



RESEARCH ARTICLE

# Evaluation of different passion fruit genotypes based on morphological, quantitative traits and molecular marker (ISSR)

Snekha S<sup>1</sup>, Geethalakshmi I<sup>1\*</sup>, Saraswathy S<sup>1</sup>, Malini N<sup>1</sup>, Sakthivel K<sup>2</sup> & Geethanjali S<sup>3</sup>

<sup>1</sup>Department of Fruit Science, Horticultural College and Research Institute for Women, Trichy 620 027, Tamil Nadu Agricultural College and Research Institute, Coimbatore, Tamil Nadu, India

<sup>2</sup>Department of Plant Breeding and Genetics, Anbil Dharmalingam Agricultural College and Research Institute, Trichy 620 027, Tamil Nadu Agricultural College and Research Institute, Coimbatore, Tamil Nadu, India

<sup>3</sup>Department of Crop Physiology and Biochemistry, Anbil Dharmalingam Agricultural College and Research Institute, Trichy 620 027, Tamil Nadu Agricultural College and Research Institute, Coimbatore, Tamil Nadu, India

\*Email: [geethalakshmi.i@tnau.ac.in](mailto:geethalakshmi.i@tnau.ac.in)



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## Abstract

Passion fruit (*Passiflora* spp.), an underutilized and unexplored fruit crop, holds significant potential for improving nutritional health in tropical and subtropical regions. This study aimed to characterize various genotypes of passion fruit including yellow (G1, G3, G5), purple (G2, G4, G10), sweet (G6, G7, G9) and giant (G8), collected from different regions in Tamil Nadu, India, based on morphological, yield and biochemical traits. A comprehensive evaluation of morphological, yield and quality traits, combined with advanced statistical analysis, including correlation studies and principal component analysis (PCA), identified genotypes with desirable traits, offering valuable resources for breeding programs to improve passion fruit cultivation. The analysis included biometric data, yield performance and quality traits, complemented by molecular diversity assessment using ISSR markers. Significant variation was observed across morphological and biochemical traits; genotype G8 showed the highest fruit weight and juice content, while genotype G7 had the highest total soluble solids and sugar content. Principal component analysis (PCA) and hierarchical clustering revealed distinct groupings, providing insights into their genetic diversity and potential for future crop improvement. These findings suggest that specific genotypes could be strategically targeted for breeding programs to enhance yield, fruit quality and resilience in passion fruit cultivation.

## Keywords

clustering; correlation; ISSR marker; passion fruit; Principal Compound Analysis (PCA)

## Introduction

Passion fruit (*Passiflora* spp.) is a perennial woody vine with axillary tendrils, first noted by Spanish missionaries in South America (1). The genus *Passiflora* comprises over 500 species within 18 genera (2). Early Christian missionaries to South America described the fruit and flowers of *Passiflora* as a strong, delicious and unusual perfume and flavour. They called it the "Symbol of the Passion of Christ" (3, 4). The majority of *Passiflora* species are found in tropical and subtropical regions, with the highest occurrence and diversity in South America (5). Two main types of passion fruit are widely cultivated: the yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener), suited to tropical conditions and the purple passion fruit (*Passiflora edulis* f. *edulis* Sims), which thrives best under subtropical regions.

The fruit is typically round or ovoid, containing numerous tiny, dark brown to black pitted seeds and a thick, smooth rind with fine white specks (6). The juice contains a balanced ratio of minerals, including sodium, magnesium, sulfur and chlorides, along with sugars, vitamins A and C, fiber, phenolic compounds and ascorbic acid (7). Research also highlights its high levels of antioxidants including flavonoids, carotenoids and polyphenols, which may possess anti-inflammatory and disease-prevention properties (8). Passion fruit has a relatively high  $\beta$ -carotene concentration, making it a good source of vitamin A precursor, which is often associated with cancer prevention. Its strong flavour and high acidity have led to comparisons to a natural concentrate. Passion fruit juice is often diluted (1:6) or used as a minor ingredient in other fruit liquids. Its high acidity limits its use as a table fruit, but the rich flavour makes it ideal for processing into a variety of value-added products, such as concentrates, juices, drinks, powders, squash, nectar, jams, jellies and sherbets. (Fig. 1).

Brazil leads global passion fruit production, followed by Australia, Peru, Venezuela, South Africa and Sri Lanka, accounting for 90% of total production. Approximately 96% of the passion fruit species are found in tropical and subtropical climates, primarily in South America. In India, passion fruit is considered a minor fruit crop, introduced in the 20<sup>th</sup> century and primarily grown in the Nilgiris, Coorg and Malabar regions of South India (6). In India, passion fruit is limited to Kerala, Tamil Nadu (including Kodaikanal, Nilgiris, Thandikudi, Yercaud), Karnataka (Coorg) and the Northeastern states of Mizoram, Nagaland, Manipur and Sikkim, covering a total area of 9.11 thousand hectares and yielding 45.82 thousand tons. Although passion fruit has high production potential in Kerala, its cultivation remains limited due to lack of scientific cultivation practices, suitable varieties and research. Studying existing genetic variability aids in the characterization and conservation of passion fruit genotypes. Phenological and pomological studies are useful to assess the different genotypes and for finding superior genotypes. There is a need

to identify and categorize superior types based on quality and morphological characteristics to facilitate the selection of suitable varieties.

Morphological, qualitative and molecular markers are the approaches used to characterize the different genotypes of passion fruit (9). Estimation of genetic variability based on polymorphisms of molecular markers is increasing among the genus *Passiflora* (10). The present study unveils the following objectives: to assess the variability of morphological characters of different genotypes collected from different parts of Tamil Nadu and to categorize them based on morphological, yield and biochemical characters and to study the molecular diversity in genotypes using ISSR markers which will further supplement to crop improvement programmes.

## Materials and Methods

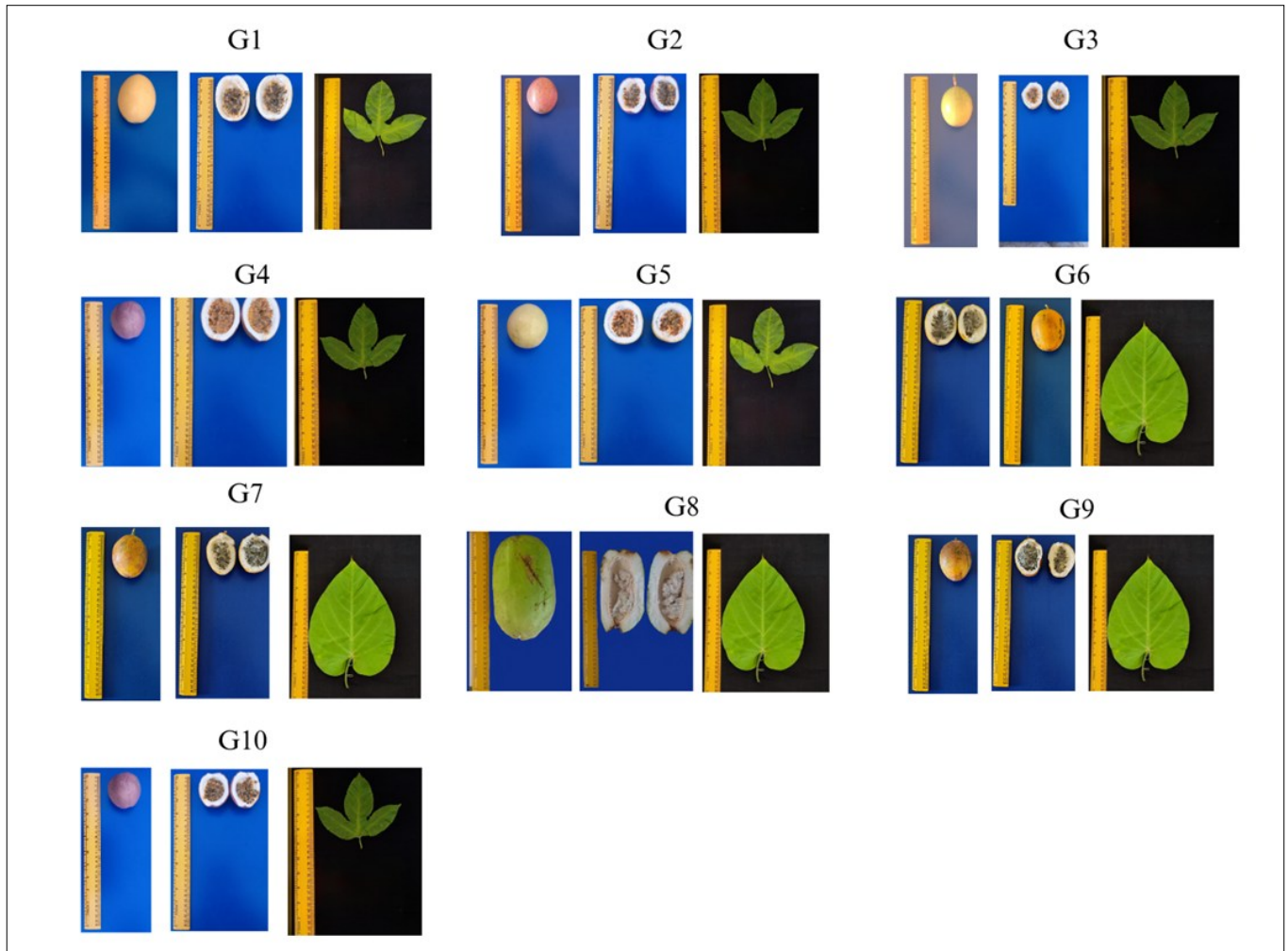
Passion fruit genotypes (yellow, purple, sweet and giant types) were collected from research stations and farmers' fields in Chinnamanur, Gudalur, Surulipatti, Thadiyankudisai, Thandikudi, Kodaikanal and Yercaud in Tamil Nadu for this experiment (Fig. 2, Table 1). The experiment was carried out during 2024 (Jan to Sept) and followed Randomized Block Design (RBD) with ten genotypes and four replications. Data collection encompassed morphological, biometric, yield and biochemical traits for genotype characterization following DUS guidelines from the International Union for the Protection of New Varieties of Plants (UPOV) (2024). The 10 passion fruit genotypes were also used to study the genetic relationships using 10 ISSR primers at the Centre for Excellence of Molecular Breeding, TNAU (Table 2).

## Statistical analysis

Data on morphological, yield and quality traits were statistically analyzed to assess diversity and genetic variation. Descriptive statistics, including mean, minimum, maximum, critical difference (CD) and coefficient of variation (CV), were



Fig.1. Major production of passion fruit in the world and in India.



**Fig. 2.** Different passion fruit genotypes (G1, G3, G5- *Passiflora edulis* f. *flavicarpa* Deg.; G2, G4, G10- *Passiflora edulis* Sim.; G6, G7, G9- *Passiflora ligularis* A. Juss; G8- *Passiflora quadrangularis* L.).

**Table 1.** List of passion fruit genotypes and their place of collection

Genotypes	Type	Scientific Name	Place of collection	Latitude °N	Longitude °E
Genotpe-1	Yellow	<i>Passiflora edulis</i> f. <i>flavicarpa</i> Deg	Chinnamanur	9.838875	77.392192
Genotpe-2	Purple	<i>Passiflora edulis</i> Sim	Gudalur	9.69333	77.253986
Genotpe-3	Yellow	<i>Passiflora edulis</i> f. <i>flavicarpa</i> Deg	Surulipatti	9.643608	77.269643
Genotpe-4	Purple	<i>Passiflora edulis</i> Sim	Thadiyankudisai	10.180421	77.462380
Genotpe-5	Yellow	<i>Passiflora edulis</i> f. <i>flavicarpa</i> Deg	Thadiyankudisai	10.320146	77.546315
Genotpe-6	Sweet	<i>Passiflora ligularis</i> A. Juss	Thadiyankudisai	10.104632	77.368023
Genotpe-7	Sweet	<i>Passiflora ligularis</i> A. Juss	Thandikudi	10.299496	77.671779
Genotpe-8	Giant	<i>Passiflora quadrangularis</i> L.	Thandikudi	10.30503	77.64699
Genotpe-9	Sweet	<i>Passiflora ligularis</i> A. Juss	Kodaikanal	10.237688	77.488702
Genotpe-10	Purple	<i>Passiflora edulis</i> Sim	Yercaud	11.432767	78.504263

**Table 2.** List of ISSR primers and its sequence

Sl. No	Primers	Primer sequence
01.	<b>UBC 807</b>	AGAGAGAGAGAGAGAT
02.	<b>UBC 808</b>	AGAGAGAGAGAGAGAC
03.	<b>UBC 810</b>	GAGAGAGAGAGAGAT
04.	<b>UBC 811</b>	GAGAGAGAGAGAGAC
05.	<b>UBC 812-F</b>	GAGAGAGAGAGAGAA
06.	<b>UBC 824</b>	TCTCTCTCTCTCTCG
07.	<b>UBC 842-F</b>	GAGAGAGAGAGAGAYG
08.	<b>UBC 857-F</b>	ACACACACACACACYG
09.	<b>UBC 873-F</b>	GACAGACAGACAGACA
10.	<b>UBC 888-F</b>	BDBCACACACACAGACA

calculated with quantitative parameters using AGRES software. Multivariate relationships among the traits were analyzed using Principal Component Analysis (PCA) in R software. Correlation studies were conducted using Pearson's correlation coefficient (11) and a dendrogram based on Agglomerative Hierarchical Clustering was conducted using Ward's method (12).

In molecular studies, each ISSR band was treated as an independent locus, with distinct, reproducible and well-resolved fragments visually scored, as absent (0) or present (1), for each of the 10 genotypes. A locus was deemed polymorphic if a consistent band was present in one or more, but not all, individuals of the population. The polymorphic information content (PIC) value for each locus was calculated (13) using the formula:

$$PIC_i = 2f_i(1-f_i)$$

where  $PIC_i$  is PIC for locus  $i$ ,  $f_i$  is the frequency of amplified fragments and  $1 - f_i$  is the frequency of non-amplified fragments. Shannon's information index (14), Nei's genetic diversity (15) and all the genetic diversity parameters were estimated using GenAEx 6.503 (16). Analysis of molecular variance (AMOVA) was performed using GenAEx 6.503 to calculate the sum of squares and variance components within and between populations. Pairwise genetic distance (17) was used to construct the dendrogram using Hierarchical Clustering on DARwin software (18)

## Results

### Variation in morphological characters

In breeding programs, morphological features are essential for variety identification, classification, plant adaptability, fruit quality, disease resistance and yield improvement (19). Qualitative characters of the *Passiflora* species revealed significant variability across the traits. Genotypes G1, G2, G3, G4, G5 and G10 exhibited a cleft shape, medium size, hastate leaf base, serrated leaf margin and trilobate leaf margin division. In contrast, genotypes G6, G7, G8 and G9 were distinct, showing a cordate shape, large size, subcordate leaf base, entire leaf margin and undivided leaf margin. White flower colour was observed in all the genotypes except G8, which exhibited a whitish pink colour. Genotypes G1, G2, G3 and G4 had oblong-shaped fruits, while G6, G7, G8 and G9 exhibited an

ellipsoid shape and G5 and G10 had round fruits. The study reported that both yellow and purple types are round to ovoid, with yellow to orange pulpy juice and excellent flavour (20). Fruit colour was yellow in genotypes G1, G3 and G5, greenish-yellow colour in G8 and yellowish orange with white speckles in G6, G7 and G9. Black seeds were observed in G1, G2, G3, G4, G6, G7, G9 and G10 genotypes, while genotype G8 exhibited dark brown seeds. Yellow pulp was observed in genotypes G1, G2, G3, G4, G5 and G10, while white pulp was present in genotypes G6, G7, G8 and G9.

### Variation in biometric characters

Quantitative traits were evaluated for different Passion fruit genotypes during the vegetative and flowering to harvesting stages. Genotype 8 exhibited significantly greater values in several traits, including stem girth (8.97 cm), seed length (0.76 cm), seed breadth (0.64 cm), seeds per fruit (170.20), 100-seed weight (4.86 g), days from flower bud initiation to anthesis (24.51) and days from anthesis to fruit set (3.81) compared to other genotypes.

The traits, leaf length (14.59 cm) and tendril length (24.3 cm) were recorded significantly high variation in the genotype 7. The angle between lateral veins recorded a higher value in genotype 3 (67.2°) and genotype 1 observed a significantly higher variation in the length of the right lateral lobe (7.4 cm) trait. Peduncle length was recorded longest in genotype 6 (7.43 cm) (Table 3).

### Variation in yield characters

The yield traits, including single fruit weight (277.9 g), fruit length (18.01 cm), fruit breadth (9.03 cm), rind thickness (9.83 mm), rind weight (166.6 g), pulp weight including seeds (111.3 g) and juice content (98.93 ml) were notably higher in genotype 8. A study on *P. quadrangularis* reported that it produces the largest fruits in the genus, greenish-yellow fruits measuring 15-20 cm in length, resembling melons (21). A study on sweet passion fruit types at both high and low elevations found that fruit weight and size were greater in plants grown at higher elevations (22). For the number of fruits per vine trait, there was a significant variation between genotype 10 (126.32) and genotype 8 (50.7). Genotype 2 recorded a significantly higher percent in fruit set (89.20%). The Pulp-to-rind ratio was significantly higher in genotype 4 (0.80 g) and the pulp recovery percentage was notably higher in genotype 9 (45.64%). A study conducted at KAU evaluated eight genotypes for yield and

**Table 3.** Descriptive statistics of biometric traits for 10 Passion fruit genotypes

TRAITS	RANGE		GENOTYPE		CD	CV
	Min	Max	Highest	Lowest		
Girth of stem (cm)	6.12	8.97	G8	G1	0.65	5.92
Leaf length (cm)	10.69	14.596	G8	G10	1.15	6.15
Leaf breadth (cm)	9.24	16.53	G5	G8	1.24	6.52
Angle between lateral veins (°)	44.5	67.2	G3	G5	5.56	6.40
Length of right lateral lobe (cm)	0.0	7.4	G1	G6	0.61	10.91
Tendril length (cm)	12.4	24.3	G7	G3	1.49	5.85
Peduncle length (cm)	2.53	7.43	G6	G8	0.46	7.29
Seed length (cm)	0.53	0.76	G8	G1	6.05	6.04
Seed breadth (cm)	0.16	0.64	G8	G6	0.03	7.69
Number of seeds per fruit	118.12	170.20	G9	G7	12.59	6.29
Weight of 100 seeds (g)	1.06	4.86	G8	G9	0.23	6.75
Days taken from flower bud initiation to anthesis	18.01	24.51	G8	G5	1.82	6.06
Days taken from anthesis to fruit set	2.33	3.81	G8	G3	0.25	6.20



quality parameters, finding that genotype 4 (purple) had the highest yield and genotype 5 (yellow) exhibited the best quality parameters (23). These high-performing genotypes could be valuable for future crop improvement programmes and commercial cultivation (Table 4).

#### Variations in biochemical characters

The biochemical characteristics of 10 passion fruit genotypes were analyzed, revealing that genotype 7 had significantly higher levels of total soluble solids (18.62 °Brix), total sugars (12.64%), reducing sugars (6.45%), non-reducing sugars (6.19%) and vitamin A (58.03 mg per 100g).

Titrate acidity (3.94%) was exhibited significantly high in the genotype 5. Vitamin C was notably higher in genotype 8 (32.92 mg per 100g) and genotype 9 recorded significantly higher in total flavonoid (28.93 mg per 100g) and antioxidant activity (22.21%). Typical yellow varieties of passion fruit generally exhibited a vivid canary-yellow rind and higher acidity levels (22).

Ascorbic acid levels in passion fruit cultivars ranged from 22.5 mg per 100 g to 48.75 mg per 100 g (24). The study reported that the concentration of ascorbic acid in fruit ranged between 16.98 and 30.50 mg per 100 g (25).

The research concluded that the highest antioxidant capacity was found in passion fruit (*Passiflora edulis*) pulp (64% of DPPH reduced) when compared to other fruits including mango, pineapple, banana and litchi which exerted lower free radical-scavenging activities (45–58%) (26) (Table 5).

#### Correlation coefficient analysis

The association of various biometric and quality traits with yield traits in passion fruit genotypes is shown in Fig. 3. The correlation coefficient provides insight into key traits for genotype assessment (27). Correlation analysis aids in designing selection strategies to improve yield, helping to identify traits that contribute to high-yield varieties.

In this study, correlation analyses were conducted for yield and fruit traits. The correlation coefficient matrix reveals a significant positive correlation between yield per vine and several traits, including rind weight (0.72), single fruit weight (0.73), pulp weight with seeds (0.75), juice content (0.73), fruit breadth (0.68), seed count per fruit (0.87) and seed breadth (0.84). A significant negative correlation was observed with peduncle length (-0.82), while other traits exhibited a non-significant correlation with yield per vine.

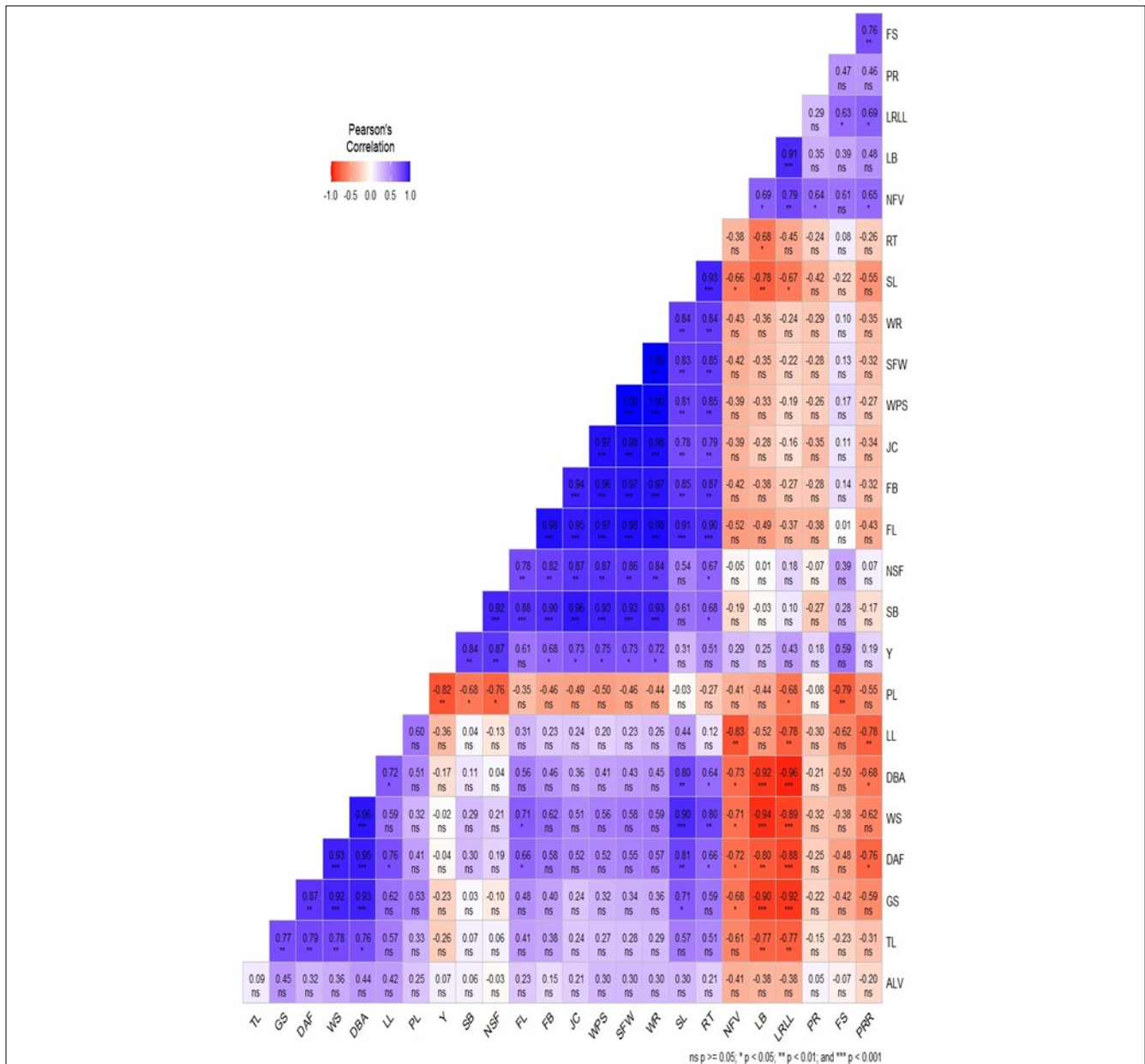
In a study, 11 yellow passion fruit genotypes from South and Southeast Brazil were evaluated for fruit characters. Positive correlation was recorded for yield/plant, number of fruits per plant, average fruit weight and yield/plant for the respective genotypes. The study also analyzed correlations between the production and quality characteristics of yellow passion fruit in southern Brazil. There were significant correlations between productivity and production per plant, productivity and number of fruits per ha, fruit weight and number of fruits per plant and fruit weight and number of fruits per ha (28). The study noted a high correlation between fruit weight and characteristics like seed number, fruit length and diameter in *P. edulis* (29).

**Table 4.** Descriptive statistics of yield traits for 10 Passion fruit genotypes

TRAITS	RANGE		GENOTYPE		CD	CV
	Min	Max	Highest	Lowest		
Number of fruits per vine	50.7	126.32	G10	G8	9.21	6.93
Fruit set (%)	71.66	89.20	G2	G6	7.30	6.13
Single Fruit weight (g)	65.4	277.9	G8	G7	11.86	7.88
Fruit length (cm)	5.63	18.01	G8	G4	0.75	7.19
Fruit breadth (cm)	5.26	9.03	G8	G6	0.53	6.06
Rind thickness (mm)	5.11	9.83	G8	G1	0.56	6.08
Pulp to rind ratio (g)	0.62	0.80	G4	G6	0.06	6.05
Weight of rind (g)	37.2	166.6	G8	G7	7.01	8.07
Weight of pulp along with seed (g)	27.0	111.3	G8	G6	4.85	7.63
Pulp recovery (%)	36.57	45.64	G9	G6	3.66	6.11
Juice content (ml/fruit)	13.31	98.93	G8	G7	4.22	9.91
Yield per vine (kg)	3.46	14.08	G8	G7	0.691	5.439

**Table 5.** Descriptive statistics of biochemical traits for 10 Passion fruit genotypes

TRAITS	RANGE		GENOTYPES		CD	CV
	Min	Max	Highest	Lowest		
Total Soluble Solids (°Brix)	13.02	18.62	G7	G8	1.440	6.065
Titrate acidity (%)	0.64	3.94	G5	G7	0.288	8.099
Total sugars (%)	8.01	12.64	G7	G1	0.930	6.108
Reducing sugar (%)	3.51	6.45	G7	G1	0.459	6.324
Non-reducing sugar (%)	4.48	6.19	G7	G10	0.480	6.279
Vitamin- C (mg/100g)	19.53	32.92	G8	G6	2.296	6.538
Total flavonoid (mg/100g)	10.01	28.93	G9	G10	1.576	6.590
Antioxidant activity (DPPH) (%)	6.42	22.21	G9	G10	1.347	7.651
Vitamin A (mg/100g)	1.02	58.03	G7	G4	3.543	10.202



**Fig. 3.** Pearson correlation coefficients between biometric and yield traits of passion fruit genotypes.

(GS- Girth of stem, LL- Leaf length, LB- Leaf breadth, ALV- Angle between lateral veins, TL- Tendril length, PL- Peduncle length, SL- Seed length, SB- Seed breadth, NSF- Number of seeds per fruit, WS- Weight of 100 seeds, DBA- Days taken from flower bud initiation to anthesis, DAF- Days taken from anthesis to fruit set, NFV- Number of fruits per vine, FS- Fruit set, SFW- Single Fruit weight, FL- Fruit length, FB- Fruit breadth, R- Rind thickness, PRR- Pulp to rind ratio, WR- Weight of rind, WPS- Weight of pulp along with seed, JC- Juice content, PR- Pulp recovery, LRLL- Length of right lateral lobe, TSS- Total Soluble Solids, TA- Titratable acidity, TS- Total sugars, RS- Reducing sugar, NRS- Non-reducing sugar, VC- Vitamin- C, TF- Total flavonoid, AA- Antioxidant activity, VA- Vitamin A, Y- Yield per vine)

### Genetic Diversity Based on Principal Component Analysis and Cluster

**Principal component analysis:** Principal Component Analysis (PCA) was conducted on 34 traits to determine the primary factors influencing overall yield and its components. Principal component analysis of passion fruit characters in 10 genotypes revealed that five principal components PC1, PC2, PC3, PC4 and PC5 had eigenvalues of more than one, which collectively accounted for 94.98% of the total variation (Table 6). PC1 contributed 45.08% of the total variation because of variation in each trait, with an eigenvalue of 15.33. PC1 recorded high positive values for traits such as days taken from flower bud initiation to anthesis (0.98), weight of 100 seed (0.97) and high negative loadings for the length of the lateral lobe (-0.94) and leaf breadth (-0.89). The fruit weight and number of seeds had strong positive loading (30). PC2 contributed 33.91% of the total variation with an eigenvalue of 11.53. The variation in this

component was contributed by traits with strong positive loadings such as seed breadth (0.94), number of seeds per fruit (0.94) and a high negative coefficient for peduncle length (-0.84). PC3 had an eigenvalue of 2.58 and contributed 7.59% to the total variation. The traits showed high positive factor values in the component of pulp recovery (0.69), fruit set (0.55) and high negative coefficient for leaf length (-0.34). PC4 accounted for 5.32% of the total variation and an eigenvalue of 1.81. This component had a high positive loading for an angle between lateral veins (0.68) and pulp recovery (0.53) and a high negative loading for reducing sugar (-0.36). PC5 accounted for 3.09% of the total variation with an eigenvalue of 1.05. PC5's variation was contributed by traits with high positive loadings, such as total soluble solids (0.42) and tendril length (0.38), with a high negative loading for the angle between lateral veins (-0.40) (Table 6).

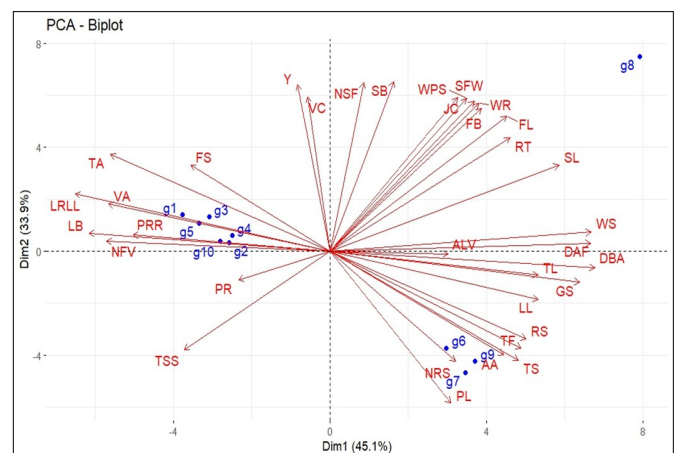
**Table 6.** Different characters of five principal components

Traits	PC1	PC2	PC3	PC4	PC5
Girth of stem	0.92	-0.17	0.17	-0.06	-0.13
Leaf length	0.77	-0.27	-0.34	0.30	0.34
Leaf breadth	-0.89	0.10	-0.18	0.23	0.24
Angle between lateral veins	0.44	-0.01	-0.07	0.68	-0.40
Tendrill length	0.77	-0.13	0.37	-0.23	0.38
Peduncle length	0.45	-0.84	-0.19	0.02	-0.12
Seed length	0.85	0.48	0.03	-0.13	-0.13
Seed breadth	0.24	0.94	-0.12	0.04	0.17
Number of seeds per fruit	0.12	0.94	0.11	0.04	0.16
Weight of 100 seeds	0.97	0.11	0.15	-0.14	-0.11
Days taken from flower bud initiation to anthesis	0.98	-0.09	0.12	0.01	-0.09
Days taken from anthesis to fruit set	0.96	0.05	0.04	-0.01	0.12
Number of fruits per vine	-0.82	0.06	0.38	0.02	-0.18
Fruit set	-0.51	0.48	0.55	0.17	0.05
Single Fruit weight	0.53	0.84	-0.03	0.11	0.03
Fruit length	0.65	0.75	-0.03	-0.04	0.04
Fruit breadth	0.56	0.80	0.06	-0.02	0.14
Rind thickness	0.67	0.63	0.29	-0.20	-0.19
Pulp to rind ratio	-0.73	0.09	0.52	-0.01	-0.02
Weight of rind	0.55	0.82	-0.05	0.11	0.04
Weight of pulp along with seed	0.50	0.85	0.00	0.11	0.02
Juice content	0.47	0.86	-0.13	0.06	0.10
Pulp recovery	-0.34	-0.16	0.69	0.53	0.01
Length of right lateral lobe	-0.94	0.32	-0.02	0.06	0.03
Total Soluble Solids	-0.54	-0.55	0.08	-0.28	0.42
Titratable acidity	-0.81	0.54	-0.08	0.05	0.10
Total sugars	0.70	-0.61	0.30	-0.18	-0.01
Reducing sugar	0.72	-0.49	0.08	-0.36	-0.17
Non-reducing sugar	0.46	-0.61	0.54	0.14	0.22
Vitamin- C	-0.08	0.86	0.33	-0.36	-0.01
Total flavonoid	0.71	-0.54	0.14	0.34	0.17
Antioxidant activity	0.64	-0.58	0.39	0.25	0.10
Vitamin A	-0.82	0.26	0.38	-0.30	-0.13
Yield per vine	-0.12	0.93	0.16	0.24	-0.07
Eigenvalue	15.33	11.53	2.58	1.81	1.05
Percentage of variance	45.08	33.91	7.59	5.32	3.09
Cumulative percentage of variance	45.08	78.99	86.57	91.9	94.98

The biplot illustrated the relationship between biometrics, yield, quality traits and genotypes (Fig. 4). Yield per vine showed a positive correlation with multiple traits, including fruit set percent, titratable acidity, vitamin A and vitamin C. Other correlated traits included number of seeds per fruit, seed breadth and pulp rind characteristics. It exhibited a negative correlation with total soluble solids, peduncle length, leaf length, the angle between lateral veins, tendrill length, the girth of the stem, days taken from anthesis to fruit set, days taken from bud initiation to anthesis, non-reducing sugar, antioxidant activity, total sugar, reducing sugar and total flavonoid.

**Cluster analysis:** Hierarchical clustering of 10 passion fruit genotypes based on 34 traits was performed using Ward's method in R. The analysis grouped the genotypes into five clusters (Table 7, Fig. 5). Among the five clusters, clusters I and II were the largest, each containing 3 genotypes followed by cluster IV with 2 genotypes. Clusters III and V were the smallest, each consisting of a single genotype (Table 8).

**Intra- and inter-cluster distances of five clusters:** Among the five clusters, the highest inter-cluster distance was observed between clusters I and V (12.77). This was followed by clusters II



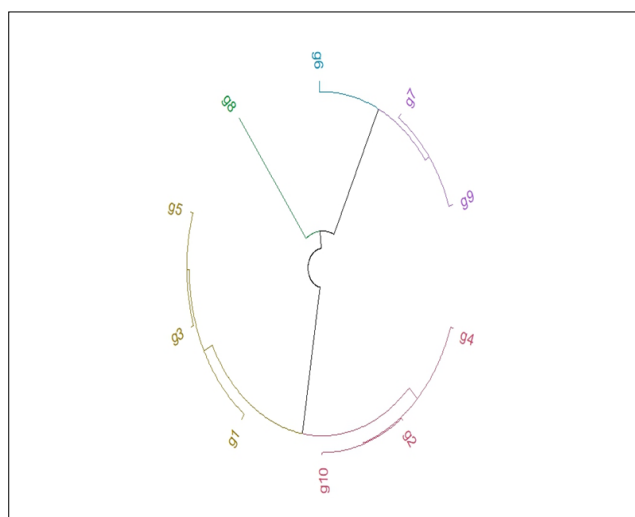
**Fig. 4.** PCA biplot depicting the relationship between biometric, yield and quality traits of 10 genotypes (GS- Girth of stem, LL- Leaf length, LB- Leaf breadth, ALV- Angle between lateral veins, TL- Tendril length, PL- Peduncle length, SL- Seed length, SB- Seed breadth, NSF- Number of seeds per fruit, WS- Weight of 100 seeds, DBA- Days taken from flower bud initiation to anthesis, DAF- Days taken from anthesis to fruit set, NFV- Number of fruits per vine, FS- Fruit set, SFW- Single Fruit weight, FL- Fruit length, FB- Fruit breadth, RT- Rind thickness, PRR- Pulp to rind ratio, WR- Weight of rind, WPS- Weight of pulp along with seed, JC- Juice content, PR- Pulp recovery, LRL- Length of right lateral lobe, TSS- Total Soluble Solids, TA- Titratable acidity, TS- Total sugars, RS- Reducing sugar, NRS- Non-reducing sugar, VC- Vitamin C, TF- Total flavonoid, AA- Antioxidant activity, VA- Vitamin A, Y- Yield per vine).

**Table 7.** Cluster mean values of five clusters for yield and quality traits

Traits	1	2	3	4	5
Girth of stem	6.52	7.22	8.11	8.63	8.97
Leaf length	12.54	11.06	13.95	14.50	14.15
Leaf breadth	15.98	13.19	10.45	11.30	9.24
Angle between lateral veins	58.47	55.47	62.00	62.40	64.50
Tendrill length	14.03	16.73	16.00	21.95	22.00
Peduncle length	3.24	3.30	7.43	6.92	2.53
Seed length	0.54	0.57	0.62	0.59	0.76
Seed breadth	0.34	0.26	0.16	0.18	0.64
Number of seeds per fruit	140.94	136.05	118.12	122.56	170.20
Weight of 100 seeds	1.21	2.06	3.07	3.19	4.86
Days taken from flower bud initiation to anthesis	18.15	19.34	22.30	22.79	24.51
Days taken from anthesis to fruit set	2.48	2.54	3.00	3.32	3.81
Number of fruits per vine	99.66	116.72	57.40	73.90	50.70
Fruit set	82.28	85.29	71.66	78.45	81.47
Single Fruit weight	101.37	79.40	65.40	71.00	277.90
Fruit length	6.29	5.88	6.02	5.83	18.01
Fruit breadth	5.90	5.75	5.26	5.65	9.03
Rind thickness	5.37	6.56	6.02	6.01	9.83
Pulp-to-rind ratio	0.71	0.75	0.62	0.67	0.63
Weight of rind	58.27	43.97	38.40	40.75	166.60
Weight of pulp along with seed	43.10	35.43	27.00	30.25	111.30
Juice content	31.09	18.96	15.01	13.38	98.93
Pulp recovery	41.21	41.95	36.57	42.47	38.24
Length of right lateral lobe	7.10	5.70	0.00	0.00	0.00
Total Soluble Solids	16.52	16.71	15.16	17.04	13.02
Titrateable acidity	3.77	2.88	0.83	0.79	1.86
Total sugars	8.74	10.11	11.46	12.69	10.51
Reducing sugar	4.05	4.99	6.34	6.35	5.58
Non-reducing sugar	4.69	5.11	5.12	6.35	4.93
Vitamin C	24.85	28.44	19.53	20.18	32.92
Total flavonoid	13.26	11.66	18.32	26.24	17.46
Antioxidant activity	7.60	10.38	13.02	20.37	12.06
Vitamin A	29.42	47.65	1.10	1.12	2.23
Yield per vine	10.10	9.24	3.75	5.34	14.08

**Table 8.** Distribution of genotypes among five clusters based on biometric, yield and quality traits

Cluster	No. of genotypes in each cluster	Genotypes
I	3	G1, G3, G5
II	3	G2, G4, G10
III	1	G6
IV	2	G7, G9
V	1	G8

**Fig. 5.** Hierarchical clustering of genotypes based on yield and quality traits using Ward's clustering method.

and V (12.49), clusters IV and V (12.48) and clusters III and V (12.09), while the shortest inter-cluster was recorded between clusters I and II (5.33). In contrast, cluster IV exhibited the highest intra-cluster distance (4.46) followed by cluster I (4.31) and cluster II (3.70), while the shortest intra-cluster distance was observed in clusters III and V (0.00).

### Mean performance of genotypes in five clusters for biometric, yield and quality traits

The mean performance of five clusters of biometric, yield and quality traits were presented in Table 9. Cluster means for the number of fruits per vine ranged from 50.7 in cluster IV (lowest) to 113.06 in cluster I (highest). The cluster analysis of passion fruit genotypes revealed significant variation in traits across clusters. Cluster I exhibited the highest leaf breadth (15.98), length of the right lateral lobe (7.10) and titrateable acidity (3.77) making it a standout for these attributes. Cluster II showed the highest values for the number of fruits per vine (116.72), fruit set percent (85.29), pulp-to-rind ratio (0.75) and vitamin A (47.65). Cluster III excelled in peduncle length (7.43). Cluster IV exhibited the highest leaf length (14.50), pulp recovery (42.47), total soluble solids (17.04), total sugars (12.69), reducing sugars (6.35), non-reducing sugar (6.35), total flavonoid (26.24) and antioxidant activity (20.37). Cluster V showed highest mean performance in girth of stem (8.97), angle between lateral veins (64.50), tendrill length (22.00), seed length (0.76), seed breadth (0.64), number of seeds per fruit (170.20), weight of 100 seeds (4.86), days taken from flower bud initiation to anthesis (24.51), days taken from anthesis to fruit set (3.81), single fruit weight (277.90), fruit length (18.01), fruit breadth (9.03), rind thickness (9.83), weight of rind (166.60), weight of pulp along with seed (111.30), juice content (98.93), vitamin C (32.92) and yield per vine (14.08).

These findings highlight the diverse genetic potential across clusters, which can be exploited for breeding programmes aimed at improving specific fruit quality traits, yield and nutritional content in passion fruit. A high degree of



fruit diversity was evident from the UPGMA dendrogram which separated the species of *Passiflora* into two main clusters. The first principal cluster consisted of *P. edulis* varieties along with *P. quadrangularis* and *P. ligularis* having large fruits (31) (Fig. 6). The study concludes that the inter- and intra-specific phenological and pomological variations observed may be attributed to changes in environmental factors such as temperature and altitude.

### ISSR Polymorphism analysis

The 10 ISSR primers produced 147 scorable bands in total. Band counts varied across populations, with population 3 showing the highest number of bands (74), followed by population 2 (43) and population 1 with the fewest bands (30) (Fig. 7).

Polymorphic Information Content (PIC) is a measure used in molecular marker studies to assess the informativeness of a marker for distinguishing between genotypes. PIC value ranges between low PIC (< 0.25) indicating low genetic differentiation; moderate PIC (0.25–0.50) indicating a moderate level of genetic variation and high PIC (> 0.50) signifying a highly informative marker (Table 9). Population 3 exhibits the highest genetic diversity, with the largest number of different alleles (1.485), effective alleles (1.528), Shannon's Information Index (0.437), diversity (0.300) and the highest percentage of polymorphic loci (72.16%). This indicates a wider genetic variation in Population 3, potentially making it more resilient to environmental changes and offering better opportunities for breeding programs. High genetic diversity is crucial for

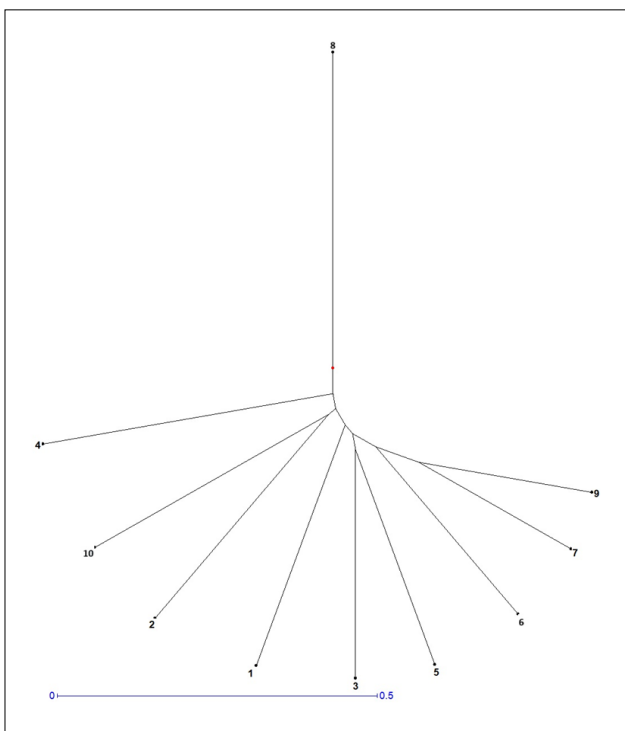


Fig. 6. Dendrogram showing the cluster pattern of 10 passion fruit genotypes.

Table 9. Passion fruit diversity obtained by primers used in ISSR polymorphism analysis

Populations	Number of genotypes in a population	Number of different alleles	Number of effective alleles	Number of bands	Shannon's Information Index	Diversity	Percentage of Polymorphic Loci	PIC
Pop1	3.000	0.608	1.239	30	0.190	0.133	29.90%	0.132875
Pop2	3.000	0.887	1.355	43	0.282	0.197	44.33%	0.197022
Pop3	4.000	1.485	1.528	74	0.437	0.300	72.16%	0.300258

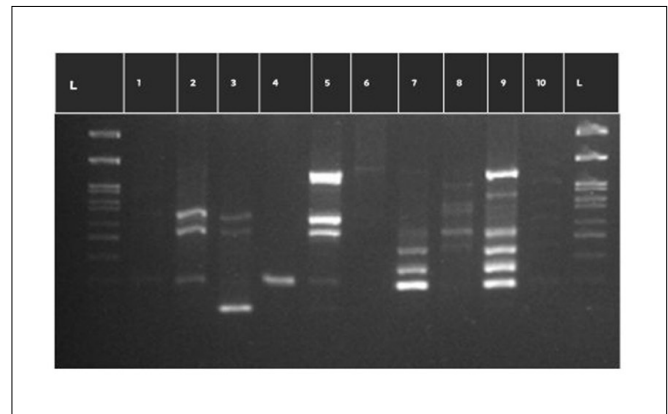


Fig. 7. ISSR profiles of passion fruit genotypes. Agarose gel of PCR products amplified with the primer UBC 573. Lane L 100 bp ladder (New England Biolabs, Beverly, MA). Lane numbers refer to the genotypes listed in Table 1.

adaptation to changing environments and for maintaining species fitness (15). According to the study, PIC values are crucial in identifying the most effective markers for genetic analysis in plant breeding programs, with higher values indicating better potential for selecting superior genotypes (32). Molecular markers with moderate to high PIC values are more useful in diversity analysis and marker-assisted selection for crop improvement (Table 9).

Population 1 exhibits the lowest diversity with only 0.608 different alleles, 1.239 effective alleles and 29.90% polymorphic loci, indicating lower genetic variation and a higher risk of inbreeding or genetic drift.

### Analysis of Molecular Variance (AMOVA)

The Analysis of Molecular Variance (AMOVA) is a statistical method used to partition genetic variation within and between populations. AMOVA helps to understand how genetic diversity is structured across populations.

AMOVA results showed that 90% of genetic variation was within populations, which is typical for outcrossing species like passion fruit, while only 10% was among populations (Table 10).

The high percentage of genetic variation within populations suggests that individual populations of passion fruit have a considerable amount of genetic diversity. This pattern is common in outcrossing species, where frequent gene flow occurs within populations. This could be due to natural pollination mechanisms in passion fruit, where insects or wind cause cross-pollination between individual plants within a population. The smaller proportion of variation between populations is not highly differentiated. This can be due to gene flow between populations, possibly through seed

Table 10. AMOVA

Source	df	SS	MS	Total Variance %
Among Pops	2	41.850	20.925	10
Within Pops	7	106.250	15.179	90
Total	9	148.100		100

or pollen dispersal. This suggests that populations have not been isolated long enough to develop strong genetic differences. Consistent genetic exchange among populations also helps maintain a relatively uniform gene pool.

These results imply that the genetic differentiation among populations was low and most of the diversity was present within the populations. This is beneficial for breeding programs, as it indicates a broad genetic base within individual populations, which can be harnessed to develop improved varieties. However, it also highlights that the populations may be vulnerable to genetic drift if population sizes decrease, as there is little differentiation to buffer against loss of diversity.

### Similarity index

The similarity index presents pairwise genetic similarity values among different passion fruit genotypes, reflecting their genetic closeness as determined by ISSR markers (Table 11). Genetic similarity within and between passion fruit populations offers valuable insights for breeding and conservation efforts. Within populations, genotypes exhibit moderate to high genetic similarity, indicating shared ancestry or evolutionary pressures. For example, population 1 (G1, G3, G5) and population 2 (G2, G4, G10) show moderate genetic similarity, with some pairs like G5 and G10 having a high similarity index of 55. In contrast, population 3 (G6, G7, G8, G9) displays more genetic diversity, with some genotypes closely related and others more distinct. Between populations, genetic similarity tends to be lower, reflecting greater genetic divergence due to reduced gene flow and different evolutionary paths. Crosses between more genetically distinct populations, such as population 1 and population 3, could introduce new traits and enhance genetic diversity. Despite this general pattern, certain genotypes from different populations, such as G5 from population 1 and G10 from population 2, exhibit high genetic similarity, which could be useful for stabilizing desirable traits across populations. The variation in genetic similarity both within and between populations suggests opportunities for selective breeding to improve vigour, disease resistance and other traits while maintaining a broad genetic base for conservation.

## Discussion

The genotypes G6, G7, G9 (Sweet type) and G8 (Giant type) display light green stems. Light stem colouration is typically associated with larger-fruited types like giant passion fruits, which was similarly reported in previous studies (33). The yellow and purple types (G1, G2, G3, G4, G5, G10) exhibit a cleft

leaf shape and medium leaf size, which is consistent with previous findings (34), where cleft leaf types were associated with better light interception, commonly seen in yellow and purple passion fruit types. Yellow and purple genotypes (G1-G5, G10) display a hastate leaf base shape with serrate leaf margins and similar observations were recorded in previous studies (35), noting these features in *Passiflora edulis* species. Heterophylly, the occurrence of different leaf shapes on the same plant, is present in yellow and purple types (G1-G5, G10), corroborating earlier findings (36), which stated that heterophylly is commonly observed in *Passiflora edulis* and its cultivars. The yellow and purple types (G1-G5, G10) generally have oblong fruit shapes, except for G5, which is round. This aligns with findings that yellow passion fruit types tend to have oblong or round shapes.

Sweet and giant types (G6-G9) exhibit ellipsoid fruit shapes, which is consistent with observations in *P. alata* and sweet passion fruit (*Passiflora alata*) (37). Most yellow and purple types (G1-G5, G10) have yellow pulp, a typical characteristic of *P. edulis* varieties and a similar observation was recorded (38), where pulp colour was yellow in both subspecies. Sweet and giant types (G6-G9), on the other hand, have white pulp, which is characteristic of sweet passion fruits, as noted by previous studies (39). White pulp in sweet type is often linked to a less acidic and sweeter taste, which is a distinguishing trait from the yellow pulp found in more common types. The yellow and purple types show typical characteristics of *Passiflora edulis*, while the sweet and giant types exhibit features aligned with *Passiflora alata* and other larger-fruited types.

Sweet genotypes (G6-G9) exhibited longer leaves, with G7 showing the highest value (14.59 cm), while purple types G4 and G10 displayed shorter leaves (10.73 cm and 10.69 cm, respectively), consistent with findings in sweet varieties (39). The larger leaves of sweet genotypes align with previous findings (39), which noted that *Passiflora alata* (sweet varieties) tend to have larger leaves compared to *P. edulis* (yellow and purple types). Studies observed that elongated lateral lobes in passion fruit are characteristic of yellow and purple genotypes (40). The weight of 100 seeds varied significantly, with G6 (Sweet) showing the highest weight at 3.07 g. This finding agrees with previous studies, which noted that heavier seeds often correlate with better nutrient reserves, potentially leading to improved seedling establishment (41).

The number of fruits per vine varied significantly among the genotypes, with G10 (Purple) producing the highest yield of 126.32 fruits per vine, while G8 (Giant) had the lowest at 50.7

**Table 11.** Similarity index

G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	
0										<b>G1</b>
16	0									<b>G2</b>
19	23	0								<b>G3</b>
14	14	21	0							<b>G4</b>
37	35	34	39	0						<b>G5</b>
10	12	19	6	41	0					<b>G6</b>
31	37	34	33	44	31	0				<b>G7</b>
29	31	30	27	40	25	28	0			<b>G8</b>
45	45	44	45	44	43	28	38	0		<b>G9</b>
36	40	43	38	55	38	45	45	49	0	<b>G10</b>

fruits. This variation in fruit set percentage (G10 at 82.00% and G6 at 71.66%) is consistent with previous findings (42), which noted that higher fruit set percentages are often associated with better pollination and environmental conditions.

The total soluble solids (TSS) content ranged from 13.02 °Brix in G8 (Giant) to 18.62 °Brix in G7 (Sweet). Higher TSS values are often associated with sweeter fruit, which is a desirable trait for consumers. This finding aligns with previous studies (43), which indicated that TSS is a critical factor in determining fruit quality and consumer acceptance. Additional studies have reported significant findings for traits such as juice content (44), antioxidant activity (45-47), flavonoids (48, 49), vitamin A (49), TSS (43, 50), titratable acidity (43, 50), vitamin C (49, 50) and both reducing and non-reducing sugars (38, 50), all of which contribute to the overall quality and nutritional profile of the fruit.

The positive correlation between yield per vine and the weight of the rind suggests that thicker or heavier rinds are associated with higher fruit yields. Previous studies also reported similar findings, indicating that rind weight positively correlates with overall fruit development, ensuring higher yields in commercial passion fruit cultivars (47). Previous findings similarly observed that selecting for higher pulp and seed weight could enhance total yield, making this trait a key target for breeding programs focused on improving juice yield in passion fruit (48). Correlation studies showed that traits like the number of fruits per vine have a significant positive correlation with fruit yield per vine (kg), emphasizing their importance in selecting yield traits. Direct selection based on these traits would lead to a simultaneous improvement of traits and fruit yield/vine in the passion fruit. Positive and significant correlations could be used to select for higher yield. This result is in accordance with the previous findings for *Passiflora edulis* f. *flavicarpa* (28, 36) and *Passiflora ligularis* A. Juss (47).

The five principal components (PC1, PC2, PC3, PC4 and PC5) explained 94.98% of the total variation, capturing the most critical variation in the data. High positive loadings on fruit weight and number of seeds imply that genotypes with heavier fruits and more seeds tend to have higher yields, a finding corroborated with previous studies (30), which also identified these traits as critical in determining fruit quality and market value. Similarly, negative correlation between excessive vegetative traits and fruit yield in passion fruit, concluding that energy devoted to vegetative growth can detract from reproductive output (48). PC2 explained 33.91% of the variation and was heavily influenced by seed breadth and the number of seeds per fruit, both showing strong positive loadings. The positive contribution of seed traits to yield is consistent with the previous findings (49, 50), which reported that seed-related traits were critical determinants of yield in passion fruit. Studies also noted that longer peduncles can detract from fruit development, as resources are allocated to vegetative growth instead of reproductive traits (45). Similarly, genotypes with larger leaves tended to have lower yields, likely due to a shift in resource allocation from fruit to leaf development.

The formation of five distinct clusters indicates significant variability in passion fruit genotypes. This clustering pattern highlights the genetic diversity within the passion fruit genotypes,

a finding corroborated with previous research (30), which also observed a high degree of diversity in *Passiflora* species when analyzing morphological traits. A study has shown that greater inter-cluster distances often correlate with increased heterotic potential, leading to hybrids with superior yield and fruit quality.

The development of ISSR molecular markers for agronomically important crops, such as passion fruit, has provided important insights into intraspecific genetic diversity (35). This information has proved invaluable for conservation and genetic improvement efforts.

The presence of unique ISSR alleles in the *Passiflora* species was verified through the existence of species-specific bands. Unique bands amplified by ISSR primers have applications in both germplasm identification and genetic improvement programs. The 18 ISSR primers produced 227 markers, with a polymorphism level of 98%. A study observed greater interspecific polymorphism (99%) compared to intraspecific polymorphism (76%) in *Passiflora* (42, 40). ISSR PCR has proved successful in assessing genetic diversity within various plant groups (48).

## Conclusion

The research on passion fruit genotypes has revealed significant findings related to their growth, yield and quality characteristics. Each genotype exhibited distinct morphological, biometric and biochemical traits, making them suitable for different cultivation and breeding objectives. The yellow genotypes (G1, G3, G5) showed a strong performance in yield-related traits, while the purple genotypes (G2, G4, G10) were prominent in quality aspects such as pulp recovery and fruit set percentage. The sweet types (G6, G7, G9) excelled in sugar content and antioxidant activity, which are critical for flavour and nutritional value. The giant type (G8) stands out for its significantly larger fruit size, weight and juice content, making it ideal for commercial juice production. In terms of genetic diversity, molecular marker analysis using ISSR primers confirmed a wide variation among the genotypes, with Population 3 showing the highest genetic diversity. This diversity provides a rich source for breeding programs aimed at enhancing yield, disease resistance and quality traits. Cluster and principal component analysis revealed that the genotypes are grouped based on traits, which can be leveraged to optimize future breeding programs. The study's findings highlight the potential for developing superior passion fruit varieties that meet consumer demands. The results contribute significantly to the ongoing efforts to improve passion fruit varieties and enhance their economic viability in tropical and subtropical regions. Future work should focus on more extensive field evaluations, particularly in different agro-climatic regions, to validate the performance of these genotypes under various environmental stresses. Additionally, integrating molecular tools such as next-generation sequencing (NGS) technologies will further accelerate the identification of beneficial traits and enhance the efficiency of breeding programs. This study forms a foundation for the improvement of passion fruit, aligning with the growing demand for higher quality, yield and suitable variety.

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## Authors' contributions

GI and SS did the planning and mobilized resources for this research. SS did the field research and data analysis. Results interpretation was done together by SS, SK, MN and GS. SS provided the first draft, whose main editing was done by SK and MN. All authors contributing to this manuscript accept this last version.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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