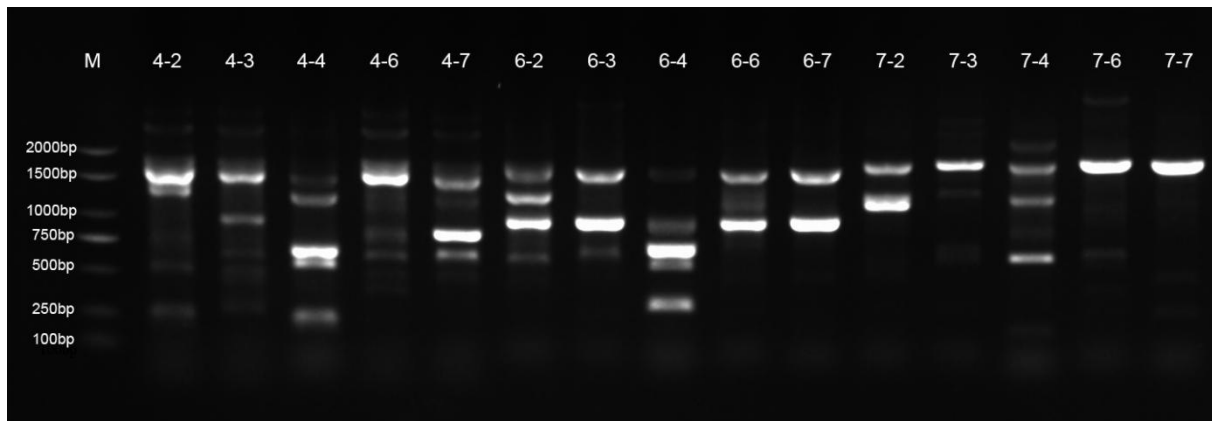


Fig. 6 Screening results of some primers

M: DL2000 Plus DNA Marker; 4-2: mE4-Em2, 4-3: mE4-Em3, 4-4: mE4-Em4, 4-6: mE4-Em6, 4-7: mE4-Em7, 6-2: mE6-Em2, 6-3: mE6-Em3, 6-4: mE6-Em4, 6-6: mE6-Em6, 6-7: mE6-Em7, 7-2: mE7-Em2, 7-3: mE7-Em3, 7-4: mE7-Em4, 7-6: mE7-Em6, 7-7: mE7-Em7

Polymorphism analysis

Using the 21 selected SRAP primer pairs and the optimized reaction system, PCR amplification was performed on the 140 passion fruit accessions. The amplification products obtained with primer pair mE5-Em3 (Fig. 7) displayed clear, sharp bands with no smearing. A total of 345 bands were detected, of which 343 were polymorphic (Table 6). On average, each primer pair amplified 16.43 bands, including 16.33 polymorphic bands. The Percentage of Polymorphic Bands (PPB) ranged from 93.30% to 100%, with an average of 99.41%. This indicates an extremely high level of genetic polymorphism in the tested germplasm as revealed by SRAP markers, with allelic variation present at nearly all loci, suggesting a broad genetic background.

Analysis using PopGen32 showed that the mean Na was 1.9942 (SD = 0.0185, CV = 0.93%), indicating stable amplification and consistent allele detection. The mean Ne was 1.4516 (SD = 0.1366, CV = 9.41%), reflecting considerable variation in allele frequencies among accessions. The mean H and I were 0.2727 and 0.4208, respectively, indicating a moderate level of genetic diversity. However, the relatively high SDs (0.0716 for H, 0.0950 for I) and CVs (26.27% for H, 22.57% for I) suggest uneven levels of genetic variation among the different germplasm accessions.

Table 6 Genetic diversity parameters of passion fruit using SRAP

Primer comb	NAB*	NPB	PPB(%)	Na	Ne	H	I
mE2-Em1	16	16	100.00%	2.0000	1.5622	0.3302	0.4965
mE2-Em2	11	11	100.00%	2.0000	1.4657	0.2832	0.4372
mE2-Em3	16	16	100.00%	2.0000	1.5149	0.3070	0.4672
mE2-Em4	19	19	100.00%	2.0000	1.5594	0.3254	0.4902
mE2-Em6	18	18	100.00%	2.0000	1.5467	0.3305	0.4990
mE3-Em2	14	14	100.00%	2.0000	1.5724	0.3347	0.5012
mE3-Em3	22	22	100.00%	2.0000	1.6562	0.3656	0.5351
mE3-Em4	15	14	93.30%	1.9333	1.4974	0.2901	0.4391
mE3-Em5	19	19	100.00%	2.0000	1.4325	0.2587	0.4018
mE3-Em6	16	16	100.00%	2.0000	1.4380	0.2659	0.4104
mE3-Em7	20	20	100.00%	2.0000	1.4616	0.2751	0.4209
mE4-Em2	19	19	100.00%	2.0000	1.2733	0.1822	0.3041
mE4-Em3	18	18	100.00%	2.0000	1.5945	0.3418	0.5071
mE4-Em4	14	14	100.00%	2.0000	1.3480	0.2088	0.3336
mE4-Em6	15	15	100.00%	2.0000	1.4060	0.2499	0.3893
mE4-Em7	13	13	100.00%	2.0000	1.3119	0.2072	0.3423
mE5-Em3	18	18	100.00%	2.0000	1.5227	0.3150	0.4816
mE5-Em4	16	16	100.00%	2.0000	1.5645	0.3437	0.5199
mE5-Em6	14	14	100.00%	2.0000	1.4324	0.2760	0.4362
mE6-Em4	14	14	100.00%	2.0000	1.1071	0.0850	0.1661
mE7-Em4	18	17	94.40%	1.9444	1.2156	0.1505	0.2578
Total	345	343	-	-	-	-	-
Mean	16.4286	16.3333	99.41%	1.9942	1.4516	0.2727	0.4208
St. Dev	-	-	-	0.0185	0.1366	0.0716	0.0950
CV	-	-	-	0.93%	9.41%	26.27%	22.57%

*NAB: Total number of amplified bands; NPB: Number of polymorphic bands; PPB: Percentage of polymorphic bands; Na: Observed number of alleles; Ne: Effective number of alleles; H: Nei's gene diversity; I: Shannon's information index; St. Dev: standard deviation; CV: coefficient of variation.

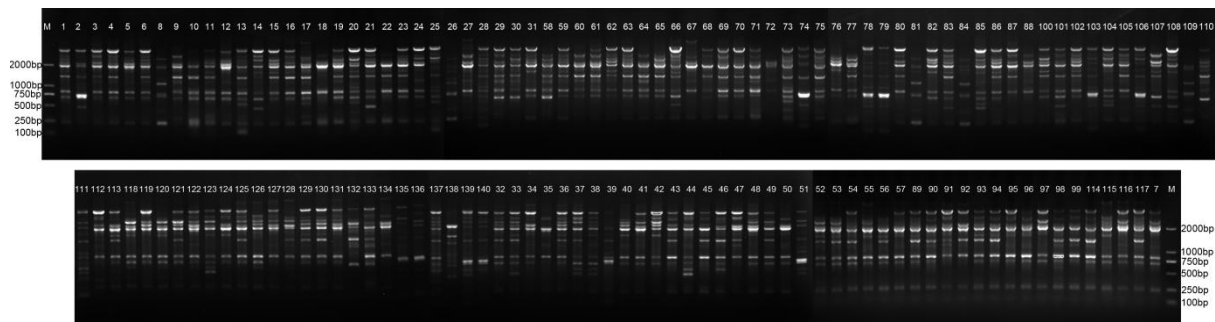


Fig. 7 Amplification results for 140 samples using SRAP primer comb mE5-Em3

M: *Trans2K*[®] DNA Marker; 1-140: No. of passion fruits

Genetic relationship analysis of 140 passion fruit accessions

The correlation between Nei's genetic identity (GI) and genetic distance (GD) across the 140 accessions is visualized in Fig. 8, and the corresponding original data are summarized in Supplementary Table S2. GI values ranged from 0.4870 to 0.9275, with corresponding GD ranging from 0.0752 to 0.7196. It indicates that the genetic differences among different germplasms vary greatly, with both highly homologous groups and materials retaining unique genetic backgrounds. In striking contrast, a subset of the germplasm accessions exhibits notably low genetic similarity. Among these, germplasm accessions 80 and 26 exhibited the lowest GI of 0.4870 and the highest corresponding GD of 0.7196, indicating pronounced genetic divergence and a distant phylogenetic relationship. Comparative analysis of variety types revealed that the five passion fruit accessions (No. 8, 25, 80, 83, 108) exhibited close pairwise genetic relationships ($GI \geq 0.6435$, $GD \leq 0.4409$). In contrast, their genetic affinities with most other accessions were relatively distant, with GI ranging from 0.51 to 0.64 and GD from 0.52 to 0.63. Similarly, germplasm 132 (*P. coccinea*) and the seven other seven *P. coccinea* $\times$ *P. edulis* var. *flavicarpa* hybrids (No. 9, 28, 29, 31, 59, 60, 63) demonstrated high genetic similarity ($GI \geq 0.7159$, $GD \leq 0.3342$). Notably, germplasm 133 (*P. trifasciata*) and 138 (*P. caerulea*) showed distant genetic relationships with the remaining 138 passion fruit accessions ($GI \leq 0.6841$, $GD \geq 0.4917$). These findings indicate that these two accessions possess unique genetic backgrounds within the tested population, exhibiting significant differentiation from other germplasm and thus can be classified as distinctive germplasm resources.

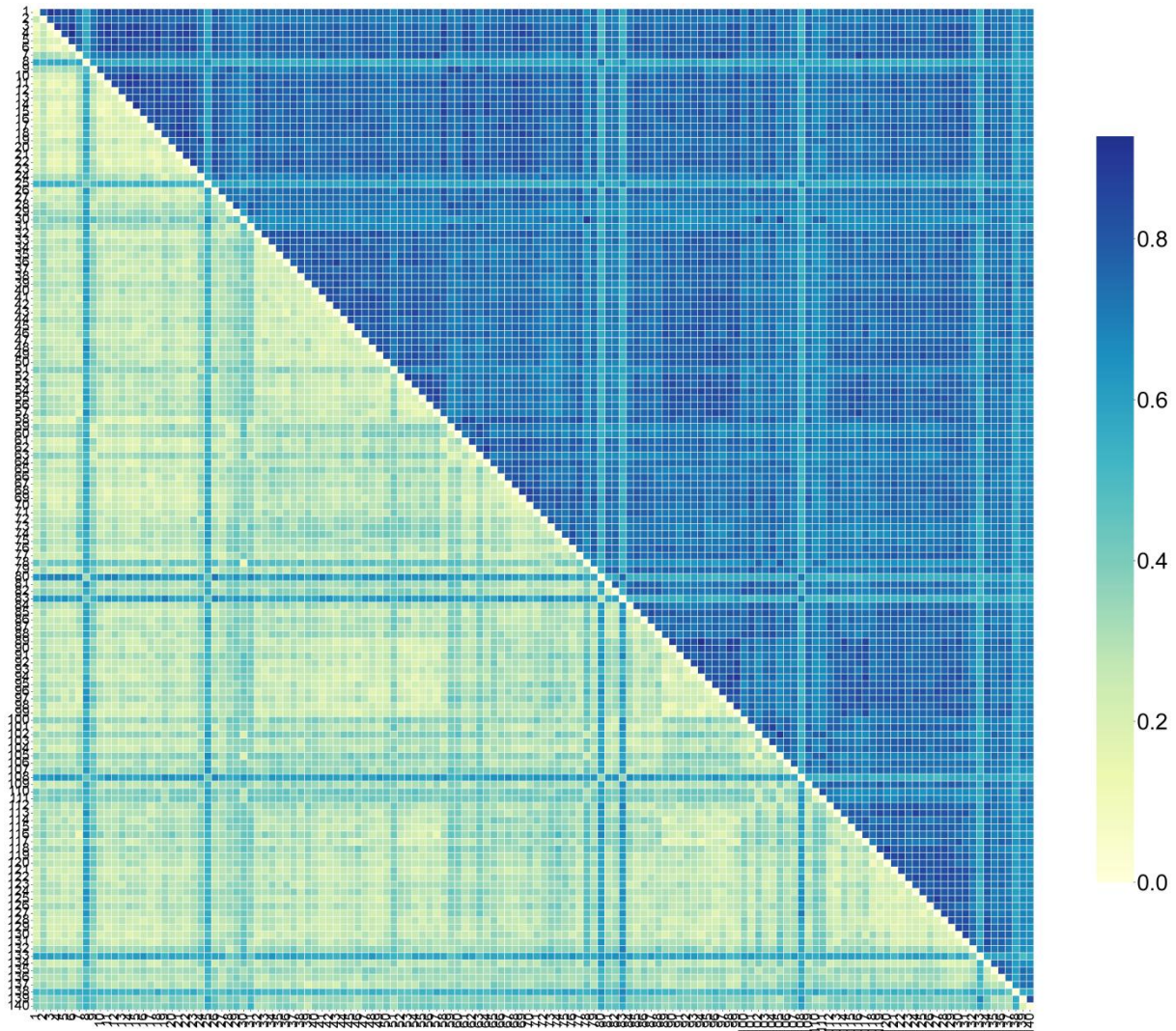


Fig. 8 Correlation plot showing Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for 140 passion fruit accessions; raw pairwise values are listed in Supplementary Table S2

Cluster analysis of 140 passion fruit accessions

Using the Neighbor-Joining (NJ) method in MEGA11 software, a clustering dendrogram was constructed for the 140 passion fruit accessions. Based on the clustering results, the tested germplasm could be divided into five major groups (Fig. 9), the characteristics of which were closely associated with fruit type and genetic background.

Group I comprised 46 yellow-fruited accessions, primarily *P. edulis* var. *flavicarpa* × *P. edulis* var. *flavicarpa* hybrids from Jinjizhai and Wanyi. Notably, accession 30 (*P. coccinea* × *P. edulis* var. *flavicarpa*) clustered here, indicating molecular affinity to yellow-fruited germplasm. Group II consisted predominantly of yellow-fruited accessions, including commercial cultivars (89, 36, 40 et al) and numerous hybrids. Several

purple-fruited accessions (137 et al) also clustered here, suggesting genetic introgression or shared background between fruit types. Group III mainly contained purple-fruited accessions, such as 102 and 136. However, yellow-fruited accession 77 (*P. edulis* var. *flavicarpa* × *P. edulis* var. *flavicarpa*) grouped here, indicating genetic proximity to purple types despite its yellow phenotype. Group IV comprised eight *P. coccinea* -related accessions. Group V, though small, contained the most distinctive materials: five *P. edulis* accessions (8, 25, 80, 83, 108) and rare ornamental species (133 *P. trifasciata*, 138 *P. caerulea*), representing a valuable resource for specialty cultivar development and germplasm conservation.

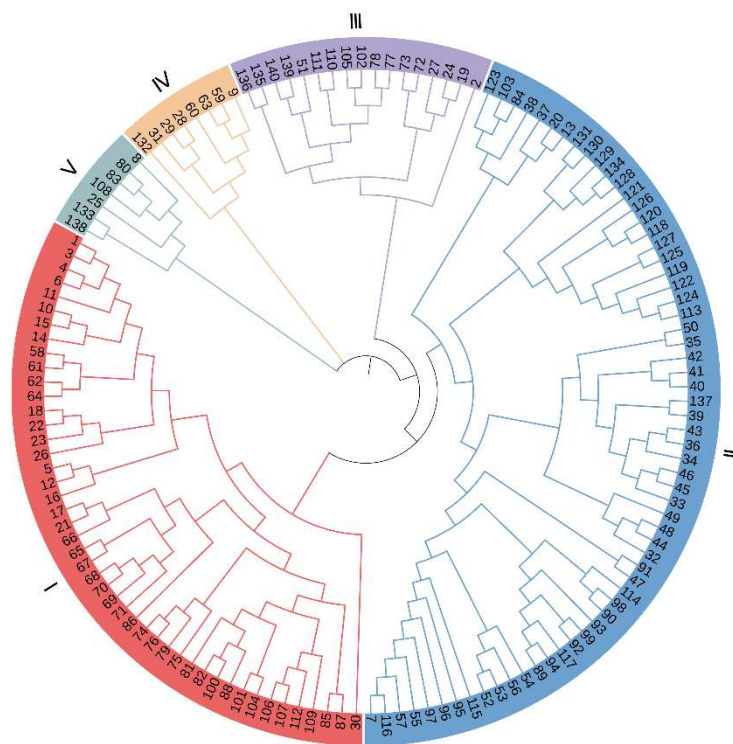


Fig.9 Cluster analysis of passion fruit based on SRAP markers

Note: 1~140:140 varieties of passion fruit 'No.' (Table S1)

Discussion

SRAP molecular markers, with advantages such as simple operation, low cost, high polymorphism, and no need for a reference genome (Li and Quiros 2001), have been widely used in the analysis of genetic diversity and variety identification of fruit trees (Işık et al. 2026; Xuan et al. 2022). *Passiflora edulis* leaves are rich in

polysaccharides, polyphenols, and viscous substances, which can easily lead to poor DNA quality and thus affect amplification efficiency (Lade et al. 2014). In this study, a combination of single-factor and orthogonal experiments was used to optimize the key components of the SRAP-PCR system (Zhang et al. 2012; Gui et al. 2025). The results showed that in a 20 μ L system, the optimal conditions were a primer concentration of 1.2 μ mol/L, 9 μ L of 2 $\times$ Taq Plus Master Mix II, and 15 ng of template DNA. This is consistent with the method of Chen et al. (2025), who employed an orthogonal design to balance efficiency and accuracy, but differs from the component concentrations used by Lin (2023) in SRAP analysis of *Prunus salicina*. These discrepancies may be attributed to variations in genome complexity and GC content among different species, highlighting the necessity of optimizing the reaction system for the target species. This combination effectively eliminated primer dimers and non-specific amplification, obtaining clear and stable bands, providing reliable technical support for large-scale analysis of *Passiflora edulis* germplasm resources.

Only primers with high polymorphism, clear bands, and good stability can accurately reflect the genetic differences among the tested germplasms (Guo et al. 2023). Based on the amplification results, 21 out of 49 SRAP primer combinations were selected using the optimized PCR system in this study. These primers exhibited favorable polymorphism and clear banding patterns, enabling effective differentiation among various passion fruit germplasms.

Genetic diversity plays a crucial role in guiding the conservation, classification, and selection of elite parents for germplasm resources (Chen et al. 2025). Unlike random markers such as RAPD (Wadood et al. 2026) and ISSR (Li et al. 2026), SRAP markers target open reading frames (ORFs) and effectively reveal polymorphisms arising from length variations in non-coding regions, including introns and promoters, thereby exhibiting higher polymorphism (Li and Quiros 2001). For instance, Xia et al. (2020) analyzed 11 passion fruit accessions using nine SRAP primers and obtained a polymorphism rate of 84.93%. Similarly, Yan et al. (2025) detected a polymorphism rate of 95.55% in 77 grape accessions using 10 SRAP primer pairs. In the present study, 21 selected SRAP primer pairs were used to amplify 140 passion fruit accessions, yielding a total of 345 clear and stable bands. Among these, 343 bands were polymorphic, corresponding to a polymorphism rate of 99.41%, with an average of 16.43 bands per primer pair. These results demonstrate that the selected SRAP primers exhibit high polymorphism and exceptional efficiency in detecting genetic variation.

Genetic diversity parameters obtained from SRAP markers vary considerably among different crops. For instance, Ma et al. (2024) analyzed 114 wild kiwifruit accessions using 13 SRAP primer pairs and reported mean genetic diversity parameters ($N_e = 1.452$, $H = 0.245$, $I = 0.439$), indicating a moderately high level of genetic

diversity among wild kiwifruit germplasms. Similarly, Işık et al. (2026) investigated 85 alfalfa accessions with 10 SRAP primer pairs and obtained comparable values ($N_e = 1.44$, $H = 0.27$, $I = 0.42$), suggesting a moderately high genetic diversity level in the tested alfalfa germplasms. In contrast, Song et al. (2025) employed SRAP markers to study 106 sugar beet accessions and reported $N_a = 1.7492$, $N_e = 1.4256$, $H = 0.2614$, and $I = 0.3772$, indicating a relatively low to moderate level of genetic diversity. However, the coefficients of variation for H (22.54%) and I (26.09%) reported in their further analysis revealed substantial differentiation in genetic diversity indices among accessions, suggesting that some germplasms may harbor unique genetic information. Genetic diversity assessments of passion fruit also vary among different studies. Araponga et al. (2025) analyzed 125 sour passion fruit accessions from Brazil using ISSR markers and reported H_e ranging from 0.166 to 0.203 and I ranging from 0.249 to 0.308, indicating a moderately low level of genetic diversity in the tested materials. Morillo et al. (2024) employed eight SSR primers to examine 84 passion fruit genotypes and obtained higher values ($H_e = 0.43$, $I = 0.71$). In contrast, Wu et al. (2024) detected even greater genetic diversity in 87 passion fruit accessions using SSR markers ($N_a = 8.625$, $N_e = 3.100$, $H_e = 0.650$, $I = 1.41$), suggesting that passion fruit possesses a considerable genetic diversity foundation. SRAP-based analysis in this study revealed $N_a = 1.994$, $N_e = 1.4516$, $H = 0.2727$, and $I = 0.4208$, indicating that alleles at specific gene regions are largely intact and conserved across most accessions. These findings further confirm the relatively high level of genetic diversity present in passion fruit germplasm resources. Meanwhile, the coefficients of variation (CV) for the estimated parameters were 0.93% (N_a), 9.41% (N_e), 26.27% (H), and 22.57% (I). These findings reveal that despite relatively conserved allele numbers across the tested accessions, uneven allele frequency distribution and substantial heterogeneity in genetic diversity among loci are evident. Compared with similar studies, Olouch et al. (2018) analyzed 22 Kenyan passion fruit accessions and reported $I = 0.344$, $H = 0.233$, and a polymorphism rate of 65.5%. Xia Ling et al. (2020) examined 11 *Passiflora* germplasms from Sanya and obtained a polymorphism rate of 84.93%. The present study detected higher genetic diversity (PPB=99.41%, $I = 0.4208$, $H = 0.2727$) than those previously reported, indicating that cultivated passion fruit germplasms possess a rich genetic foundation.

NJ cluster analysis based on SRAP markers grouped the passion fruit germplasm accessions into five distinct clusters (Fig. 9). Cluster I consisted exclusively of yellow passion fruit, primarily comprising seedlings and hybrids. Cluster II was composed predominantly of yellow-fruited hybrids, along with a small number of purple-fruited interspecific hybrids. Cluster III contained mostly purple passion fruit. Cluster IV consisted entirely of accessions related to *P. coccinea*. Cluster V comprised seedlings of passion fruit with distinctive fruit

colors. This clustering pattern fully demonstrates the high resolution and effectiveness of SRAP markers in assessing genetic diversity among passion fruit germplasms. Genetic similarity analysis further supported the classification: the five *P. edulis* and seven *P. coccinea* accessions all showed low genetic distances. This consistency with the NJ clustering results confirms the reliability of the inferred genetic relationships.

Conclusion

This study systematically characterized the genetic diversity of 140 *Passiflora edulis* accessions. A 98% polymorphic band ratio and moderately high genetic diversity parameters (Nei's $H = 0.27$, Shannon's $I = 0.42$) confirm that passion fruit germplasm holds rich genetic variation. Distinctive accessions (e.g., No. 77, 133, 138) identified via cluster analysis and coefficient of variation screening exhibit significant genetic divergence from mainstream cultivars, qualifying them as core parents for expanding breeding genetic bases and developing novel germplasm. These findings provide theoretical underpinnings and technical support for scientific conservation, efficient utilization, and precision breeding passion fruit germplasm resources.

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Data availability Other data required for this study are provided in the Supplemental.

Ethics declarations

Conflict of interest The authors declare no conflict of interest.

Consent to publication The authors have readed the manuscript and consent to its publication.

References

- Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Rep* 27(4): 617–631. <https://doi.org/10.1007/s00299-008-0507-z>
- Araponga CS, Silva GS, de Melo CAF, Souza MM (2025) Genetic diversity and population structure of sour passion fruit in Brazil. *J Genet Eng Biotechnol* 23(4):100607. <https://doi.org/10.1016/j.jgeb.2025.100607>
- Arshad MT, Basher NS, Ibrahim NA, Ikram A, Maqsood S, Rasheed A, Naveed F et al (2025) Nutritional, Pharmacological and Industrial Applications of Mangosteen and Passion Fruit: A Review. *Food Science & Nutrition*, 13(7): e70574. <https://doi.org/10.1002/fsn3.70574>
- Betancur-Ancona D, Pérez-Navarrete C, Chel-Guerrero L, Sosa-Crespo I & Sandoval-Peraza VM (2025) Functional, bioactive, and sensory properties of nutraceutical beverages enriched with passion fruit (*Passiflora edulis*) peel fiber. *Food Chemistry Advances*, 101005. <https://doi.org/10.1016/j.focha.2025.101005>
- Budak H, Shearman RC, Parmaksiz I, Gaussoin RE, Riordan TP, Dweikat I (2004) Molecular characterization of Buffalograss germplasm using sequence-related amplified polymorphism markers. *TAG Theoretical and Applied Genetics* 108(2):328–334. <https://doi.org/10.1007/s00122-003-1428-4>
- Carneiro MS, Camargo LEA, Coelho ASG, Vencovsky R, Júnior RPL, Stenzel NMC, Vieira MLC (2002) RAPD-based genetic linkage maps of yellow passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.). *Genome* 45(4):670–678. <https://doi.org/10.1139/g02-035>
- Chen T, Wei X, Feng B, Chen L (2025) Recent Advances in Crop Genetics and Breeding. *Life* 15(12):1891. <https://doi.org/10.3390/life15121891>
- Chen WY, Xu CY, Qian ZY, Wu H, Wang YF, Qin RG, Luo ZS (2025) Optimization of SRAP-PCR system for Polygonati Rhizoma and screening of effective primers. *Shandong Chemical Industry* 54(6):4–8. <https://doi.org/10.19319/j.cnki.issn.1008-021x.2025.06.071>
- de Jesus ON, Lima LKS, Soares TL, da Silva LN, dos Santos IS, Sampaio SR, de Oliveira EJ (2022) Phenotypic diversity and alternative methods for characterization and prediction of pulp yield in passion fruit (*Passiflora* spp.) germplasm. *Scientia Horticulturae* 292:110573. <https://doi.org/10.1016/j.scienta.2021.110573>

- El-Shahed AA, Abdellatif KF, Ibrahim IA, Mohamed AM, Abdelsalam IZ, Elsehrawy OA (2017) Efficiency of sequence related amplified polymorphism (SRAP) and target region amplified polymorphism (TRAP) markers in detecting banana somaclonal variants. *African Journal of Biotechnology* 16(16):879–888. <https://doi.org/10.5897/ajb2017.15893>
- Faleiro FG, Junqueira NTV, Junghans TG, de Jesus ON, Miranda D, Otoni WC (2019) Advances in passion fruit (*Passiflora* spp.) propagation. *Revista Brasileira de Fruticultura* 41: e155. <https://doi.org/10.1590/0100-29452019155>
- Guo YZ, Huang Q, Ye XR, Chen Z, Huang Z, Chen HL, Yan DW, Xue ZZ (2023) Morphological and SRAP markers-based genetic diversity determination on *Phaseolus lunatus* L. germplasms. *Fujian Journal of Agricultural Sciences* 38(2):166–173. <https://doi.org/10.19303/j.issn.1008-0384.2023.02.006>
- Grover A, Sharma PC (2016) Development and use of molecular markers: past and present. *Critical Reviews in Biotechnology* 36(2):290–302. <https://doi.org/10.3109/07388551.2014.959891>
- Gui TQ, Li CY, Wei Y, Wu ZL, Zhang SP (2025) Orthogonal design optimization of SRAP-PCR reaction system and primer screening for *Bletilla striata*. *Fujian Agricultural Science and Technology* 56(3):18–24. <https://doi.org/10.13651/j.cnki.fjnykj.2025.03.003>
- Işık Mİ, Türkoğlu A, Eren B, Koç A, İleri O, Haliloğlu K, Sarıtaş Y, Bocianowski J (2026) Assessment of genetic diversity and population structure in alfalfa (*Medicago sativa* L.) using sequence-related amplified polymorphism (SRAP) markers. *Genetic Resources and Crop Evolution* 73(1):8. <https://doi.org/10.1007/s10722-025-02692-6>
- Lade BD, Patil AS, Paikrao HM (2014) Efficient genomic DNA extraction protocol from medicinal rich *Passiflora foetida* containing high level of polysaccharide and polyphenol. *SpringerPlus* 3:457. <https://doi.org/10.1186/2193-1801-3-457>
- Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *TAG Theoretical and Applied Genetics* 103(2-3):455–461. <https://doi.org/10.1007/s001220100570>
- Li X, Cao X, Xie L, Liu Y, Yin Y, Lu B, Shi X, Meng H (2026) Analysis of genetic diversity of Tartary buckwheat (*Fagopyrum tataricum*) in Yunnan province based on inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers. *Genetic Resources and Crop Evolution* 73(2):117. <https://doi.org/10.1007/s10722-026-02747-2>

- Lin XJ (2023) Genetic diversity analysis of Nai Plum (*Prunus salicina*) resources and genetic identification of superior varieties based on SCoT and SRAP markers. Fujian Agriculture and Forestry University.
<https://doi.org/10.27018/d.cnki.gfjnu.2023.001193>
- Liu B, Ding P, Ye R, Li Y, Ou S, Gentile A, Ma X, Deng Z (2023) Genetic Diversity Analysis of Guangxi Kumquat (*Fortunella Swing*) Germplasm Using SRAP Markers. *Horticulturae* 9(6):689.
<https://doi.org/10.3390/horticulturae9060689>
- Letunic I, Bork P (2024) Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research* 52(W1): W78–W82. <https://doi.org/10.1093/nar/gkae268>
- Maciel KS, de Lima PAM, Madalon FZ, Ferreira MF da S, Alexandre RS, Lopes JC (2019) Genetic diversity in passion fruit plants at different altitudes. *Australian Journal of Crop Science* 13(7):1083–1093.
<https://doi.org/10.21475/ajcs.19.13.07.p1545>
- Ma X, Han Z, Li T, Yang Y, Su W, Li W (2024) Genetic diversity and population structure analysis of wild kiwifruit (*Actinidia* spp.) using SRAP markers. *Biotechnology & Biotechnological Equipment* 38(1).
<https://doi.org/10.1080/13102818.2024.2373845>
- Morillo AC, Manjarres EH, Morillo Y (2024) Diversity and genetic structure of yellow passion fruit in Boyacá-Colombia using microsatellite DNA markers. *Brazilian Journal of Biology* 84: e282426.
<https://doi.org/10.1590/1519-6984.282426>
- Mwaniki LM, Wanjala BW, Runo S, Omwenga G, Simiyu ES (2025) Evaluating Genetic Diversity in Kenyan Pineapple (*Ananas comosus*) Germplasm Using Sequence-Related Amplified Polymorphism (SRAP) Markers. *American Journal of Plant Sciences* 16(04):509–524. <https://doi.org/10.4236/AJPS.2025.164038>
- Oluoch P, Nyaboga EN, Bargul LJ (2018) Analysis of genetic diversity of passion fruit (*Passiflora edulis* Sims) genotypes grown in Kenya by sequence-related amplified polymorphism (SRAP) markers. *Annals of Agrarian Science* 16(4):367–375. <https://doi.org/10.1016/j.aasci.2018.08.003>
- Viana AJC, Souza MM, Araújo IS, Corrêa RX, Ahnert D (2010) Genetic diversity in *Passiflora* species determined by morphological and molecular characteristics. *Biologia Plantarum* 54(3):535–538.
<https://doi.org/10.1007/s10535-010-0094-z>
- Rajan N, Debnath S, Perveen K, Khan F, Pandey B, Srivastava A, Khanam MN et al (2023) Optimizing hybrid vigor: a comprehensive analysis of genetic distance and heterosis in eggplant landraces. *Frontiers in Plant Science* 14:1238870. <https://doi.org/10.3389/fpls.2023.1238870>

- Roodt R, Spies JJ, Burger TH (2002) Preliminary DNA fingerprinting of the turf grass *Cynodon dactylon* (Poaceae: Chloridoideae). *Bothalia* 32(1):117–122. <https://doi.org/10.4102/abc.v32i1.474>
- Robarts DWH, Wolfe AD (2014) Sequence-related amplified polymorphism (SRAP) markers: a potential resource for studies in plant molecular biology. *Applications in Plant Sciences* 2(7):1400017. <https://doi.org/10.3732/apps.1400017>
- Rosado RDS, Rosado LDS, Borges LL, Bruckner CH, Cruz CD, dos Santos C E M (2019) Genetic diversity of sour passion fruit revealed by predicted genetic values. *Agronomy Journal* 111(1):165–174. <https://doi.org/10.2134/agronj2018.05.0310>
- Cerqueira-Silva C B M, Santos E S L, Conceição L D H C S, Cardoso-Silva C B, Pereira A S, Oliveira A C, Corrêa R X (2012) Genetic variation in a wild population of the 'sleep' passion fruit (*Passiflora setacea*) based on molecular markers. *Genetics and Molecular Research* 11(1):731–738. <https://doi.org/10.4238/2012.March.22.3>
- Song Y, Li J, Li S, Wu Z, Pi Z (2025) Genetic diversity analysis of sugar beet multigerm germplasm resources based on SRAP molecular markers. *Horticulturae* 11(8):988. <https://doi.org/10.3390/horticulturae11080988>
- Su X, Wang X, Li R, Zhou C, Chen L, Chen S, Cai N, Xu Y (2024) Identification and evaluation of diploid and tetraploid *Passiflora edulis* Sims. *Plants* 13(18):2603. <https://doi.org/10.3390/plants13182603>
- Wadood A, Hameed A, Saleem MY, Khan AR, Akram S (2026) Multivariate analysis highlights genetic structure in Olive (*Olea Europaea* L.) germplasm using Resource-Efficient RAPD marker system. *Molecular Biology Reports* 53(1):379. <https://doi.org/10.1007/s11033-026-11548-w>
- Wei XQ, Li L, Xiong YQ, Xu L, Zhang XJ, Chen ZF, Song QJ, Xu JH (2022) Research on the identification of passion fruit varieties in Fujian province and construction of fingerprint based on SSR markers. *Fujian Journal of Agricultural Sciences*, 37(10):1288–1297. <https://doi.org/10.19303/j.issn.1008-0384.2022.010.007>
- Wu FC, Cai GJ, Xi PY, Guo YL, Xu M, Li AD (2024) Genetic diversity analysis and fingerprint construction for 87 passionfruit (*Passiflora* spp.) germplasm accessions on the basis of SSR fluorescence markers. *International Journal of Molecular Sciences* 25(19):10815. <https://doi.org/10.3390/ijms251910815>
- Wu Y, Tao Q, Li B, Xu D (2013) An optimal SRAP-PCR system of *Rhododendron hybridum* and its genetic diversity analysis with SRAP marker. *Journal of Zhejiang A&F University* 30(6):844–851. <https://doi.org/10.11833/j.issn.2095-0756.2013.06.007>

- Xia L, Gong JJ, Liang XJ, Wang FT, Ma YL, Cao M (2020) Genetic diversity analysis of *Passiflora* germplasm resources based on SRAP marker and ITS sequence. *Molecular Plant Breeding* 18(15):5157–5163. <https://doi.org/10.13271/j.mpb.018.005157>
- Xuan DTK, Nguyen QT, Khang NHM, Mai HNX, Trung DDM, Chau NNB, Thao NP, Quoc NB (2022) Molecular characterization of coconut (*Cocos nucifera* L.) varieties in Vietnam using sequence-related amplified polymorphism (SRAP) markers. *Biologia* 77:3087–3097. <https://doi.org/10.1007/s11756-022-01136-1>
- Yan XW, Wei YJ, Tang F, Wang J, Liang D (2025) Genetic diversity analysis of grape germplasm resources based on SRAP markers. *Northern Horticulture* 21:8–15. <https://doi.org/10.11937/bfy.20251465>
- Zhang AM, Lu HD, Xue JP, Tao XK, Xue T, Sheng W, Zhu YF (2012) Study on optimization of SRAP-PCR reaction system for *Pinellia ternata* in Suzhou. *China Journal of Chinese Materia Medica* 37(24):3815–3818. <https://api.semanticscholar.org/CorpusID:25998517>
- Zhang JQ, Zhang ZJ, Xu YQ, Zhu QQ, Zhang YL, Lin ZY, Wu SH, Lin QJ, Jiang L, Du LJ, Shi ZQ, Niu XQ, Li YY (2026) Genetic diversity of passion fruit germplasms analyzed by using start codon targeted (SCoT) markers. *Genetic Resources and Crop Evolution* 73:191. <https://doi.org/10.1007/s10722-026-02795-8>

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