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Article

Morpho-Physiochemical and Seed Protein Profiling of *Passiflora* species Found in the North Eastern Himalayan Region of India

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Abstract: Passion fruit is an underutilised fruit in northeastern India, known for its unique flavour and health benefits. This study analysed 15 genotypes (P1 to P15) to explore their morphological and biochemical traits related to fruit quality and yield. *P. quadrangularis* L. (P15) exhibited maximum traits such as flower length, fruit size, weight, juice content, shelf-life, and yield. *P. edulis* f. *flavicarpa* (P3, P5, P2) had the highest seed count per fruit and antioxidant activity, along with greater chlorophyll and anthocyanin levels. *Passiflora edulis* Sim (P8, P11) showed superior total soluble solids, carotenoids, and vitamin C. The study found that fruit shelf life positively correlated with seed weight, while the number of fruits per vine negatively correlated with seed traits and peel weight. Additionally, certain traits, such as total carotenoids, had strong positive correlations with reducing sugar and flavonoids. Principal component analysis revealed distinct trait relationships, particularly for genotypes P7 and P10. SDS-PAGE protein profiling indicated a significant distance between P3 and P14, emphasising genetic diversity. In conclusion, this research highlights the diverse morphological and biochemical characteristics of passion fruit genotypes, paving the way for the region's improved fruit quality, yield, and breeding strategies.

Keywords: biochemical; correlation; morphology; pca; *Passiflora*; sds-page

1. Introduction

Passion fruit is a significant yet underexploited fruit abundantly found in the northeast region of India [1]. It is a perennial, vigorous, climbing, woody vine that produces round or oval-shaped fruits [2]. Fruits have a tough, smooth, waxy, dark purple/yellow coloured rind with faint, fine white specks [3]. Fruit contains orange-coloured pulpy juice with many small, hard, dark brown to black pitted seeds. It belongs to the family Passifloraceae, which comprises 16 genera with more than 700 species [4] and about 520 species of genera *Passiflora*, the majority of which are distributed to Neotropics and Africa [5]. The genera *Passiflora* shows significant variations within and between species [6–8]. The genotypes of different species also varied in physiochemical characteristics, as Ramaiya et al. [9] reported, as the purple type recorded the highest fruit length, width, and weight. In contrast, the yellow type recorded the highest juice weight. The fruit shape varies from round to ovoid [10]. The *Passiflora incarnata* and *Passiflora coccinea are* different in their filament colour [11]. Even differences among the different passion fruit species were observed in pulp, peel, and seeds [12,20]. The

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morpho-physiochemical and seed protein of *Passiflora foetida* and *Passiflora edulis* indicated unique bands, indicating the diversity in protein molecular weight [21].

The northeastern region of India has a rich diversity of underexploited fruits, including different passion fruit species, especially *Passiflora edulis* Sim (purple type), *Passiflora edulis* f. *flavicarpa* Deg (yellow type), *Passiflora quadrangularis* L (giant granadilla) as well as *Passiflora ligularis* Juss (sweet granadilla) [27]. The total area dedicated to passion fruit cultivation in India during 2023 was 0.011 million hectares, yielding a total production of 0.056 million metric tons [22], which is mainly dominated by yellow and purple types. Passion fruit juice [3], leaves [23], rind residue (5-6% protein), and seed (23 % oil; similar to soybean and sunflower oil) [1] have industrial importance. In Nagaland, India, fresh passion fruit leaves are boiled to extract their medicinal properties, which are often used for treating dysentery and hypertension [24]. In contrast, in South America, it is used as a sedative, diuretic, anthelmintic, anti-diarrheal, stimulant, and tonic and for the treatment of hypertension, signs of menopause, and infant colic [25]. The 21st century has witnessed continued research, technology, marketing systems, and conservation efforts for underutilised crops, including passion fruit [26]. However, improved passion fruit cultivars could be an important source of income for marginal to large farmers because of their nutritional and industrial value and short maturity period. As a diversified crop in the Himalayan region of India, evaluation and physicochemical characterisation have not been systematically explored. Patel et al. [18] conducted a study on six genotypes of three species *viz*., *Passiflora edulis*, *P. edulis* f. *flavicarpa* and *P. alata* at NEH region, Megalaya, India and studied the physiochemical changes. In our study, the fifteen different genotypes of *Passifora edulis* f. *flavicarpa* Deg, *Passifora edulis* Sim, *Passifora ligularis* Juss, and *Passifora quadrangularis* L., collected from Arunachal Pradesh, Manipur, Assam, Tripura, Mizoram, Nagaland, Sikkim, and Mizoram were evaluated based on morpho-physicochemical properties of fruit juice, leaves, and tendrils and validated based on seed proteins using SDS-PAGE (sodium sodecyl sulphate polyacrylamide gel electrophoresis). Moreover, By doing so, we aim to elucidate the genetic variation present within *Passifora* species endemic to the northeastern region of India. The outcomes of this research endeavour will serve as valuable resources for future investigations focused on understanding and harnessing the potential of these species.

2. Materials and Methods

2.1. Details of Genotypes

Fifteen genotypes of Passiflora species were collected from seven states in the eastern region of India, namely Arunachal Pradesh, Assam, Manipur, Nagaland, Mizoram, Sikkim, and Tripura. These genotypes include *P. edulis* f. *flavicarpa* Deg, *Passiflora edulis* Sim, *Passiflora ligularis* Juss, and *Passiflora quadrangularis* L. (Table 1, Figure 1).

Table 1. List of Passifora edulis f. flavicarpa Deg, Passifora edulis Sim, Passifora ligularis Juss, and Passifora quadrangularis L and their sources.

Species	Code	Sources	Latitude (N)	Longitude (E)	Altitude
P. edulis f. *flavicarpa* Deg	P1	Andro, Manipur	24⁰73'	94⁰04'	815 m
P. edulis f. *flavicarpa* Deg	P2	West Imphal, Manipur	24⁰47'	93⁰58'	906 m
P. edulis f. *flavicarpa* Deg	P3	Sutamura, west Tripura, Tripura	23⁰62'	91⁰26'	20 m
P. edulis f. *flavicarpa* Deg	P4	College of Agriculture, Biswanath Cherali, Assam	26⁰43'	93⁰08'	82 m
P. edulis f. *flavicarpa* Deg	P5	Notun Basti, Dimapur, Nagaland	25⁰55'	93⁰43'	154 m
P. edulis f. *flavicarpa* Deg	P6	CHF, Pasighat, Arunachal Pradesh	28⁰04'	95⁰19'	162 m
P. edulis Sim	P7	Kangpokpi, Manipur	24⁰42'	93⁰46'	1510 m
P. edulis Sim	P8	ICAR-NOFRI, East Sikkim	27⁰17'	88⁰36'	882 m
P. edulis Sim	P9	Aizawl, Mizoram	23⁰43'	92⁰44'	786 m
P. edulis Sim	P10	CHF, Campus, Pasighat, Arunachal Pradesh	28⁰04'	95⁰19'	168 m
P. edulis Sim	P11	Ziro, Lower Subansiri, Arunachal Pradesh	27⁰32'	93⁰48'	1566 m
P. edulis Sim	P12	Pasighat, Arunachal Pradesh	28⁰03'	95⁰20'	154 m
P. ligularis Juss	P13	Lunghar Village, Ukhrul, Manipur	25⁰16'	94⁰42'	1633 m
P. ligularis Juss	P14	Sakhabama, Kohima, Nagaland	25⁰39'	94⁰11'	1077 m
P. quadrangularis L.	P15	Pasighat, Arunachal Pradesh	28⁰03'	95⁰20'	156 m

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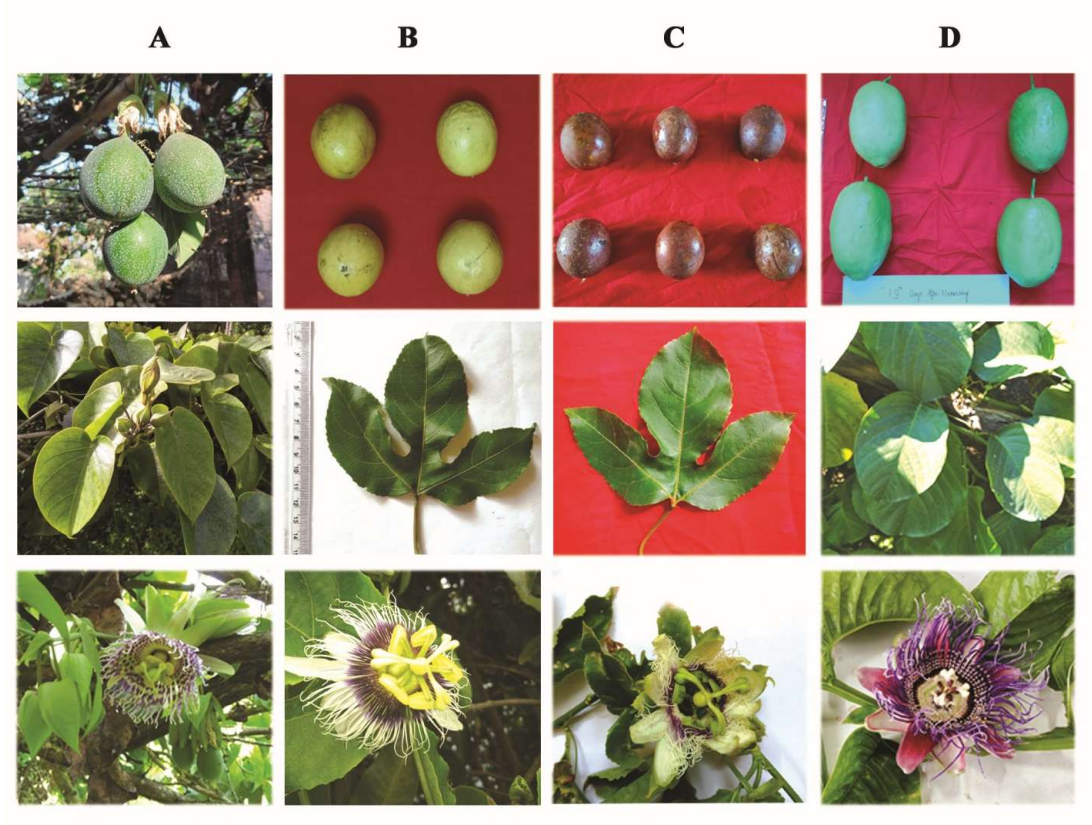

Figure 1. Fruits, leaves and flowers of (A) *Passiflora ligularis* Juss, (B) Passiflora edulis f. flavicarpa, (C) *Passiflora edulis* Sim and (D) *Passiflora quadrangularis* L.

2.2. Morphological Characterisation

The morphological characterisation such as the angle between lateral veins (°), leaf length (cm), leaf width (cm), leaf shape, leaf size, leaf base size, leaf colour, leaf margin shape, division of leaf lamina, presence of leaf nectaries, sinus depth, presence of heterophylly, presence of stipule, petiole length (cm), tendril length (cm), length of the right lateral lobe (cm), peduncle length (cm), flower length (cm), filament length (cm), stamen length (cm), number of flowers per node, fruit length (cm), fruit breadth (cm), fruit weight (g), number of fruits per vine, fruit yield (kg per vine), peel weight (g), shelf-life (days), the weight of 100 seeds (g), seed length (cm), seed width (cm), number of seeds per fruit, seed weight per fruit, stem colour, flower colour, petal colour, ovary colour, fruit colour, fruit shape and seed colour, were recorded following the morpho-agronomic descriptors for *Passiflora* spp. [28] (Figure 7 supplementary file) and the tropical fruit descriptor developed by Bioversity International (IPGRI) (supplementary files). The leaves, tendrils, and ripe fruits were collected for the study. The Image J software was used to calculate the angle between lateral veins [29].

2.3. Sample Preparation

The fruits were harvested at full ripeness, retaining their natural skin colour. The fruits were washed in running water, halved, and divided into pulp, peel (albedo and flavedo), and seeds. The parts were packed into plastic bags and stored in a freezer at -20 °C until further analysis.

2.4. Biochemical Characterisation

The biochemical attributes of pulp, leaves, and tendrils were analysed following standard protocol. The total soluble solid content (TSS) was determined in pulps with a °Brix refractometer; total carotenoid content (mg100g^{-1}) as per [30]; total flavonoids (mg100g^{-1}) as per [31]; antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl, DPPH) (%) [32]; titratable acidity (%) as per [33]; total carbohydrates (%) [34]; reducing sugar (%) [35] and non-reducing sugar (%) [36]. The anthocyanin content (mg 100g^{-1}) in petioles and tendrils was determined using [37]. The vitamin C (mg 100g^{-1}) [37], phenol (mg g^{-1}) [31] and chlorophyll content (mg g^{-1}) [38] content in leaves were determined following standard methods (Table 2).

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Table 2. Quality parameters studied following standard protocol.

Plant Part	Characters	References
Leaf	I.Anthocyanin content (mg/100 g)	[37]
	II.Vitamin C content (mg/100 g)	[37]
	III.Phenol content (mg/100 g)	[31]
	IV.Chlorophyll content (mg/g)	[38]
Petiole	V.Anthocyanin content (mg/100 g)	[37]
Tendril	VI.Anthocyanin content (mg/100 g)	
Fruit	VII.Vitamin C (mg/100 g)	[37]
	VIII.Total Soluble Solids (°Brix)	Hand refractometer
	IX.Total carotenoid (mg/100 g)	[30]
	X.Total flavonoids (mg/100 g)	[31]
	XI.Antioxidant activity (DPPH) (%)	[32]
	XII.Titratable acidity (%)	[33]
	XIII.Total carbohydrates (%)	[34]
	XIV.Reducing sugar (%)	[35]
	XV.Non-reducing sugar (%)	[36]

2.5. Protein Extraction and Estimation

Seeds weighing 0.2 grams were extracted from the fully ripened fruits of the selected genotypes of the *Passiflora* species. These extracted seeds had been pre-soaked in a phosphate buffer solution (pH 7.0) for 24 hours before use. Seeds have been crushed with a solution of Tris-HCl 0.06 M (PH 7.4), 10 mM urea, 1 mM EDTA, 0.1% TCA, 2.5% glycerol, 0.5% SDS and 1.25% β-mercaptoethanol. After crushing volume, make up to 2 mL and centrifuged at 8000 rpm for 20 minutes at 4 ^{0}C. Estimation of protein was done as per Lowry's method [39] to quantify the amount of protein present in the sample.

2.6. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis was carried out in the presence of denaturing agent (SDS/SLS) as per the procedure described by [40] with some modifications, and RF value and the molecular weight of each protein band were determined to observe variation among the *Passiflora* species found in the north-eastern Himalayan region of India (Table 3).

Table 3. The formulation for 15% acrylamide separating gel, 5% acrylamide stacking gel and sample preparation.

	formulation for 15% acrylamide separating gel	formulation for 5% acrylamide stacking gel
Water	6.9 mL	5.5 mL
30% Acrylamide mixture	15 mL	1.3 mL
Separating gel buffer (1.5M Tris-HCl, pH 8.8)	7.5 mL	1.0 mL
2% SDS	0.3 mL	0.1 mL
10% Ammonium persulphate	0.3 mL	0.1 mL
TEMED	0.012 mL	0.008 mL
Sample preparation		
0.6 M Tris-HCl	5.0 mL	
1% SDS	0.5 g	
0.5% Bromophenol blue solution	5 mL	
10% sucrose	5.0 g	

2.7. Staining Solution

Silver staining was used to stain the protein bands as per [42] which comprised of fixing Solution (50% ethanol, 12% glacial acetic acid, 0.05 mL formaldehyde), sensitising solution (0.02% aqueous solution of sodium thiosulphate), silver stain solution (0.2% silver nitrate and 0.076% formaldehyde), developing solution (6% sodium carbonate, 0.004% sodium thiosulphate and 0.05% formaldehyde solution) and terminating/stopping solution (12% acetic acid).

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2.8. Statistical Analysis

Data analysis was performed using variance analysis with the SAS software. In addition, the Pearson correlation matrix and principal component analysis (PCA) were conducted using Python packages, including scikit-learn, bioinfokit, seaborn, scipy, numpy, pandas, and matplotlib. The gels were scored as presence (+) or absence (-) of protein polypeptide bands for protein banding pattern. Depending upon the presence or absence of polypeptide bands, the similarity index (SI) [42] between the genotypes was calculated by the following formula:

$$SI= \frac{2Z}{X+Y} \times 100$$

Where Z= Number of similar bands between the genotypes, and X+Y =Total number of bands in the two genotypes compared.

3. Results

3.1. Morphological Parameters

Variations in morphological characteristics were evident among the collected genotypes, as illustrated in Figure 1 and detailed in Tables 4 and 5. The genotype P10 of *P. edulis* Sim displayed the highest leaf length at 15.13 cm, while genotypes P13 (14.45 cm) and P14 (14.38 cm), belonging to P. ligularis Juss., exhibited similar leaf lengths. Notably, the maximum leaf width was recorded in genotype P1 (16.07 cm), followed by P4 (15.67 cm) and P2 (15.56 cm), all of which are classified under *Passiflora edulis* f. *flavicarpa* Deg. In terms of flower length, genotype P15 of *Passiflora quadrangularis* L. displayed the longest at 9.20 cm, followed by P10 (7.73 cm) of *Passiflora edulis* Sim, which was comparable to P10 (7.40 cm) of *Passiflora edulis* f. *flavicarpa* Deg. The genotype P4 of *Passiflora edulis* f. *flavicarpa* Deg exhibited the longest stamen and filament lengths, measuring 2.02 cm and 1.37 cm, respectively. Conversely, genotype P15 of *Passiflora quadrangularis* L. demonstrated superior metrics in various parameters, including fruit length (14.48 cm), breadth (9.30 cm), fruit weight (496.67 g), seed length (0.79 cm), the angle between lateral veins (64.52°), seed width (0.62 cm), seed weight per fruit (9.37 g), and shelf life of fruit (27 days at room temperature), when compared to *Passiflora edulis* f. *flavicarpa* Deg, *Passiflora edulis* Sim, and *Passiflora ligularis* Juss genotypes under study. Furthermore, fruit shapes exhibited diversity ranging from oblate (genotypes P1, P2, P3, P4, P6, P7, P8, P9, P10, P11, and P12 of *Passiflora edulis* f. *flavicarpa* Deg) to ellipsoid (genotype P5 of *Passiflora edulis* f. *flavicarpa* Deg, and genotypes P13 and P14 of *Passiflora ligularis* Juss), and oblong (genotype P15 of *Passiflora quadrangularis* L.). The length of the right lateral lobe varied significantly, with *Passiflora edulis* f. *flavicarpa* Deg (genotype P1) exhibiting the maximum at 7.17 cm, while absent in genotypes P13 and P14 of *Passiflora ligularis* Juss and genotype P15 of *Passiflora quadrangularis* L. Notably, a higher number of fruits per vine were observed for genotypes P12 (174.67) and P7 (166.67), both belonging to *Passiflora edulis* f. *flavicarpa* Deg. In contrast, genotype P15 (52.33) of *Passiflora quadrangularis* L. exhibited the least. Genotype P15 displayed the maximum number of flowers per node (2.67), followed by genotypes P13 and P14 (1.67), while other genotypes bore only one flower per node. In terms of fruit yield (kg per vine), genotype P15 (26.23 kg per vine) of *Passiflora quadrangularis* L. and genotype P6 (10.47 kg per vine) of *Passiflora edulis* f. *flavicarpa* Deg demonstrated the highest yields. In contrast, genotype P13 (4.62 kg per vine) of *Passiflora ligularis* Juss exhibited the lowest. These findings underscore the significant variability in morphological traits and fruit productivity among different *Passiflora* genotypes, suggesting the potentiality of these regions.

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Table 4. Morphological characters of fifteen genotypes of Passiflora edulis f. flavicarpa Deg, Passiflora edulis Sim, Passiflora ligularis A. Juss and Passiflora quadrangularis L.

Species	Code	Angle between lateral veins (°)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Tendril length (cm)	Length of right lateral lobe (cm)	Peduncle length (cm)	Flower length (cm)	Filament length (cm)	Stamen length (cm)	Number of flowers per node
P. edulis f. *flavicarpa* Deg	P1	63.38ab	12.56bc	16.07a	2.21bc	13.39g	7.17a	3.27de	6.93d	0.83d	1.80abc	1.00b
P. edulis f. *flavicarpa* Deg	P2	63.02ab	12.21bcd	15.56ab	1.94cd	12.70g	7.03ab	3.20de	6.87d	0.80d	1.77abcd	1.00b
P. edulis f. *flavicarpa* Deg	P3	59.70abc	11.23cdef	14.10bcd	1.78de	16.13e	6.90b	3.27de	5.60i	0.83d	1.63abcde	1.00b
P. edulis f. *flavicarpa* Deg	P4	63.38ab	12.70b	15.67ab	2.22bc	20.23c	5.90de	3.10de	5.60i	1.37a	2.02a	1.00b
P. edulis f. *flavicarpa* Deg	P5	57.21bc	10.20f	12.68de	1.76de	14.57f	6.60c	3.07de	6.73d	0.83d	1.50bcdef	1.00b
P. edulis f. *flavicarpa* Deg	P6	42.24d	11.27cdef	14.80abc	3.02a	22.40b	5.80e	3.23de	7.40c	0.90cd	1.67abcde	1.00b
P. edulis Sim	P7	58.32abc	10.68ef	13.24cde	1.73de	20.54c	5.43g	2.93de	6.30e	1.13b	0.70h	1.00b
P. edulis Sim	P8	57.12bc	10.86def	13.92bcd	1.79de	19.13d	5.27h	3.30d	5.93fgh	1.13b	0.80gh	1.00b
P. edulis Sim	P9	57.10bc	10.66def	13.80bcd	1.75de	19.11d	5.19h	3.27d	5.81fgh	1.11b	0.78gh	1.00b
P. edulis Sim	P10	57.97abc	15.13a	12.07ef	2.49b	22.13b	6.00d	5.07c	7.73b	0.80d	1.12fgh	1.00b
P. edulis Sim	P11	54.07c	10.41f	12.64de	1.96cd	20.00c	5.40gh	2.93de	5.77hig	0.67e	1.28def	1.00b
P. edulis Sim	P12	58.79abc	10.42f	13.28cde	1.89cde	19.20d	5.30gh	2.87e	5.87fgh	1.13b	1.39cdef	1.00b
P. ligularis Juss	P13	62.17ab	14.45a	10.51fg	2.42b	14.27f	0.00i	7.23a	6.00f	0.97c	1.57abcdef	1.67c
P. ligularis Juss	P14	63.55ab	14.38a	11.58ef	1.88cde	16.13e	0.00i	6.43b	5.97fg	0.83d	1.97ab	1.67c
P. quadrangularis L.	P15	64.52a	11.95bcde	9.44g	1.56e	28.13a	0.00i	2.40f	9.20a	0.83d	1.42cdef	2.67a

Table 5. Morphological characters of fifteen genotypes of Passiflora edulis f. flavicarpa Deg, Passiflora edulis Sim, Passiflora ligularis A. Juss and Passiflora quadrangularis L.

Species	**Code**	**Fruit length (cm)**	**Fruit breadth (cm)**	**Fruit weight (g)**	**Number of fruits per vine**	**Fruit yield (kg per vine)**	**Peel weight (g)**	**Shelf-life (days)**	**Weight of 100 seeds (g)**	**Seed length (cm)**	**Seed width (cm)**	**Number of seeds per fruit**	**Seed weight per fruit**
P. edulis f. *flavicarpa* Deg	P1	6.16cd	5.33cd	78.75b	126.34ef	9.94bc	42.85b	9.33cde	1.17f	0.54cd	0.35b	146.33bcd	1.92ef
P. edulis f. *flavicarpa* Deg	P2	6.63bc	6.16b	65.1b	138.33de	9.01bcde	42.32b	8.33de	0.90g	0.56bc	0.38b	231.33a	2.08def
P. edulis f. *flavicarpa* Deg	P3	6.16cd	5.33cd	69.98b	144.00cde	10.07bc	43.34b	6.33e	1.32f	0.54cd	0.35b	193.00ab	2.54cdef
P. edulis f. *flavicarpa* Deg	P4	6.51bcd	5.64bc	77.69b	118.00f	9.15bcd	43.29b	7.00e	2.00de	0.52de	0.37b	166.33bcd	3.03cd
P. edulis f. *flavicarpa* Deg	P5	5.95cd	5.02cd	45.18b	128.00ef	5.78cde	20.59b	11.67bcd	1.95de	0.52de	0.18c	171.00bcd	3.27c
P. edulis f. *flavicarpa* Deg	P6	6.51bcd	5.64bc	77.69b	134.92ef	10.473b	47.96b	6.00e	2.00de	0.52de	0.37b	152.00bcd	3.04cd
P. edulis Sim	P7	5.95cd	5.02cd	45.18b	166.67ab	7.56bcde	24.59b	10.33bcde	2.31c	0.51de	0.20c	140.00bcd	3.23c
P. edulis Sim	P8	4.79e	4.27ef	32.7b	159.67abc	5.22de	16.30b	10.00bcde	1.88e	0.52de	0.21c	123.33de	2.31cdef
P. edulis Sim	P9	4.68e	4.21f	31.78b	152.67bcd	4.85de	15.90b	12.00bcd	1.85e	0.49e	0.19c	87.67e	1.61f
P. edulis Sim	P10	6.16cd	5.33cd	33.08b	161.33abc	5.35de	16.17b	9.33cde	2.17cd	0.54cd	0.35b	146.33bcd	3.15cd
P. edulis Sim	P11	5.69d	4.86de	43.89b	157.67abc	6.95bcde	22.86b	9.83bcde	2.16cd	0.52de	0.22c	136.33cde	2.97cde
P. edulis Sim	P12	5.72d	4.89de	43.99b	174.67a	7.68bcde	22.23b	13.00bc	2.18cd	0.51de	0.20c	141.67bcd	2.92cde
P. ligularis Juss	P13	7.17b	5.38cd	53.41b	86.67g	4.62e	32.31b	14.00b	3.20b	0.60b	0.17c	164.33bcd	5.27b
P. ligularis Juss	P14	7.16b	5.36cd	53.23b	87.33g	4.67e	31.94b	12.00bcd	3.20b	0.60b	0.17c	180.33bc	5.25b
P. quadrangularis L.	P15	14.48a	9.30a	496.67a	52.33h	26.23a	360.00a	27.33a	5.45a	0.79a	0.62a	172.33bcd	9.37a

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3.2. Contributions of Morphological Characters Towards Diversity in Passiflora Species

The morphological characters such as angle between lateral veins (°), leaf length (cm) leaf width (cm), petiole length (cm), tendril length (cm), length of right lateral lobe (cm), peduncle length (cm), flower length (cm), filament length (cm), stamen length (cm), number of flowers per node, fruit length (cm), fruit breadth (cm), fruit weight (g), number of fruits per vine, fruit yield (kg per vine), peel weight (g), weight of 100 seeds (g), seed length (cm), seed width (cm), number of seeds per fruit, seed weight per fruit were studied towards diversity. Among the different variable seed length contributed the highest percent towards genetic divergence (55.24%), followed by the seed weight per fruit (22.86%), number of seeds per fruit (8.57%) and filament length (1.90%). In contrast, the length of the right lateral lobe and seed breadth (5.71%) contributed to the same percentage (Figure 2).

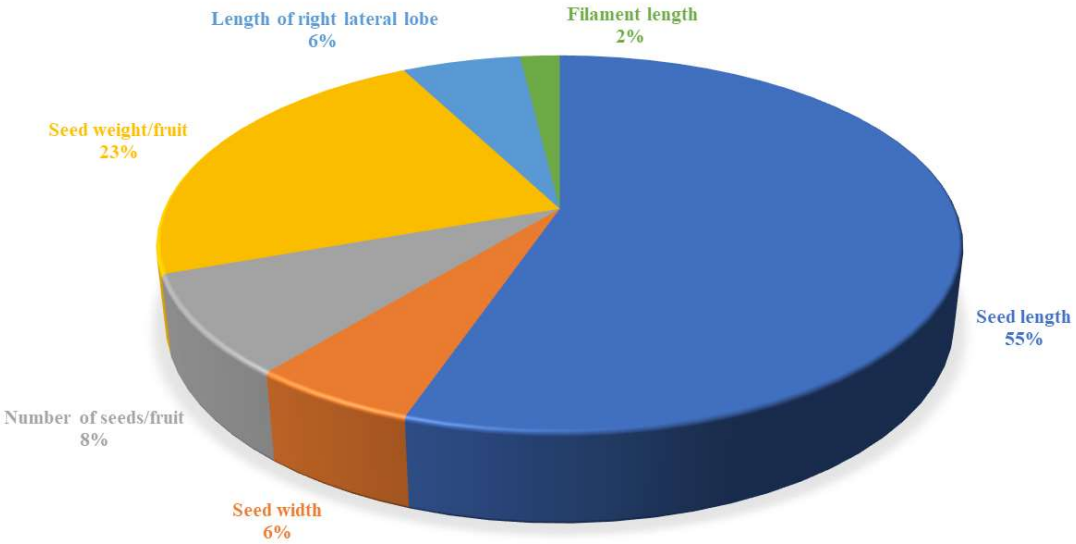

Figure 2. Maximum percentage contributions of morphological characters towards diversity.

3.3. *Biochemical Characteristics of Fruit Juice, Leaves, Petioles and Tendrils*

Significant differences were observed in the biochemical attributes of fruit juice, leaves, tendrils, and petioles among *Passiflora edulis* f. *flavicarpa* Deg, *Passiflora edulis* Sim, *Passiflora ligularis* A. Juss and *Passiflora quadrangularis* L. collected from the northeastern Himalayan region of India under study (Tables 6 and 7). Among the horticultural characteristics, a significant result was recorded for maximum juice content (mL per fruit) in genotype P15 (117.92 mL) followed by P6 (34.20 mL). In comparison, the lowest value was registered in genotype P9 (10.94 mL). The highest TSS content was recorded for P8 (18.28 °Brix), a purple type (*Passiflora edulis* Sim) and P5 (18.13 °Brix), a yellow type (*Passiflora edulis* f. *flavicarpa* Deg). In contrast, the lowest TSS was recorded for P15 (13.54 °Brix), a giant granadilla (*Passiflora quadrangularis* L.). The highest titratable acidity content was recorded for P2 (3.91%) and P1 (3.56%), while the lowest was recorded in P14 (0.63%). The highest vitamin C content in fruit juice was recorded in P11 (0.320 mg g^{-1}) and P15 (0.309 mg g^{-1}), whereas the lowest values were recorded for P13 (0.134 mg g^{-1}) and P14 (0.128 g^{-1}). The genotype P10 contained the highest amount of total carbohydrates (12.88%), followed by P7 (12.36%), while the lowest value was recorded for P6 (7.81 %). The highest percentage of reducing sugar was recorded in genotype P8 (6.92%), which was comparable to P5 (6.88%), while the lowest was registered in genotype P13 (3.51 %) of *Passiflora ligularis* Juss. The highest percentage of non-reducing sugar was recorded in P2 (6.54%) and P4 (6.52%), while the lowest was in P6 (3.05%) of *Passiflora edulis* f. *flavicarpa* Deg. Among the different genotypes, the total carotenoid content was highest in genotypes P8 (0.397 mg g^{-1}) and P5 (0.30 mg g^{-1}). Both genotypes belong to *Passiflora edulis* Sim, while the lowest was registered in genotypes P13 and P14 (0.0001 mg g^{-1}), and both species belong to *Passiflora ligularis* Juss. The genotype P5 contained a significantly higher amount of total flavonoid and antioxidant activity (0.355 mg g^{-1}; 22.15%), followed by P10 (0.261 mg g^{-1}; 14.76%), while the lowest was registered in genotype P9 (0.077 mg g^{-1}) for flavonoids and genotype P15 (6.28%) for antioxidant activity. The maximum vitamin C content of leaves was recorded for P5 (1.749 mg g^{-1}) and P12 (1.500 mg g^{-1}), while it was the least in genotype P10 (0.489

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mg g^{-1}). Significantly higher phenol content in the leaves was recorded for P10 (4.985 mg g^{-1}), P15 (3.489 mg g^{-1}), P14 (3.113 mg g^{-1}) and P13 (3.108 mg g^{-1}) while least in genotype P3 (1.432 mg g^{-1}). The maximum total chlorophyll content of leaves was recorded for P2 (2.91 mgg^{-1}).

3.4. Maximum Percentage Contributions of Biochemical Characters Towards Diversity in Passion Fruit Species

The biochemical parameetrs such as Vitamin C, total soluble solids, total carotenoid, total flavonoids, antioxidant activity, titratable acidity, total carbohydrates, reducing sugar, non-reducing sugar in fruit juice, anthocyanin content in leaves, petioles and tendrils, phenol, total chlorophyll and vitmain C content in leaves were subjected towards diversity contribution. Among the traits studied, total flavonoid content contributed to the highest percentage towards genetic divergence (63.81%), followed by chlorophyll content in leaves (17.14%), total carbohydrates content (8.57%), anthocyanin content in petioles (3.81%) and total carotenoid content (1.90%) (Figure 3).

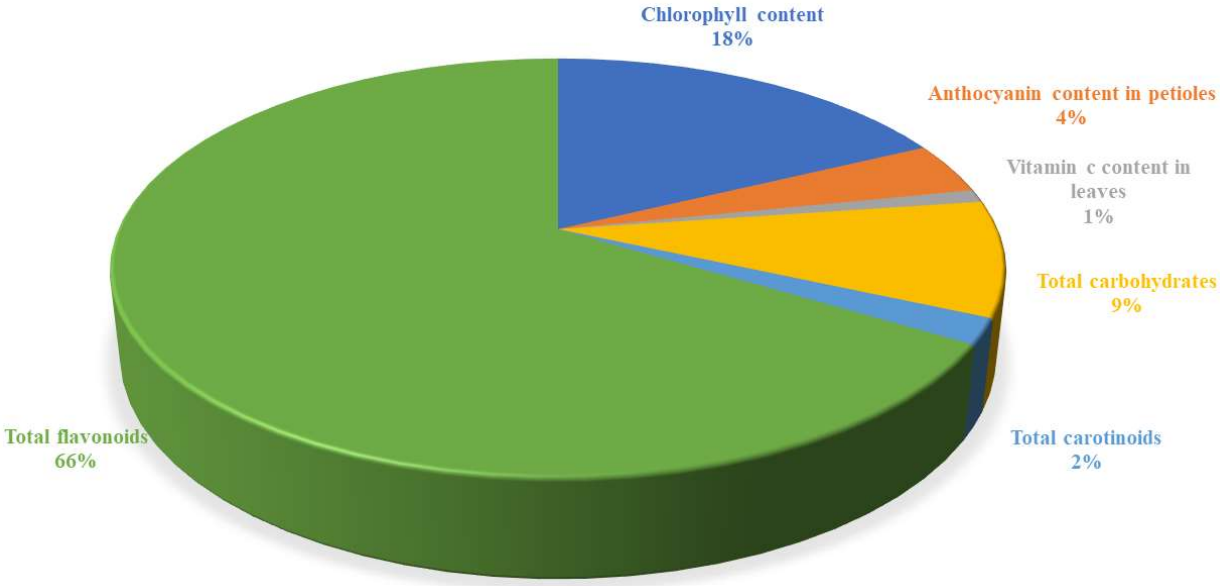

Figure 3. Maximum percentage contributions of biochemical characters towards diversity.

3.5. Characterisation of Passiflora Species Through Seed Protein Profiles (SDS-PAGE)

The protein banding pattern of fifteen genotypes of *Passiflora edulis* f. *flavicarpa* Deg, *Passiflora edulis* Sim, *Passiflora ligularis* A. Juss and *Passiflora quadrangularis* L collected from different states of the the northeastern Himalayan region of India was generated by SDS-PAGE (Figure 4). Cluster analysis of the banding pattern of fifteen genotypes based on similarity index and UPGMA resulted in distinct clusters (Figure 5). A total of 89 protein bands, as per

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Table 6. Biochemical parameters of fruit juice of Passiflora edulis f. flavicarpa Deg, Passiflora edulis Sim, Passiflora ligularis A. Juss and Passiflora quadrangularis L.

Species	Genotypes	Vit C (mg g^{-1})	Total soluble solids (^{0}Brix)	Total carotenoid (mg g^{-1})	Total flavonoids (mg g^{-1})	Antioxidant activity (DPPH) (%)	Titratable Acidity (%)	Total carbohydrate (%)	Reducing sugar (%)	Non-reducing Sugar (%)	Fruit juice content (mL/fruit)
P. edulis f. *flavicarpa* Deg	P1	0.238bcd	16.17d	0.100hi	0.114def	10.85cde	3.56ab	10.14de	4.92cdef	5.20abcd	34.17b
P. edulis f. *flavicarpa* Deg	P2	0.26.1abc	15.97d	0.090ij	0.113def	11.70bcd	3.91a	11.14abcd	4.63defg	6.54a	20.70bcd
P. edulis f. *flavicarpa* Deg	P3	0.262abc	15.13e	0.116h	0.09ef	12.93bcd	3.41abc	12.12ab	4.93cdef	5.79abc	24.09bcd
P. edulis f. *flavicarpa* Deg	P4	0.262abc	16.03d	0.08.7ij	0.11def	11.48bcd	3.32bc	10.31cde	4.10fg	6.52a	31.35bc
P. edulis f. *flavicarpa* Deg	P5	0.265abc	18.13a	0.300c	0.35a	22.15a	1.15gh	10.84bcd	6.88a	4.72abcd	21.31bcd
P. edulis f. *flavicarpa* Deg	P6	0.214cd	17.53c	0.077j	0.16cde	12.50bcd	2.43e	7.81f	4.35efg	3.05d	34.20b
P. edulis Sim	P7	0.292ab	18.07a	0.265f	0.11def	11.96bcd	2.78de	12.36ab	6.36ab	6.28ab	17.35d
P. edulis Sim	P8	0.226cd	18.28a	0.397a	0.24bc	12.60bcd	2.59e	10.59bcd	6.92a	3.42cd	14.09d
P. edulis Sim	P9	0.207cd	17.94ab	0.196g	0.07g	9.80def	2.52e	12.06abc	5.54bcd	5.99abc	10.94d
P. edulis Sim	P10	0.214cd	17.27c	0.240b	0.26b	14.76b	3.19bcd	12.88a	5.98abc	6.40ab	13.75d
P. edulis Sim	P11	0.320a	14.70ef	0.285e	0.10ef	13.70bc	2.91cde	12.18ab	6.41ab	5.96abc	18.14cd
P. edulis Sim	P12	0.177de	17.53bc	0.145d	0.20bcd	10.33cdef	1.20g	10.14de	5.35bcde	3.86bcd	18.78cd
P. ligularis Juss	P13	0.134e	14.43f	0.0001k	0.11ef	7.52efg	0.64h	8.41f	3.51g	4.25abcd	16.77d
P. ligularis Juss	P14	0.127e	14.64bc	0.0001k	0.11def	7.17fg	0.63h	8.80ef	4.03fg	4.50abcd	16.77d
P. quadrangularis L.	P15	0.308a	13.54g	0.0172k	0.17cde	6.28g	1.81f	10.32cde	5.52bcde	4.92abcd	117.92a

Table 7. Biochemical parameters in leaves, petioles and tendrils Passiflora edulis f. flavicarpa Deg, Passiflora edulis Sim, Passiflora ligularis A. Juss and Passiflora quadrangularis L.

Species	Genotypes	Leaf Vit. C (mg g^{-1})	Leaf Phenol (mg g^{-1})	Leaf chlorophyll (mg g^{-1})	Petiole anthocyanin (µg g^{-1})	Tendril anthocyanin (µg g^{-1})
P. edulis f. *flavicarpa* Deg	P1	1.151cd	1.538h	2.84ab	20.30bcde	28.90ab
P. edulis f. *flavicarpa* Deg	P2	1.1807c	1.527h	2.90a	18.60cde	33.90a
P. edulis f. *flavicarpa* Deg	P3	0.981de	1.432h	1.56de	30.50ab	26.90abc
P. edulis f. *flavicarpa* Deg	P4	1.218c	1.795g	1.50de	15.70def	19.80abcd
P. edulis f. *flavicarpa* Deg	P5	1.748a	1.942g	1.87cde	28.10abc	21.40abcd
P. edulis f. *flavicarpa* Deg	P6	1.256c	1.798g	1.16e	9.30ef	24.50abcd
P. edulis Sim	P7	1.172cd	2.709e	2.16bcd	19.0cde	31.40ab
P. edulis Sim	P8	1.101cd	2.813de	1.57de	35.40a	21.10abcd
P. edulis Sim	P9	1.183c	2.242f	1.64cde	05.90f	20.40abcd
P. edulis Sim	P10	489g	4.985a	2.60ab	10.10def	31.10ab
P. edulis Sim	P11	1.258c	2.968cd	1.32e	4.30f	14.10bcd
P. edulis Sim	P12	1.500b	3.059c	2.38abc	5.90f	06.70d
P. ligularis Juss	P13	0.882ef	3.108c	1.23e	21.50bcd	26.40abc

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P. ligularis Juss	P14	0.752f	3.113c	1.10e	20.40bcde	16.0abcd
P. quadrangularis L.	P15	0.493g	3.489b	1.65cde	17.80cde	09.30cd

P1 (2.84 mg g^{-1}) and P10 (2.60 mg g^{-1}) while the least was in P13 (0.0012 mg g^{-1}). The anthocyanin in petioles of P8 (35.40 µg g^{-1}), P3 (30.50 µg g^{-1}) and P5 (28.10 µg g^{-1}) contained a significantly higher amount. In comparison, genotype P2 showed a higher amount of anthocyanin in tendrils (33.90 µg g^{-1}), followed by P7 (31.40 µg g^{-1}) and P10 (31.1 µg g^{-1}). The lowest anthocyanin content was recorded in genotype P11 (4.30 µg g^{-1}) in petioles and genotype P12 (6.70 µg g^{-1}) in tendrils.

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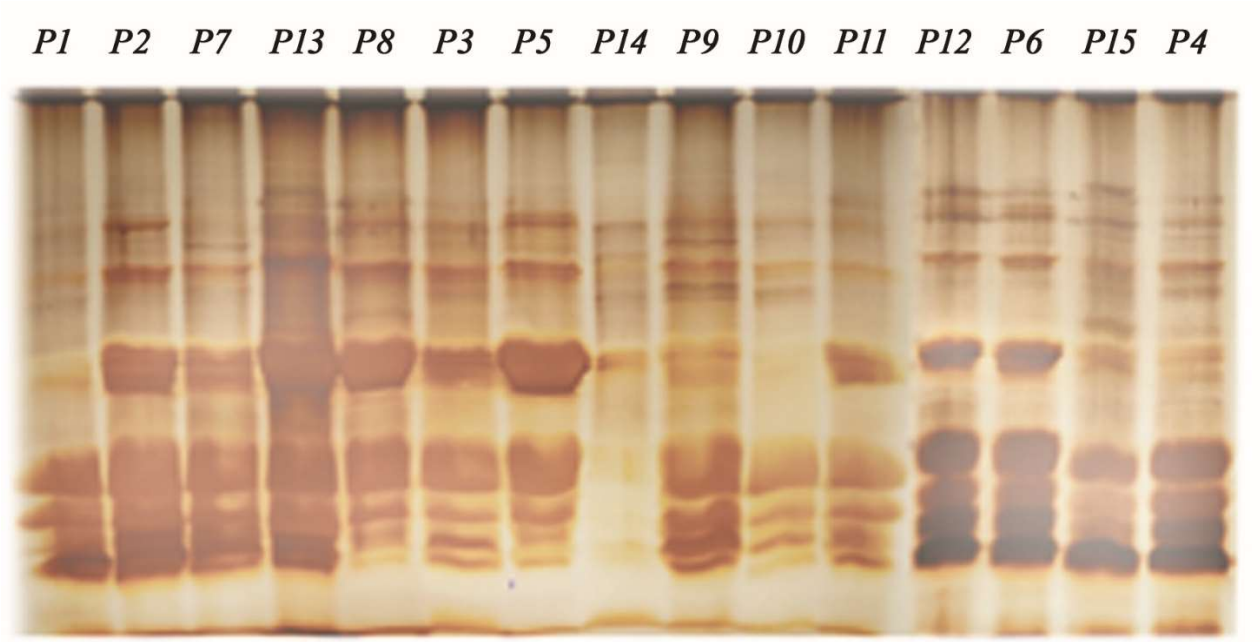

Figure 4. Seed protein profiling of 15 genotypes of passion fruit under study.

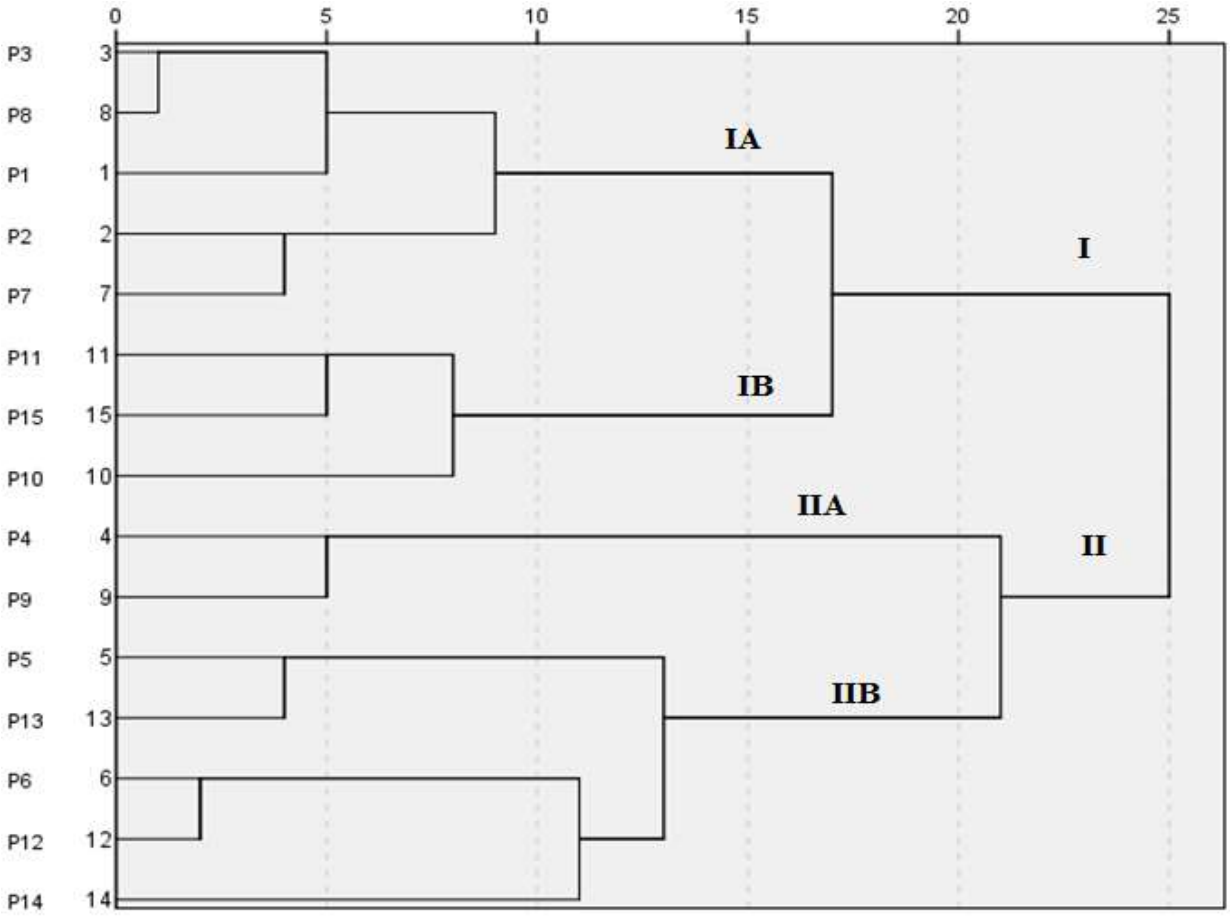

Figure 5. Dendrogram using ward linkage of seed protein of fifteen passion fruit genotypes.

Rm values, were identified through silver staining. The genotypes displayed significant variation in the number of protein bands, ranging from 6 to 11. Among them, P13 and P14 showed the highest number (11) of protein bands, while the lowest number (6) of bands was present in P1 (*P. edulis* f. *flavicarpa* Deg). *P. ligularis* Juss (genotypes P13 and P14) was characterised by three specific protein bands at bands 2, 10 and 13. In contrast, band number 30 was found only in P1 (*P. edulis* f.

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Flavicarpa Deg) and P13 (*P. ligularis* Juss). Band number 2 (Rm=0.11) was exclusively present in genotypes P8; similarly, band number 9 (Rm=0.18) was found in genotypes P11 and P14, whereas it was absent in all other genotypes studied. The same protein bands were observed in *P. edulis* f. *Flavicarpa* Deg (P2, P3 and P4) and *P. edulis* Sim (P8, P9, P10 and P11).

3.6. Correlation Analysis

Among the morphological traits studied, the fruit length was maximum positively correlated with fruit breadth, fruit weight,peel weight and seed length; fruit weight with fruit yield and peel weight; weight of 100 seeds with seed weight/fruit. Whereas, number of fruits/vine was maximum negatively correlated with seed weight/fruit; fruit length, breadth and weight with number of fruits/vine; weight of 100 seeds, seed length, seed weight/fruit and angle between lateral veins with leaf width and length of right lateral lobe; flowe length with filament length; length of right lateral lobe with number of inflorescence/node and peduncle length; tendril length with stamen length, peduncle lenth and length of right lateral lobe, seed weight with leaf width and length of right lateral lobe; fruit yield with pendulce length, length of right lateral lobe, leaf width and petiole length.The number of fruit/vine indicated negative correlation with most of the studied parameters except for length of right lateral lobe, leaf width and filament lenght (Figure 6). Regrading biochemical traits, total carotenoid content was significantly positively correlated with reducing sugar, total flavonoids, and antioxidant activity; total soluble sugar with total carotenoid, total flavonoid and antioxidant activity of fruit juice as well leaf vitamin C content. However, leaf vitamin C content showed a negative correlation with leaf phenol content, while total soluble sugar content showed a maximum negative correlation with fruit juice content (Figure 7).

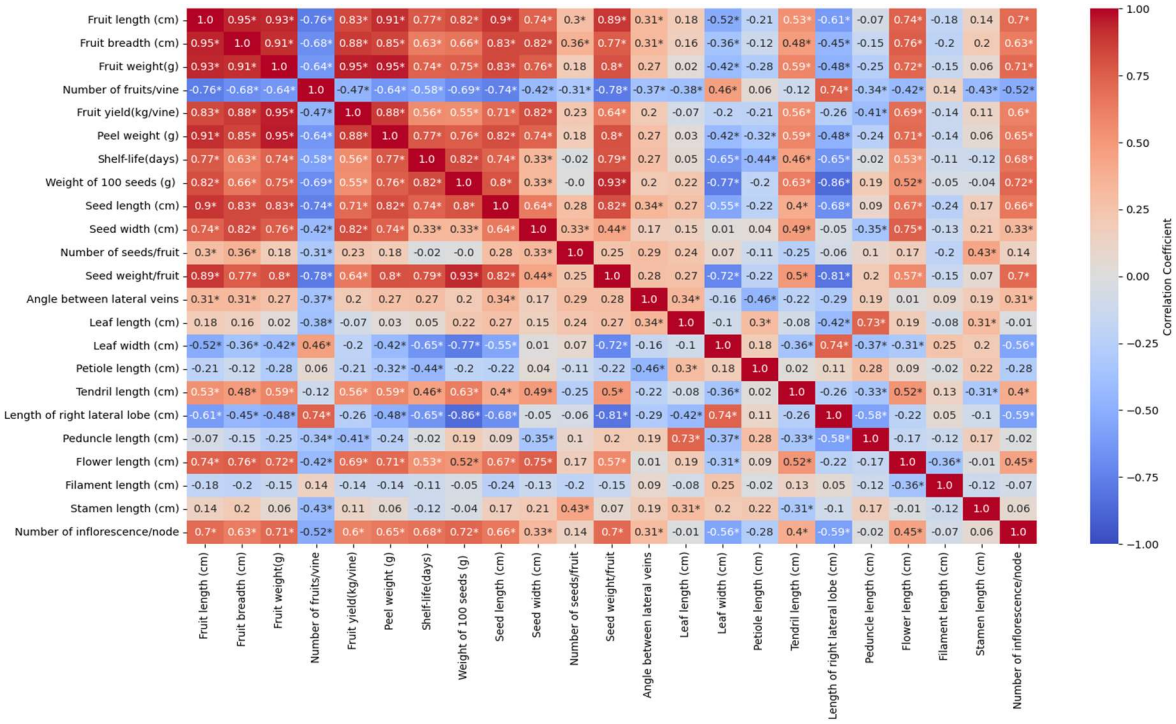

Figure 6. Correlation study of morphological traits.

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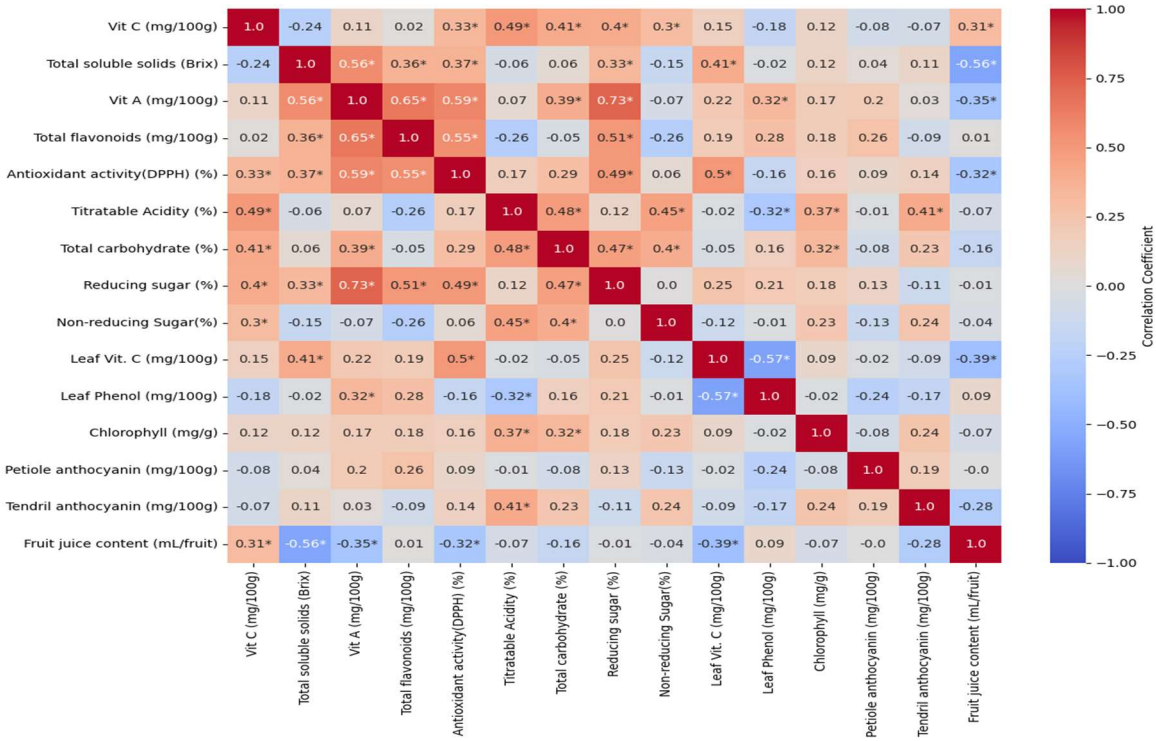

Figure 7. Correlation study of biochemical traits.

3.7. Principal Components Analysis

The principal component analysis was performed for morphological and biochemical traits across the genotypes to understand the relationship between critical variables (Figures 8 and 9). The principal component 1 accounted 47.7 % of the variation, while PC2 accounted for 13.8 % of the variation in the morphological traits studied. Petiole length exhibited a negative correlation with *P. edulis* f. *flavicarpa* Deg (P1) and *P. edulis* Sim (P7 and P10) in PC1, whereas most traits showed a positive correlation in PC2 in other passion fruit genotypes under study. Regarding biochemical traits, a total variation of 43% was accounted for, with most traits showing a positive correlation. The genotype P5 (*P. edulis* f. *flavicarpa* Deg) accounted for total solube sugar while genotypes P1 (*P. edulis* f. *flavicarpa* Deg), P14 (*P. ligularis* Juss) and P15 (*P. quadrangularis* L.) were associated with fruit juice content.

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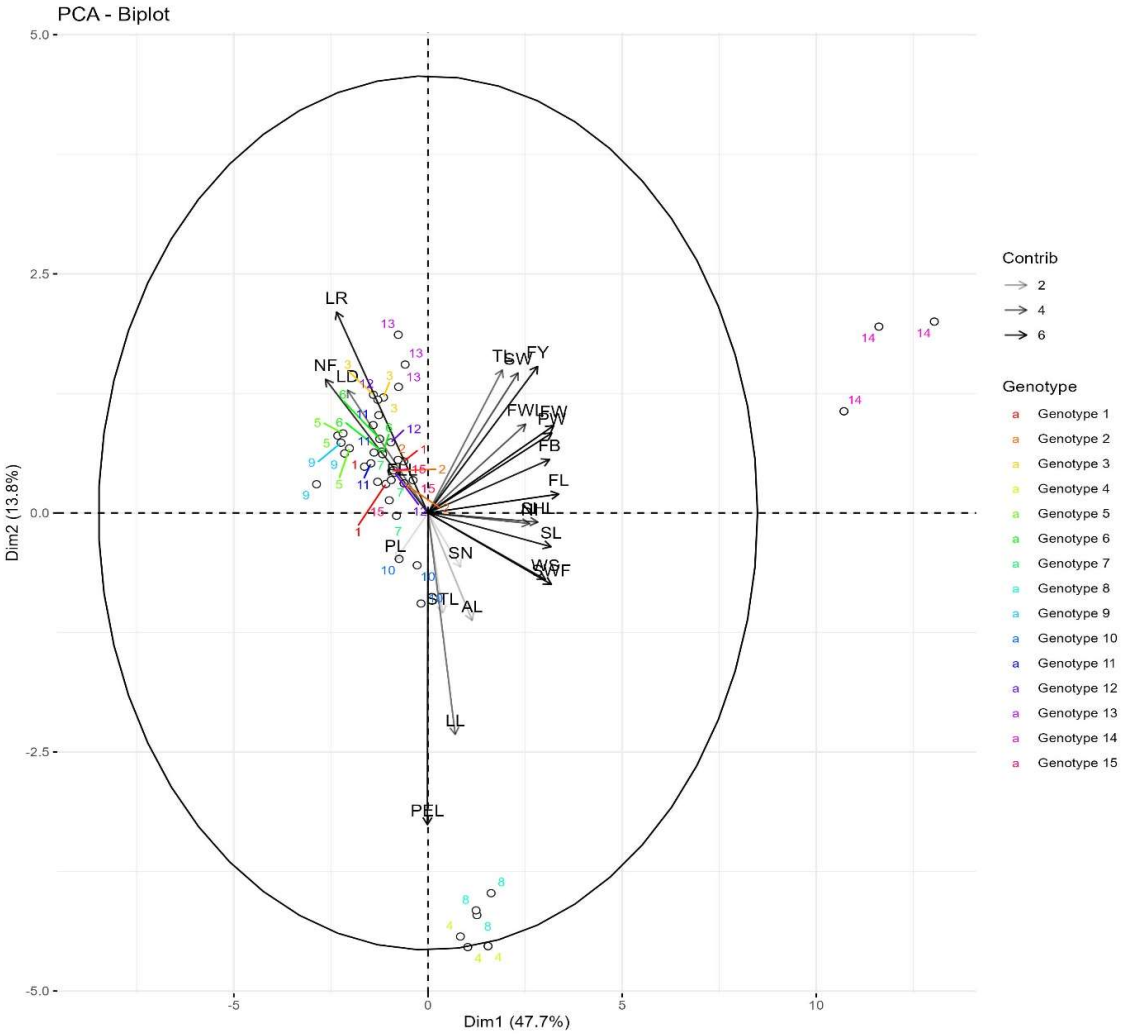

Figure 8. Principal component analysis for morphological traits.

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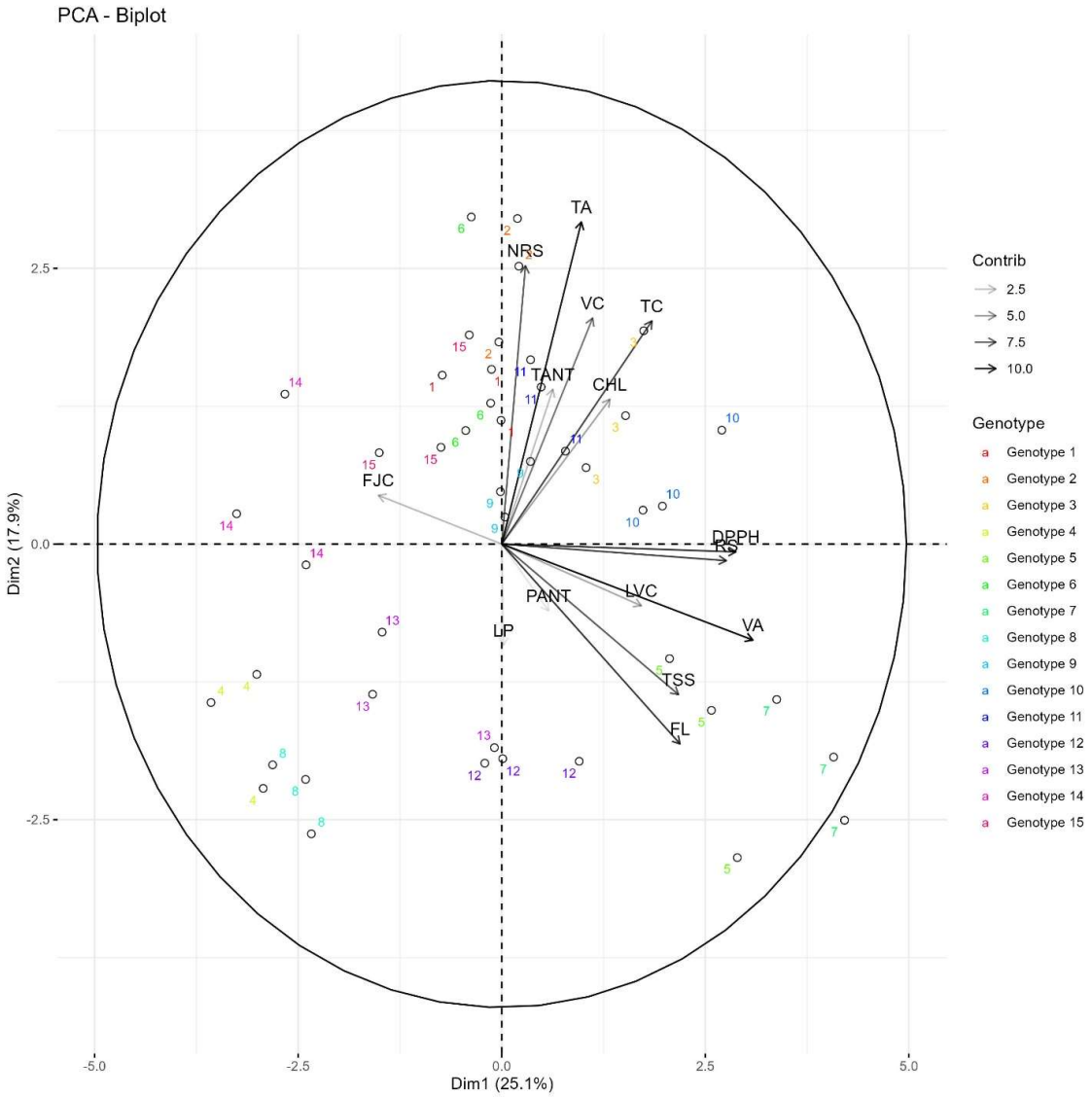

Figure 9. Principal component analysis for biochemical traits.

4. Discussion

Understanding the genetic diversity within collections of genotypes is crucial for effectively conserving and managing germplasm resources and utilising them in breeding programs for passion fruit [43]. Genetic variation among genotypes is essential for developing new crop varieties that enhance productivity and resilience against biotic and abiotic stresses. In this study, we used different morpho-physicochemical traits and seed proteins to estimate diversity among fifteen genotypes belonging to *P. edulis* f. *flavicarpa* Deg, *P. edulis* Sim, *P. ligularis* Juss and *P. quadrangularis* L., collected from Arunachal Pradesh, Manipur, Assam, Tripura, Mizoram, Nagaland, Sikkim and Mizoram states located in northeastern Himalayan region of India during 2018-2020. Differences in leaf and stamen characters were observed among the genotypes, as they belong to different species, while even within the species of *Passiflora edulis* Sim (genotype P10) variations were evident. These differences arose due to the diverse origins of the different species from the seed. Variability in morphological characters was also noted by [23,44] indicating significant diversity among the collected *Passiflora* species. The genotype P15 belongs to *Passiflora quadrangularis* L. represented the maximum fruit length, fruit breadth, fruit weight, seed length, angle between lateral veins, seed width, and seed weight per fruit compared to *Passiflora edulis* f. *flavicarpa* Deg, *Passiflora edulis* Sim and *Passiflora ligularis* Juss genotypes. This phenomenon can be attributed to the giant nature of *Passiflora quadrangularis* L., as reported by [46]. Regarding flower length, *Passiflora quadrangularis* L. exhibited the maximum, while stamen and filament length were greatest in *Passiflora edulis* f. *flavicarpa*, in agreement with findings by [45]. In some cases, different passion fruit species might be preferred for specific markets or

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vegetable purposes. Additionally, factors such as variety selection, growing conditions, and cultural practices also influence fruit size and overall yield. The ultimate goal for breeders and growers is to achieve higher fruit yield per vine. Improving passion fruit directly correlates with selecting elite genotypes that exhibit higher fruit yield and quality. Similar findings from the data align with those of [15], which resulted in higher yields. The maximum TSS content was recorded for P8 (18.28 °Brix), a purple type (*Passiflora edulis* Sim), and P5 (18.13 °Brix), a yellow type (*Passiflora edulis* f. *flavicarpa* Deg). In contrast, the lowest content was observed in P15 (13.54 °Brix), a giant granadilla (*Passiflora quadrangularis* L). The total soluble sugar content varies according to species, geographical origin, genetic factors, environmental conditions, cultural practices, and harvesting techniques [16]. For instance, genotype P8, belonging to *Passiflora edulis* Sim (purple type), was collected from East Sikkim at an altitude of 882 meters, resulting in higher soluble sugar content. Data on TSS content align with findings from [17,18]. It is therefore, the passion fruit genotypes with high total soluble sugar content are valued for their superior flavor, marketability, nutritional benefits, processing qualities, and ability to meet consumer preferences. As a result, genotypes that consistently produce fruits with high sugar content are highly sought after in the passion fruit industry [19]. The concentration of ascorbic acid in *Passiflora* species highlights the potential use of the fruit as a good source of ascorbic acid. Not only are the fruit juice rich in vitamin C, but the vitamin C content of the leaves was also significant, with the highest reported in genotype P5, which is of the yellow type. Hence, these assortments could serve as a promising reservoir of ascorbic acid, offering nutritional, medicinal, and commercial advantages. Utilised in traditional remedies for ailments such as dysentery and hypertension [24], fresh passion fruit leaves also boast properties as a sedative, diuretic, anthelmintic, anti-diarrheal, and stimulant [25]. Moreover, they are enriched with antioxidant potential due to their high vitamin C content.. These findings are consistent with the results of [47] regarding ascorbic acid content. Additionally, genotypes P8 and P5 contained higher amounts of total carotenoid content compared to the other studied genotypes. This finding is consistent with the one reported by [12]. The variation observed among the genotypes is not only due to geographical effects but also influenced by factors such as the maturation stage of the fruits at harvest, crop year, variety, and storage conditions [13]. It has been reported that temperature can impact the total carbohydrate or sugar content in passion fruit [14], which is reflected in the results of the yellow, purple passion, sweet, and giant granadilla fruit collected from different altitudes and temperature conditions in the northeastern Himalayan region of India. Studies have found a range from 5.25 to 6.98 g/100 g of total sugars in yellow passion fruit [15]; however, they have also reported that the total sugar varied from 5.75 to 13.97 g/100 g for *P. edulis* f. *flavicarpa* harvested in two different places, and these values are consistent with those detected in our study due to variation in geography. Flavonoids possess antioxidant, pharmacological, and cytotoxic properties [48], and they demonstrate the capacity to shield against DNA damage induced by reactive oxygen species (ROS). The genotype P5 (*P. edulis* f. *flavicarpa* Deg) and P10 (*P. edulis* Sim) recorded maximum flavonoid content among other genotypes under study. Carmona-Hernandez et al. [49] documented a flavonoid content of 0.29 mg catechin g^{-1} for *P. edulis* f. *flavicarpa* and 0.28 mg catechin g^{-1} for *P. edulis* f. *edulis*. These reported values are almost similar than those observed in our study which also includes same species. Our findings highlight P5 and P10, as a promising genotype for breeding due to its notably higher flavonoid content and exhibits considerable potential. Our outcomes were also in line with the findings of [50]. The antioxidant potential is influenced by more than just the phenolic composition. It is also shaped by various bioactive compounds, their interactions, and synergistic effects [52]. The DPPH value showed that genotype P5 of *Passiflora edulis* f. *flavicarpa* exhibited the highest antioxidant activity. de Oliveira et al. [52] documented a total antioxidant activity of 7.20 micromoles of Trolox equivalents per gram of fresh weight in ripe yellow passion fruit cultivated under conventional management. Reis et al. [12] found a correlation between antioxidant activity and carotenoid content, a relationship also observed in our study, which contributed significantly to the total antioxidant activity. Our result are also inline with the work of [57,58] who reported the higher naioidcanr acivity in *Passiflora edulis* leaves. The giant granadilla (*Passiflora quadrangularis* L) holds nutritional importance as it can serve as a source of fresh vegetables, with its green stage being suitable for cooking [53]. Additionally, the leaves of *Passiflora edulis* f. *flavicarpa* Deg and *Passiflora edulis* Sim can be utilised as a rich source of vitamin C. SDS-PAGE method used to discern the diversity of seed proteins within the germplasm. In genetic variation studies, seed proteins serve as crucial genetic markers due to their direct correlation with structural genes [54,55]. Any modification in a gene's coding sequence typically translates into a corresponding alteration in the

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protein's primary structure it encodes [56]. According to Beena and Beevy [21], SDS–PAGE protein profiling indicates variations within and between Passiflora species. The low TF% of 40% observed in *Passiflora edulis* varieties suggests the cultivated taxa have evolved. The presence of unique protein bands at 65.66 KDa *in Passiflora foetida* and 17.50 KDa in *Passiflora edulis* confirms their interspecific variation. Our study resulted in a maximum distance between genotype P3 (*Passiflora edulis* f. *flavicarpa* Deg) and P14 (*Passiflora ligularis* Juss), indicating their potential for breeding programs.

5. Conclusions

From the study, it can be concluded that fifteen genotypes from four *Passiflora* species collected from various parts of the northeastern region of India exhibit significant variation in morphological and biochemical characteristics, comprising thirty-nine morphological traits and seventeen biochemical characters. SDS-PAGE protein profiling also revealed variations at both the intra- and interspecific levels among *Passiflora* species found in northeast India. Based on the mean performance of all the traits studied, genotypes P2, P8, P3, P5, P11, and P6 were identified as superior for most of the yield components and fruit quality traits. These genotypes could serve as valuable parental sources in future breeding programs.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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