



RESEARCH ARTICLE

Mapping and characterisation of mandarin (*Citrus reticulata* Blanco) genotypes across Eastern India

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Abstract

Citrus is one of the most significant fruit crops in the Rutaceae family. The Northeastern Himalayan region of India and the foothills of the Central and Western Himalayan regions, often referred to as the “treasure house of *citrus* germplasm” are rich sources of *citrus* genetic diversity. The present study aimed to assess the morphological and biochemical variabilities among different mandarin (*Citrus reticulata* Blanco) germplasms cultivated/grown in Eastern India. Following ICAR-National Bureau of Plant Genetic Resources (NBPGR) descriptors, a comprehensive survey and exploratory collection were conducted from native populations across West Bengal, Sikkim and Nagaland during 2024–25, covering diverse altitudinal and agro-climatic conditions. Mandarins collected from Dzongu (North Sikkim) exhibited the highest fruit weight (112.98 g) and total soluble solids (TSS: 10.13 ± 1.08 °Brix), along with superior vitamin C content (33.48 ± 4.44 mg/100 mL) and TSS:Acid ratio (20.87 ± 3.97), suggesting superior sweetness and nutritional quality. In contrast, Tseminyü (Nagaland) recorded the lowest fruit weight (61.05 g) and highest titratable acidity (1.04 ± 0.08 %), while Rüsoma (Nagaland) showed the highest reducing sugar (4.67 ± 1.98 %) and large fruit size (fruit length 57.62 mm, width 63.38 mm). Overall, the study revealed considerable genetic variability among the collected accessions, identifying Dzongu and Rüsoma as promising sources for quality planting material and breeding programs aimed at fruit quality improvement in mandarin. High-quality planting material can be produced using the accession.

Keywords: *Citrus*; fruit quality; germplasm characterisation; mandarin; Northeast India

Introduction

Citrus is one of the most important fruit crops that belongs to the family Rutaceae and the majority of its species are native to China (1). Some species viz. *Citrus indica*, *C. jambhiri*, *C. karna*, etc. are of Indian origin. The total production of *citrus* fruits in the world is 139.80 million tons from an area of 9.08 million hectares, in which India ranks third globally in the *citrus* production followed by China (35.47 million tons) and Brazil (19.07 million tons) (2).

Citrus groupings have extremely complex taxonomic relationships. The frequent occurrence of spontaneous mutations and interspecific hybrids in nature may be the cause of this intricacy in relationships. *Citrus* crop improvement initiatives primarily rely on the representation of the morphological, cytological and genetic traits of different cultivars (3, 4). The validation of phenotypic variety is thought to be significantly hampered by morphological flexibility. However, it has been shown that several genes control the transmission of citrus agronomic qualities, which are solely assessed based on their morphological characteristics (5).

Morphological descriptions are used extensively because they provide the fundamental genotype passport information under the specified environmental conditions (6, 7). For crop conservation and genetic advancement, it is important to identify superior genotypes in the collected germplasm (8, 9). According to several

studies morphological and genetic diversity are distinct from one another (10–12).

In India, Northeastern Himalayan region and foothills of the Central and Western Himalayan tracts are rich sources of *citrus* genetic diversity (13). It is designated as a treasure house of *citrus* germplasms because of the existence of 23 species, 1 subspecies and 68 varieties (14). It boasts a rich diversity of flora, with its unique geographical and climatic conditions supporting the cultivation of a wide range of fruits. Among these, mandarin (*Citrus reticulata* Blanco) holds significant promise as a high-value crop. Mandarin, a *citrus* fruit renowned for its sweet flavour, easy-peel rind and nutritional benefits, has garnered increasing attention from both farmers and consumers alike. *Citrus* fruits are well known for their dietary, nutritional, medicinal and cosmetic properties and are also good sources of citric acid, flavonoids, phenolics, pectins, limonoids, ascorbic acids, etc (15, 16). They are also known to have a good shelf life (17–19). The cultivation of mandarin orange in various parts of Northeastern region dates back to time immemorial. Consequently, this crop has evolved distinct characteristics across various geographical regions in respect to its colour, texture, sweetness and longevity. Therefore, the oranges available in Nagaland is different in various forms with Khasi mandarin, Darjeeling mandarin and Sikkim mandarin. However, while mandarin cultivation is widespread in several regions across India,

such as Punjab, Haryana and Maharashtra, its potential, exploration, evaluation and characterisation in the Northeastern states remain relatively underexplored.

Genotype-by-environment interaction ($G \times E$) represents a critical concern in *citrus* breeding, as it affects the comparative performance and ranking of genotypes across diverse growing environments, influencing heritability, genetic gain and the strategic deployment of genetic material. To overcome these challenges and optimise breeding programs in Eastern India, molecular marker technologies utilising DNA markers have emerged as powerful tools for genetically dissecting quantitative traits and identifying desirable *citrus* genotypes (20).

Simple sequence repeat (SSR) markers serve as valuable genomic tools for analysing genetic diversity and variability of *citrus* species, providing discriminatory power to accurately estimate genetic distance between accessions. Complementing molecular approaches, high-throughput phenotyping (HTP) technologies have emerged as indispensable tools, enabling non-destructive, rapid assessment of critical traits like root architecture, chlorophyll content and canopy temperature in controlled and field environments (21). The integration of these advanced molecular and phenotypic approaches provides a comprehensive framework for characterising mandarin genotypes, understanding their $G \times E$ interactions and supporting evidence-based selection strategies tailored to the distinct agroclimatic conditions of Eastern India (22).

The topography of the Northeastern states of India encompasses a diverse range of landscapes, including mountains, valleys, plateaus and plains, which profoundly influence the cultivation of mandarin (*C. reticulata*) in the region. Understanding the interplay between topography and Mandarin cultivation is crucial for optimising production, enhancing resilience and harnessing the fruit's economic potential. Topography influences rainfall distribution, with orographic effects causing precipitation variations across different elevations. The rich soil condition, high rainfall and existence of different altitude

situations from almost sea level to 1500 m above place the region in a unique position for the adaption of almost all the *Citrus* species and varieties of commercial importance (23). This study endeavours to bridge this gap by undertaking a comprehensive exploration, evaluation and characterisation of mandarin varieties found in the Northeastern states. By delving into the diverse array of mandarin cultivars thriving in this region, we aim to unearth valuable insights that can inform cultivation practices, enhance productivity and promote sustainable indigenous knowledge management (IKM) agricultural development.

Materials and Methods

Collection of plant material

Extensive surveys were conducted in the states of Sikkim, West Bengal and Nagaland during the year 2024–25. Random sampling was carried out across major *Citrus* growing regions of these states. Samples were collected from the major orange producing district from each state during the harvesting season, from November to February. At each location, three representative orchards were selected for sampling. From each orchard, ten mature and healthy fruits were randomly collected (Fig. 1, Table 1). Thus, a total of 30 fruits were collected from each location and 210 fruits from all seven locations combined to guarantee that diversity is represented in every area. Trees selected for sampling were approximately 10–15 years old and maintained under farmer-managed conditions. For ease of identification and analysis, each collection site was assigned a unique accession code, which was used throughout the study.

Morphological characterisation

Morphological characterisation of the collected mandarin germplasm was carried out following the *Citrus* descriptors prescribed by the ICAR-National Bureau of Plant Genetic Resources (NBPGR). Observations were recorded on three representative trees (orchards) per accession. Quantitative traits included tree height,

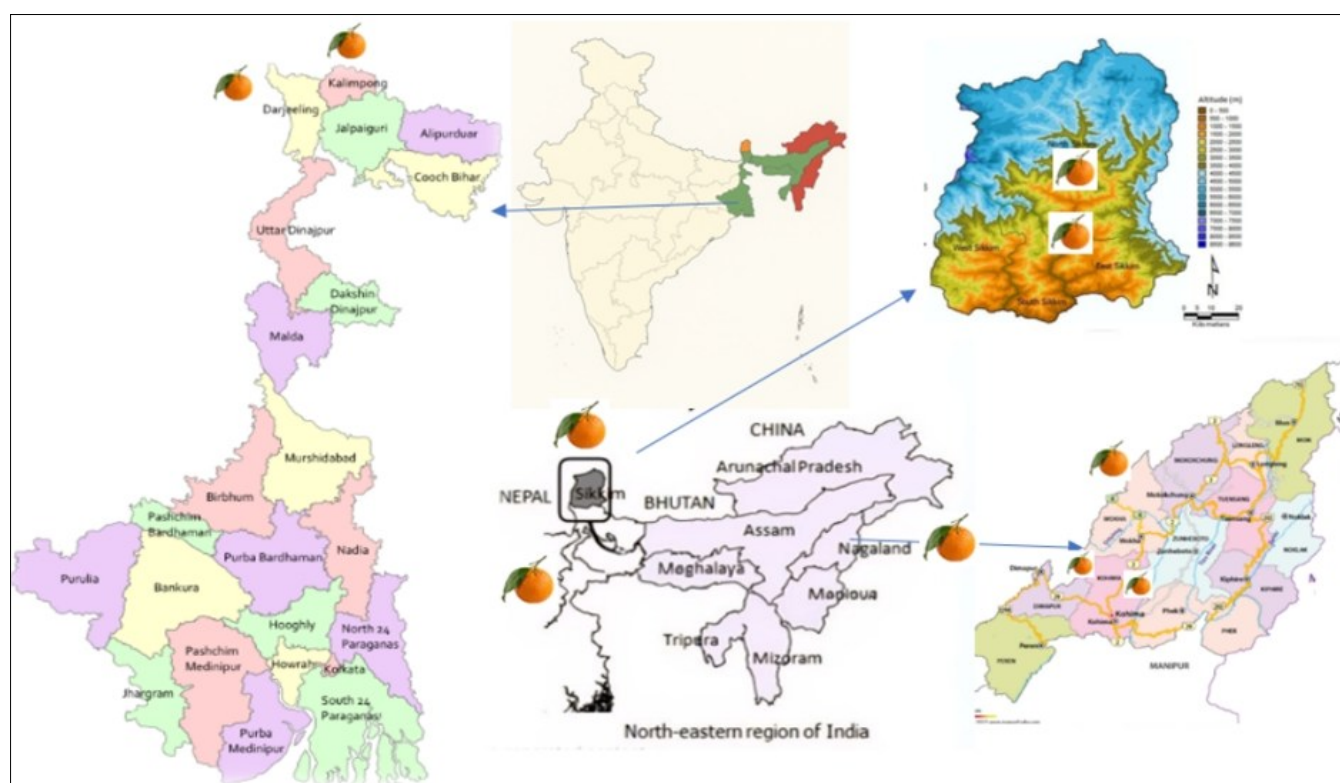


Fig. 1. Biodiversity mapping of mandarin ecotypes in Eastern India [Source: <https://share.google/9QWx1wZc3TfMBLgID>].

Table 1. Geographic origin, altitude and accession codes of mandarin germplasm collected from Eastern India

Sl. No.	Collection accession code	State	District	Village	Latitude	Longitude	Altitude
1	SKM-01	Sikkim	Mangan	Dzongu	27.446396	88.508335	1436 m
2	SKM-02		Gangtok	Khamdong	27.259857	88.499158	1187 m
3	WB-01	West Bengal	Darjeeling	Mirik	26.873739	88.201585	1079 m
4	WB-02		Kalimpong	Kalimpong	27.063033	88.459586	1075 m
5	NAG-01	Nagaland	Kohima	Rüsoma	25.714724	94.147759	1316 m
6	NAG-02		Tseminyü	Tseminyü	25.981083	94.230938	1312 m
7	NAG-03		Wokha	Bluehill	26.1024	94.2522	1399 m

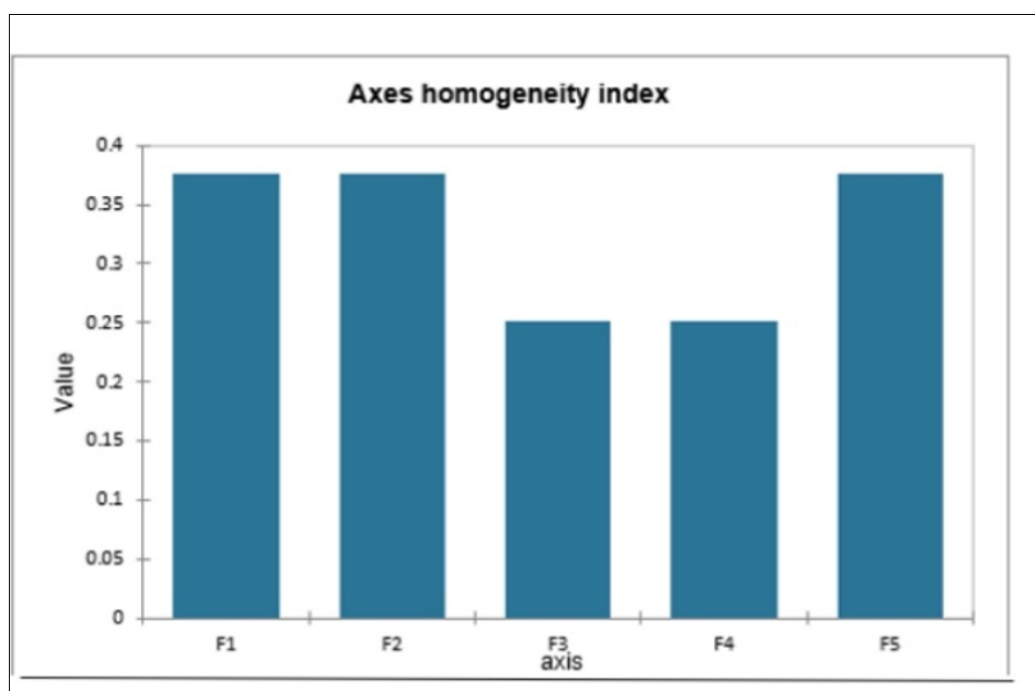
trunk girth, canopy spread (East-West and North-South), leaf length, leaf width, fruit length, fruit width, fruit weight, rind thickness, juice content, number of seeds per fruit and seed weight were recorded according to the NBPGR descriptor scale. Mean values of quantitative traits were used for statistical analyses including analysis of variance (ANOVA), principal component analysis (PCA) and correlation analysis (Fig. 2, Table 2).

Biochemical characterisation

The fruit samples were brought to the Department of Horticulture, Sikkim University and Department of Horticulture, Nagaland University laboratory for analysis (Fig. 3).

Fruit juice sample preparation

Fresh fruits were washed with distilled water and air dried. Juice was

**Fig. 2.** Principal component axes homogeneity index.**Table 2.** Eigenvalues, factor and contribution of first five principal components axes in mandarin genotypes for 17 traits

Variables	PC1	PC2	PC3	PC4	PC5
Total soluble solids	0.016	0.379	-0.317	-0.053	0.100
pH	0.037	-0.127	0.360	-0.225	0.528
Vitamin C	0.070	0.316	-0.283	-0.336	0.173
Titrateable acidity	-0.333	0.154	0.146	-0.324	-0.130
Total sugar	0.161	-0.284	-0.287	-0.360	-0.038
Reducing sugar	-0.004	0.107	-0.106	0.574	-0.340
TSS/Acid ratio	0.354	-0.027	-0.298	0.128	0.213
Age of plant	-0.339	0.196	-0.161	-0.099	0.094
Height of plant (m)	0.254	0.280	0.211	-0.061	-0.039
Tree girth (cm)	0.367	0.235	0.030	0.097	0.114
East–West spread	0.361	0.248	-0.006	-0.035	0.053
North–South spread	0.221	0.391	0.150	0.002	-0.008
Leaf length (cm)	0.288	-0.221	0.005	-0.253	-0.371
Leaf width (cm)	0.280	-0.349	-0.072	-0.112	0.036
Fruit length (mm)	0.065	-0.026	0.533	0.185	0.230
Fruit width (mm)	-0.257	0.006	-0.240	0.130	0.419
Rind thickness (mm)	-0.075	0.256	0.213	-0.318	-0.329
Eigenvalue	8.363	1.858	1.662	1.531	1.184
Variance explained (%)	46.462	10.322	9.235	8.504	6.580
Cumulative variance (%)	46.462	56.784	66.019	74.523	81.103

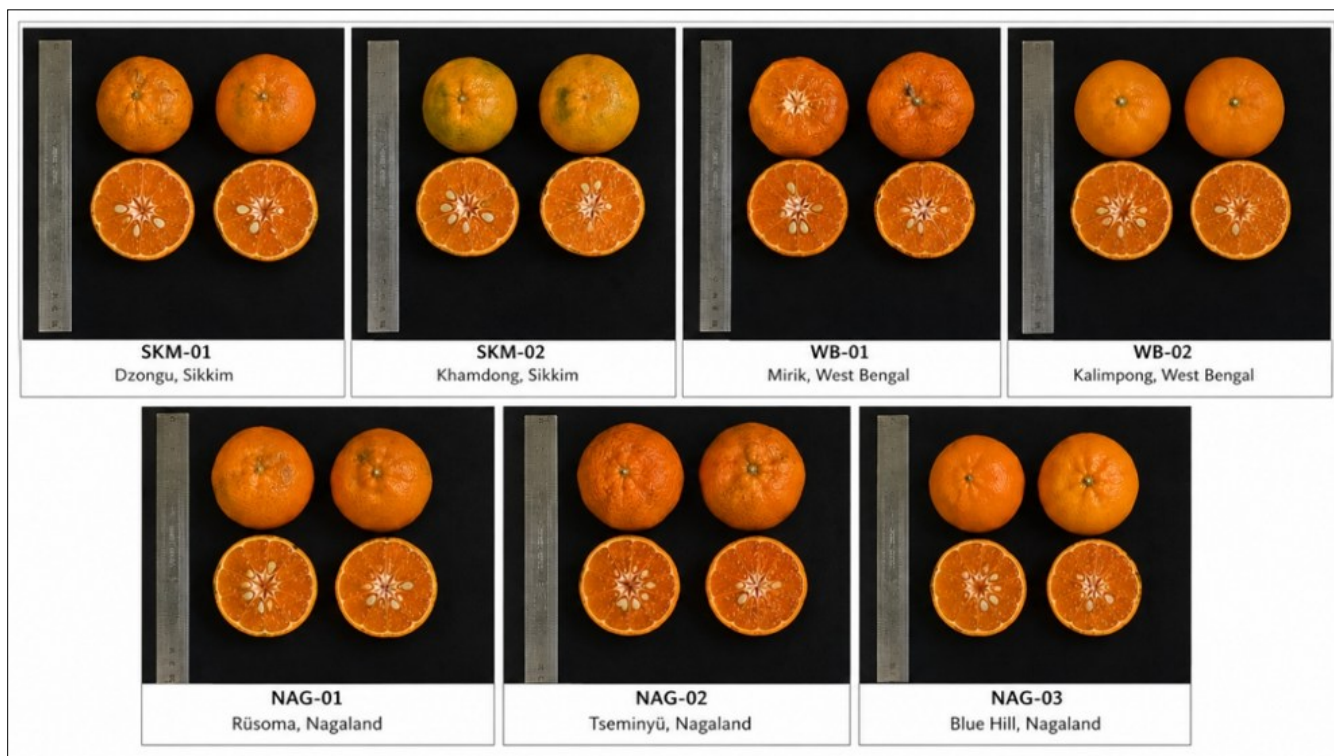


Fig. 3. Fruit morphology of seven mandarin accessions.

Top row, left to right: SKM-01 (Dzongu, Sikkim), SKM-02 (Khamdong, Sikkim), WB-01 (Mirik, West Bengal), WB-02 (Kalimpong, West Bengal). Bottom row, left to right: NAG-01 (Rüsoma, Nagaland), NAG-02 (Tseminyü, Nagaland), NAG-03 (Blue Hill, Nagaland).

extracted manually and filtered through muslin cloth. The extracted juice was immediately used for biochemical analysis. Three analytical replicates were maintained for each accession. The fruits collected from each orchard were considered one biological replicate. Juice extracted from pooled fruit samples was analysed in triplicate for biochemical parameters.

Fruit width/equatorial diameter (cm) was measured using vernier calliper (Generic Digital Digimatic Vernier Calliper, VC-01). Maturity index was determined using the TSS: TA ratio.

Fruit biochemical trait measurement

The total soluble solids (TSS) content of mandarin juice was measured using a hand refractometer (Atago PAL-1 digital refractometer) (24). The instrument was calibrated with distilled water before use. A few drops of freshly extracted juice were placed on the prism surface and the TSS value was recorded directly in °Brix at room temperature. The prism was cleaned thoroughly after each reading to ensure measurement accuracy.

Titrateable acidity: It was determined by the standard acid-base titration method. A 10 mL of freshly extracted juice was transferred to a 100 mL volumetric flask and the volume was made up to 100 mL with distilled water (25). Subsequently, 10 mL of the diluted juice was transferred to a conical flask and 2–3 drops of phenolphthalein indicator were added. The sample was titrated against 0.1 N sodium hydroxide (NaOH) until the appearance of a faint pink colour that persisted for approximately 15 sec. The volume of NaOH consumed was recorded and titrateable acidity was calculated and expressed as percentage citric acid.

$\text{Titrateable acidity (\%)} = (\text{Titre value} \times 0.1 \times 64 \times 10) / \text{Aliquot volume} \times 1000$

Ascorbic acid (Vitamin C): The colorimetric approach used by a 2.5 mL aliquot of fruit juice was transferred to a 25 mL volumetric

flask and the volume was made up to 25 mL using 3 % metaphosphoric acid (26). Subsequently, 5 mL of the diluted extract was titrated against standard 2,6-dichlorophenol indophenol dye until a light pink colour persisted for approximately 15 sec. The ascorbic acid content was calculated using the following formula:

$$\text{Vitamin C (mg/100 g)} = \text{Titre Value} \times \text{Dye Factor} \times 200$$

Total sugar: Total sugar content was estimated using the Fehling's solution method (27). A 10 mL of fruit juice was transferred to a 100 mL conical flask, followed by the addition of 8–10 drops of concentrated hydrochloric acid and 15–20 mL of distilled water. The mixture was gently boiled for 8–10 min to hydrolyse non-reducing sugars. After hydrolysis, the solution was neutralised and the volume was made up to 100 mL with distilled water. The prepared solution was titrated against freshly prepared Fehling's solution A and B (2 mL each) until the endpoint was reached. Total sugar content was calculated using the formula.

$$\text{Total sugar (\%)} = (a \times V \times 100) / (T \times 1000 \times W)$$

where:

a = Fehling factor

V = Volume made up in volumetric flask (100 mL)

T = Titre value (Burette reading, BR)

W = Volume of juice initially taken (10 mL)

Reducing sugar: Reducing sugar content was determined using the Fehling's solution method (27). A 2 mL each of Fehling's solution A and B were transferred to a 100 mL conical flask and diluted with approximately 15 mL distilled water. The mixture was heated to boiling and 2 drops of methylene blue indicator were added. The hot Fehling's solution was titrated against the diluted juice sample (10 mL juice diluted to 100 mL with distilled water) until the endpoint was reached, indicated by the disappearance of the blue colour and

the formation of a brick-red precipitate. The titration was completed rapidly (that is about 15 sec) while maintaining boiling conditions. The initial and final burette readings were recorded and reducing sugar content was calculated using the formula.

$$\text{Reducing sugar (\%)} = (a \times V \times 100) / (T \times 1000 \times W)$$

where:

a = Fehling factor

V = Volume made up in volumetric flask (100 mL)

T = Titre value (Burette reading, BR)

W = Volume of juice initially taken (10 mL)

Statistical analysis

OPSTAT software was used to statistically analyse the quantitative data from each genotype's leaves and fruits. Each characteristics mean was determined and data were subjected to one-way ANOVA using OPSTAT software. Mean comparisons were performed at $p \leq 0.05$. Origin Pro software (version 9.9) was used to conduct PCA and correlation analysis (28).

Results

The mandarin (*C. reticulata*) germplasms collected from West Bengal, Sikkim and Nagaland exhibited significant morphological and biochemical variability. Fruit weight (112.98 g), juice percentage (66.69 %), pulp weight (86.40 g), seed weight (2.31 g) and total soluble solids (10.13 ± 1.08 °Brix) were all highest in accessions from Dzongu (North Sikkim). Additionally, Dzongu had the highest TSS:Acid ratio (20.87 ± 3.97) and the highest vitamin C (33.48 ± 4.44 mg/100 mL), indicating better sweetness and nutritional quality. Tseminyü (Nagaland), on the other hand, had the highest titratable acidity (1.04 ± 0.08 %) and the lowest fruit weight (61.05 g), whereas Rüsoma (Nagaland) recorded the highest reducing sugar content (4.67 ± 1.98 %) and larger fruit dimensions (57.62 mm length, 63.38 mm breadth). According to these results, there is significant genetic variation among the accessions that were

gathered and Dzongu and Rüsoma show promise as sources of superior planting materials.

Principal component analysis (PCA)

Principal component analysis (PCA) was performed using 17 morphological and biochemical traits of seven mandarin accessions collected from Eastern India (Table 2). The first five principal components (PCs) had eigenvalues greater than 1.0 and together accounted for 81.10 % of the total variation observed among the accessions (Table 2). PC1 explained the largest proportion of variation (46.46 %), followed by PC2 (10.32 %), PC3 (9.24 %), PC4 (8.50 %) and PC5 (6.58 %). PC1 was positively associated with tree girth (0.367), east-west canopy spread (0.361), TSS/acid ratio (0.354), leaf length (0.288) and leaf width (0.280), whereas age of plant (-0.339) and titratable acidity (-0.333) exhibited negative loadings. PC2 was characterised by high positive loadings for north-south canopy spread (0.391), TSS (0.379), vitamin C (0.316) and plant height (0.280). PC3 was mainly influenced by fruit length (0.533) and pH (0.360), while PC4 was dominated by reducing sugar (0.574). PC5 showed strong positive associations with pH (0.528) and fruit width (0.419).

The PCA biplot revealed clear differentiation among accessions based on fruit quality and vegetative traits, indicating substantial phenotypic diversity within the studied mandarin germplasm. The high cumulative variance explained by the first five principal components suggests that the selected traits effectively captured the majority of variability present among the evaluated accessions (Fig. 4 and 5).

Pearson's correlation analysis revealed several significant relationships among the evaluated morphological and biochemical traits (Fig. 6). Strong positive correlations were observed among total soluble solids (TSS), total sugar and reducing sugar, indicating a close association among fruit quality attributes. Fruit size traits, particularly fruit length, fruit width and rind thickness, also exhibited positive correlations with biochemical parameters, suggesting coordinated development between fruit growth and biochemical composition.

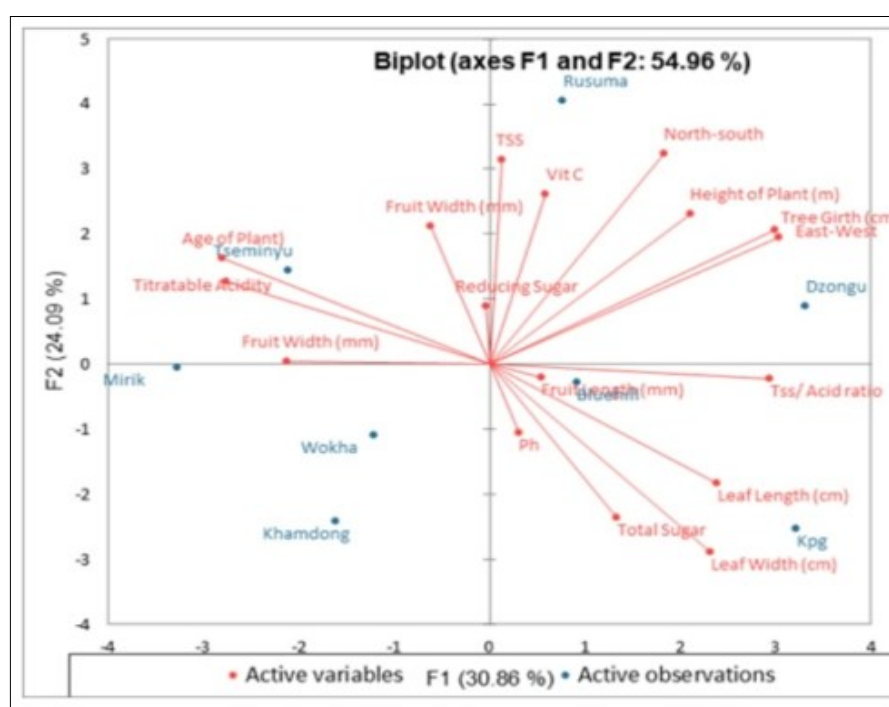


Fig. 4. Principal component analysis biplot for morphological and biochemical traits for individuals.

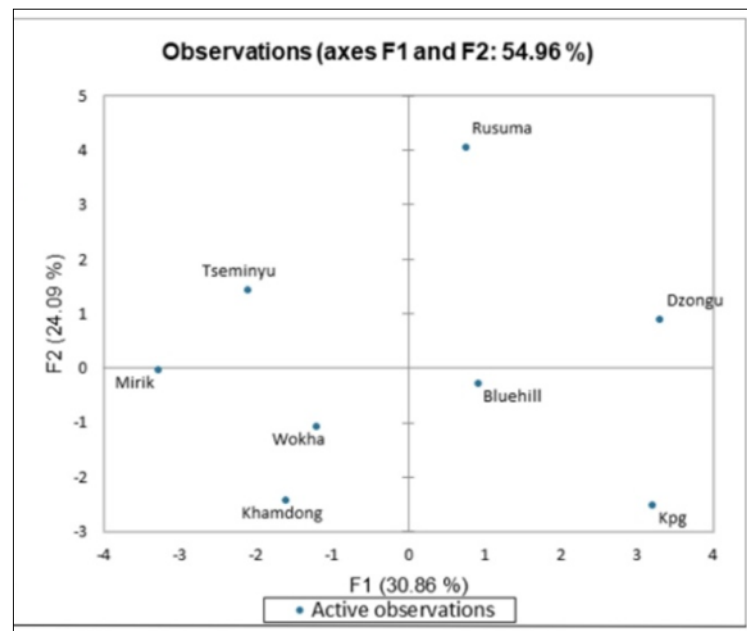


Fig. 5. Principal component analysis biplot for mandarin.

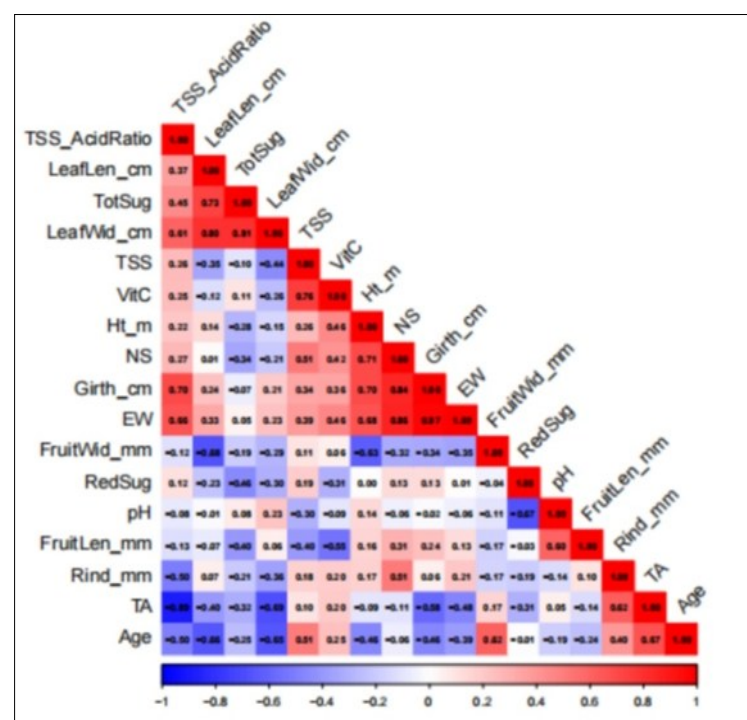


Fig. 6. Correlation analysis of different morphological and biochemical parameter.

A significant negative correlation was observed between pH and titratable acidity, which is consistent with the expected inverse relationship between acidity and fruit juice pH. In contrast, plant age, canopy orientation (East-West and North-South spread) and leaf traits generally showed weak or non-significant correlations with most fruit quality parameters, indicating a comparatively lower contribution to variation in fruit biochemical characteristics.

The correlation analysis complemented the PCA results by demonstrating that fruit quality attributes and fruit size characteristics were the major contributors to variability among the studied mandarin accessions. These findings suggest that biochemical traits, together with key fruit morphological characteristics, are important descriptors for germplasm characterisation and accession differentiation (Fig. 7).

Discussion

The predominance of biochemical characteristics in the PCA, such as pH, TSS/acid ratio, vitamin C and total sugars, highlights their importance for determining fruit quality and differentiating across genotypes. Principal component analysis demonstrated that fruit quality attributes were the major contributors to accession differentiation, with the first five principal components explaining 81.10 % of the total variation. The strong influence of traits such as TSS/acid ratio, vitamin C, total soluble solids (TSS), reducing sugar and pH indicates that biochemical characteristics represent the most important sources of variability within the studied germplasm. These findings suggest that fruit quality traits are highly informative for the characterisation and discrimination of mandarin accessions.

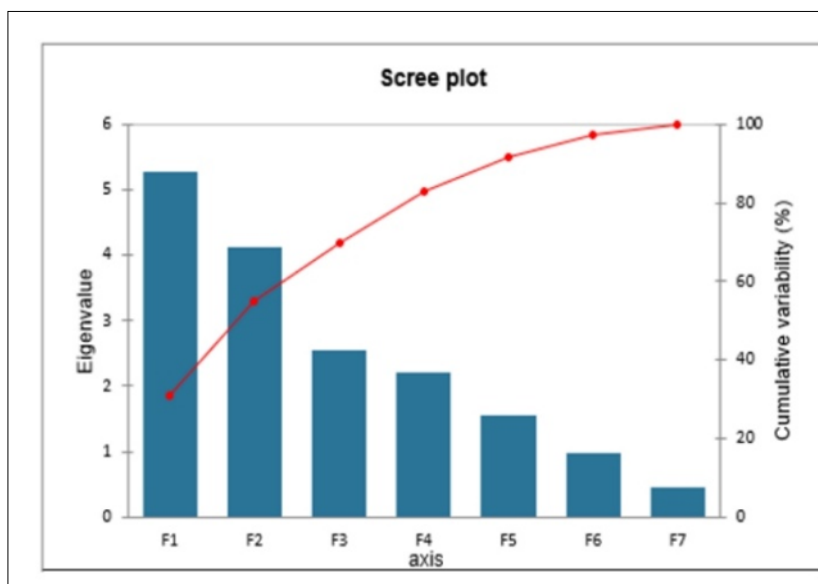


Fig. 7. Principal component analysis scree plot for morphological and biochemical traits.

Among the evaluated accessions, SKM-01 (Dzongu) exhibited superior biochemical characteristics, including higher TSS, vitamin C content and TSS/acid ratio, indicating its potential as a valuable source of fruit quality traits. In contrast, NAG-01 (Rüsoma) was distinguished by desirable fruit size and morphological characteristics. The separation of these accessions in the PCA biplot reflects their distinct phenotypic profiles and highlights the existence of substantial diversity within mandarin germplasm from Eastern India. Such diversity is essential for germplasm conservation and provides opportunities for the identification of promising parental materials for future breeding programmes.

The contribution of fruit length, fruit width and rind thickness to the principal components further demonstrates the importance of fruit morphology in accession differentiation. Although biochemical traits accounted for a greater proportion of variability, fruit size characteristics also played an important role in distinguishing accessions. Similar findings have been reported in tomato, peach and banana germplasm, where fruit quality and morphological traits were identified as major determinants of diversity and genotype classification (29–31). The present findings therefore confirm the usefulness of integrating both biochemical and morphological descriptors in mandarin germplasm evaluation.

The correlation analysis complemented the PCA results by revealing strong positive associations among TSS, total sugar and reducing sugar, indicating coordinated sugar accumulation during fruit development. Positive relationships between fruit size traits and biochemical characteristics suggest that improvements in fruit growth may be accompanied by enhanced fruit quality. A strong negative correlation between pH and titratable acidity was also observed, which is consistent with the expected physiological relationship between fruit acidity and juice pH.

Vegetative traits such as canopy orientation, plant age and leaf dimensions generally exhibited weaker associations with fruit biochemical characteristics and contributed less to accession differentiation than fruit quality traits. Nevertheless, these characteristics remain useful descriptors for overall germplasm characterisation and orchard evaluation. The high cumulative variance explained by the first five PCs demonstrates that the selected morphological and biochemical traits effectively captured the majority of variation present among the studied accessions.

The distinct patterns observed in the heatmap support this interpretation. Fruit size measurements show strong positive relationships that indicate coordinated developmental processes; larger fruits typically grow proportionately in several dimensions. Similarly, the strong correlations between sugar-related characteristics can be explained by their tight clustering, which represents common metabolic pathways throughout fruit development. The hypothesised physiological link, in which rising acidity naturally decreases pH, is supported by the strong negative association between pH and titratable acidity. This interpretation is supported by other investigations. A network of 74 significant relationships between fruit characteristics, with dry matter, pH and °Brix serving as the three main interconnected nodes was discovered (32). In a similar vein, it was found that a negative correlation between fruit size parameters and biochemical features, indicating an inverse relationship between metabolite concentrations and fruit dimensions (33). There is very strong evidence for the physiological connection between pH and acidity. Sugar molecules had a strong link with total soluble solids, while organic acids such as citric and quinic acid exhibited significant positive correlations with titratable acidity (34).

Overall, the results indicate that biochemical traits, particularly TSS, vitamin C, reducing sugar, total sugar and TSS/acid ratio, together with fruit size characteristics, are the most informative parameters for the characterisation and selection of mandarin germplasm. The superior performance of SKM-01 (Dzongu) and NAG-01 (Rüsoma) suggests that these accessions represent valuable genetic resources for future conservation, selection and breeding programmes aimed at improving fruit quality and productivity in mandarin.

These results collectively imply that biochemical traits and particular fruit morphology parameters should be given priority in selection, characterisation and breeding because they are both highly variable and closely related, whereas non-significant traits might be viewed as less important for genotype differentiation. It was emphasised that the importance of different morphological and biochemical traits in identifying and differentiating *Citrus* genotypes (35–39).

Conclusion

Mandarin (*Citrus reticulata*) accessions collected from West Bengal, Sikkim and Nagaland exhibited substantial morphological and biochemical variability. Multivariate analyses revealed that biochemical traits, particularly TSS/acid ratio, vitamin C, total sugar, reducing sugar and pH, together with key fruit size characteristics, were the principal contributors to variability among the evaluated accessions. Among the studied germplasm, SKM-01 (Dzongu, Sikkim) and NAG-01 (Rüsoma, Nagaland) exhibited desirable fruit quality and fruit morphological attributes, highlighting their potential as valuable genetic resources for germplasm conservation, selection and future breeding programmes aimed at improving mandarin fruit quality. The combined principal component analysis (PCA) and correlation analyses provided a robust framework for identifying superior accessions and key traits associated with fruit quality. However, further multi-location and multi-year evaluations are required to validate the performance and stability of these accessions before their large-scale utilisation in breeding or cultivation programmes.

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Authors' contributions

NC carried out the genetic studies, participated in the data collection, data analysis and drafted the manuscript. US participated in the design of the study and edited the manuscript. CSM and LS participated in the data analysis. AS participated in the supervision and design of the study. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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