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RESEARCH ARTICLE

Genetic diversity assessment among Darjeeling mandarin accessions through fruit quality traits grown in Eastern Himalaya regions of India

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Abstract

This study aims to assess the genetic diversity among mandarin accessions grown in the Darjeeling and Kalimpong regions through the analysis of key fruit quality traits. A comprehensive evaluation of 17 orchards over two seasons (2020-21 and 2020-22) measured 18 traits, including fruit weight, volume, pulp weight and total phenol content. The results revealed considerable variation in the studied traits, with high coefficients of variation observed for total phenol content (68.2 %) and number of seeds (33.4 %). Significant correlations were identified among fruit traits, highlighting genetic factors as primary drivers of diversity, with minimal environmental influence. Principal Component Analysis (PCA) and cluster analysis further classified the germplasm into six distinct clusters, emphasizing the genetic distinctiveness of accessions such as DL and KR. Correlation results depicts that fruit traits such as fruit weight is not associated with biochemical traits such as Total Soluble Solids (TSS), Total Phenol Content (TPC), Total Flavonoid Content (TFC) and antioxidants through 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and by Ferric Reducing Antioxidant Power (FRAP) assay. In other words, bigger fruit size does not necessarily have high biochemical traits. Also, huge variability exists within the mandarin accessions. These high variability, diversity and structure could be utilized for citrus breeding programme, which may be helpful in breeding varieties with high yield and nutritional properties. Thus, genetic variability provides a valuable resource for breeding programs aimed at improving fruit quality and supporting agricultural sustainability in the region.

Keywords: accessions; diversity; mandarin; physio-chemical traits; variability

Introduction

Multiple nutrient shortages (including iron, zinc, iodine and vitamin A) arise in a diet high in calories and energy but low in nutrients. A synonym for this is "hidden hunger". Over two billion people globally are impacted by reliance on low-cost staple foods and inadequate dietary diversity (1). Thus, nutritional security which refers to the intake of food enriched with essential nutrients in an adequate amount is a topic of grave concern from the health perspective of human beings and livestock (2, 3). Citrus fruits are one such fruit known for high nutritional values and various health promoting effects which are due to their abundance of nutrients and bioactives (4). Citrus fruits have significant nutritional benefits due to the presence of carbohydrates, minerals, vitamins and dietary fibre. Among the citrus fruits, mandarins are a good source of organic acid and phenolic compounds. The nature and concentration of these compounds play a significant role in determining taste and overall organoleptic quality (5).

Citrus, a key fruit crop globally, is cultivated in over 130 countries, predominantly in tropical and subtropical regions (6). According to the horticultural field, the worldwide citrus fruit is divided into four groups: sweet orange, mandarin, grapefruit & pummelo and the common acid members (lemon, lime and citron) (7). Due to the utilization and global marketing of the hard to peel citrus fruits such as orange, grapefruit and pummelo, there is a reduction in consumption of such fruit with an increase in consumption of simple to peel mandarin (8). Over the past decade, there has been a steady increase in consumption and worldwide marketing of mandarins that are easy to peel, with an anticipated annual production of over 24 million tons. In India, the loose skinned mandarins represent about 45 % of total citrus fruit area (9) (NHB, 3rd Estimate). It is the most economically important and popular fruit intended for the fresh market. The Eastern Himalayan region of India, with its diverse climatic conditions, offers a unique environment for the cultivation of mandarin accessions. Mandarins, a major citrus species, exhibit significant variability in fruit quality traits, which can be utilized for improving breeding programs and enhancing agricultural sustainability. The Eastern Himalayan region also has a large variability of mandarin

germplasm and is the primary gene centres of citrus worldwide. These variations in plant types, fruit quality parameters and other characters vary from different location and from single location. The observed variation was not only from environmental factors but also genetic basis which was reflected in the Random Amplified Polymorphic DNA (RAPD) profile from different locations (10). Reports suggest that large genetic differences in fruit colour and carotenoid pigments, bioactive compounds and aroma volatile contents between different citrus groups and even among varieties (11-15). However, to the best of our knowledge, no comprehensive systematic study has yet been conducted that explicitly assessed the variation in fruit quality attributes among different mandarin subgroups and types. Recent developments in citrus genome sequencing and genomics (16, 17) make it essential to do a high throughput phenotypic analysis of citrus quality traits to provide accurate comparisons between phenotypic and genomic data. Also, to provide a scientific basis for farmers and consumers to plant and choose citrus varieties with excellent nutritional quality, a comprehensive evaluation and comparison of the properties of citrus fruits are necessary. The study of genetic diversity through fruit quality traits helps in identifying superior accessions that are well-adapted to regional environmental conditions. This research focuses on assessing the genetic diversity of mandarin accessions based on key fruit quality traits, grown in the Eastern Himalayan region. Therefore, a comprehensive study of fruit quality traits in mandarin accession from Darjeeling and Kalimpong hills was conducted. The result provided reflects the significant variation in fruit quality characteristics among the mandarin accessions.

Material and Methods

In the present investigation, fruits of mandarin were collected during harvesting season (December to January) 2020-21 and 2021-22 from 17 mandarin orchards (Fig. 1). Fully mature/ripe fruits were collected when it has developed full orange colour and most important flavour. The fruits were harvested manually by hand picking with the help of a ladder (where harvesting of citrus fruit is done by pulling or clipping from the stem) with twist, jerk and pull method. The details of place of collection are given in Table 1. Fruits from all directions were collected from each tree, a total number of 10 fruits (4 big, 3 medium and 3 small size) were harvested from each tree and from an orchard total 50 fruits were collected.

The age of the orchards ranged from 20 to 25 years. Fruits and trees after collection were labelled. The fruits were then washed and dried for analysis. Analysis was carried out at the Biochemistry Laboratory of IARI regional station, Kalimpong, West Bengal.

Methodology for fruit morphological traits

The fruit weight (FW), pulp weight (PuW), peel weight (PW) and seed weight (SW) were recorded using an electronic weighing balance (QUINTIX224, Sartorius Lab instruments GmbH & Co. KG Goettingen, Germany) and expressed in grams. Fruit diameter, fruit breadth and peel thickness were measured using Vernier calipers (Mitutoyo Absolute, Kawaski, Japan) and expressed in millimetres.

Preparation of juice sample for biochemical analysis

The mandarin fruit juice was extracted by cutting the fruit in half and careful hand-squeezing to obtain the juice. The juice was passed through a strainer to remove pulp and seeds. The freshly squeezed juice was centrifuged at 3000 g for 10 min and the supernatant was stored at -20 °C for further analysis.

Methodology for fruit biochemical trait measurement

For the biochemical trait measurement TSS (Total Soluble Solids), Titrable Acidity (TA) and ascorbic acid content were measured.

To determine TSS (Total Soluble Solids) a drop of mandarin juice was placed on the prism of the digital refractometer (HI 96801, Hanna Instrument Inc., Romania) and expressed in degree Brix (°Brix) (18).

Titrable acidity was expressed as percent acid (titrating with 0.1 N NaOH). Pipette out 1mL of juice sample to that add 4 mL of distilled water and again dilute it 5 mL of distilled water. To the diluted sample add 30 µL of phenolphthalein indicator solution and titrate with 0.1 N sodium hydroxide solution until the pink colour develops (19).

Table 1. List of mandarin accession, collection site along with coordinates

S. NO	CODE	LATITUDE°	LONGITUDE°	ELEVATION(MSL)	COLLECTION SITE
1	BTL	26.8995	88.2113	1045.5	Silu Goan, Mirik
2	OL	26.9001	88.2099	975.6	Silu Goan, Mirik
3	STL	26.8996	88.2119	1050.61	Silu Goan, Mirik
4	LSHG	26.8971	88.1739	1578.88	Silu Goan, Mirik
5	PTL	26.9008	88.2108	975.79	Silu Goan, Mirik
6	CS	26.9697	88.3683	1173.83	Labda, Mungpoo
7	TT	26.9662	88.3669	1173.92	Labda, Mungpoo
8	PC	26.9693	88.3677	1173.85	Labda, Mungpoo
9	RP	26.9668	88.3686	1038.91	Labda, Mungpoo
10	STL	26.8996	88.2118	1050.61	Labda, Mungpoo
11	KR	26.9291	88.3731	971.83	Sittong –I, Kurseong
12	PR	26.9292	88.3736	668.93	Sittong –I, Kurseong
13	DL	26.9292	88.3734	966.34	Sittong –I, Kurseong
14	DY	26.9018	88.2117	891.06	Sittong –I, Kurseong
15	SS	26.8961	88.2023	1191.22	Mirik
16	AS	26.8959	88.2022	1182.12	Mirik
17	YK	27.0592	88.4715	1158.23	Barbot, Bong Busty

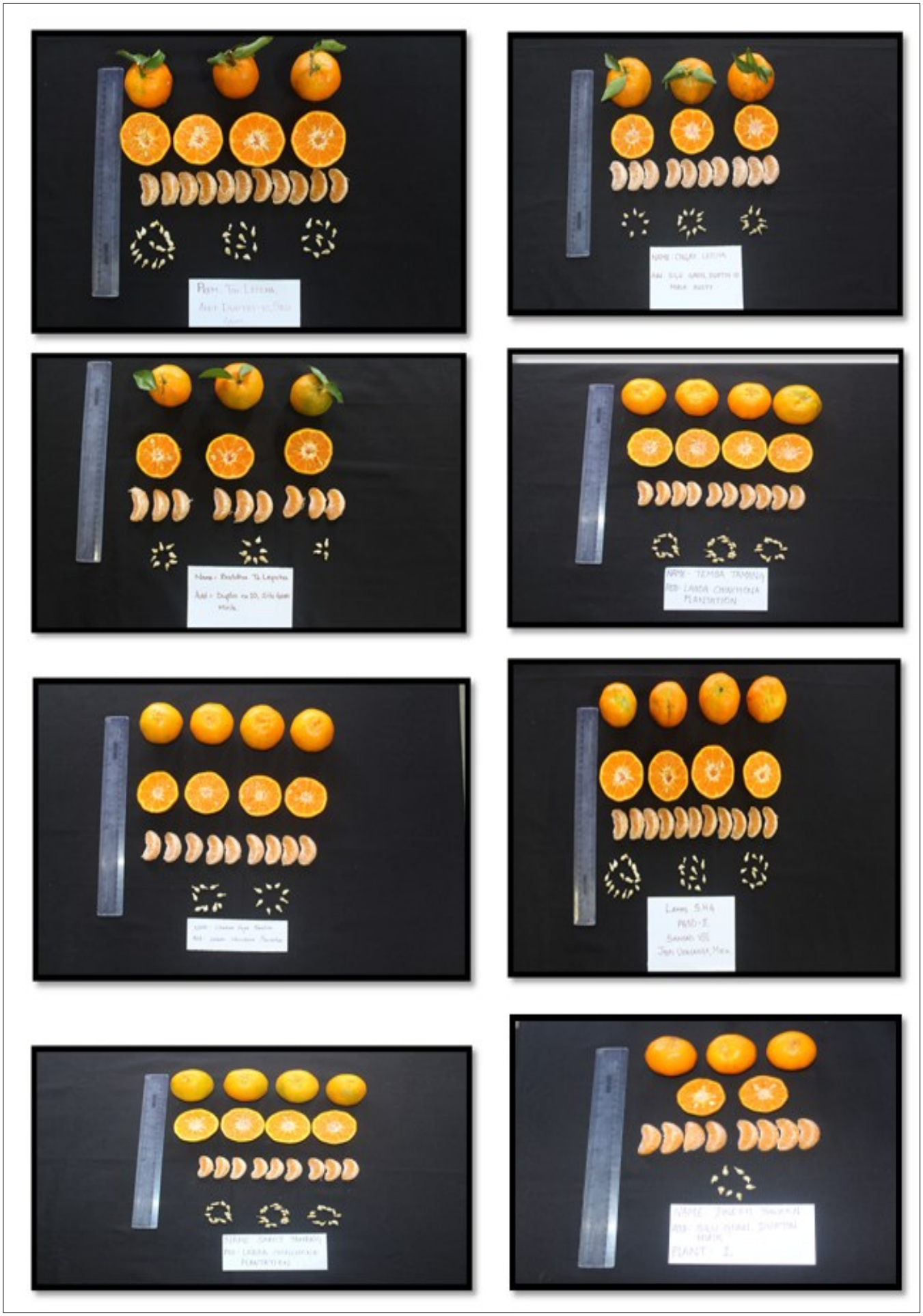

Fig. 1 Superior accessions of Darjeeling mandarin accessions from Darjeeling and Kalimpong hills.

$$\% \text{ acid} = \frac{\text{ml 0.1 N NaOH X factor}}{\text{Volume of sample in ml}} \text{ X 100}$$

Vitamin C (ascorbic acid) content in the juice was determined by titration against a dye (2,6-dichlorophenolindophenol) also known as DCPIP, as described previously (20). The chemical composition is $C_{12}H_7Cl_2NO_2$. Dissolve 100 mg ascorbic acid in 100 mL of 4 % oxalic acid solution in a standard flask, the concentration of the stock standard solution is 1 mg/mL. Dilute 10 mL of stock solution to 100 mL with 4 % oxalic acid. The concentration of working standard is 100 µg/mL. Now, pipette out 5 mL of the working standard solution into a 100 mL of conical flask to that add 10 mL of 4 % oxalic acid and titrate against the dye (V_1 mL). End point is the appearance of pink colour which persists for a few minutes. Extract five grams of sample in 4 % oxalic acid and make up to a known volume (100 mL) and centrifuge. Pipette out 5 mL of this supernatant, add 10 mL of 4 % oxalic acid and titrate against the dye (V_2 mL).

Amount of ascorbic acid mg/100mL sample =

$$\frac{0.5 \text{ mg}}{\text{V1 ml}} \times \frac{\text{V2ml}}{\text{5ml}} \times \frac{\text{100 ml}}{\text{Wt. of the sample}} \times 100$$

Total Phenol Content

The total phenolic content in the sample was calculated using a modified Folin-Ciocalteu (FC) method (21). 50 µL of methanolic extract was taken and was diluted with 450 µL of distilled water, to this 150 µL of FC reagent (diluted 1:1 v/v) was added and the solution was vortexed. After adding 500 µL of 20 % (w/v) Na_2CO_3 the mixture was left in the dark for one hour. The greenish-blue colour formed was analysed at 650 nm using a microplate reader (BIO-RAD, iMARKTM, Japan) to determine the absorbance. All the reagents were added to 500 µL of methanol, except for the plant extract, which was used as a blank. To ascertain the phenolic content of the samples, gallic acid was used as a reference. Total phenol content in the sample was expressed as milligrams per grams of Gallic acid equivalent (mg/g GAE).

Total Flavonoid Content

Total flavonoid content in the mandarin fruits were analysed using aluminium chloride ($AlCl_3$) method (22). In the 100 µL of sample (filtered fruit juice), 400 µL methanol was added to dilute the concentration. Then, 10 % aluminium chloride solution (100 µL) was added and thoroughly mixed. Again, 100 µL of 1M sodium acetate was added and the solution mixture was incubated in the dark at room temperature for 45 min. After 45 min, the golden-yellow colour solution mixture was measured at 415 nm (BIO-RAD, iMARKTM, Japan). For the measurement of blank, in 500 µL of methanol all the reagents were added except for plant extract and incubate at dark for 45 min and absorbance was taken at 415 nm. Quercetin was used as standard to determine the number of flavonoids in the plant sample and expressed in milligram per gram equivalent of quercetin.

Antioxidant studies

Several methods have been reported for assessing plant sample antioxidant capability. Antioxidant activity was assessed in this study using the DPPH (2,2′-diphenyl-1-picrylhydrazyl) and FRAP (Ferric reducing antioxidant power) methods.

DPPH radical scavenging assay

In the samples, antioxidant activity was assessed using a modified version of the DPPH (2,2′-diphenyl-1-picrylhydrazyl) method (23). To the 25 µL sample 575 µL methanol was added to a sample to make total volume to 600 µL. After thoroughly mixing, 200 µL of 0.004 % DPPH solution (4 mg of DPPH dissolved in 100 mL of methanol) was added. The reaction mixture was incubated in the dark for 30 minutes and the absorbance was measured at 517 nm. 600µl of methanol and 200 µl of 0.004 % DPPH was used for control. The standard used for DPPH antioxidant assay was butylated hydroxyanisole (BHA).

Percentage of antioxidant activity was calculated using the formula:

Radical scavenging activity (%) = (A (control)-A (sample))/ (A (control)) ×100

where, control was 600 µL methanol + 200 µL DPPH solution. Sample was plant extract made up to 600 µL using methanol + 200 µL DPPH solution.

FRAP method

Total antioxidant activity is measured by Ferric Reducing Antioxidant Power (FRAP) assay. In a 100 µL of sample extracts, 900 µL of methanol was added and 2.5 mL of Phosphate Buffer Saline (PBS) of 0.2M concentration, pH 6.6. The contents were thoroughly mixed, then 2.5 mL of 1 % Potassium ferricyanide solution was added. The reaction mixture was vortexed well using a vortex shaker. The mixture was incubated at 50 °C for 20 min. After incubation was over, 2.5 mL of 10 % Trichloroacetic acid (TCA) was added and was centrifuged at 3000 rpm for 10 min. 2.5 mL of supernatant was collected from the centrifuged tubes into separate test tubes and 2.5 mL of deionized water was added. In the sample test tube 0.5 mL of ferric chloride was added which would give a bluish colour formation and the absorbance was measured at 700 nm. For the positive control antioxidant molecule like ascorbic acid was taken and for the blank only distilled water with all the reaction mixture was taken (24). The FRAP value was calculated using the following equation.

Frap value $=(A_1-A_0)/(A_C-A_0) \times 2$

Where, A_C= absorbance of the positive control

A_1= absorbance of the sample

A_0= absorbance of the blank

Statistical analysis

Web Agri Stat Package-2 (WASP-2) created by ICAR Complex Goa, India was used for analysing descriptive statistics, including the minimum, maximum, mean, standard deviation and coefficient of variation. The R statistical tool (version 4.3.3, The R foundation) was used to analyse the data for correlation, PCA, K-means cluster plots and dendrograms of Mandarin accessions. Pearson correlation was analysed by coorplot (25). Principal Component analysis was conducted

using the most recent versions of FactoMineR, Factoextra and ggplot2 (26, 27). The cluster, factoextra, dendextend and ggplot2 programs were used to perform the cluster analysis (28).

Results and Discussion

When choosing the desired lines, which form the foundation for creating the breeding program, genetic variety and genetic diversity are crucial.

Descriptive statistical analysis

The descriptive statistics for the mean, minimum, maximum, standard deviations and coefficient of variation for eighteen fruit trait including morphological and fruit quality traits are shown in Table 2. Fruit weight ranges from 53.10 g to 153.80 g, with an average of 103.69 g and a relatively high standard deviation (24.14), indicating noticeable variation among the accessions. The CV of 23.28 % also reflects moderate variability. Peel weight has the second-highest coefficient of variation (26.63 %), which suggests considerable variation in peel thickness or heaviness among the accessions. The pulp weight shows relatively less variability with a CV of 24.85 % and an average of 74.22 g. Number of seeds shows higher variability (CV = 33.45 %). Juice volume shows moderate variability (CV = 28.25 %), indicating differences in juiciness among accessions. Ascorbic acid (Vitamin C) content is an important nutritional trait. The CV of 24.38 % shows moderate variability in the vitamin C content across accessions Total phenol content, which is often linked to antioxidant properties, has the highest coefficient of variation (68.27 %). This indicates substantial variability in the phenolic content, which could be a key factor for breeding or selecting varieties with higher health benefits. Antioxidant activity shows moderate variability (CV = 23.51 %).

Correlations among the traits

Pearson correlation coefficients among 18 fruit traits are given in Fig. 2. Correlation ranges from -1.0 to +1.0. The inter-correlation coefficients showed highly positive as well as negative correlations

Highly significant positive correlations were observed among FW and PuW (1.0), FW and JV (1.0), PuW and JV (1.0), FB and JV (1.0). Similarly, significant positive correlations were observed for the following traits: FW and PW (0.9), FW and FD (0.9), FV and PW (0.9), PW and FB (0.9), PW and PT (0.9), FD and FB (0.9), PUW and FB (0.9), FW and FV (0.8), PW and PuW (0.8), PuW and FD (0.8), PW and FD (0.8), FV and FB (0.8), FV and PT (0.8), SW and JV (0.8), FD and JV (0.8), PW and JV (0.8), PT and FV (0.8). Number of seed (NOSD) showed no correlation or minimum correlation with the following fruit traits such as PuW (0), PW (0), FW (0.1), FV (0.2), FB (0.2) and NoS (0.3). It is not necessary for big fruits to have more seeds or for tiny fruits to have fewer seeds, as this association shows that big fruit weight does not correlate with more seeds.

The following fruit biochemical traits showed no correlation or negative correlation with the fruit morphological traits:

TSS (Total Soluble Solids) showed negative correlation with FW (-0.5), FV (-0.6), PW (-0.5), PuW (-0.5), FD (-0.5), FB (-0.6), NOS (-0.4), NOSD (-0.1), SW (-0.2), JV (-0.5) and PT (-0.5). No correlation with TPC (0), FRAP assay (0) however a positive correlation was observed for TA (0.2) TPC (0.6) and DPPH (0.1).

TPC (Total phenol content) depicted negative correlation with PT (-0.2), JV (-0.2), SW (-0.3), NOSD (-0.2), NOS (-0.2), FB (-0.2), FD (-0.1), PuW (-0.1), PW (-0.3), FV (-0.2) and FW (-0.2). No correlation with TSS (0) and a correlation of 0.1 and 0.2 for AA and TA respectively. TFC (Total Flavonoid content) displayed a negative correlation with 12 fruit morphological traits and positive correlation with TSS (0.6) and DPPH (0.3).

Principal component analysis and grouping germplasm based on PCA biplot

In the present investigation, the PCA (Principal Component Analysis) was performed using 18 quantitative traits. The results from the eight components revealed 94.04 % of the total variation. This shows a large amount of variation exists among the Darjeeling Mandarin germplasm (Table 3). The first component accounts for 46.47 % of variance, showing

Table 2. Descriptive statistics for 18 fruit attributes in mandarin accessions

Trait	Maximum	Minimum	Range	Average	Standard Deviation	Variance	Standard Error of Mean	Co-efficient of Variation
Fruit weight (g)	153.80	53.10	100.70	103.69	24.14	582.86	5.86	23.28
Fruit Volume (ml)	148.90	36.70	112.20	93.04	23.89	570.67	5.79	25.68
Peel weight (g)	39.70	10.90	28.80	27.71	7.38	54.43	1.79	26.63
Pulp weight (g)	112.20	38.80	73.40	74.22	18.44	340.20	4.47	24.85
Fruit Diameter (mm)	59.53	41.73	17.80	52.67	4.09	16.74	0.99	7.77
Fruit Breadth (mm)	71.30	46.63	24.67	60.09	5.68	32.26	1.38	9.45
Number of segment	9.90	8.70	1.20	9.35	0.37	0.13	0.09	3.91
Number of seed	27.60	2.70	24.90	16.97	5.68	32.22	1.38	33.45
Seed weight (g)	3.12	1.51	1.61	2.22	0.45	0.21	0.11	20.48
Juice Volume (ml)	68.90	19.10	49.80	42.55	12.02	144.50	2.92	28.25
TSS (Total Soluble Solids)° Brix	15.29	9.61	5.68	11.13	1.27	1.61	0.31	11.42
Peel Thickness (mm)	2.87	1.40	1.47	2.28	0.37	0.14	0.09	16.33
Total Acidity (%)	1.76	0.47	1.29	1.29	0.33	0.11	0.08	25.28
Total phenol comtent (mg/g GAE)	3.46	0.31	3.15	1.07	0.73	0.54	0.18	68.27
Ascorbic Acid mg/100 ml sample	74.60	32.40	42.20	41.58	10.14	102.71	2.46	24.38
Total flavonoid content (mg/g Quecetin)	11.76	5.69	6.07	7.79	1.78	3.16	0.43	22.80
DPPH Assay % scavenging activity	34.04	12.06	21.99	23.28	5.47	29.94	1.33	23.51
FRAP value	2.52	1.78	0.74	2.05	0.20	0.04	0.05	9.63

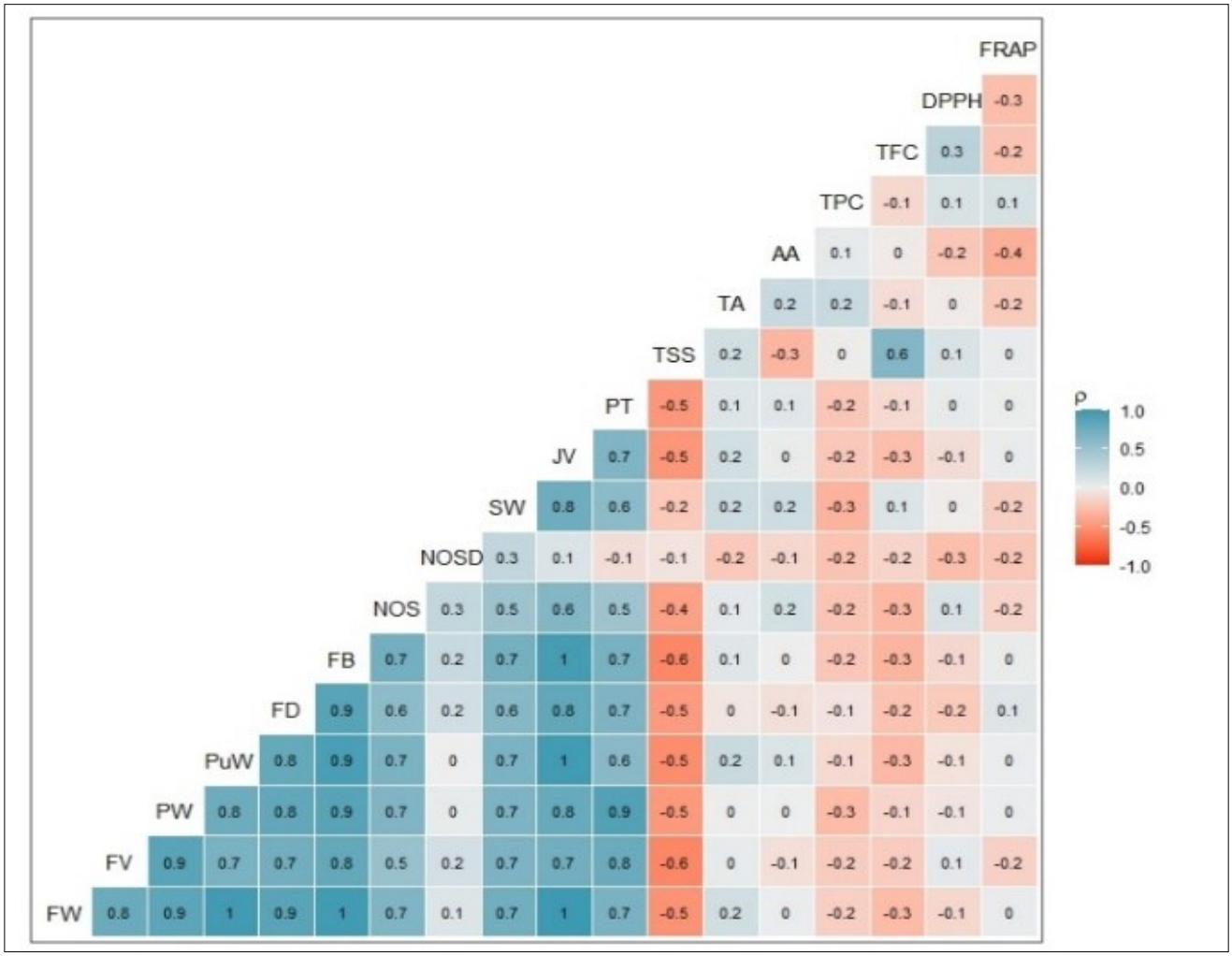

Fig. 2. Pearson correlation coefficient among 18 quantitative fruit traits in 16 mandarin germplasm collected from different elevations. TA, Titrable Acidity; AA, Ascorbic Acid; TFC, Total Flavonoid Content; TSS, Total Soluble Solids; DPPH, (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay; JV, Juice Volume; FW, Fruit weight; PW, Pulp weight; FB, Fruit Breadth; FD, Fruit Diameter; SW, Seed weight; PT, Peel thickness; PW, Peel weight; FV, Fruit Volume; NOS, Number of Segment; NOSD, Number of seed, FRAP assay.

Table 3. First 8 components from the PCA Analysis of 18 quantitative traits in 17 Darjeeling Mandarin germplasm

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Fruit weight (g)	0.33	0.02	0.03	0.07	0.13	0.07	0.03	0.10
Fruit Volume (ml)	0.30	-0.09	-0.07	0.06	-0.17	0.02	-0.27	-0.37
Peel weight (g)	0.32	-0.07	-0.08	0.13	-0.04	-0.19	-0.05	-0.13
Pulp weight (g)	0.32	0.03	0.13	0.07	0.09	0.07	0.13	0.24
Fruit Diameter (mm)	0.30	0.09	-0.10	0.09	0.07	0.05	-0.29	0.16
Fruit Breadth (mm)	0.34	0.04	-0.05	0.00	0.04	0.09	0.03	0.08
No. of segment	0.25	-0.06	0.02	-0.15	-0.22	0.20	0.41	0.33
No. of seed	0.06	0.00	-0.40	-0.52	0.06	0.44	-0.22	-0.02
Seed weight (g)	0.27	-0.30	-0.02	-0.09	0.24	-0.02	-0.12	0.09
Juice Volume (ml)	0.33	0.00	0.02	0.06	0.14	0.07	0.09	0.13
TSS° Brix	-0.21	-0.32	-0.15	0.19	0.46	0.17	0.08	0.09
Peel Thickness (mm)	0.27	-0.09	0.03	0.17	-0.10	-0.31	-0.10	-0.36
Total Acidity (%)	0.04	-0.14	0.54	0.04	0.43	0.32	0.20	-0.41
Total phenol	-0.08	0.23	0.41	0.26	-0.09	0.37	-0.61	0.24
Ascorbic Acid	0.03	-0.07	0.51	-0.44	-0.01	-0.42	-0.06	0.31
Total flavonoid	-0.11	-0.56	-0.14	0.17	0.16	-0.23	-0.29	0.31
DPPH Assay	-0.03	-0.39	0.06	0.36	-0.55	0.28	0.20	0.11
FRAP Assay	-0.01	0.47	-0.19	0.41	0.26	-0.17	0.17	0.21
Standard deviation	2.89	1.36	1.29	1.24	1.09	1.00	0.84	0.79
eigenvalue	8.36	1.86	1.66	1.53	1.18	1.01	0.70	0.63
percentage of variance	46.47	10.35	9.23	8.49	6.54	5.61	3.88	3.48

strong positive contributions from traits like Fruit weight, Fruit volume, Peel weight and Pulp weight. PC2 explains 10.35 % of the variance, with contributions from traits such as FRAP Assay and a strong negative contribution from Total flavonoid and DPPH Assay. PC3 to PC8 capture smaller amounts of variance but highlight unique relationships between traits like Ascorbic acid, Total phenol and Total acidity. The PCA biplot illustrated that principal component 1 and 2 accounted for a large portion of the total variation in the data, specifically 46.5 % and 10.3 %, respectively (Fig. 3). Dimension 1 explains 46.5 % of the total variance, while Dimension 2 explains 10.3 %. This suggests that almost 57 % of the total variance in the data us captured by these two components. The blue dots represent individual accessions/ germplasm, each labelled with abbreviations like “KR”, “PTL”, “RP” etc. These points are plotted based on their coordinates in the new principal component spaces. Accessions that are located near each other have similar characteristics, whereas those that are far are more dissimilar. DL is positioned far from the other points, indicating it has unique or distinct characteristics relatability to most of the accessions which are clustered near the centre. The arrows represent the variables or features in the dataset, showing how each variable contributes to the two principal components. The direction and length of the arrows indicate the correlation between the variables and the principal components. Longer arrows suggest a stronger contribution to the variability explained by the component. Variables such as "NCS", "PC", "FD" and "PW" contribute heavily to Dim1, while others like "SS", "SW" and "FV" may influence Dim2. Samples like "PTL," "STL1" and "STL2" are closely grouped, indicating they are more similar in terms of the measured variables. Conversely, samples like "DL" and "KR" are far from the main cluster, suggesting they have distinct characteristics.

Cluster analysis

Based on quantitative data, the PCA classified the cultivars into six main clusters Fig. 4 shows a hierarchical clustering diagram often used in genetic studies to show the relationships or similarities between different samples or accessions based on a set of traits. Each branch of the dendrogram represents a group of accessions that are more like each other than to those in other branches. The dendrogram shows several clusters, represented by different coloured branches. The cluster dendrogram divided the germplasm into 6 groups. Accessions LSHG and PTL are closely related, forming a distinct cluster. The other cluster includes accessions OL, STL1, PC and SS. Among these, OL and STL1 show the closest relationship. Comprising AS, STL2, KR and PR, this cluster shows STL2 and KR as the most similar. This last group includes BTL and SAM as the closest pair, followed by RP. A small cluster involving DY and CS, showing a relatively close relationship.

The K means clustering carried out in 17 different germplasm of mandarin based on 18 quantitative data divided the germplasm into 6 different clusters (Fig. 5). Cluster I consists of two germplasm they are LSHG and PTL, Cluster II includes germplasm PC, SS, STL1, Cluster III had CS, DY, TT and BTL germplasm. In the Cluster IV there was only one germplasm i.e DL, Cluster V contains PR, STL2, SAM, AS, KR, RP and DY from cluster III. The germplasm DL was included in Cluster IV. Fruit weight was recorded maximum of 153.08 g, minimum of 53.10 g with an average of 103.69 g. This indicates variability in fruit size, with the standard deviation being 24.14, suggesting a moderate spread. The CV of 23.28 % indicates a moderate variation in fruit weight across the samples. Total phenol (68.2 %) had the highest CV values, followed by the number of seeds (33.4 %). In the Indian mustard accessions highest CV of 48.57 % was recorded by seed weight (29). While in the remaining fruit

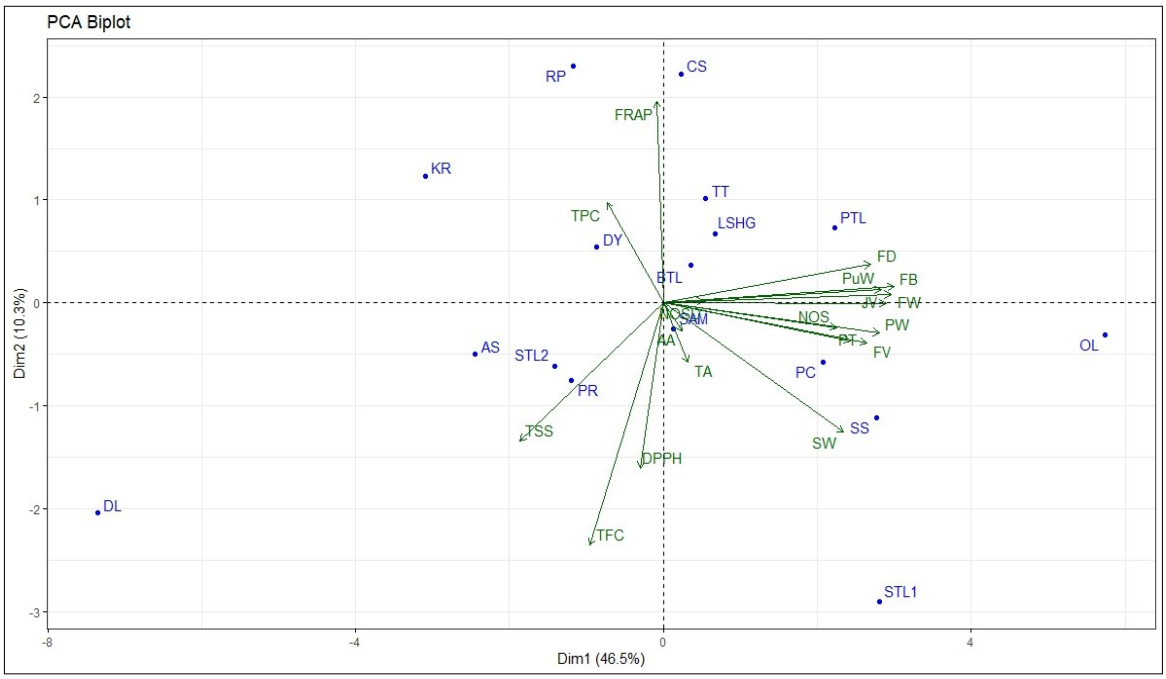

Fig. 3. The PCA biplot graph showing PCA score and loading of 18 quantitative data.

The abbrevations of the variables are as following: TA: Titrable Acidity, AA: Ascorbic Ac-id, TPC: Total Phenol Content, TFC: Total Flavonoid Content, TSS: Total Soluble Solids, DPPH: DPPH antioxidant assay, JV: Juice Volume, FW: Fruit weight, PuW: Pulp weight, FB: Fruit Breadth, FD: Fruit Diameter, SW: Seed weight, PT: Peel thickness, PW: Peel weight, FV: Fruit Volume, NOS: Number of Segment, NOSD: Number of seed, FRAP: anti-oxidant assay.

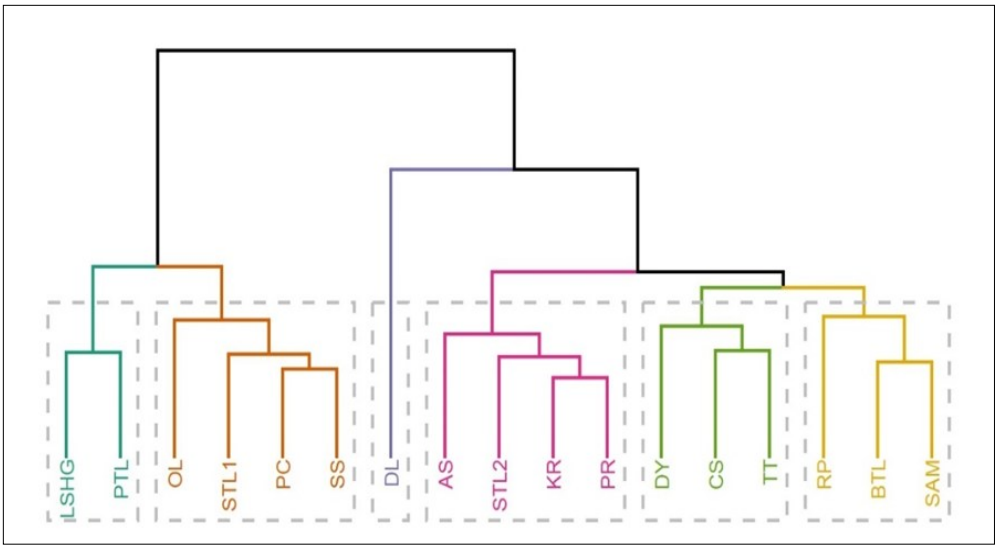

Fig. 4. Cluster analysis of 17 mandarin germplasm based on 18 qualitative data.

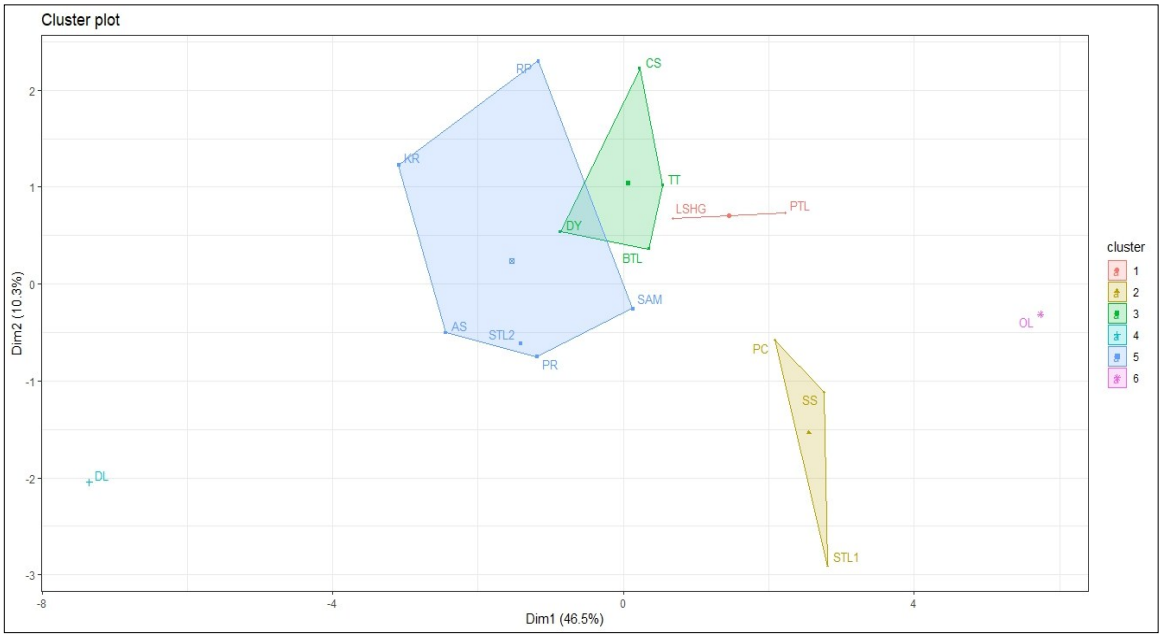

Fig. 5. K means cluster plotted against PCA Scatter plot (Component 1 on the x-axis and Component 2 on the Y axis, K-3).

traits medium CV values were observed ranging from 28.25 to 20.48 %. Fruit traits such as Number of segment (3.91 %), Fruit diameter (7.77 %) and FRAP assay (9.63 %) recorded lowest CV values. The range in CV values revealed a greater degree of variation in the germplasm under investigation. Comparable results were observed, with statistically significant variations in the coefficient of variation for both genotypic and phenotypic characteristics in Mandarin germplasm (30). The analysis revealed higher CV values for the variables under investigation as well as broader and more substantial diversity. These variations in plant genetic diversity offers breeders the chance to create new and improved varieties with desirable features, including traits that are valued by both farmers and breeders (31). Similarly, significant differences were observed for fruit volume ranging from 148.90 mL to 36.70 mL and number of seed (27.6 to 2.7). Significant differences in the quantity of seeds were also noted for mandarin cultivars in all PCA cluster (32). This demonstrated that the environment has a minimal impact on these traits and that genotype plays an important part (33, 34). Fruit weight showed significant genetic variety, as previously reported (35). "Minneola tangelo" a hybrid between Dancy tangerine and a Duncan grapefruit tree recorded the largest fruit (219.0 g), while "Nour Clementine" had the smallest (58.0 g). Peel weight has a variation with a maximum (39.70 g), minimum of (10.90 g) and an average of (20.71 g) with a CV of 35.56 % showing a higher relative variability compared to the total fruit weight. Highly significant positive correlations were observed among traits i.e. fruit weight with pulp weight, juice volume and significantly positive correlations of fruit weight with peel weight and fruit diameter. These findings align with previous reports of similar results in mandarins from the Sikkim and Darjeeling hills (36). Additionally, it was revealed that *Psidium cattleyanum* fruit weight exhibited a robust and favourable connection with morphological fruit descriptors such as peel weight (0.99), number of seeds per fruit (0.70), pulp weight (0.99) and seed weight (0.96) (37). Regarding PCA, PCA of 94.04 % showed high variation from the eight components. Similarly, 93.3 % of the overall variance in the guava germplasm was reported using the six PCA components which indicated that there is a large diversity in the germplasm under study (38). Principal Component Analysis indicates that the first two principal components account for

nearly 57 % of the total variance, which is significant for the analysis of fruit quality traits in mandarin accessions. Similar findings on genetic diversity and fruit quality traits were investigated in mandarin germplasm (39). Their findings showed significant genetic diversity in traits like fruit weight, total soluble solids and acidity contributing to variability among mandarin accessions. The biplot provides insights into how the variables (features) differentiate these samples and how strongly they contribute to explaining the variance. The PCA biplot helps identify patterns in the data and the relationships between samples and variables. Based on the plot: Dim1 seems to capture the most variability, with most of the samples aligning along this axis. This indicates that most of the variance in the dataset can be explained by variables heavily contributing to Dim1. Dim2 captures less variability but may reveal secondary relationships that are not apparent from the first dimension alone. The dendrogram indicates a clear genetic diversity among the accessions, with some groups having closer relationships and others being more distantly related. Previous study revealed high average similarity among accessions, supporting the hypothesis that all mandarins may be variants of a single clone. Thus, mandarin in Bhutan may represent a variant of a single clone (40, 41). The classification of accessions into distinct clusters through PCA and K-means clustering offers valuable insights into the unique characteristics of certain accessions, which could be harnessed for future breeding programs. This genetic diversity provides an essential resource for the development of superior mandarin varieties with improved nutritional quality and adaptability to regional climatic conditions. The findings from this research provide valuable insights for the development of high-quality, nutritionally rich mandarin varieties, contributing to both agricultural sustainability and improved nutritional security in the region.

Conclusion

This study successfully highlights the significant genetic diversity among mandarin accessions grown in the Eastern Himalayan region of India through the assessment of key fruit quality traits. The results demonstrated substantial variability in traits such as fruit weight, total phenol content and number of seeds, underscoring the genetic richness within the mandarin germplasm of this region. The observed correlations between fruit morphological and biochemical traits further emphasize the importance of genetic factors in shaping fruit quality characteristics with limited influence from environmental factors.

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Authors' contributions

Designing the experiment, fruit quality traits analysis, biochemical analysis, data analysis and result Interpretation were done by NG. The manuscript was written by NG and RMS. Manuscript correction and editing was done by SS. The final draft of the manuscript was revised and finalized by RMS and SS.

Compliance with ethical standards

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

Ethical issues: None

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