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RESEARCH ARTICLE

Unravelling the fruit quality, bioactive potential and genetic insights of Indian coffee plum [*Flacourtia jangomas* (Lour.) Raeusch.] plants under *Terai* natural vegetation of West Bengal

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Abstract

The present study aimed to unveil the morpho-biochemical characters of fruits, the presence of bioactive compounds and genetic diversity of different Indian coffee plum plants selected from the natural vegetation of the Terai region of West Bengal during the years 2022-23 and 2023-24. A wide array of variation in fruit morphology and quality aspects concerning fruit length (14.7 to 22.4 cm), fruit diameter (17.1 to 23.7cm), fruit weight (4.37 to 7.82g), number of seeds (5.8 to 11.1), TSS (6.2 to 10.9ºBrix), acidity (0.40 to 0.52 %), reducing sugar (4.02 to 5.80 %) and ascorbic acid content (100.5 to 156.7 mg/100g)wasnoticed. Fruits of selected plants also possessed comprehensive variation in antioxidants (63.9 to 88.7 % of DPPH inhibition), total phenols (145.7 to 278.6 µgGAE/g), flavonoids (85.1 to 163.9 mgQE/g), anthocyanin content (33.5 to 56.4 µg/100g) and carotenoid content (1.135 to 1.6114 mg/100g). A positive correlation was noted among fruit size, quality parameters such as TSS, acidity and ascorbic acid content. Ascorbic acid content was found to be positively correlated with fruit size, total sugar, antioxidant activity, phenol, flavonoid and carotenoids. A remarkable negative correlation was also noted between the number of fruits per cluster and the number of seeds per fruit, with other parameters. The entire population of Indian coffee plum plants represented three major clusters, comprising 8, 3 and 9 different plants, based on three different parameter clusters, to create such variation. ICPG-J5 is ideal for the commercial fresh fruit market due to its large, heavier fruit and excellent biochemical properties, ascorbic acid and strong antioxidant activity. ICPG-J7 stands out for processing purposes, as it has the largest fruit size, highest weight and a balanced sweet-tangy profile. ICPG-J2 is the best choice for functional food applications, rich in flavonoids, anthocyanins and strong antioxidant properties, making it valuable for health-focused products.

Keywords: bioactive compounds; fruit morphology; genetic diversity; Indian coffee plum; quality parameters

Introduction

The Indian coffee plum [*Flacourtia jangomas* (Lour.)Raeusch.], or Indian Cherry, is commonly known as Panial (Assamese), Lukluki (Bengali), Talispatri (Hindi), Takhob (Thai), LobiLobi (Indonesian) and Botoko Plum (Philippino). It is a small to medium-sized (6–10 m height) deciduous tree belonging to the Salicaceae family. Being native to the Indian subcontinent and Southeast Asia, this fast-growing tree thrives in tropical and subtropical climates (1, 2). It is characterized by ovate to lanceolate, glossy green leaves that turn reddish as they mature. This plant bears tiny, greenish-yellow flowers and is dioecious. The tree produces small, round, reddish-purple to brownish-purple fruits with a sweet-tart flavour (3, 4). Adaptable to various soil types, the Indian coffee plum is primarily found in natural vegetation and forests and is rarely planted in orchards. However, it is often planted as a natural fence due to its thorny branches (5, 6). Although the fruits have much nutritional and medicinal value, the plant is not under cultivation and moreover, its thorny nature, small-sized fruit and growth under forest or natural vegetation have placed this fruit plant in the underutilized category (7, 8). Ripe fruits are relished fresh and are also used to prepare good quality jelly and jam (9, 10).

Beyond its edible and ornamental value, the Indian Coffee Plum is also known for its medicinal properties. The fruit has been traditionally valued for its medicinal properties, offering a range of health benefits (3,5). Packed with antioxidants and essential nutrients, the fruit is known for its ability to support digestion and strengthen the immune system (9,11). Due to its astringent nature, it is often used to ease digestive issues such as diarrhoea and dysentery (1,12). Additionally, the fruits' high vitamin C and high antioxidant content help boost immunity, promote healthy skin and fight against cancer (13,14). The bark and leaves contain

antimicrobial and anti-inflammatory compounds, making them helpful in treating wounds, infections and fevers (6, 15). Herbal remedies often utilize bark extracts to help regulate blood sugar levels and manage hypertension (16, 17).The plant is also recognized for its liver-protective properties, which aid in detoxification and enhance overall circulation (1, 10). In folk medicine, decoctions made from the leaves and roots are used to relieve joint pain and rheumatism (5, 12). Some traditional practices also apply crushed leaves to soothe skin irritations and insect bites (4, 18). The fruits' natural analgesic properties can help alleviate mild pain, while its antioxidant-rich composition contributes to overall wellness (11, 14).

The Indian coffee plum is naturally distributed across tropical and semitropical regions of India, particularly in the North eastern states and *Terai* forests of North Bengal, Western Ghats and Eastern Ghats (2, 6). It thrives in diverse ecological conditions, including forest edges and riverbanks (1). Studies on its distribution reveal its adaptability to varying climatic and soil conditions. Diversity assessments, based on morphological, biochemical and genetic markers, indicate significant variation among populations (17). These findings emphasize the species' potential for conservation, domestication and genetic improvement. Its rich genetic diversity makes it valuable for breeding programs focused on fruit quality, stress tolerance and adaptability to different agro-climatic zones of India (18).

The Terai region of West Bengal falls under the humid subtropical climate (Cfa) according to the Köppen climate classification. This region, located at the foothills of the Eastern Himalayas, experiences distinct seasonal variations. The high humidity and significant rainfall support dense forests, tea plantations and rich biodiversity, making the Terai region ecologically significant (5, 7). Natural vegetation and the contiguous forest area play as the reservoir of flora diversity, including many underutilized fruits (15, 16). Distribution of Indian coffee plum in natural greenery, household gardens and roadside fallow jungles is common in the districts of Jalpaiguri, Alipurduar and Cooch Behar (6, 7). Although the existence of diversity in Indian coffee plum in this region has been primarily reported, no attention has been paid to this neglected fruit in terms of its quality aspects and the profiling of bioactive compounds (7, 9). Despite the lack of scientific research, the fruit is locally popular for its unique sweet and sour taste, rich nutritional and medicinal properties (3, 10). Considering the importance of the fruit and the availability of a wide base population, the present study aimed to assess the diversity analysis of selected Indian coffee plum plants collected from *the Terai* natural vegetation of West Bengal, focusing on fruit morpho-biochemical traits and bioactive compounds.

Materials and methods

Selection of plants and collection of fruits

As the Indian coffee plum is naturally distributed across in the North eastern states and *Terai* forests of North Bengal, the present study was performed on 20 different Indian coffee plum individuals (selected through transect sampling), aged between 10 to 15 years, from natural vegetation of Cooch Behar, Alipur Duar and Jalpaiguri district of West Bengal which come under *Terai* region, during the year 2022-23 and 2023-24. The GPS coordinates of the plants were recorded using a handheld Garmin GPS 12H device (Table 1 and Fig. 1). The fully mature, ripe fruits were brought to the Department of Horticulture and Post-harvest Technology, Institute of Agriculture, Visva-Bharati, Sriniketan, West Bengal, India for further morphological and biochemical analysis as detailed below. Five composite samples containing ten fruits from each selected plant have been taken into account for observation of all fruit morphological, biochemical and bio-active properties of all Indian coffee plum trees under the present experiment.

Morphological characters

The morphological traits of the fruit samples were assessed using standard physical measurement techniques to ensure consistency and accuracy. For each composite sample, data were recorded from five randomly selected fruit units or fruit clusters, depending on the trait being measured. The average of these five values of each observation was used to represent each parameter.

Table 1. Geographical position of the selected Indian coffee plum plants

Sl. No.	Indian Coffee plum plant	GPS Location		Place
		Latitude (°N)	Longitude (°E)	
1.	ICPG-C1	26.35	89.68	Tufanganj, Cooch Behar, WB, India
2.	ICPG-C2	26.36	89.47	Pundibari, Cooch Behar, WB, India
3.	ICPG-C3	26.43	89.39	Cooch Behar Town, Cooch Behar, WB, India
4.	ICPG-C4	26.15	89.48	Dinhata, Cooch Behar, WB, India
5.	ICPG-C5	26.09	89.45	Baro Atia Bari, Cooch Behar, WB, India
6.	ICPG-C6	26.19	89.18	Sitalkhuchi, Cooch Behar, WB, India
7.	ICPG-C7	26.37	89.22	Angarkata, Cooch Behar, WB, India
8.	ICPG-J1	26.54	89.02	Dhupguri, Jalpaiguri, WB, India
9.	ICPG-J2	26.56	88.82	Maynaguri, Jalpaiguri, WB, India
10.	ICPG-J3	26.51	88.72	Jalpaiguri Town, Jalpaiguri, WB, India
11.	ICPG-J4	26.58	88.67	Denguajhar, Jalpaiguri, WB, India
12.	ICPG-J5	26.64	88.44	Binnaguri, Jalpaiguri, WB, India
13.	ICPG-J6	26.88	88.73	Malbazar, Jalpaiguri, WB, India
14.	ICPG-J7	26.77	88.50	Junglee Mahal, Jalpaiguri, WB, India
15.	ICPG-A1	26.58	89.78	Raydaak, Alipurduar, WB, India
16.	ICPG-A2	26.74	89.39	Hasimara, Alipurduar, WB, India
17.	ICPG-A3	26.72	89.16	Birpara, Alipurduar, WB, India
18.	ICPG-A4	26.54	89.22	Gairkata, Alipurduar, WB, India
19.	ICPG-A5	26.52	89.54	Falakata, Alipurduar, WB, India
20.	ICPG-A6	26.62	89.03	Bara Saulmari, Alipurduar, WB, India

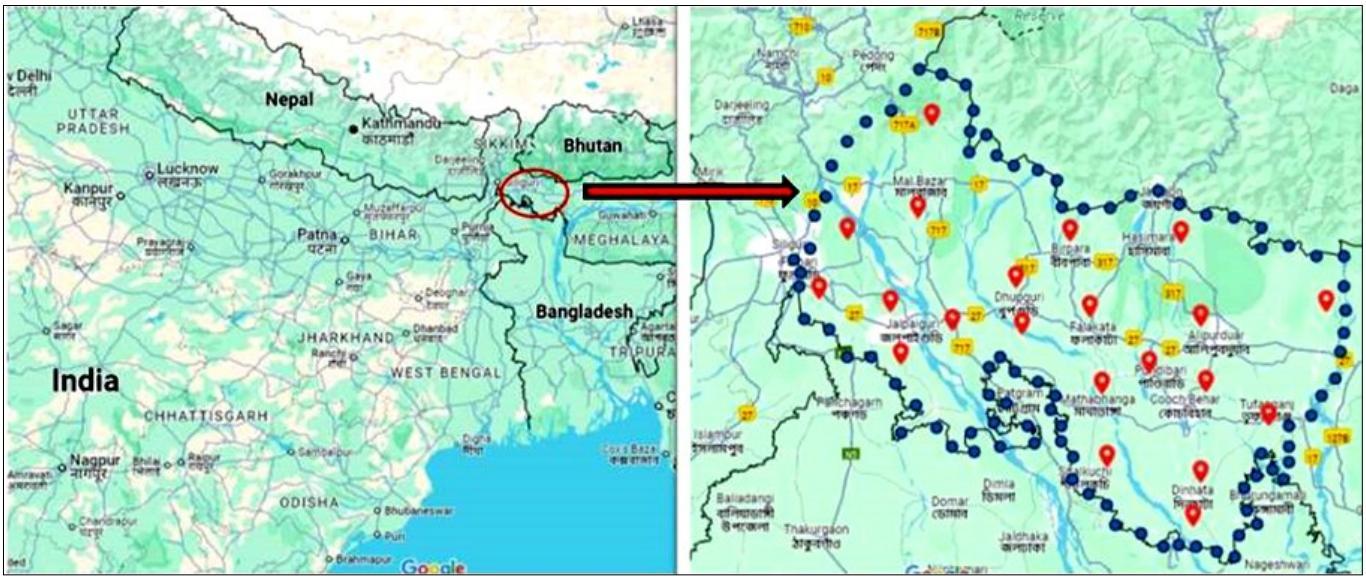

Fig. 1. Geographical locations of Indian coffee plum plants under present study.

Fruit length and diameter

Fruit length and diameter were measured using a precision vernier caliper (±0.01 mm accuracy). The length was recorded as the distance from the fruit base to the apex, while the diameter was measured at the widest part of the fruit, perpendicular to its length. Each fruit was measured individually and values were averaged to obtain a representative dimension.

Fruit weight

Fruit weight was determined using a calibrated digital balance (accuracy ±0.01 g). Each of the five selected fruits was weighed separately and the mean value was calculated to express the fruit weight for the composite sample.

Seed count per fruit

Seed count per fruit was obtained by manually extracting and counting the seeds from each of the five sampled fruits. Care was taken to ensure that all seeds, including partially developed ones, were included in the count.

Number of fruits per bunch

The number of fruits per bunch was evaluated by selecting five representative clusters. The total number of fruits in each cluster was counted and the mean value was calculated.

Biochemical parameters

Following biochemical parameters of the collected fruits were observed:

Total soluble solids (TSS)

TSS was measured using a digital refractometer (Konica Minolta, Japan; range: 0-65 ° Brix). A drop of freshly extracted juice from each fruit sample was placed on the prism surface of the refractometer and the reading was recorded in degrees Brix (°B), which reflects the concentration of soluble sugars and other dissolved solids. The instrument was calibrated with distilled water before each session to ensure accuracy.

Titratable acidity

Titratable acidity was determined following the acid-base titration method outlined by Rangana (19). A known volume (10 mL) of fruit juice was diluted with distilled water and titrated against 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator. The endpoint was noted by the appearance of a faint pink colour, which persisted for at least 30 seconds. The acidity was calculated as a percentage of citric acid equivalent per 100 mL of juice.

Reducing sugar

Reducing sugars were quantified using the standard method (19). The procedure involved reaction with Fehlings' solution under heat, where reducing sugars reduce the copper ions to form a red precipitate. The volume of juice required to reduce a known volume of Fehlings' solution was recorded and the results were calculated and expressed as a percentage of reducing sugars by weight.

Ascorbic acid

Ascorbic acid content was estimated using the indophenol dye method as described by (20). Fresh plant tissue (5 g) was homogenized in 3 % metaphosphoric acid, filtered and titrated against standard indophenol dye until a pink endpoint was reached. Results were expressed in mg/100 g fresh weight.

Antioxidant activity

Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, as described (21). One millilitre of methanolic extract was mixed with 3 mL of 0.1 mM DPPH solution and incubated in the dark for 30 minutes. Absorbance was recorded at 517 nm using a double-beam UV-Visible spectrophotometer (LABMAN, LUV2000T, India). The percentage inhibition of DPPH radicals was calculated to assess antioxidant potential.

Bioactive compounds

Different bioactive compounds of Indian coffee plum fruits have been estimated as follows:

Total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method (22). In brief, 0.5 mL of extract was mixed with 2.5 mL of 10 % Folin-Ciocalteu reagent and 2 mL of 7.5 % sodium carbonate. After incubation in the dark for 30 minutes, absorbance was read at 765 nm. A standard curve using quercetin was used to express results as mg of quercetin equivalents per 100 grams of sample (mg QE/100 g).

Flavonoid content

Flavonoid content was quantified using the aluminium chloride colourimetric method (19, 53, 54). 1 mL of methanolic fruit extract was mixed with 0.3 mL of 5 % $NaNO_2$, followed by the addition of 0.3 mL of 10 % $AlCl_3$ after 5 min. Subsequently, 2 mL of 1 M NaOH was added after 6 minutes and the final volume was adjusted to 10 mL with distilled water. Absorbance was measured at 510 nm using a UV-Visible spectrophotometer (LABMAN, LUV2000T, India). Quercetin was used as a standard and results were expressed as mg quercetin equivalents per gram (mg QE/g) of fresh weight.

Anthocyanin content

Anthocyanin content was estimated following the pH differential method (23). Fruit extract was diluted separately in buffers at pH 1.0 (potassium chloride) and pH 4.5 (sodium acetate). Absorbance was measured at 520 and 700 nm by a UV -visible spectrophotometer (LABMAN, LUV2000T, India) and total anthocyanin content was calculated using the molar extinction coefficient of cyanidin-3-glucoside and expressed as µg per 100 g FW.

Carotenoid content

Carotenoid content was determined according to the method of Lichtenthaler and Wellburn (24). Fresh tissue (1 g) was homogenized in 80 % acetone and centrifuged. Absorbance of the supernatant was read at 470 nm by a UV-visible spectrophotometer (LABMAN, LUV2000T, India) and carotenoid concentration was calculated and expressed as mg per 100 g of sample.

Statistical analysis of data

The observed data (2022-23 and 2023-24) were processed through descriptive statistics and analysis of variance, following the method proposed and using statistical software SPSS (Statistical Package for Social Sciences, IBM SPSS Version 27) for correlation, PCA, Bi-plot and cluster analysis (25).

Results and Discussions

Fruit physical parameters

The genotypic variations in fruit physical parameters reveal valuable information for selecting plants suitable for different horticultural and commercial purposes (Table 2).

Number of fruits per cluster

The fruits clustering pattern of Indian coffee plum individuals (Table 2) varied significantly among plants, ranging from 1.34 (ICPG-J7) to 3.58 (ICPG-J4)fruits per cluster. Some of the plants expressed very high numbers of fruits per cluster, such as ICPG-J4 (3.58 fruits/cluster), ICPG-A4 (3.50) and ICPG-A1 (3.49), which appeared to be more prolific in fruit production. In contrast, plants like ICPG-J7 and ICPG-J5 have very few fruits per cluster, indicating larger fruit size and better resource allocation. Higher fruit clustering ability in Indian coffee plum plants, indicating a high fruit-bearing capacity, is in line with findings in *Flacourtia indica*, where notable fruit set was associated with enhanced reproductive success and higher economic returns (26). On the contrary, Indian coffee plum plants with a minimum number of fruits per cluster, but larger fruits, were similar to *Flacourtia montana*, where fewer but larger fruits were found to have superior market preference (27). The recorded variation in fruit clustering suggests an inverse relationship between the number of fruits per cluster and individual fruit size, a common trade-off in fruit crops influenced by source-sink dynamics (28).

Fruit size

A high diversity of fruit size (length and diameter) was also exhibited among the Indian coffee plum plants in the present study (Table 2). The longest (22.4 mm) and widest (23.7 mm) fruits wereobserved in the plant ICPG-J7, followed by ICPG-J5 (21.1 mm length, 23.2 mm diameter, Fig. 2) and ICPG-C5 (20.5 mm length, 22.0 mm diameter), which may be considered ideal for table use. Conversely, the smallest fruits were observed in ICPG-A4 and ICPG-A1 (14.7 mm in length with a 16.0 mm diameter and 14.9 mm in length with a 16.2 mm diameter, respectively). The overall mean fruit length across all plants (17.74 mm) and the mean fruit diameter (19.10 mm) indicated that the majority of the plants fall within anintermediate fruit size. The suitability of larger fruits of Indian coffee plum aligns with consumer preference for table use, which aligns with reports on *Flacourtia jangomas* accessions from northeastern India (18). On contrary, smaller-sized fruits as recorded in ICPG-A5 and ICPG-A1, may be advantageous where high fruit numbers per plant are desirable for breeding or processing purpose, as seen in *Flacourtia rukam* (29). The inverse relationship between fruit clustering and fruit size, as observed in some Indian coffee plum plants, is similar to that noted in Flacourtia montana, where a high fruit load reduces individual fruit development due to reduced resource allocation (18).

Fruit weight

The average fruit weight across plants was 5.96 g, with a standard deviation of 0.92 g, indicating moderate variation in fruit weight (Table 2). The heaviest fruit was noted in ICPG-J7 (7.82 g), followed by ICPG-J5 (7.54 g) and ICPG-C5 (7.10 g). These plants, combined with their larger size, are preferable for table use and the processing of value-added products. In contrast, lightest fruits were noted in the plants like ICPG-A4 (4.37 g), ICPG-A1 (4.75 g) and ICPG-J4 (4.88 g). These plants may be suitable for applications where the quantity per tree is of greater importance.ICPG-J7, ICPG-J5 and ICPG-C5 exhibited the highest fruit weight, making them suitable for both fresh markets and processing industries, similar to findings in *Flacourtia indica*, where heavier fruits had better pulp yield and processing quality (30). Lighter fruits, such as those in ICPG-A4 and ICPG-A1, may be preferred for high-density orchards, as observed in *Flacourtia rukam*, where small-fruited plants demonstrated higher cumulative yields per tree (31). The variation in fruit clustering suggests the involvement of genetic factors controlling inflorescence architecture and fruit set, a finding similar to those in *Flacourtia indica*, where genotypic differences influenced reproductive traits (32). Fruit size and weight, which exhibit significant variability, are known to be controlled by quantitative trait loci (QTLs) that regulate cell division and expansion. Similar QTLs have been identified in *Flacourtia rukam* and other minor fruit crops, influencing fruit elongation and width (33).

Table 2. Fruit morphological diversity of different Indian coffee plum plants under Terainatural vegetation of West Bengal

Indian coffee plum plants (ICPG)	No. of fruits/ cluster	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	No. of seeds/ fruit	10 seed weight (g)
ICPG-C1	2.35	18.5	19.8	6.28	7.8	2.31
ICPG-C2	2.69	17.4	18.5	5.79	6.6	2.35
ICPG-C3	2.09	18.7	19.8	6.65	7.7	2.18
ICPG-C4	2.95	17.1	18.6	5.77	6.8	2.12
ICPG-C5	1.48	20.5	22.0	7.10	9.2	2.10
ICPG-C6	2.96	17.0	18.2	5.70	6.4	2.20
ICPG-C7	3.31	15.8	17.1	5.02	6.1	2.16
ICPG-J1	3.46	16.8	18.2	5.36	6.3	2.20
ICPG-J2	1.86	19.2	20.5	6.88	8.7	2.28
ICPG-J3	2.88	17.4	18.7	5.82	6.8	2.02
ICPG-J4	3.58	15.3	16.4	4.88	5.8	2.41
ICPG-J5	1.42	21.1	23.2	7.54	9.6	2.40
ICPG-J6	3.00	16.2	17.6	5.14	6.3	2.27
ICPG-J7	1.34	22.4	23.7	7.82	11.1	2.01
ICPG-A1	3.49	14.9	16.2	4.75	5.5	2.39
ICPG-A2	3.07	16.6	18.0	5.49	6.5	2.26
ICPG-A3	2.54	18.1	19.5	6.11	7.5	2.15
ICPG-A4	3.50	14.7	16.0	4.37	5.6	2.39
ICPG-A5	1.77	19.6	21.2	6.92	8.9	2.20
ICPG-A6	2.66	17.6	18.9	5.98	6.9	2.22
SD	0.71	2.01	2.11	0.92	1.46	0.11
Mean	2.62	17.74	19.10	5.96	7.30	2.23

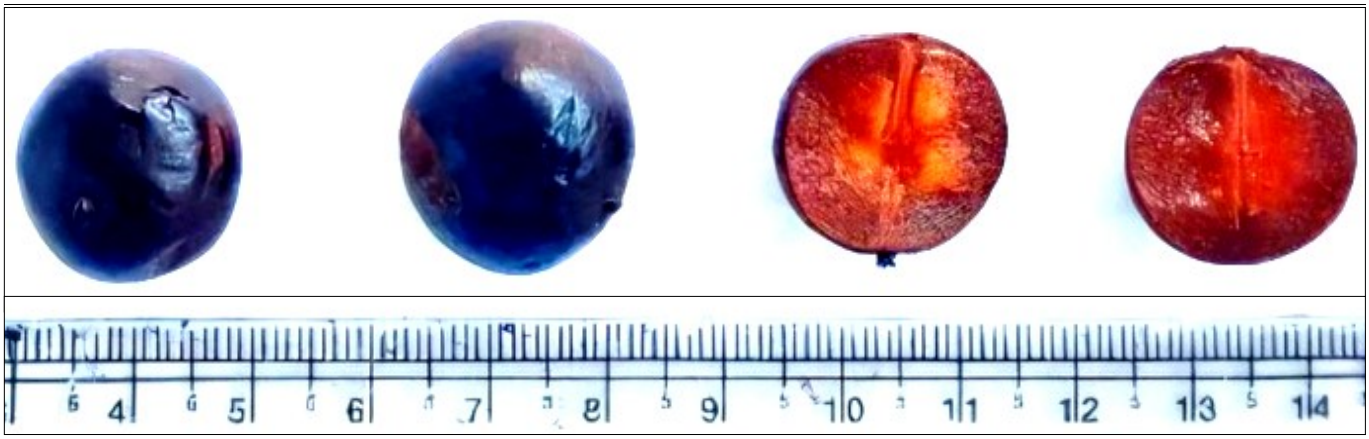

Fig. 2. Normal and cross sectional view of the fruits of Indian coffee plum plant ICPG-J2.

Seed count and seed weight

The number of seeds per fruit of Indian coffee plum plants (Table 2) ranged from 5.5 (ICPG-A1) to 11.1 (ICPG-J7). Higher seed counts are primarily associated with larger fruit sizes, as observed in ICPG-J7, ICPG-J5 (9.6) and ICPG-C5 (9.2). Plants ICPG-A1 (5.5), ICPG-J4 (5.8) and ICPG-A4 (5.6) have fewer seeds per fruit, exhibited them potentially suitable for direct consumption. On the other hand, 10-seed weight has been observed to be relatively stable across Indian coffee plum plants, ranging between 2.01 g (ICPG-J7) and 2.41 g (ICPG-J4), with a mean of 2.23 g. This indicated variability in the number of seeds per fruit, along with consistency in individual seed weight. Plants with higher seed counts have also been reported in *Flacourtia jangomas* and fruits with high seed content are favourable for germplasm conservation and breeding (34). Although plants that produce fewer seeds per fruit are desirable for direct consumption, similar to findings in *Flacourtia indica* (35). The relatively stable 10-seed weight across plants (2.01 g to 2.41 g) suggests uniformity in seed development, which is beneficial for breeding programs focused on seedling vigour and genetic improvement (32). Seed number, a crucial determinant of consumer preference, also showed genotypic variation, suggesting genetic control over ovule fertilization and seed development, as reported in *Flacourtia indica*(31).

Fruit biochemical parameters

The analysis of biochemical characters and bioactive compounds across 20 Indian coffee plum plants from the Terai region of West Bengal provides significant insights into their nutritional and functional properties (Table 3).

Total Soluble Solids (TSS)

The Indian Coffee Plum plants exhibited an average TSS content of 8.48 °Brix (table 3). The sweetest among them were ICPG-J2 (10.9 °Brix), ICPG-J7 (10.8 °Brix) and ICPG-J5 (10.7° Brix), making them ideal for fresh consumption. In contrast, ICPG-J4 (6.2 °Brix) and ICPG-C7 (6.4 °Brix) recorded the lowest TSS, indicating a comparatively milder sweetness. The observed range of 6.2 to 10.9 °Brix aligns with findings in other *Flacourtia* species, where TSS variation was linked to genetic factors and ripening stages (18). Higher TSS levels, as recorded in ICPGJ2 and ICPG-J7, make these plants ideal for fresh consumption, similar to high-TSS selections in *Flacourtia indica* (31). Lower TSS plants, such as ICPG-11, may be more suitable for processing applications that incorporate additional sugar.

Acidity

With an average acidity of 0.45 % and a low standard deviation of 0.03, the plants displayed relatively stable acidity levels (Table 3). The highest acidity was observed in ICPG-J7 (0.52 %) and ICPG-C5 (0.51 %), contributing to a tangy flavour profile.

Meanwhile, ICPG-7 (0.40 %) had the lowest acidity, making it a milder variety. The recorded range (0.40-0.52 %) suggests moderate acidity, similar to values reported in *Flacourtia montana* (36). ICPG-J7 and ICPG-C5 exhibited the highest acidity, providing a tangy taste desirable for processing. Lower acidity in ICPG-C7 suggests a milder flavour, a trait preferred for direct consumption, aligning with studies on *Flacourtia rukam* (26).

Reducing sugar

The plants demonstrated a mean reducing sugar content of 4.60 %, with moderate variability (SD = 0.52) (Table 3). ICPG-J2 (5.80 %), ICPG-J7 (5.39 %) and ICPG-J3 (5.25 %) stood out with the highest sugar levels, enhancing their natural sweetness. In contrary, ICPG-C1 and ICPG-J1 contained the least amount of reducing sugars (4.02 % and 4.09 % respectively). The high levels of reducing sugar in ICPG-J2 and ICPG-J7 suggest a sweeter taste, similar to that of high-sugar plants in *Flacourtia jangomas* from northeastern India (34).

Ascorbic acid

Considerable variation has been observed in the ascorbic acid content of Indian coffee plum plants, with an average value of 130 mg/100g (Table 3). ICPG-J5 and ICPG-C4 emerged as excellent sources of ascorbic acid (156.7 mg/100g and 155.6 mg/100g), while ICPG-J4and ICPG-A1 contained the lowest amounts of ascorbic acid (100.5 mg/100g and 107.1 mg/100g). This aligns with reports in *Flacourtia indica*, where ascorbic acid-rich plants exhibited enhanced antioxidant properties (37). ICPG-J5 and ICPG-C4 demonstrated superior vitamin C content, supporting their potential as functional foods for boosting immunity.

Antioxidant activity

The antioxidant activity of the Indian coffee plum plants has exhibited high variation (Table 3). ICPG-J5 and ICPG-C4 possessed the highest antioxidant potential (88.7 and 88.45 % inhibition), making them suitable for functional food applications. On the contrary, ICPG-J4 recorded the lowest inhibition rates (63.9%).

Bioactive compounds

Total phenolics

A notable range in total phenolic content was observed in the present study for Indian coffee plum plants, with an average of 235.5 mg GAE/g (Table 3). The highest phenolic concentrations were recorded in ICPG-J5 (278.6 mg GAE/g) and the lowest content was found in ICPG-J4 (145.7 mg GAE/g). The high antioxidant activity in ICPG-J5 and ICPG-C4 mirrors findings in *Flacourtia montana* and *Flacourtia jangomus*, where strong DPPH inhibition was associated with high phenolic content (30, 38, 39). Phenolic content, ranging from 145.7 to 278.6 mg GAE/g, further reinforces the functional potential of these plants.

Flavonoid content

Flavonoid content of Indian coffee plum plants varied moderately with average value of 35.44 mg QE/g (Table 3). ICPG-A2 had the highest flavonoid levels(168.7 mg QE/g) denotes greater antioxidation capabilities. The lowest flavonoid amount was noted in ICPG-A4 (99.6 mg QE/g).

Anthocyanin content

The determination of anthocyanin revealed significant variation, with an average value of 44.3 µg/100 g (Table 3). The most pigment-rich Indian coffee plum plants were ICPG-J2 (56.4 µg/100g) and ICPG-16 (54.9 µg/100g), made them valuable fruit rich in natural colour. Meanwhile, ICPG-J4 had the very low anthocyanin content(33.5 µg/100g).

Carotenoid content

The maximum carotenoid content has been recorded in ICPG-A2 (1.611 mg/100g), indicating its higher nutritional value (Table 3). On contrary, ICPG-15 evidenced the least carotenoid content(1.135 mg/100g). The very high variability of various bioactive compounds, such as ascorbic acid, total phenolics and antioxidation capacity, in the present experiment indicates strong genotypic differences and potential for selective breeding in Indian coffee plum plants. The plants like

Table 3. Biochemical characters and bioactive compound diversity of different Indian coffee plum plants under Terai natural vegetation of West Bengal

Indian coffee plum plants (ICPG)	TSS (°Brix)	Acidity (%)	Reducing sugar (%)	Ascorbic acid (mg/100g)	Antioxidant activity (DPPH % inhibition)	Total phenolics (mg GAE/g)	Flavonoid content (mgQE/g)	Anthocyanin content (µg/100g)	Carotenoid content (mg/100g)
ICPG-C1	9.2	0.47	4.02	118.3	73.5	225.6	87.1	41.5	1.314
ICPG-C2	8.3	0.41	4.17	134.9	78.9	243.2	138.5	43.8	1.148
ICPG-C3	8.5	0.42	4.30	128.5	80.3	266.8	163.9	52.3	1.497
ICPG-C4	9.6	0.45	4.56	155.6	88.4	253.2	152.2	45.0	1.560
ICPG-C5	8.8	0.51	4.35	133.4	85.3	272.8	156.3	51.6	1.543
ICPG-C6	6.7	0.43	5.11	111.5	73.1	216.7	120.6	36.1	1.311
ICPG-C7	6.4	0.40	4.20	118.2	71.6	209.1	111.8	36.3	1.201
ICPG-J1	7.8	0.47	4.09	136.8	76.5	231.3	132.5	41.7	1.346
ICPG-J2	10.9	0.48	5.80	143.7	82.5	267.5	158.9	56.4	1.528
ICPG-J3	7.2	0.46	5.25	115.4	69.6	198.4	85.1	37.1	1.162
ICPG-J4	6.2	0.44	4.28	100.5	63.9	145.7	99.6	33.5	1.147
ICPG-J5	10.7	0.48	4.44	156.7	88.7	278.6	158.3	48.7	1.565
ICPG-J6	8.4	0.44	5.03	142.9	82.6	243.7	127.4	39.2	1.475
ICPG-J7	10.8	0.52	5.39	152.4	87.4	261.0	152.1	50.5	1.419
ICPG-A1	6.9	0.45	4.24	107.1	68.3	182.9	91.2	40.2	1.135
ICPG-A2	9.3	0.48	4.38	126.8	81.6	272.2	168.7	54.9	1.611
ICPG-A3	7.8	0.46	5.19	138.7	80.9	239.7	122.3	40.1	1.311
ICPG-A4	6.3	0.47	4.13	110.2	76.5	221.5	118.4	39.6	1.177
ICPG-A5	8.6	0.46	5.21	140.5	81.6	237.0	141.7	42.4	1.415
ICPG-A6	7.9	0.43	5.08	129.4	78.0	230.3	129.2	42.7	1.323
SD	1.50	0.03	0.52	16.8	7.42	36.28	3.07	7.03	0.367
Mean	8.48	0.45	4.60	130.1	78.3	235.5	35.44	44.3	1.372

ICPG-J5, ICPG-C4 and ICPG-J2 are cognizant with higher levels of multiple bioactive compounds, fitting them ideal for applications in pharmaceuticals and functional food. The considerable presence of flavonoids, anthocyanins and carotenoids contributes to the antioxidant and nutraceutical properties of Indian coffee plum plants. The highest flavonoid levels, as found in ICPG-16 and ICPG-9, are similar to those in flavonoid-rich selections of *Flacourtia rukam* (40). The anthocyanin-rich Indian coffee plum plants, such as ICPG-9 and ICPG-16, show potential for natural colourant applications, as observed in *Flacourtia montana* (41). The carotenoid-rich plants of Indian coffee plum (ICPG-A2 and ICPG-J5)emerge as strong pro-vitamin A sources, aligning with studies on *Flacourtia indica* (42).

The notable variation in TSS and reducing sugars indicates genetic divergence in carbohydrate metabolism of Indian coffee plum plants, governed by enzymes such as sucrose synthase and invertase. Plants with high TSS, such as ICPG-J2 (Fig. 3) and ICPG-J7, May likely possess allelic variations that favour higher sugar accumulation. Similar finding has been reported in high-sugar selections of *Flacourtia jangomas* (34). Acidity, as the critical parameter for flavour balance, exhibits relatively low variation, indicating a strong genetic component that regulates organic acid biosynthesis. A similar report has been observed in *Flacourtia montana* by Patel et al. (36). The diverse array of ascorbic acid and antioxidant activity in Indian coffee plum plants exhibits differential expression of genes involved in the biosynthesis of ascorbic acid and phenolic metabolism. Plants ICPG-J5 and ICPG-C4 exhibited the highest antioxidant activity, likely characterized with enhanced expression of polyphenol oxidase and flavonoid biosynthetic genes, as reported in *Flacourtia indica* (30, 43). The genetic variability in the secondary metabolite pathway was also noted in Indian coffee plum fruits under the present experiment, with high total phenolics and flavonoids. The relation between antioxidant activity and phenolic content are similar to the *Flacourtia rukam*, where polyphenol accumulation was influenced by genetic variation (40). The considerable high carotenoid levels in the Indian coffee plum plants of the present study suggest divergences in terpenoid biosynthesis, aligning with studies in *Flacourtia indica* (42).

Correlation analysis

The correlation analysis of Indian coffee plum plants in the present study revealed significant interrelationships among physical and biochemical traits, providing valuable insights for selection and breeding programs (Table 4 and Fig. 4). A strong negative correlation was observed between the number of fruits per cluster with fruit length (-0.966), fruit diameter (-0.964) and fruit weight (-0.973) expressing plants producing more fruits per cluster tend to have smaller size. A strong positive correlation has been noted between the number of seeds per fruit and fruit length (+0.982) and weight (+0.966), implying that larger fruits tend to have more seeds. Total soluble solids (TSS) established a positive correlation with fruit diameter (+0.791) and fruit weight (+0.794), indicating that larger fruits are generally sweeter. Acidity of fruits exhibited a moderate positive correlation with the number of seeds per fruit (+0.643), suggesting that more-seeded fruits might have slightly more acidity. Sugar accumulation may have little influence on the dimensions of fruits, as evidenced by the positive correlation between sugar content and TSS (+0.338). A strong positive

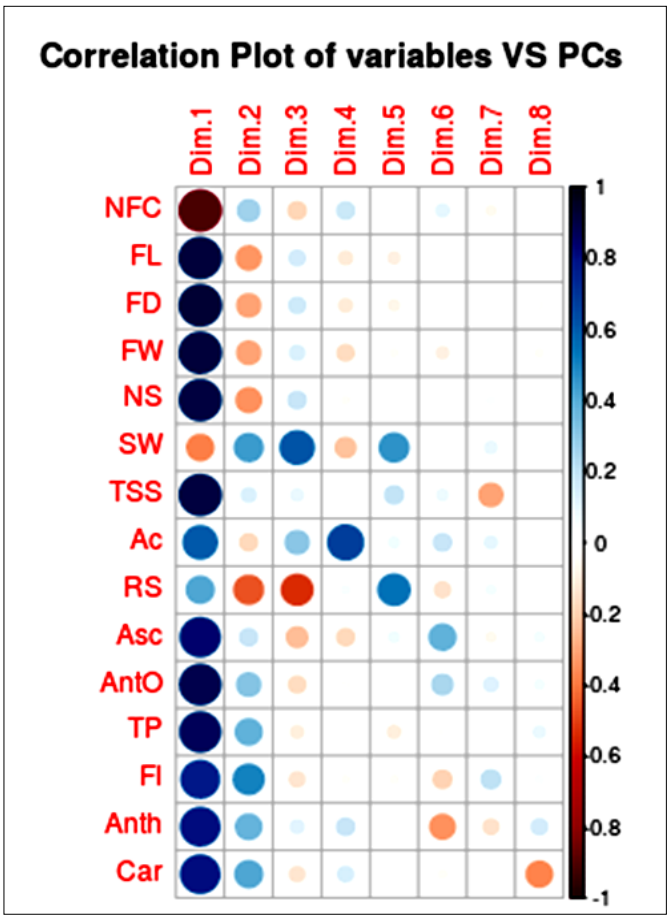

Fig. 4. Correlation plot of variables (different fruit physical, biochemical parameters and bioactive compounds) against different principal components.

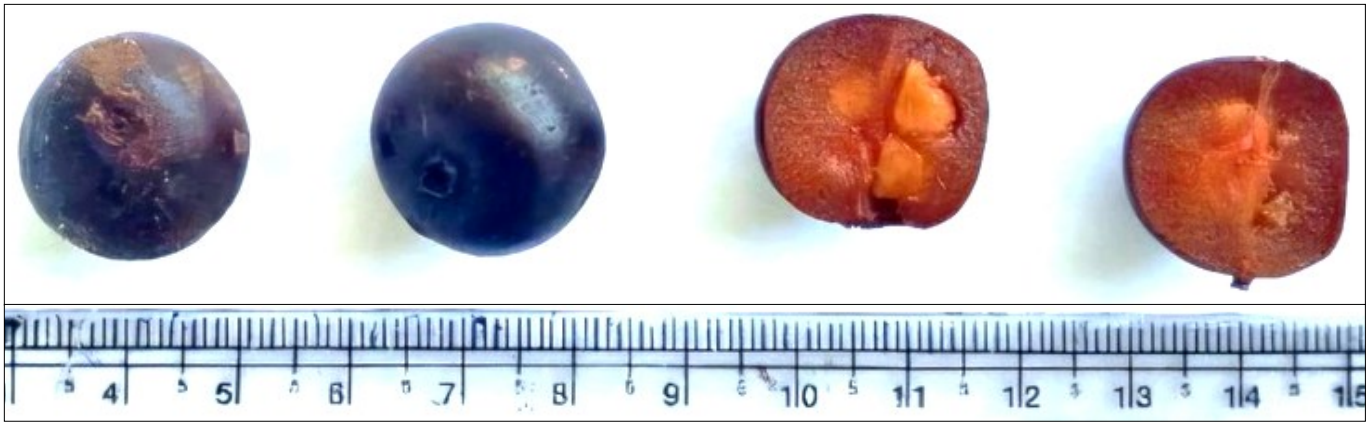

Fig. 3. Normal and cross sectional view of the fruits of Indian coffee plum plant ICPG-J5.

Table 4. Correlation analysis of different morphological, biochemical parameters and bioactive compounds of Indian coffee plum plants *Terai* natural vegetation of West Bengal

	No. of fruits/ cluster	Fruit length	Fruit diameter	Fruit weight	No. of seeds/ fruit	10 seed weight	TSS	Acidity	Reducing sugar	Ascorbic acid	Antioxidant	Total phenolics	Flavonoid content	Anthocyanin content	Carotenoid content
No. of fruits/ cluster	1														
Fruit length	-0.966*	1													
Fruit diameter	-0.964*	0.996*	1												
Fruit weight	-0.973*	0.989*	0.985*	1											
No. of seeds/ fruit	-0.959*	0.982*	0.979*	0.966*	1										
10 seed weight	0.319	-0.423	-0.397	-0.396*	-0.389	1									
TSS	-0.769*	0.781*	0.791*	0.794	0.787*	-0.173	1								
Acidity	-0.520	0.579	0.594	0.521	0.643*	-0.228	0.569	1							
Reducing sugar	-0.408	0.401	0.387	0.429	0.428	-0.446*	0.338	0.223	1						
Ascorbic acid	-0.638	0.675	0.701*	0.666*	0.659*	-0.311*	0.818*	0.357	0.359	1					
Antioxidant	-0.685*	0.676	0.701*	0.663	0.670*	-0.284	0.804*	0.460*	0.272	0.915*	1				
Total phenolics	-0.689*	0.660	0.674*	0.663	0.626*	-0.264	0.792*	0.408	0.191	0.784*	0.915*	1			
Flavonoid content	-0.556	0.535	0.543	0.556	0.520	-0.164	0.674	0.297	0.165	0.705*	0.830*	0.856*	1		
Anthocyanin content	-0.645	0.605	0.600	0.635	0.608	-0.141	0.807*	0.510*	0.163	0.566	0.704*	0.836*	0.829*	1	
Carotenoid content	-0.579	0.559	0.584	0.586	0.549	-0.232	0.770*	0.449*	0.227	0.709*	0.816*	0.834*	0.823*	0.769*	1

(Level of significance P=0.05*).

correlation was noted between antioxidant activity and total phenolic content (+0.915), revealing the significant positive role of phenolic compounds in determining the antioxidant potential of the Indian coffee plum plants. Additionally, flavonoid content and anthocyanin content have demonstrated strong positive correlation (+0.830 and +0.704) with antioxidant activity. Carotenoid content of Indian coffee plum plants exhibited a considerable positive correlation with total phenolic content (+0.834) and flavonoid content (+0.823), which denotes the co-expression of bioactive compounds in certain plants. Moreover, anthocyanin content is also valued for its strong positive correlation with total phenolics (+0.836), further demonstrating its potential for antioxidant properties.

The correlation analysis in Indian coffee plum (*Flacourtia jangomas*) plants highlights the probable genetic influences, interactions and co-expression of various fruit morphological, biochemical composition and bioactive compounds. The strong negative correlation between fruit clustering and fruit size (-0.966 to -0.973) suggests a possible genetic trade off controlled by regulatory genes influencing sink strength and resource partitioning, similar to findings in *Flacourtia indica* (32). The strong positive correlation between fruit weight, length and diameter (+0.989, +0.985) indicates posiibility of genetic co-regulation of cell expansion genes, as reported in *Flacourtia rukam* (37). The association of TSS with fruit size (+0.791) suggests probable genetic linkage between sugar metabolism and fruit growth, aligning with studies on *Flacourtia montana* (8). The high correlation between antioxidant activity and phenolic content (+0.915) suggests shared genetic pathways governing secondary metabolite biosynthesis, similar to minor fruit crops like *Garcinia indica* (44). Marker-assisted selection targeting these traits can optimize breeding for high-quality fruit production.

Principal component analysis and Bi-plot

Key genetic variability in Indian coffee plum plants was revealed by Principal Component Analysis (PCA) for trait-based selection in breeding programs (Table 5). It is evident from the Scree Plot (Fig. 5) that the first principal component (PC1) accounts for the highest variance (~60-70%), followed by the second principal component (PC2), which exhibits a notable decrease. The variance contribution is minimal from the third principal component (PC3) onwards, forming an elbow that retains essential information. The PC1 expresses the most variation, positively correlating with fruit length, fruit diameter, fruit weight, total soluble solids, total phenols and antioxidants, representing overall fruit size and quality. PC2 differentiates plants based on reducing sugar (RS) and seed weight (SW), indicating a sugar-seed weight trade-off. The independent variability of seed weight was observed in the third and fifth principal components (PC3 and PC5).

In contrast, the fourth principal component (PC4) showed a strong reflection of acidity, distinguishing between acidic and non-acidic plants. Fruit morphological and biochemical traits, including bioactive properties, are interrelated, with larger fruits generally having higher phenolic and antioxidant content. On contrary, sugar accumulation (reducing sugar) is negatively correlated to flavonoids and anthocyanins, suggesting a biochemical trade-off between sweetness and pigmentation. Considering the breeding

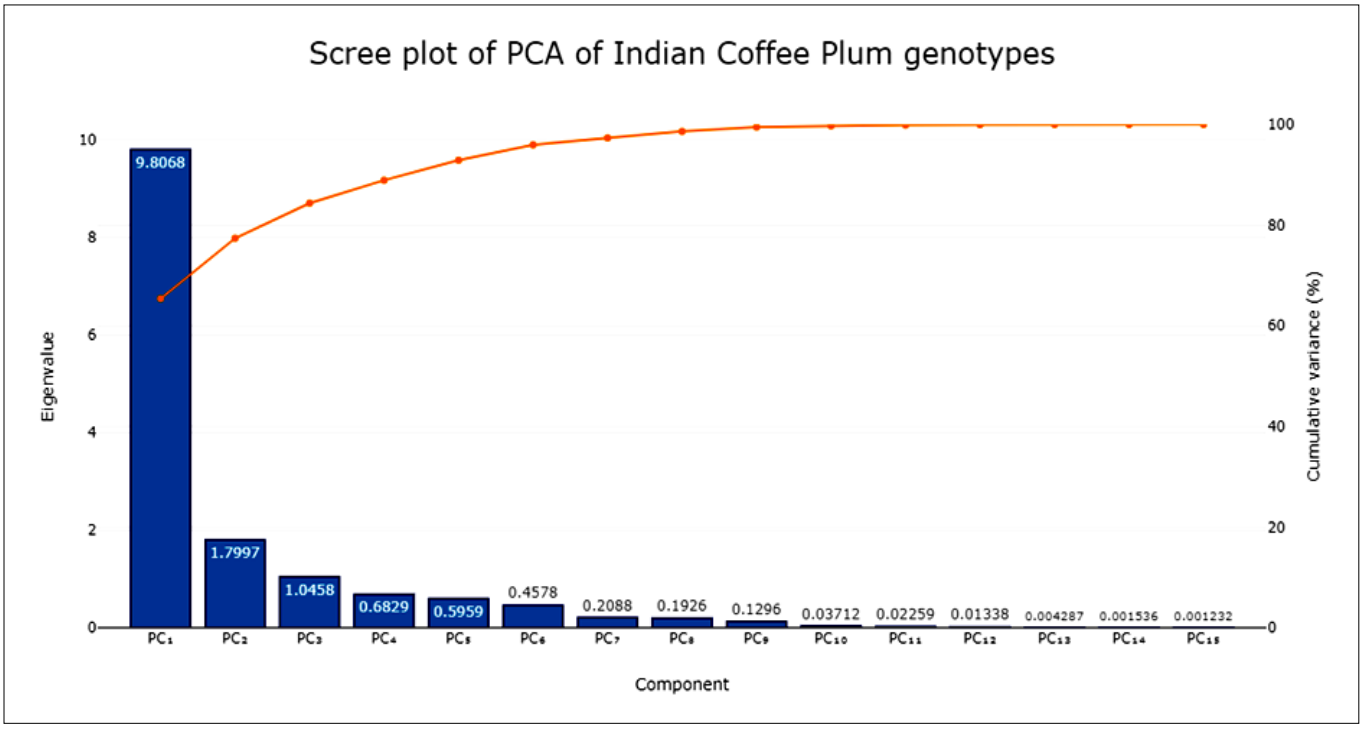

Fig. 5. Scree plot of Principal Component Analysis of Indian coffee plum plants.

Table 5. Principal component analysis of of different morphological, biochemical parameters and bioactive compounds of Indian coffee plum plants *Terai* natural vegetation of West Bengal

Components	PC_1	PC_2	PC_3	PC_4
Eigenvalue	9.80	1.79	1.04	0.68
% of Variance	65.37	11.99	6.97	4.55
Cumulative (%)	65.37	77.37	84.34	88.90
ICPG-C1	-0.516	-0.9198	2.027	0.008
ICPG-C2	-0.985	0.9658	0.445	-1.735
ICPG-C3	1.671	1.1216	-0.114	-0.950
ICPG-C4	1.396	1.6857	-1.604	0.125
ICPG-C5	3.930	-0.2613	0.808	0.983
ICPG-C6	-2.103	-0.9198	-0.886	-0.292
ICPG-C7	-3.528	-0.1479	-0.794	-0.862
ICPG-J1	-1.178	0.8298	-0.113	0.734
ICPG-J2	3.819	0.2639	-0.245	0.338
ICPG-J3	-2.087	-2.8159	-1.028	0.748
ICPG-J4	-5.359	-0.7571	1.229	0.021
ICPG-J5	4.821	0.664	1.771	-1.131
ICPG-J6	-0.632	1.0725	-1.339	-0.186
ICPG-J7	5.901	-2.261	0.015	0.686
ICPG-A1	-4.537	0.007545	1.093	0.467
ICPG-A2	1.026	2.7607	-0.077	1.507
ICPG-A3	0.335	-0.9572	-0.973	-0.060
ICPG-A4	-3.708	1.1631	0.742	0.932
ICPG-A5	2.188	-1.1995	-0.177	-0.693
ICPG-A6	-0.452	-0.295	-0.776	-0.640

strategies, PC1 denotes selection for high-yield, antioxidant-rich plants, PC2 for sweeter fruits andPC4 for acidity-based preferences. Finally, the PCA effectively classified genotypic variations, aiding in the selection of superior Indian coffee plum cultivars with desirable fruit characteristics. The strong loadings of fruit size, TSS and antioxidants in PC1 align with previous studies that emphasise the co-expression of morphological and biochemical parameters in other crops(45). The PC2 sugar-seed trade-off suggests metabolic regulation, consistent with genetic linkage of carbohydrate metabolism and seed traits(46).This PCA Bi-plot guided selection will help breeders to enhance fruit quality and yield through multi-trait selection strategies(47).

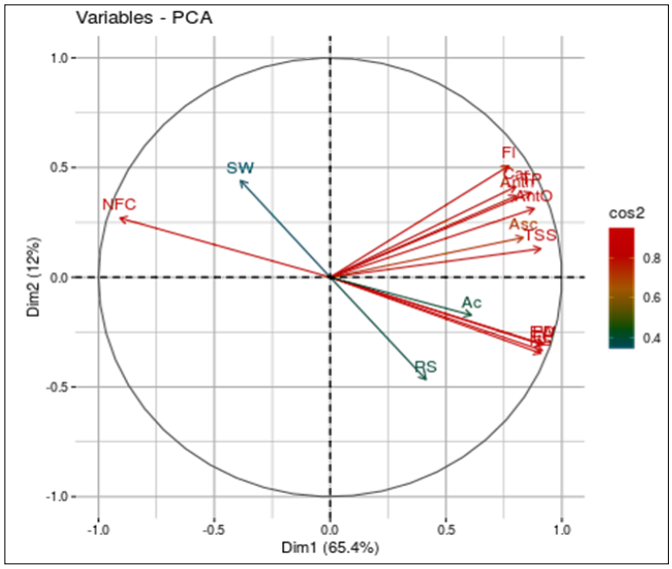

Fig. 6. Bi-plot of variables (different fruit physical, biochemical parameters and bioactive compounds) against two major principal components.

The PCA Bi-plot effectively visualises the variation and relationships among different traits and plants of Indian coffee plum (Fig. 6-8). The first dimension (Dim1: 65.4 %) accounts for the highest variance, while the second dimension (Dim2: 12 %) adds further differentiation. Traits such as fruit length (FL), fruit diameter (FD) and total soluble solids (TSS) are closely clustered, indicating a strong correlation. This suggests that selecting for one of these traits may simultaneously influence the others. In contrast, seed weight (SW) and the number of fruit clusters (NFC) exhibit a distinct spread, implying a more independent inheritance. Plants like ICPG-J7 (ICPG-14) and ICPG-A2 (ICPG-16) are positioned far from the centre, indicating unique trait expressions, making them valuable for breeding programs. Their distinctiveness suggests genetic divergence, which could be explored for hybridization or conservation strategies. Research indicates that PCA helped identify trait-group relationships and genetic improvement strategies.

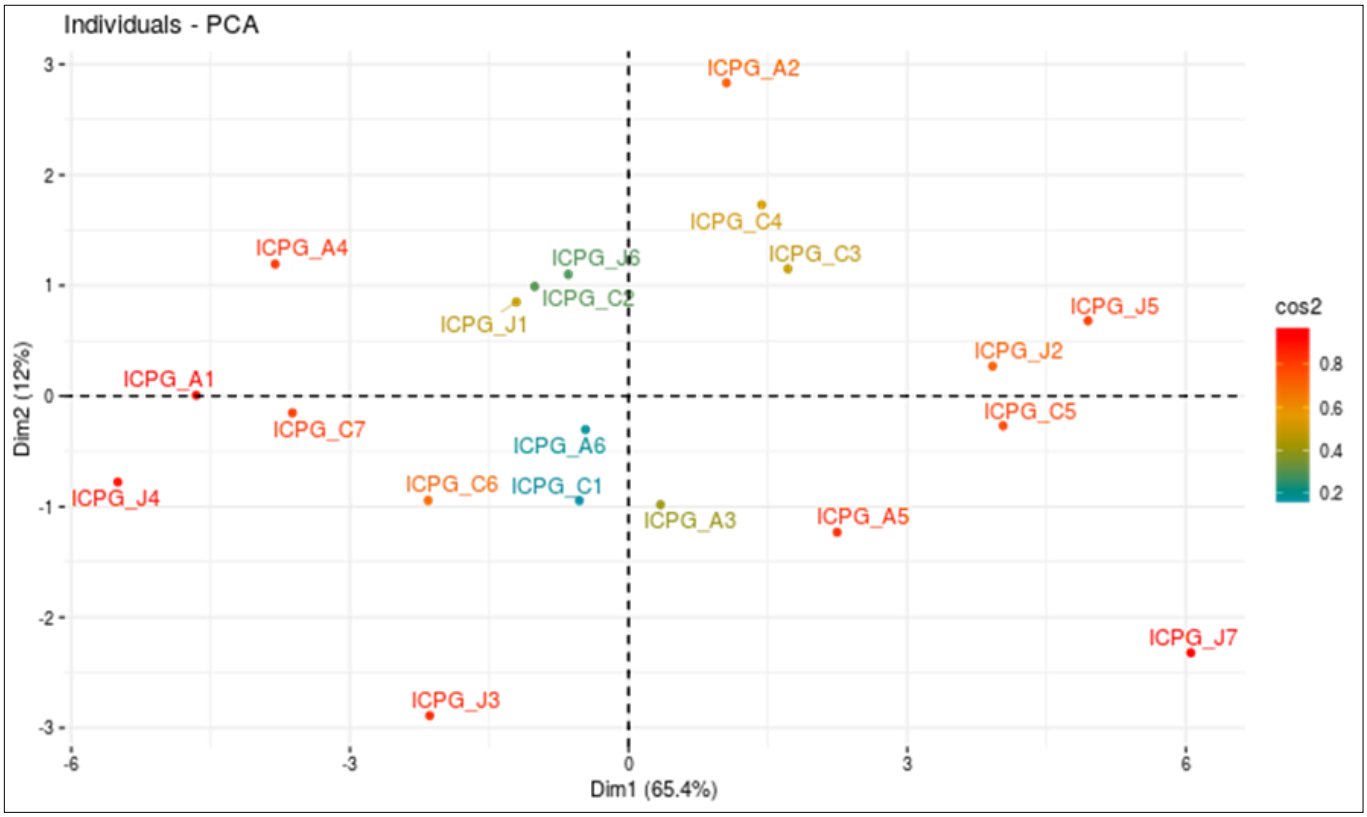

Fig. 7. Bi-plot of different Indian coffee plum plants against two major principal components.

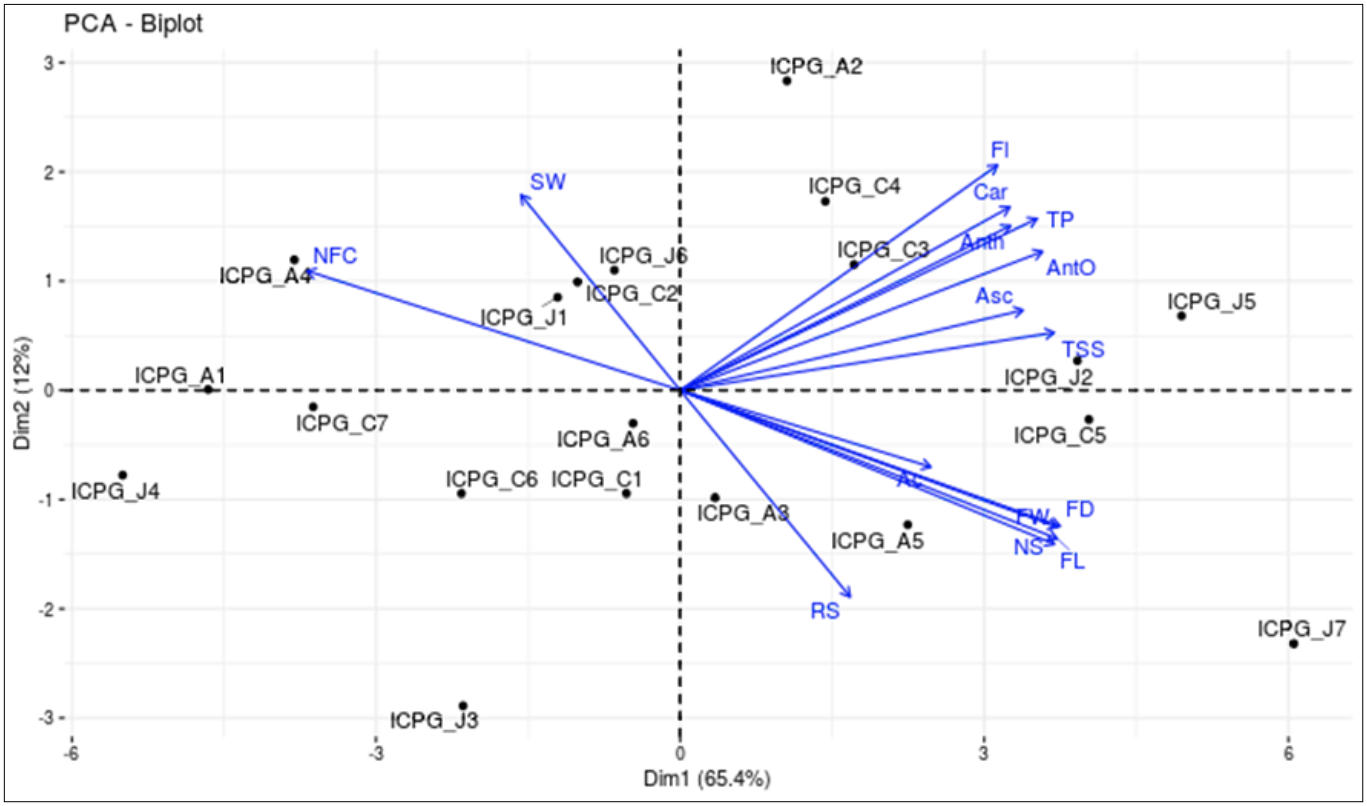

Fig. 8. Bi-plot of different variables (different fruit physical, biochemical parameters and bioactive compounds) and different Indian coffee plum plants against two major principal components.

Additionally, integrating molecular markers enhanced selection efficiency by linking PCA data with genetic variations (44, 48, 49). As no direct PCA studies on *Flacourtia jangomas* have been found, this study suggests its usefulness in evaluating genetic diversity, aiding in conservation and enhancing breeding strategies for yield and quality (50).

Hierarchical clustering

Hierarchical clustering analysis was of Indian coffee plum plants based on their fruit morphological and biochemical characteristics segmented the dendrogram into three distinct clusters (Fig. 9), as represented by different colours (red, green and blue). The clustering pattern is strongly influenced by the correlation between fruit physical and biochemical properties including bioactive compounds (Fig. 10). Cluster-I (Red) comprises the Indian coffee plum plants with larger fruit size and high yield and includes eight different plants viz.ICPG-J7, ICPG-

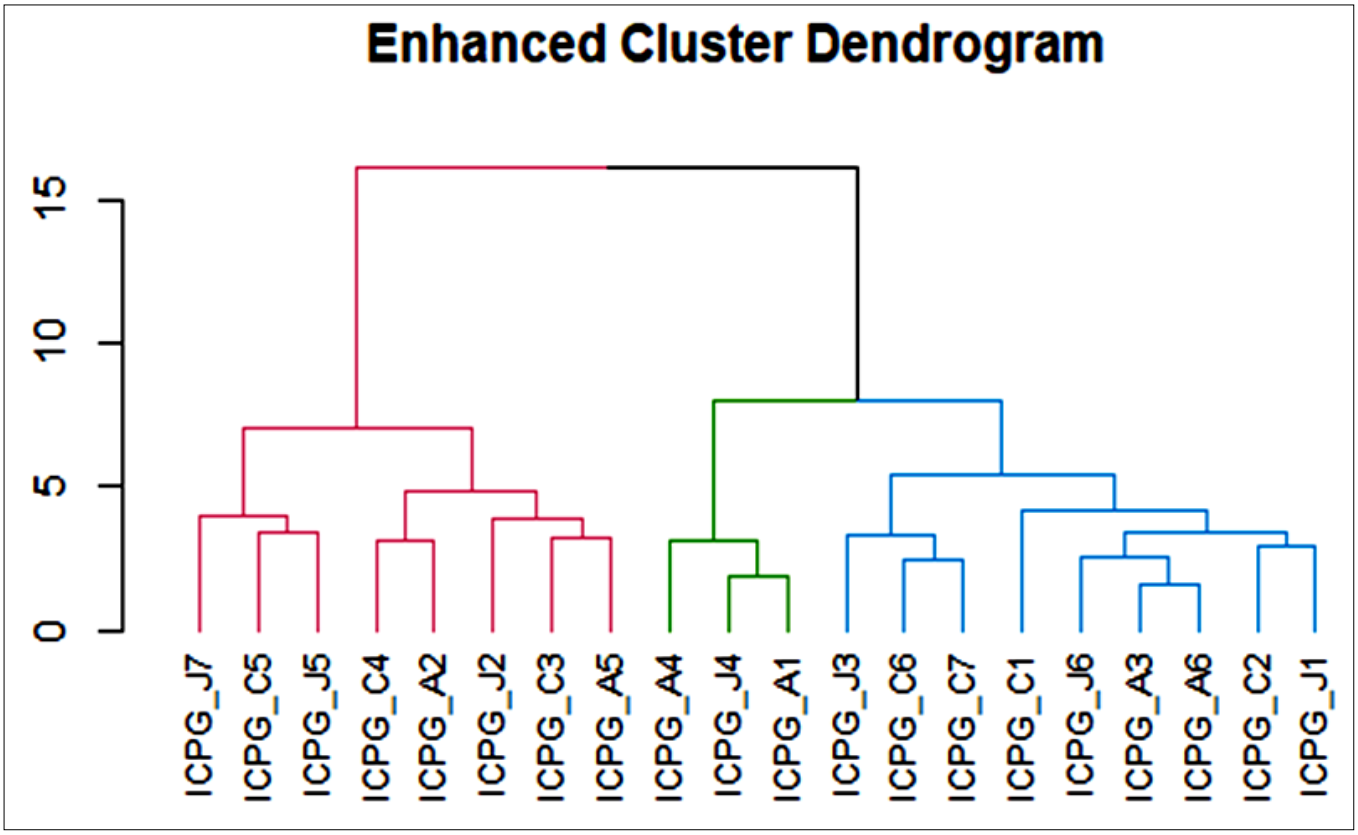

Fig. 9. Enhanced cluster Dendogram of different Indian coffee plum plants.

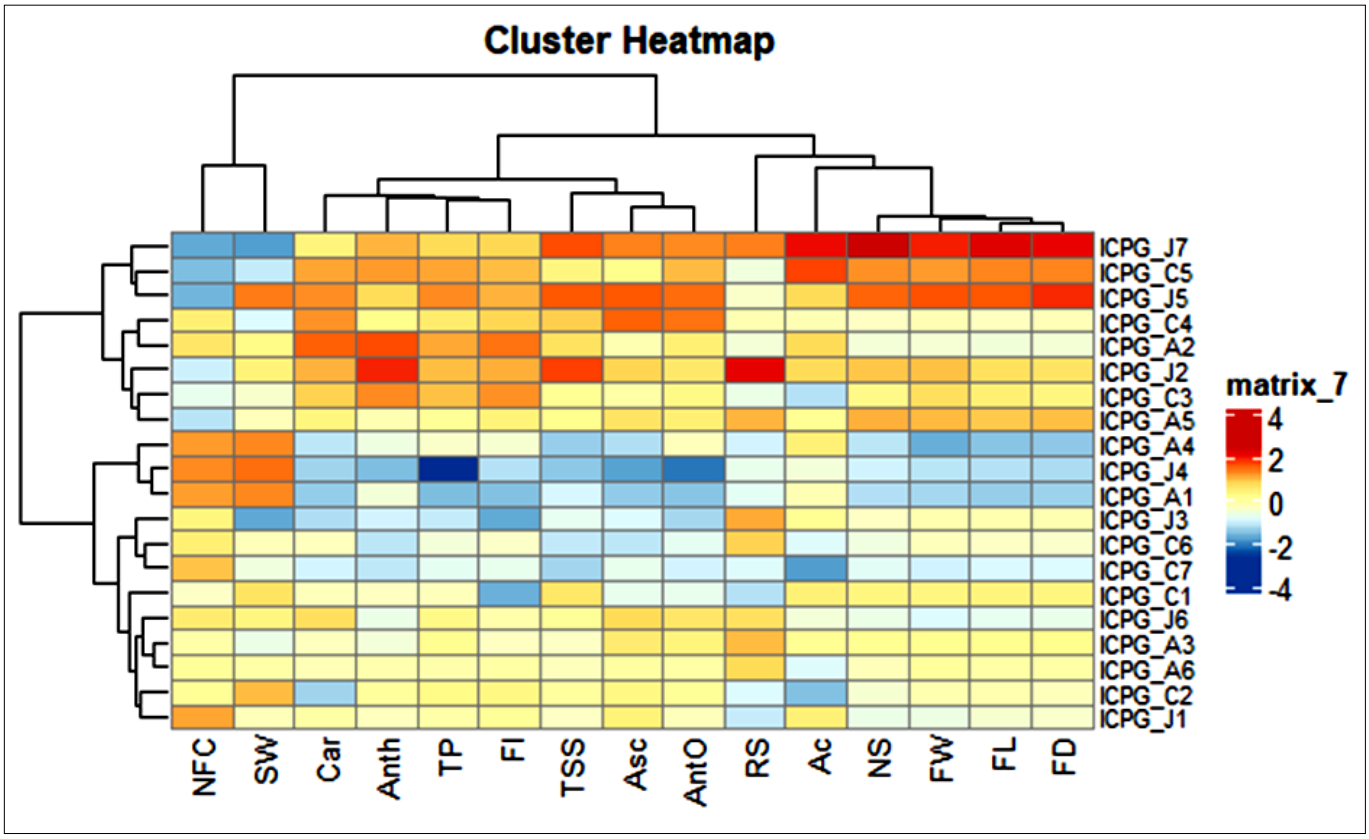

Fig. 10. Two way Hierarchical Clustering heat map of of different Indian coffee plum plants.

C5, ICPG-J5, ICPG-C4, ICPG-A2, ICPG-J2, ICPG-C3 and ICPG-A5. These plants possessed higher number of fruits per cluster but relatively smaller fruit size. Cluster-II (Green) is intermediate group of Indian coffee plum with balanced traits. This cluster includes three plants likeICPG-A4, ICPG-J4 and ICPG-A1,serve as a bridge between the high-yielding varieties and those with superior biochemical composition. Moderate fruit size and weight, made them ideal for fresh consumption and processing purpose. Cluster-III (Blue) is composed of Indian Coffee Plum plants with higher biochemical quality and includes eight plants namely ICPG-J3, ICPG-C6, ICPG-C7, ICPG-C1, ICPG-J6, ICPG-A3, ICPG-A6, ICPG-C2 and ICPG-J1.These plants exhibit a strong positive correlation with TSS, antioxidants, total phenolics, flavonoids and carotenoid content. Lower number of fruits per

cluster but higher content of bioactive compounds expressed them suitable for pharmaceuticals, applications in functional foods and processing for value-added products.

Hierarchical clustering of Indian coffee plum plants in the present study revealed three distinct clusters with respect to genetic divergence in fruit development, metabolic pathways and bioactive compound accumulation. Cluster-I comprised high -yielding plants with smaller fruit size, reflecting strong genetic control over fruit set and resource partitioning, as observed in *Flacourtia indica* (32). Cluster-II involved intermediate plants with balanced traits of yield and quality likely governed by polygenic inheritance, aligns to findings in *Flacourtia rukam* (37). Cluster-III integrating the plants with higher bioactive compounds likely having genetic regulation of secondary metabolite pathways, similar to antioxidant-rich accessions in *Flacourtia montana* (8, 51, 52). Three major clusters based on fruit size, sweetness and fibre content has been identified in recent clustering studies in jackfruit and many other crop species where fruit morphology and biochemical traits defined the groups (55, 56, 57), similar to the clustering observed in Indian coffee plum in the present experiment. A clustering study on ber (*Ziziphus mauritiana*) has been based on antioxidant content and yield, comparable to Cluster-III in Indian coffee plum, where bioactive-rich plants were grouped separately (18). Similar clustering were also reported in citrus (*Citrus spp.*), plants based on vitamin C, acidity and phenolic content (35, 58).

Conclusion

The study of Indian coffee plum plants revealed significant variations in the physical and biochemical parameters of their fruit, impacting their suitability for commercial and horticultural applications. ICPG-J5 is ideal for the commercial fresh fruit market due to its large heavier fruit and excellent biochemical properties, ascorbic acid and strong antioxidant activity. ICPG-J7 stands out for processing purposes, as it has the largest fruit size, highest weight and a balanced sweet-tangy profile. ICPG-J2 and ICPG-A2 are the best choices for functional food applications, due to their richness in flavonoids, anthocyanin content and strong antioxidant properties, making them valuable for health-focused products. Correlation analysis indicated that higher fruit clustering resulted in smaller fruit size, while larger fruits were heavier and had higher sweetness. Antioxidant activity strongly correlated with total phenolic content. Hierarchical clustering grouped plants into three clusters: high-yielding but smaller-fruited varieties, balanced plants and those with superior biochemical properties suited for nutraceutical applications. The study highlights the potential of selective breeding for optimizing fruit size, yield and nutritional value in Indian coffee plum plants.

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Authors' contributions

PD[1] conceptualized, conducted experimentation, analysed data and prepared the manuscript. PM, PD[2], UT, PS, AM and NB collected the fruit samples and conducted experimentation. PD[1]:Prahlad Deb and PD[2]: Payel Das.

Compliance with ethical standards

Conflict of interest: All authors declare no conflict of interest.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work/manuscript the author(s) used ChatGPT to improve the language and correct grammatical mistakes. After using this tool/service, the author (s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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