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Integrative RAPD and SSR marker analysis reveals genetic diversity associated with fruit shape variation in pepper (*Capsicum* spp.)

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Abstract. *Zakiyah 'AS, Siswanto D, Utami DW, Arumingtyas EL. 2026. Integrative RAPD and SSR marker analysis reveals genetic diversity associated with fruit shape variation in pepper* (Capsicum *spp.*). *Biodiversitas 27 (4): d270422. https://doi.org/10.13057/biodiv/d270422*. The shape of pepper (*Capsicum* spp.) fruit significantly influences consumer preferences, which, in turn, affects its commercialization and economic value. In nature, peppers exhibit a wide range of fruit shapes that may be caused by genetic or environmental factors. Information confirming the causal factors underlying this variation, particularly at the molecular level, remains limited. Molecular markers are essential tools for assessing genetic diversity within germplasm. This research aims to identify the genetic profiles of pepper with various fruit shapes using RAPD and SSR markers. The findings will be beneficial for future studies concerning the genetic mechanisms underlying fruit shape in peppers. The study examined ten *Capsicum* spp. genotypes characterized by elongated, triangular, and blocky fruit shapes. A total of nineteen RAPD and seventeen SSR markers were employed for analysis. The data were processed using the UPGMA method based on Jaccard's similarity coefficient for RAPD, and Euclidean similarity coefficient for SSR. The results from both RAPD and SSR markers indicated polymorphism in alleles. Specifically, OPU-10 and Chr12SSR10 show higher polymorphism, as indicated by PIC values. The dendrogram generated from both markers demonstrated that the clustering was closely related to fruit morphology, particularly in distinguishing elongated, triangular, and blocky fruit shapes. The RAPD and SSR markers are reliable for identifying genetic diversity in *Capsicum* spp. Specifically, the OPU-10 and Chr12SSR10 markers may have potential for future studies on genetic diversity related to fruit morphology.

Keywords: *Capsicum* spp., fruit shape, genetic diversity, RAPD, SSR

INTRODUCTION

Capsicum spp., commonly known as chili or pepper, is an important crop with major roles in agricultural production, the food industry, human diets, and cultural practices worldwide (IPGRI 1995; Wang et al. 2024). In addition to its pungency, color, and flavor, *Capsicum* is valued for its health-related compounds, including vitamins, antioxidants, and capsaicinoids, which contribute to its nutritional and economic importance (Wijaya et al. 2020; Agarwal et al. 2021; Dhamodharan et al. 2023).

The diversity of *Capsicum* spp. is clearly reflected in fruit morphology, including variation in size, shape, color, texture, and pungency. Fruit shapes range from elongated and conical to rounded, bell-shaped, and blocky forms, and these differences are strongly influenced by genetic factors (IPGRI 1995; Wang et al. 2024). According to the descriptors of the International Plant Genetic Resources Institute, at least six fruit-shape categories can be recognized in *Capsicum* spp., namely elongated, triangular, rounded, bell-shaped, cuboid or blocky, and intermediate forms (IPGRI 1995). This broad variation reflects underlying genetic diversity, adaptation to different growing conditions, and responses to consumer preferences and market demands (Andrade et al. 2020; Liu et al. 2023; Zulkarnain et al. 2023; Wang et al. 2024).

The wide phenotypic diversity of *Capsicum* indicates a rich reservoir of genomic variation shaped by natural processes and breeding activities (Andrade et al. 2020; Liu et al. 2022). However, fruit morphology is a complex quantitative trait controlled by multiple genes and their interaction with environmental factors (Sharmin et al. 2018). Therefore, reliable molecular markers are needed to assess genomic diversity and to identify variation associated with desirable fruit-shape characteristics.

DNA-based molecular markers have become effective tools for detecting genomic variation (Amiteye 2021). Among them, Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) markers are widely used because they can reveal polymorphisms across the genome and generate informative multilocus profiles (Dwinianti et al. 2019; Pahlevi et al. 2021; Šutković et al. 2022; Satyawan et al. 2024). RAPD is a dominant marker system that uses arbitrary primers to amplify anonymous genomic regions and can detect polymorphism rapidly and cost-effectively (Amiteye 2021; Pahlevi et al. 2021). In contrast, SSR markers are co-dominant, multiallelic, and highly polymorphic, making them particularly useful for genetic diversity studies, population genetics, and linkage

analysis (Rahmattullah et al. 2020; Terryana et al. 2020; Zhong et al. 2021; Adeyemo et al. 2023).

Previous studies on *Capsicum* have commonly applied a single molecular marker system for purposes such as species identification in *Capsicum* from different regions (Viáfara-Vega et al. 2025), genomic characterization of *Capsicum* mutants (Wartono et al. 2019), and analysis of genomic variation in cayenne peppers with potential flood resistance (Pahlevi et al. 2021). Only one study specifically reported RAPD markers associated with elongated fruit shape in *Capsicum annuum* L. (Cvikic et al. 2009). Overall, earlier work has mainly addressed diversity at the species or population level, rather than examining the relationship between genomic variation and fruit morphology. This gap supports the need to use both dominant and co-dominant markers to identify genomic variation associated with fruit shape.

This study aims to quantify the genetic similarity associated with various fruit morphologies among 10 *Capsicum* genotypes, and to compare the ability of RAPD and SSR markers in capturing the fruit shape diversity. Furthermore, this research seeks to employ both dominant (RAPD) and co-dominant (SSR) markers in combination to illuminate relationships among varieties and identify genomic variation associated with desirable fruit morphology. The analysis focuses on genetic diversity among *Capsicum* spp. genotypes differing in fruit shape, without assessing environmental effects or population-level variation. The results are expected to identify effective molecular markers for distinguishing pepper fruit shapes, provide useful genomic information for breeding programs.

MATERIALS AND METHODS

Sample collection

The plant material comprises 10 chili pepper varieties with different fruit shapes. The chili fruits are commercial cultivars with the local names Bejo, Katokon, Pilar, Sios Tavi, Elegance, Lestari, Kamilia, Bird Eye Chili, Cakra Hijau, and chili pepper mutant line G1M16. The samples were obtained from several farms located in Malang, Batu City, Tulungagung, and Pasuruan, East Java, Indonesia (Figure 1, Table 1). All samples were collected from December 2024 to January 2025, with a single sample per site and no replication, as this study focused on identifying genetic relationships among cultivars with distinct fruit shapes rather than on genetic variation within populations. The observed chili samples exhibit different fruit shapes: blocky, elongated, and triangular. The samples' fruit shapes are categorized according to the fruit shape classification in the *Capsicum* spp. descriptor (IPGRI 1995).

Table 1. List of *Capsicum* samples used in this study

Local name	Species	Fruit shape	Origin	GPS coordinate
Bejo	*Capsicum annuum* L.	Blocky	Pasuruan	7°53'10.8"S 112°47'44.9"E
Katokon	*Capsicum annuum* L.	Blocky	Malang	7°56'18.4"S 112°34'51.7"E
Pillar	*Capsicum annuum* L.	Elongated	Malang	7°53'50.8"S 112°41'36.6"E
Elegance	*Capsicum annuum* L.	Elongated	Malang	7°53'50.8"S 112°41'36.6"E
Sios Tavi	*Capsicum annuum* L.	Elongated	Tulungagung	8°09'35.6"S 111°58'24.3"E
Lestari	*Capsicum frutescens* L.	Long slim-Triangular	Tulungagung	8°09'36.7"S 111°58'24.7"E
Kamilia	*Capsicum frutescens* L.	Long slim-Triangular	Batu	7°54'07.7"S 112°31'13.7"E
Cakra Hijau	*Capsicum frutescens* L.	Short-slim Triangular	Malang	7°56'18.4"S 112°34'51.7"E
G1M16	*Capsicum frutescens* L.	Triangular	Malang	7°56'18.4"S 112°34'51.7"E
Bird Eye Chili	*Capsicum frutescens* L.	Small-Triangular	Malang	7°56'18.4"S 112°34'51.7"E

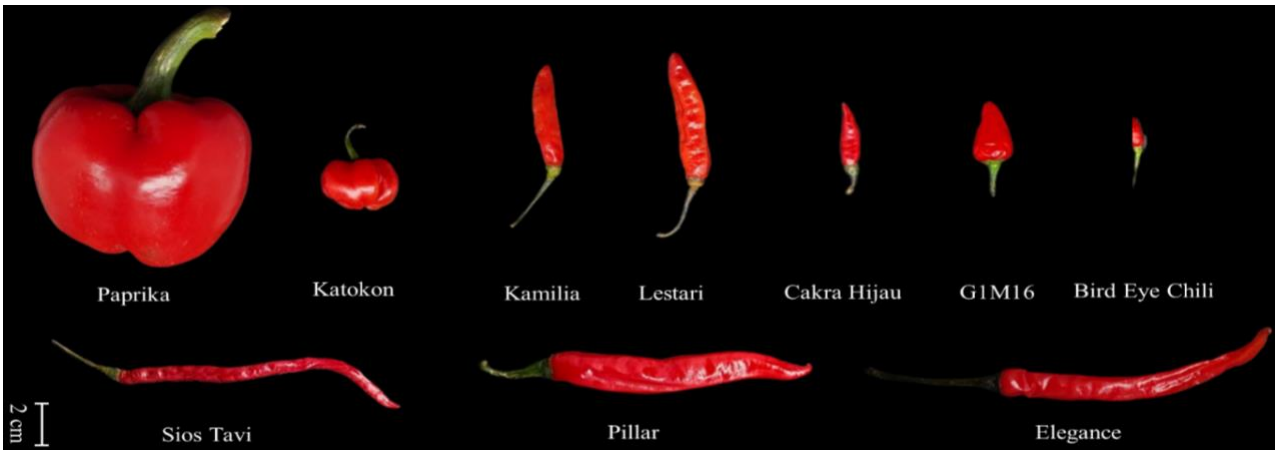

Figure 1. Variation in the fruit shape of pepper obtained from different planting location. Bejo and Katokon (Blocky), Sios Tavi, Pillar, and Elegance (elongated), Kamilia, Lestari, Cakra Hijau, G1M16, and Bird Eye Chili (triangular)

DNA extraction

Genomic DNA was isolated from 500 mg of young leaves from each of the observed chili plants per genotype. DNA isolation was performed using the CTAB method with some modifications (Doyle and Doyle 1990). The leaves were pulverized with liquid nitrogen, then 700 µL of extraction buffer (2% CTAB; 0.1 M Tris-Cl; 0.2 M EDTA; 1.4 M NaCl; 2% PVP; and 2% β-mercaptoethanol) was added. The samples were then homogenized using a vortex and incubated at 65°C for 1 hour. Next, the homogenate was centrifuged for 10 min at 13000 rpm. The supernatant was transferred to a new tube, and a chloroform-isoamyl alcohol (24:1) solution was added. The homogenate was mixed with a vortex, then centrifuged for 5 min at 10000 rpm. The supernatant was transferred to a new tube and added with cold isopropanol (0.6 v/v). The homogenate was then incubated at -20°C for one hour. The incubated homogenate was centrifuged at 10000 rpm and 4°C for 10 min, and the supernatant was discarded. The pellet was added with 500 µL of cold 70% ethanol. DNA was centrifuged again, and the DNA pellet obtained was dried at room temperature. The DNA pellet was dissolved in 50 µL of Tris-EDTA buffer, pH 7.6. The genomic DNA isolate obtained can be stored at -20°C.

Quantification of genomic DNA isolation was performed using a NanoPhotometer N60 (IMPLEN) at wavelengths of A260 nm and A280 nm. The absorbance value at the A260/A280 wavelength ratio indicates the purity of the isolated DNA. DNA is considered pure if the ratio value (A260/A280) ranges between 1.8 and 2.0 (Aboul-Maaty and Oraby 2019). A qualitative analysis of genomic DNA isolation results was performed using 1% gel agarose electrophoresis with RedSafe nucleic acid staining solution (iNtRON). Electrophoresis was performed at 50 volts. The size of the genomic DNA band was determined using a 1 Kb DNA ladder with Sizer™-1000 Plus DNA Marker Solution (iNtRON). DNA concentrations >50 ng/µL, with purity ranging from 1.8 to 2.0, were used for downstream analysis.

RAPD and SSR amplification

Amplification of RAPD and SSR molecular markers was performed by Polymerase Chain Reaction (PCR) using 19 RAPD primers and 17 SSR primer pairs. Each primer-genotype combination was run once. The PCR reaction mixture used for the RAPD marker amplification process was composed of 50 ng genomic DNA, 10 µL MyTaq HS Redmix (Meridian Bioscience, No Cat: BIO-25048), 1 µL RAPD primer, and 3 µL Nuclease Free Water (NFW) with a total final reaction of 20 µL. The PCR reactions were run in a TaKaRa PCR Thermal Cycler machine (TP650) with a program consisting of 1 cycle of initial denaturation at 95°C for 1 min. Then 45 cycles of denaturation at 94°C for 1 min, annealing at 33-41°C (Table 2), extension at 72°C for 1 min, final extension at 72°C for 5 min. PCR amplification results were visualized by electrophoresis using a 1.5% agarose gel stained with RedSafe Nucleic Acid Staining Solution (iNtRON). Visualization of electrophoresis results was performed using a UV transilluminator.

The amplification process of SSR molecular markers was carried out using the PCR method with a total PCR reaction volume of 10 µL, consisting of 5 µL MyTaq HS Redmix (Meridian Bioscience), 0.5 µL forward and 0.5 µL reverse SSR primers (Table 3), 1 µL genomic DNA with a DNA concentration of 50 ng, and 3 µL Nuclease Free Water (NFW). The amplification steps were carried out for 32 cycles, with 1 cycle for the pre-denaturation stage (94°C for 1 min). The next stage was carried out as many as 32 cycles, starting with the denaturation stage at 94°C for 1 min, the annealing stage at a specific temperature based on the type of primer (Table 3) for 1 min, the extension stage at 72°C for 1 min, and the final extension at 72°C for 5 min. Amplification results were visualized by electrophoresis using a 1.5% agarose gel.

Genetic diversity analysis

Visualization data from amplification of RAPD and SSR molecular markers was carried out in the form of a dendrogram based on Jaccard's coefficient similarity with the Unweighted Pair Group Method with Arithmetic Means (UPGMA) for RAPD (Pahlevi et al. 2021), and Euclidean coefficient similarity with the UPGMA method to show similarity between all the samples (Mas'udah et al. 2019). The Effective Multiplex Ratio (EMR) and Marker Index (MI) are used to evaluate the effectiveness of RAPD markers (Wahyudi et al. 2020). The band presence was scored as '1' and band absence was scored as '0' (Zhong et al. 2021). Only clear and unambiguous RAPD and SSR bands were used in the scoring. All analyses were conducted using PAST 4.09 (Paelontological Statistical Software version 4.09) software. The Polymorphic Information Content (PIC) for each primer was calculated using the formula from Roland-Ruiz et al. (2000) for dominant markers and from Botstein (1980) for co-dominant markers (Serrote et al. 2020).

Table 2. List of RAPD primer

Primer	Sequence (5'-3')	Annealing temp. (°C)	Source
OPW-04	CAG AAG CGG A	35	Zakiyah et al. (2023)
OPD-13	GGG GTG ACG A	38	Arumingtyas et al. (2020)
OPB-11	GTA GAC CCG T	33	Zakiyah et al. (2023)
OPL-05	ACG CAG GCA C	41	Zakiyah et al. (2023)
OPB-03	CAT CCC CCT G	35	Zakiyah et al. (2023)
OPB-04	GCA CTG GAG T	33	Zakiyah et al. (2023)
OPA-17	GAC CGC TTG T	35	Zakiyah et al. (2023)
OPU-10	ACC TCG GCA C	40	Arumingtyas et al. (2020)
OPD-19	CTG GGG AGT G	33	Zakiyah et al. (2023)
OPU-19	GTC AGT GCG G	38	Zakiyah et al. (2023)
OPW-03	GTC CGG AGT G	36	Zakiyah et al. (2023)
OPA-11	CAA TCG CCG T	36	Pahlevi et al. (2021)
OPA-01	CAG GCC CTT C	37	Arumingtyas et al. (2017)
OPA-02	TGC CGA GCT G	40	Arumingtyas et al. (2020)
OPB-06	TGC CGA GCC C	40	Zakiyah et al. (2023)
OPF-09	CCA AGC TTC C	33	Zakiyah et al. (2023)
OPF-11	TTG GTA CCC C	33	Zakiyah et al. (2023)
OPF-14	TGC TGC AGG T	38	Zakiyah et al. (2023)
OPF-18	TTC CCG GGT T	37	Zakiyah et al. (2023)

Table 3. List of SSR primer

Primer	Sequence	Annealing temperature (°C)	Source
SSR AVRDC PP-18	F: 5'-GCTAGGCTTGATCCTTCACC-3' R: 5'-CGCTTGAAATCATGCTCACT-3'	50	Terefe et al. (2022)
SSR EPMS376	F: 5'-ACCCACCTTCATCAACAACC-3' R: 5'-ATTTGTGGCTTTTCGAAACG-3'	54.4	Terefe et al. (2022)
SSR AVRDC PP-67	F: 5'-TATTCCTTCTTCACCCCTCC-3' R: 5'-GAAAGAGGCGCTAACTGGAC-3'	55	Terefe et al. (2022)
Chr7SSR15	F: 5'-GCCTGGCATGTTTTGTATTTCTA-3' R: 5'-TTTGGTGACCGACAATATAAAGG -3'	55	Zhong et al. (2021)
Chr3SSR6	F: 5'-CTCTCTAGAATGAAAAGTGCCGA-3' R: 5'-TTGTCGATTTGTTCTTCTTCCAT-3'	50	Zhong et al. (2021)
Chr4SSR20	F: 5'-AAAACAACACGACACACAGCTTA-3' R: 5'-TATATTTTTCTTGGGAACGAGCA-3'	55	Zhong et al. (2021)
Chr1SSR18	F: 5'-TTAGTGTTGTCAAAATACCCGTG-3' R: 5'-CAATAAACATATCACACGTGCAAC-3'	55	Zhong et al. (2021)
Chr9SSR16	F: 5'-CCCCACCGATGAATTTAGTAGA-3' R: 5'-TGATGATGTGTCATGGTGTATGA-3'	55	Zhong et al. (2021)
Chr11SSR16	F: 5'-ACGAGTGAACACGTACATGAAAA-3' R: 5'-GGTAGGAGCTAGGAGTAGTGACG-3'	54.4	Zhong et al. (2021)
Chr12SSR10	F: 5'-AAAAGGGACTTGTTTTCCTATCA-3' R: 5'-AAATGGGAATGCGATTATCTAAA-3'	54.4	Zhong et al. (2021)
Ca 19	F: 5'-CCGCAATGGCAGTATGATCT-3' R: 5'-CGGCTCTATCTACAACGGTG-3'	55.1	Rahmattullah et al. (2020)
Ca 26	F: 5'-CGCATATAGGCAGATCAAAT-3' R: 5'-TGACTCAAATGCTCTCTGAA-3'	50.6	Rahmattullah et al. (2020)
Ca 27	F: 5'-GCAGAGGACCAGTTAGCATA-3' R: 5'-TGTTCTGAGTCCACGATGCT-3'	52	Juliandari et al. (2019)
Ca 52	F: 5'-TAGCAGAGGACCAGTTAGCA-3' R: 5'-ATGTTCTGAGTCCACGATGC-3'	55.1	Rahmattullah et al. (2020)
Ca 56	F: 5'-CTTCGCATATGGCAGATCA-3' R: 5'-TCTCTGTGGCTGACTCAAAT-3'	52.7	Rahmattullah et al. (2020)
Ca 62	F: 5'-CGCATATAGGCAGATCAAAT-3' R: 5'-GGTCAGACTACGACTCTCTCA-3'	51	Juliandari et al. (2019)
Ca 96	F: 5'-CGCATATAGGCAGATCAAAT-3' R: 5'-AATCTCTGTGGCTGACTCAA-3'	51.8	Rahmattullah et al. (2020)

Effective Multiplex Ratio (EMR)

$$EMR = \eta \times \beta$$

Where, η: Total number of fragment from each primer. β: The fraction of polymorphic fragment.

Marker Index (MI)

$$MI = PIC \times EMR$$

Where, PIC: Polymorphic Information Content, EMR: Effective Multiplex Ratio.

PIC for dominant marker

$$PIC = 1 - (p^2 + q^2)$$

Where, *p*: The band frequency, *q*: No band frequency.

PIC for co-dominant marker

$$PIC_j = 1 - \sum_{i=1}^{n} P_i^2$$

Where, *i*: *i*-th allele of the *j*-th marker, *n*: The number of the *j*-th marker's alleles, *P*: The allele frequency.

RESULTS AND DISCUSSION

RAPD molecular marker profile

The genetic diversity among *Capsicum* spp. exhibiting distinct fruit shapes was evaluated using RAPD molecular markers. A total of 19 RAPD primers were tested. All the primers except the OPF-11 primer generated between 8-14 bands for each genotype, resulting in 185 scorable bands (Table 4). Primer OPD-13 produced the highest number of amplified fragments (Figure 2), whereas primers OPB-03, OPB-04, OPA-02, OPF-09, and OPF-18 produced the lowest number of bands, with eight bands from each primer. The percentage of polymorphic bands ranges from 0% to 100%, with primers OPU-10 and OPF-18 showing the highest polymorphism (100%), while OPF-11 produced no amplification band. The primer that did not produce an amplification band was excluded from downstream analysis.

To evaluate the efficiency of RAPD markers in To evaluate the efficiency of RAPD markers in detecting genetic variation, the PIC, EMR, and MI were calculated. Among the 19 primers analyzed, OPU-10 showed the highest PIC value, indicating its strong discriminatory capacity for detecting genetic polymorphisms. EMR values ranged from 0 to 144, with OPU-10 presenting the highest value and OPF-11 the lowest. Similarly, MI values ranged from 0 to 50.4, with OPU-10 again exhibiting the highest index. In contrast, OPF-14 showed the lowest MI values (Table 4), suggesting limited effectiveness in detecting polymorphic loci.

Clustering analysis based on RAPD molecular marker

The genetic similarity among the ten *Capsicum* spp. with distinct fruit shapes ranged from 0.463 to 0.926 (Table 5). The lowest genetic similarity was observed between Katokon and the Elegance genotype, which exhibits markedly different fruit shapes—Katokon has a blocky fruit shape, whereas the Elegance has an elongated fruit shape. In contrast, the highest Similarity Index was observed between the Pillar and Elegance genotypes, and between the Cakra Hijau and its mutant line, G1M16. The high similarity between Pillar and Elegance was consistent with the fruit shape, namely, an elongated, large-fruited chili. Likewise, the close genetic relationship between Cakra Hijau and G1M16 is expected, as G1M16 was derived from Cakra Hijau through mutagenesis.

The clustering analysis, based on Jaccard similarity coefficients constructed from RAPD marker data, further elucidated the genetic profiles among the genotypes (Figure 3). The resulting dendrogram grouped the ten genotypes into two major clusters. Group 1 consists of Pillar, Elegance, Sios Tavi, Cakra Hijau, G1M16, and Bejo, while group 2 comprises Katokon, Lestari, Kamilia, and Bird Eye Chili pepper genotypes. Within Group 1, the Bejo was identified as an outgroup (Subgroup 1B), clearly separated from the other genotypes. Pillar, Elegance, and Sios Tavi formed a distinct subcluster characterized by elongated fruit morphology, indicating that clustering corresponded well with phenotypic fruit shape. Meanwhile, the Cakra Hijau genotype and its mutant (G1M16) clustered separately from the elongated fruit shape and Bejo genotypes.

In group 2, two subclusters were identified—subcluster 2A consisted of the Katokon genotype only, which formed an independent clade associated with its blocky fruit morphology. Meanwhile, subcluster 2B consisted of the Lestari, Kamilia, and Bird Eye Chili pepper genotypes, all of which are characterized by triangular fruit shapes. These results collectively suggest that the clustering pattern derived from RAPD markers reflects, to a considerable extent, the morphological diversity observed among *Capsicum* spp. genotypes.

Table 4. The Polymorphic Information Content (PIC), Effective Multiplex Ratio (EMR) and Marker Index (MI) of RAPD molecular markers

Primer	nB	nPB	PB%	PIC	EMR	MI
OPW-04	13	10	76.9	0.23	130	29.9
OPD-13	14	9	64.2	0.28	126	35.28
OPB-11	9	6	66.6	0.25	54	13.5
OPL-05	13	10	76.9	0.26	130	33.8
OPB-03	8	5	62.5	0.19	40	7.6
OPB-04	8	5	62.5	0.23	40	9.2
OPA-17	11	7	63.6	0.27	77	20.79
OPU-10	12	12	100	0.35	144	50.4
OPD-19	14	9	64.2	0.28	126	35.28
OPU-19	11	8	72.7	0.24	88	21.12
OPW-03	10	8	80	0.28	80	22.4
OPA-11	10	8	80	0.28	80	22.4
OPA-01	9	8	88.8	0.21	72	15.12
OPA-02	8	7	87.5	0.31	56	17.36
OPB-06	10	3	30	0.11	30	3.3
OPF-09	8	5	62.5	0.21	40	8.4
OPF-11	0	0	0	0.00	0	0
OPF-14	9	2	22.2	0.09	18	1.62
OPF-18	8	8	100	0.33	64	21.12
Total	185	130				
Mean			65	0.23	73.42	19.40

Note: nB: Number of Band, nPB: Number of Polymorphic Band, PB%: Percent of Polymorphic Band, PIC: Polymorphic Information Content, EMR : Effective Multiplex Ratio, MI: Marker Index

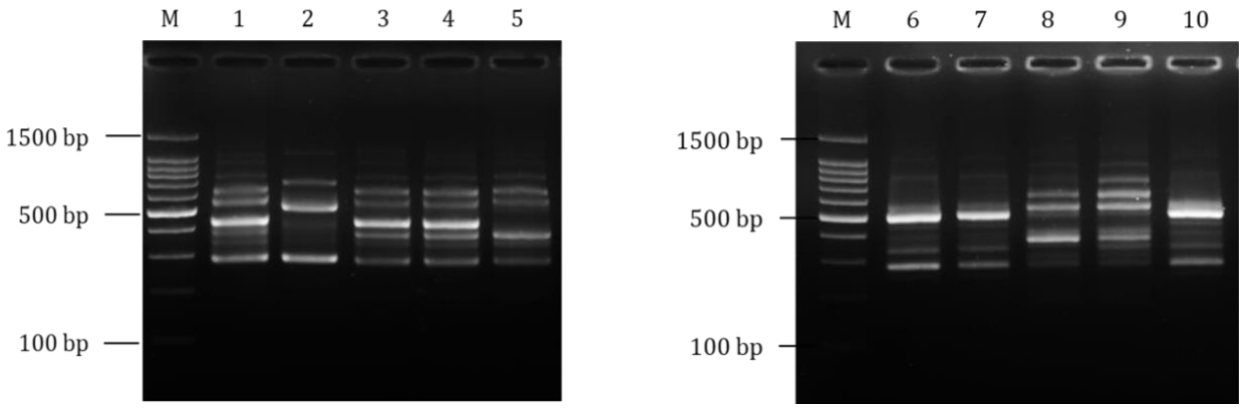

Figure 2. Amplification results from 10 pepper genotypes generated by OPU-10 primer. M: DNA ladder 100 bp, 1-10: samples

Table 5. Genetic similarity matrix from 10 chili pepper varieties using RAPD marker

Accessions	Bejo	Katokon	Pillar	Elegance	Sios Tavi	Lestari	Kamilia	Cakra Hijau	G1M16	Bird Eye Chili
Bejo	1.000									
Katokon	0.481	1.000								
Pillar	0.803	0.478	1.000							
Elegance	0.800	**0.463**	**0.926***	1.000						
Sios Tavi	0.773	0.472	0.850	0.890	1.000					
Lestari	0.529	0.580	0.536	0.538	0.529	1.000				
Kamilia	0.543	0.563	0.570	0.562	0.542	0.882	1.000			
Cakra Hijau	0.763	0.519	0.813	0.809	0.809	0.549	0.563	1.000		
G1M16	0.769	0.494	0.777	0.774	0.802	0.553	0.567	**0.926***	1.000	
Bird Eye Chili	0.576	0.552	0.560	0.541	0.552	0.744	0.710	0.574	0.579	1.000

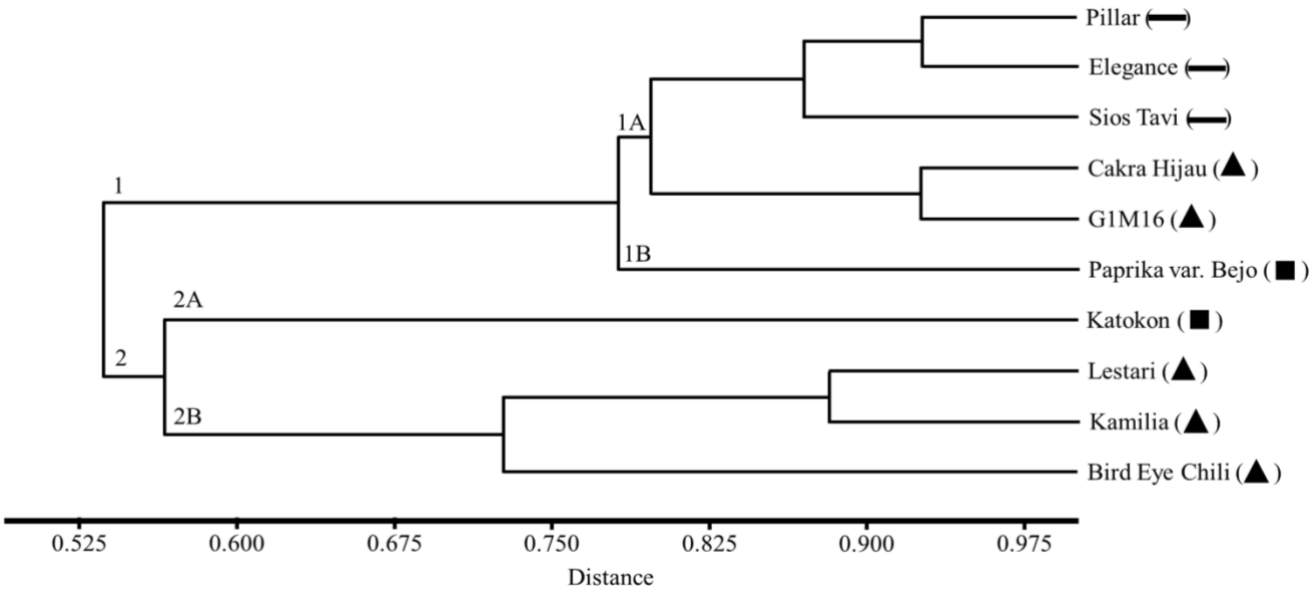

Figure 3. Dendrogram based on RAPD molecular marker from 10 *Capsicum* spp. genotypes constructed using UPGMA and Jaccard similarity coefficient. Note. Fruit shape annotation: Elongated (▬), Blocky (■), and Triangular (▲)

SSR marker polymorphism analysis

Genetic variation among the ten *Capsicum* genotypes exhibiting distinct fruit morphologies was analyzed using 17 Simple Sequence Repeat (SSR) markers. The SSR primers produced clear and polymorphic amplification profiles (Figure 4). All SSR primers amplified consistently; 13 of 17 loci were polymorphic, while four were monomorphic across the 10 genotypes. A total of 79 alleles were found across all genotypes, with the size ranging from 150 to 1700 bp (Table 6). The number of alleles per locus (Na) varied from 1 to 10. The expected heterozygosity (He) ranged between 0 and 0.89, with an average of 0.45, while the observed heterozygosity (Ho) ranged from 0 to 1, with a mean score of 0.64. The Minor Allele Frequency (MAF) values were between 0.03 and 1.0. The Polymorphism Information Content (PIC) values ranged from 0 (EPMS376, Chr3SSR6, Chr1SSR18, and Chr9SSR16) to 0.42 (Chr12SSR10), with an average PIC of 0.23 (Table 3).

Genetic similarity and phylogenetic analysis based on SSR markers

The genetic similarity among the ten *Capsicum* genotypes was determined using the Euclidean similarity coefficient. The genetic similarity values ranged from 1.414 to 5.831 (Table 7), with the highest value observed between the Bejo and Katokon genotypes (5.831), suggesting a high degree of genetic diversity. On the other hand, the lowest genetic similarity (1.414) was found between Pillar and Elegance, and between the Lestari and Kamilia genotypes, indicating a close genetic relationship.

Phylogenetic analysis based on SSR marker data revealed two major clusters among the ten *Capsicum* genotypes (Figure 5). The first cluster consisted of nine pepper varieties with elongated, triangular, and blocky fruit shapes, while the second cluster comprised solely the Katokon genotypes, which appear as an outgroup. Within the first cluster, two subgroups were identified: subgroup 1A, containing six genotypes with elongated (Pillar, Elegance, and Sios Tavi), blocky (Bejo), and triangular fruit shapes (Cakra Hijau, G1M16), whereas subgroup IB

consisted only of triangular fruit shape genotypes (Lestari, Kamilia, Bird Eye Chili). In general, genotypes with similar fruit shapes tended to cluster together, except for Bejo and Katokon, which, despite both possessing blocky fruit morphology, were separated into distinct groups, suggesting distinct genetic profiles.

Table 6. The SSR genetic character for 10 *Capsicum* spp. genotypes

Primer	Allele size range (bp)	Na	Heterozigosity		MAF	PIC
			He	Ho		
SSR AVRDC PP-18	100-1700	10	0.89	0.8	0.08	0.17
SSR EPMS376	250	1	0	0	1	0
SSR AVRDC PP-67	200-300	5	0.68	1	0.09	0.32
Chr7SSR15	130-160	5	0.72	1	0.1	0.36
Chr3SSR6	150	1	0	0	1	0
Chr4SSR20	150-1100	8	0.70	1	0.05	0.23
Chr1SSR18	100	1	0	0	1	0
Chr9SSR16	150	1	0	0	1	0
Chr11SSR16	150	2	0	0.1	1	0.18
Chr12SSR10	150	2	0	0.7	1	0.42
Ca 19	100	2	0	0.2	1	0.32
Ca 26	100-1500	5	0.68	1	0.04	0.32
Ca 27	50-1300	9	0.82	1	0.03	0.31
Ca 52	50-1500	7	0.78	1	0.03	0.29
Ca 56	100-1500	7	0.78	1	0.03	0.22
Ca 62	50-1600	5	0.71	1	0.1	0.41
Ca 96	100-1500	8	0.81	1	0.03	0.34
Total		79				
Mean			0.45	0.64	0.45	0.23

Note: Na: Number of alleles, He: Gene diversity, Ho: Observed heterozygosity, MAF: Minor Allele Frequency, PIC: Polymorphic Information Content

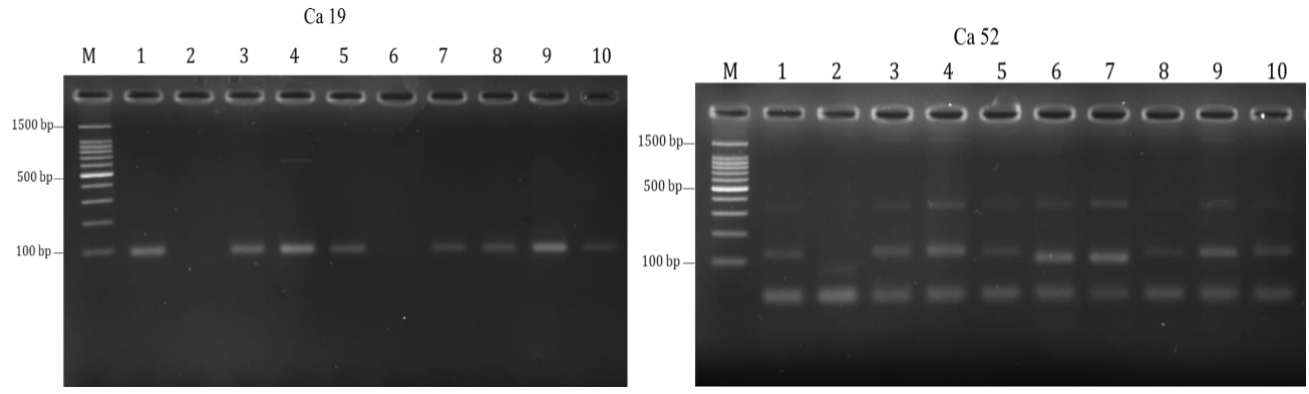

Figure 4. Representative SSR amplification results for 10 *Capsicum* spp. genotypes. M: ladder 100 bp, 1-10 : *Capsicum* spp. samples

Table 7. Genetic similarity matrix from 10 chili pepper varieties using SSR marker

Accessions	Bejo	Katokon	Pillar	Elegance	Sios Tavi	Lestari	Kamilia	Cakra Hijau	G1M16	Bird Eye Chili
Bejo	0.000									
Katokon	**5.831**	0.000								
Pillar	3.162	5.657	0.000							
Elegance	3.162	5.657	**1.414***	0.000						
Sios Tavi	3.317	5.568	2.236	1.732	0.000					
Lestari	5.196	5.568	5.385	5.385	5.292	0.000				
Kamilia	5.000	5.568	5.196	5.196	5.099	**1.414***	0.000			
Cakra Hijau	4.243	4.472	3.742	3.742	3.606	5.000	4.796	0.000		
G1M16	3.873	4.583	3.317	3.317	3.464	5.099	4.899	1.732	0.000	
Bird Eye Chili	5.196	5.196	5.000	5.000	4.899	3.742	3.464	4.123	4.472	0.000

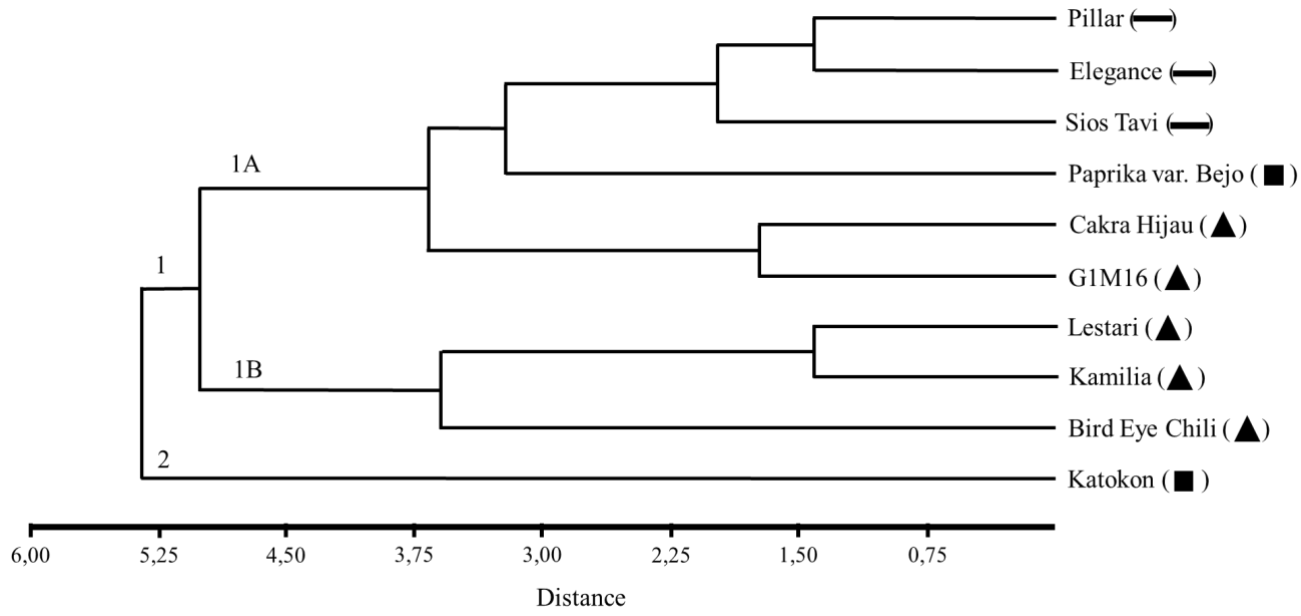

Figure 5. Clustering of the 10 *Capsicum* spp. generated by SSR molecular marker using UPGMA method with Euclidean similarity coefficient. Fruit shape annotations: Elongated (▬), Blocky (■), and Triangular (▲)

Discussion

The shape of the *Capsicum* fruit is one of the important factors in consumer preferences and the utility of peppers in the community. This study's results demonstrate the ability of RAPD and SSR molecular markers to assess genetic diversity among the 10 *Capsicum* spp. genotypes with various fruit shapes. All observed samples exhibited varying fruit shapes: blocky, elongated, and triangular. The effectiveness of RAPD and SSR primers in identifying genetic variations associated with fruit shape was also assessed using several parameters.

In this study, the effectiveness of RAPD primers in detecting genetic variation among the analyzed *Capsicum* genotypes was evaluated using three parameters: PIC, EMR and MI. The PIC values for dominant markers are classified as low (0-0.10), moderate (0.10-0.25), high (0.30-0.40), and very high (0.40-0.50) (Serrote et al. 2020). We found the PIC value varied among primers, ranging from 0.00 to 0.35, indicating that the primers exhibited low to high informativeness in distinguishing genetic variability within the tested samples. A low PIC value indicates a locus that tends to be monomorphic, as evidenced by a small number of amplified bands. This is demonstrated by the OPF-14 marker, which produced 9 bands; only 2 were polymorphic. The OPF-11 produces no amplified bands, suggesting it cannot detect sample polymorphisms. Therefore, it is not recommended for use in determining genetic diversity associated with fruit shape. Conversely, the OPU-10 primer produces the highest PIC value (0.35). OPU-10 produces 12 identifiable polymorphic bands, indicating that the OPU-10 primer effectively identifies gene polymorphisms in the sample. Molecular markers with a PIC value greater than 0.5 are classified as highly informative and are particularly valuable in genotyping studies (Jiang et al. 2010; Chesnokov and Artemyevaa 2015; Amiteye 2021). Furthermore, variations in EMR and MI values were evident in RAPD markers, reflecting the degree of polymorphism and the efficiency of each primer in amplifying diverse loci across genotypes. OPU-10 exhibited the highest EMR, MI, and PIC values, highlighting its superior discriminatory capacity. These results demonstrate that PIC, EMR, and MI are interrelated parameters that, together, provide a robust metric for assessing the effectiveness of RAPD markers in genetic diversity analysis, mapping, and phylogenetic studies (Powell et al. 1996; Elgohary et al. 2025).

For SSR molecular marker analysis, in addition to the PIC parameter, the number of alleles, gene diversity/expected heterozygosity (He), observed heterozygosity (Ho), and Minor Allele Frequency (MAF) were calculated. Of the 17 SSR primers used in this study, 13 showed high levels of polymorphism based on those parameters. The SSR primers AVRDC PP-18, Ca 27, Chr4SSR20, Ca 96, Ca 52, and Ca 56 produced a high number of alleles per locus (>5) compared to the number of alleles from the primers analyzed. In this study, the Chr4SSR20 primer produced 8 alleles per locus, whereas the study conducted by Zhong et al. (2021) yielded only 6 alleles (Zhong et al. 2021). The differences in the number of alleles are due to differences in the genetic backgrounds of the samples used (Adeyemo et al. 2023). Otherwise, the observed variation in the number of alleles is also influenced by the type and number of SSR primers employed (Zhong et al. 2021; Terefe et al. 2022).

Significant variations across all markers, revealing the differences in allele number and distribution among samples. Additional analyses, including He, Ho and MAF, are beneficial for SSR marker analysis. The low Ho values found in this study indicate that the observed genotypes are

homozygous, as found by Terefe et al. (2022), which showed a direct correlation between low Ho values and the homozygous alleles in the observed accessions (Terefe et al. 2022). On the other hand, the high number of loci with heterozygous alleles indicates that these loci have the potential to be utilized in the process of breeding *Capsicum* spp. Meanwhile, MAF can significantly represent genetic diversity information from individuals (Adeyemo et al. 2023). The PIC values for SSR markers ranged from 0.00 to 0.42, with the highest PIC observed at the Chr12SSR10 primer and classified as moderately informative. The mean PIC (0.23) was lower than the average PIC reported in previous studies (Chhapekar et al. 2020; Adeyemo et al. 2023), indicating relatively low marker effectiveness in identifying genetic variation. Nevertheless, the SSR markers used produced clustering of the observed samples, which tended to group by fruit shape. Therefore, this study remains relevant for identifying markers associated with *Capsicum* fruit shape, despite a relatively small sample size. Moreover, the RAPD OPU-10 primer and the SSR Chr12SSR10 primer are potential primers for revealing genetic diversity associated with fruit shape across several parameters.

The genetic distance among the 10 *Capsicum* genotypes was evaluated using Jaccard's similarity coefficient, providing insight into the extent of genetic divergence and its relationship with phenotypic traits. A lower similarity coefficient indicates greater genetic differentiation, which generally enhances the potential for successful hybridization and the emergence of new phenotypic combinations. Clustering analysis using the UPGMA method based on Jaccard similarity coefficients further revealed consistent patterns of genetic grouping. There was a tendency for samples with elongated fruit shapes to group in both RAPD and SSR marker clusters. Likewise, in triangular fruit shapes, most genotypes clustered together, especially in Cakra Hijau and its mutant genotype (G1M16), as well as in Lestari and Kamilia. Meanwhile, blocky fruit shapes (Bejo and Katokon) tended to be scattered. However, this pattern should be interpreted cautiously due to the limited sample size and the small number of fruit shape categories that were analyzed.

In this study, both RAPD and SSR analyses revealed the highest genetic similarity between the Pillar and Elegance genotypes, both characterized by large, elongated fruits. This strong similarity suggests a close genetic relationship consistent with their comparable fruit morphology. Conversely, the greatest genetic distance was recorded between Katokon and Elegance based on RAPD data, and between Katokon and Bejo in the SSR analysis. Katokon, a *C. annuum* genotype with blocky fruit morphology, showed substantial genetic divergence from other accessions, likely reflecting its distinct genetic profile related to fruit shape differentiation (Wijaya et al. 2020).

The observed alignment between RAPD and SSR-based clustering and fruit morphological traits supports the hypothesis that molecular diversity within *Capsicum* reflects, at least in part, phenotypic variation. Similar relationships between genetic diversity and fruit morphology have been documented in previous studies, where RAPD and other molecular markers effectively distinguished *Capsicum* species and cultivars based on domestication level, fruit shape, and size (Ince et al. 2010; Yumnam et al. 2012). Likewise, SSR markers have also been proven to effectively discriminate *Capsicum* accessions according to species origin and fruit morphological variation (Carvalho et al. 2017; Wartono et al. 2019; Zhong et al. 2021). The relatively high genetic diversity observed in the current study, as reflected by mean He and PIC values, indicates that the examined genotypes possess a broad genetic base suitable for breeding. The separate placement of Katokon as an outgroup may reflect unique allelic compositions or potential interspecific introgression, consistent with findings in local chili landraces (Nagy et al. 2007; Taranto et al. 2016). Collectively, these results confirm that both RAPD and SSR markers are robust tools for differentiating *Capsicum* genotypes and elucidating the genetic relationships underlying fruit morphological diversity. This underscores the reliability of RAPD and SSR markers for assessing genetic relationships and for guiding the selection of parental lines in breeding programs targeting desirable morphological traits.

In conclusion, this study shows that RAPD and SSR markers effectively reveal genetic diversity related to fruit shape variation in *Capsicum* spp. The clustering pattern obtained from the UPGMA analysis indicates a tendency for samples to cluster by fruit shape, distinguishing elongated, triangular, and blocky shapes. The RAPD marker OPU-10 and the SSR marker Chr12SSR10 were identified as highly informative markers. This indicates the potential of RAPD and SSR markers for germplasm selection and characterization, especially for fruit shape. The present findings highlight substantial genetic polymorphism among 10 *Capsicum* genotypes representing contrasting fruit shapes, providing valuable insights into the genetic architecture of fruit morphology and its implications for pepper breeding. Understanding genetic relationships among diverse genotypes facilitates selecting parental lines with greater genetic distance, thereby increasing heterosis and expanding the pool of genetic variation available for improvement. The limited sample size and number of molecular markers in this study necessitated a follow-up study. Future research should focus on developing novel molecular markers targeting loci associated with fruit shape diversity by integrating GWAS and QTL mapping. Elucidating the genomic regions underlying fruit morphology will enhance the potential for Marker-Assisted Selection (MAS) and contribute to the more effective utilization of *Capsicum* spp. germplasm in breeding programs to produce varieties that meet agronomic, market, and consumer demands.

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