



Exploring altitude-driven diversity: morphological, biochemical, and genetic diversity analysis of Khasi mandarin (*Citrus reticulata* Blanco) in Meghalaya, India

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Abstract Khasi Mandarin (*Citrus reticulata* Blanco) is one of the most extensively cultivated and commercially important fruit crop of Northeast India, and it is widely used for culinary purposes due to its fragrance and unique taste. This study investigated the genetic variability of elite Khasi mandarin plants from diverse altitudes in the northeastern state of Meghalaya, India. Morphological, biochemical and molecular analyses were conducted on fruits collected from nine locations spanning various altitudes. Significant variation was observed in tree morphology, fruit characteristics, and biochemical composition. Higher altitudes were associated with distinct fruit quality traits, such as higher total soluble

solids (TSS), titratable acidity, sugar and ascorbic acid content, with correlation research showing that higher altitudes generally corresponded to better overall fruit quality parameters. This study provides insights for optimizing fruit production across altitudinal gradients. Eleven out of 40 simple sequence repeat markers used for genetic diversity analysis exhibited polymorphism. Cluster analysis grouped genotypes by altitude, and the analysis of molecular variance (AMOVA) confirmed significant genetic distinctions between populations (74%). Positive correlations were observed between genetic and geographical distances ($r=0.230$) and between genetic distances based on molecular and morphological data ($r=0.506$). These insights are crucial for future conservation and crop improvement efforts.

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Introduction

Citrus fruits, botanically classified under the genus *Citrus*, are esteemed for their gustatory qualities, vivid pigmentation, and diverse health advantages. The genesis of citrus species has been traced to the southeastern Himalayan foothills, which encompass the states of Meghalaya and eastern Assam, India (Mahato et al. 2019). This region,

dating back millennia, witnessed the domestication of citrus, eventually disseminating this horticultural treasure globally via migratory movements. Notable citrus fruits include Oranges, Grapefruits, Lemons, Pummelos, and Mandarins, with the latter, *Citrus reticulata*, enjoying particular distinction. Mandarins hold prominence in China, India, Japan, and the Mediterranean region (Liu et al. 2012). *Citrus reticulata* Blanco, commonly referred to as Mandarin, belongs to the Rutaceae family and has a chromosomal count of $2n=18$. Notable global exporters of Mandarins include China, India, Spain, Brazil, Turkey, and Egypt. Within India, Mandarin stands as the preeminent citrus fruit crop, yielding 6,046.16 thousand metric tons across an expanse of 476 thousand hectares (India production of Mandarin, APEDA, 2021–22). The leading Mandarin producers in India include Madhya Pradesh, Punjab, Maharashtra, Rajasthan, Haryana, Assam, Karnataka, Arunachal Pradesh, Mizoram, Manipur, and Meghalaya. Among these states, Khasi mandarin from Meghalaya boasts exceptional consumer acceptance.

Khasi Mandarin (*Citrus reticulata* Blanco) constitutes a pivotal and economically significant citrus cultivar, primarily within the Meghalaya state of Northeast India. Historically, it is believed to be a central hub for the global dissemination of citrus (Deka et al. 2022). Khasi Mandarin significantly contributes to India's citrus production, accounting for approximately 43.6% of the total citrus output and covering nearly 38.2% of the total citrus cultivation area (Deka et al. 2022). The cultivation of Khasi Mandarin plays a pivotal role in uplifting the socioeconomic landscape of the region (Singh et al. 2016a, b). Mandarin, particularly *Citrus reticulata*, is esteemed for its nutritional and sensory attributes and is consumed both fresh and processed into juices (Li et al. 2019). Recognized for its sweet-tangy flavour, it has experienced a surge in popularity due to its desirable characteristics (Tarancón et al. 2021). Beyond their palatable attributes, mandarins are nutritional powerhouses, rich in vitamin C and dietary fibre, while also containing antioxidants such as beta-carotene and flavonoids.

Altitude significantly influences mandarin fruit quality, impacting attributes such as size, weight, acidity, and physiological weight loss (Rokaya et al. 2016). Genetic diversity further contributes to the

variability of these fruit quality traits. Molecular markers, particularly simple sequence repeats (SSR), have proven invaluable in elucidating genetic diversity within mandarin species (Barkley et al. 2006). The present study addresses the need to identify elite Khasi Mandarin plants suitable for large-scale propagation and assessed the impact of altitude on fruit quality. This research employed SSR markers to evaluate genetic diversity among elite Khasi Mandarin plants cultivated at varying altitudes within Meghalaya, offering insights into their genetic makeup and potential for commercial cultivation. By examining the interplay of climate, altitude, and genetic diversity, this study can aid growers and breeders in optimizing fruit quality and aligning production with consumer preferences.

Materials and methods

Collection of plant materials

Khasi Mandarin (*Citrus reticulata* Blanco) genotypes were systematically selected from various locations in Meghalaya, India, for this comprehensive study (Fig. S1). To determine the ecological significance of altitude in shaping citrus diversity, a multi-altitude approach was employed. Plant materials were collected from nine distinct locations spanning a range of altitudes during the harvesting season, from January to February 2023 (Table S1). To ensure representation of diversity within each location, plant samples were collected from five different trees at each site, which were selected based on their robust growth and the production of organoleptically high-quality fruits.

Morphological characterization

The initial phase of the investigation involved a thorough morphological characterization of the collected Khasi Mandarin genotypes. Following guidelines from the International Plant Genetic Resources Institute (IPGRI) citrus descriptors (1999), various morphological traits were systematically documented. These included observations of tree growth patterns, leaf morphology, fruit characteristics, and other pertinent parameters (Tables S2 and S3). This holistic approach provided

valuable insights into the phenotypic diversity of the genotypes.

Biochemical characterization

The juice content (%), total soluble solids (TSS) (⁰B), titratable acidity (TA) (in %), TSS/TA, ascorbic acid (mg/100 g), reducing sugar (%) and total sugars (%) of the collected fruit samples were analysed. TSS in the juice was measured with an Atago PAL-1 digital refractometer (Ranganna 1986). The TSS:TA ratio was calculated as a maturity index. Titratable acidity was measured by titrating the juice against N/10 NaOH and expressed as percent acidity (AOAC 1995). The ascorbic acid content was estimated following the colorimetric method adopted by Jagota and Dani (1982). Total sugars and reducing sugar were measured according to the protocols of Hedge and Hofreiter (1962), Nelson (1944), and Somogyi (1952). For ascorbic acid, reducing sugar and total sugar estimation, spectrophotometric analysis was performed by using a double-beam UV visible spectrophotometer (UV Plus, Motras Scientific).

DNA extraction and PCR amplification

High-quality genomic DNA was extracted from mandarin leaves using the cetyl trimethyl ammonium bromide (CTAB) method by Doyle and Doyle (1990). Following extraction, the DNA concentration was quantified using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer and electrophoresis on 0.8% agarose gel.

A panel of 40 SSR markers known for their utility in citrus genetic research was selected (Singh et al. 2016a, b and Kaur et al. 2022). These markers were chosen based on their polymorphic nature and wide distribution across the citrus genome. PCR amplification was performed on the extracted DNA samples using these selected SSR markers.

PCR amplification was conducted in a total volume of 10 µl. This reaction mixture included 1.0 µl of 10X PCR buffer, 1.75 mM MgCl₂, 0.5 µM forward and reverse primers, 200 µM dNTPs, 0.6 U of Taq polymerase, DNA (50 ng), and 4.0 µl of deionized distilled water. The thermal cycling was performed using a Bio-Rad thermal cycler with an initial denaturation step at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer

annealing at a temperature ranging from 48 to 62 °C for 1 min (adjusted individually for each primer), and primer extension at 72 °C for 1 min. A final extension step was conducted at 72 °C for 7 min. During the analysis, samples were loaded onto a 2.0% agarose gel, along with a standard 100 bp DNA ladder from TAKARA, Japan. Agarose gel electrophoresis was performed at a constant voltage of 80 V for 2–3 h in 0.5×TBE buffer. The gels were visualized under UV light and captured using a photo documentation system (AlphaImager™ MINI). To ensure marker repeatability, all markers were tested across the entire collection, and any null alleles were confirmed through a second amplification process.

Genetic diversity analysis

The quantitative data from the leaves and fruits of each genotype were statistically analysed using SPSS software (version 27.0). The mean for each characteristic was calculated, and groups were compared using one-way ANOVA for descriptive analysis. Correlation analysis and principal component analysis (PCA) were performed using Origin Pro software (version 9.9).

The genetic diversity analysis was performed using software, including GenAlEx and NTSYS. The key statistical parameters calculated included the polymorphism information content (PIC), Shannon's information index and Nei's genetic distance. PIC quantified the informativeness of each SSR marker in distinguishing between genotypes, while Nei's genetic distance allowed us to construct a genetic distance matrix depicting the genetic relationships among the Khasi Mandarin genotypes.

Results and discussion

Morphological and biochemical characterization

Analysis of variance

Analysis of variance (ANOVA) was conducted for all morphological and biochemical traits to measure variation among the different Mandarin plants (Tables S2 and S3). The results revealed significant differences in the morphological traits of trees at different altitudes. Specifically, trees at

higher altitudes exhibited shorter spine lengths and larger leaves compared to those at lower altitudes. Fruits from higher altitudes were smaller in size, had thinner rinds, and displayed higher levels of total soluble solids (TSS), titratable acidity (TA), sugar content, and vitamin C (AsA) compared to fruits from lower altitudes.

There is always a chance that variations in these fruit quality parameters would be influenced by the area's climatic factors, which includes temperature, rainfall, humidity, sunshine, phasing of sites/slopes, air currents, soil nutritional status, soil moisture condition, and pH (Anand et al. 2022). In this case, the variation in geography was directly related to altitude.

Correlation study of morphological and biochemical traits

Altitude and morphological traits Altitude exhibited a significant positive correlation with tree diameter ($r=0.652^{**}$) and circumference ($r=0.652$) (Fig. 1). This finding aligns with previous findings by Khalid et al. (2011) in Kinnow mandarins, reinforcing the notion that tree structural attributes respond to altitude-induced environmental variations.

Conversely, negative correlations were observed between altitude and attributes such as canopy diameter ($r=-0.351^*$), spine length ($r=-0.507^{**}$), fruit weight ($r=-0.486^{**}$), fruit length ($r=-0.632^{**}$), fruit diameter ($r=-0.632^{**}$), rind thickness (-0.795^{**}), TSS/TA ratio ($r=-0.880^{**}$), and juice volume ($r=-0.773^{**}$) (Fig. 1). These findings suggest that as altitude increases, trees tend to have smaller canopies, shorter spines, and lighter, more compact fruits. Mandarin weight decreases with

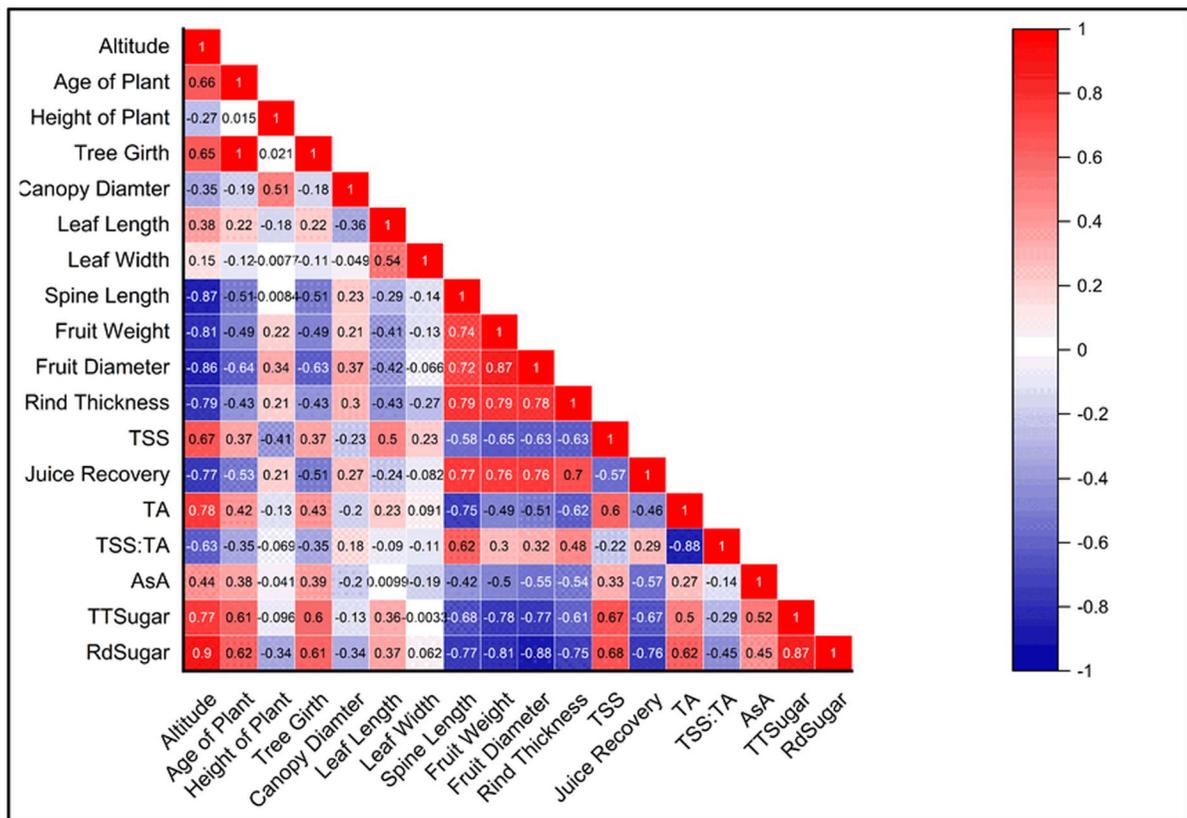


Fig. 1 Correlation analysis of different morphological and biochemical parameters

altitude, primarily due to lower temperature and reduced cell division and dry matter accumulation at higher altitudes (Rokaya et al. 2016). Catabolic activities and dry matter loss in the fruit sacs further contribute to weight loss (Rokaya 2017). The negative correlation between altitude and rind thickness aligned with previous research in Sikkim and Nepal Himalayas, indicating that environmental factors impact fruit development (Timilsina and Tripathi 2019), (Pradhan and Sharma. 2019). Furthermore, the negative correlation with the TSS/TA ratio indicates a potential shift in the balance of soluble solids to acidity, likely influenced by environmental factors. Deshmukh et al. (2016) reported that as the altitude increases and temperatures rise, the TSS:acid ratio decreases. This is due to longer fruit maturation periods at higher altitudes, resulting in the formation of more acidic compounds as reducing sugars breakdown during later stages of development.

Additionally, spine length (SL) exhibited a negative correlation with altitude ($r = -0.514^{**}$), emphasizing that trees at higher altitudes are more likely to be spineless or have shorter spines. This finding supports the notion that herbivore pressure may influence the development of defensive structures in mandarin trees.

Altitude and fruit biochemical traits

Altitude exhibited significant positive correlations with various biochemical traits, including TSS ($r = 0.673^{**}$), ascorbic acid (AsA) content ($r = 0.441^{**}$), total sugar (TS) content ($r = 0.769^{**}$), and reducing sugar (RdS) content ($r = 0.902^{**}$) (Fig. 3). These findings indicate that fruits at higher altitudes have increased sweetness, enhanced ascorbic acid levels, and elevated sugar content. This trend aligns with previous research by Rokaya et al. (2016), Shrestha et al. (2016), and Alhassan et al. (2014). Cooler, high-altitude environments likely reduce respiratory rates (Shrestha et al. 2016), leading to slower organic acid utilization and higher retention (Rokaya et al. 2016). Additionally, cooler conditions favors ascorbic acid synthesis and retention (Shrestha et al. 2016). These factors contribute to the observed increase in acidity and ascorbic acid content in Khasi mandarins at higher altitudes. This is further supported by Pradhan and Sharma (2019), who highlighted the

impact of altitude on biochemical parameters in Sikkim mandarins. The enzymatic degradation of L-ascorbic acid into 2–3 dioxy-L-gluconic acid, may be the cause of a drop in ascorbic acid level at lower altitudes (Mapson 1970).

Furthermore, the negative correlation between titratable acidity and TSS aligns with the general understanding of citrus fruit quality, where higher acidity corresponds to lower TSS. Similar findings were observed by Hangsing et al. (2016) and Rokaya et al. (2016) on mandarin oranges. Additionally, a positive correlation between plant age and certain morphological traits, such as tree diameter and fruit weight, was noted, consistent with the observations of Khalid et al. (2011). One notable finding in our study is the potential indirect indicator of fruit quality with leaf width, as it positively correlated with attributes like fruit weight. Moreover, the strong positive correlation between total sugar and reducing sugar content was consistent with the typical sugar composition in citrus fruits.

Notably, Ascorbic Acid (AsA) content showed positive correlations with both TSS ($r = 0.379$) and total sugar (TS, $r = 0.623$) contents (Fig. 3). This suggests a potential synergistic relationship between ascorbic acid and sugar content, indicating that fruits with higher sugar levels may also exhibit elevated ascorbic acid content.

Principal component analysis

Principal component analysis (PCA) revealed that the first two principal components contributed significantly to the total variability (54.99%). Key variables, such as altitude, reducing sugar, total sugar, juice content, TSS, TSS/TA ratio, fruit weight, fruit diameter, and rind thickness, were identified as important for distinguishing between genotypes (Fig. 2). This suggests broad genetic diversity among citrus genotypes based on quantitative traits, which could be exploited for future citrus selection and breeding efforts. These findings align with previous research by Shrestha et al. (2012), Roussos et al. (2012), Debabi et al. (2013), Campos et al. (2005), Malik et al. (2012), and Susandarini (2013), which highlighted the significance of various morphological and biochemical traits in discriminating and characterizing citrus genotypes.

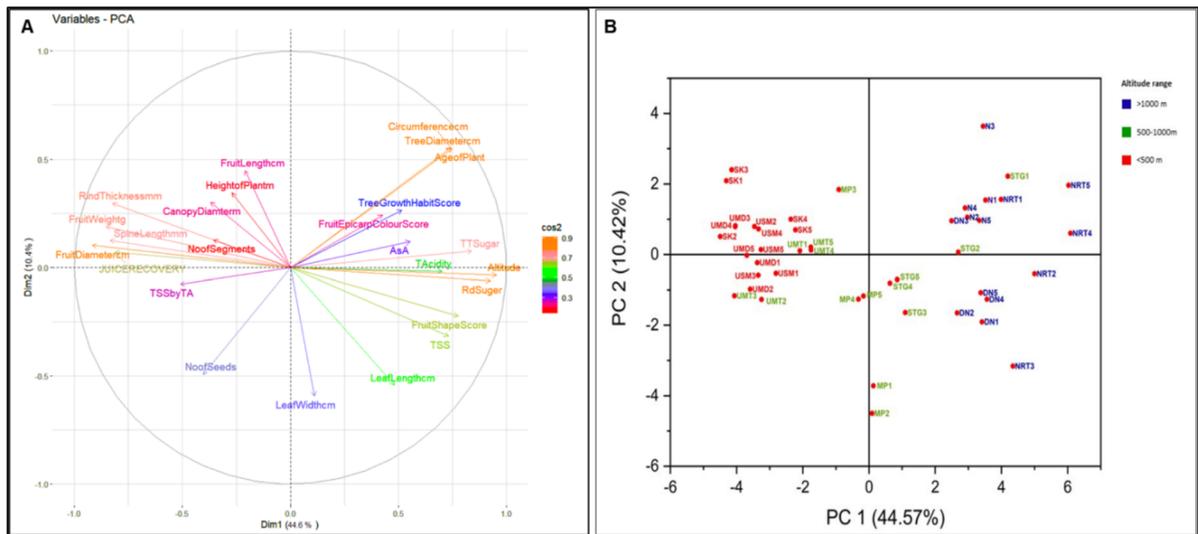


Fig. 2 Principal component analysis (PCA) results: **A-** PCA biplot for morphological and biochemical traits; **B-** PCA biplot for Khasi mandarin individuals

Molecular diversity, genetic distance and cluster analysis

SSR-based genetic distance and cluster analysis

In the present study, 40 SSR markers previously employed in citrus diversity analysis were applied to assess the genetic diversity of 45 Khasi mandarin genotypes. Among these markers, 11 exhibited polymorphic band patterns (Fig. S2). The number of observed alleles (N_a) and the effective number of alleles (N_e) were evaluated for these polymorphic markers (Table 1). CMS 30 and CMS 47 displayed N_a values of 3, indicating three unique alleles, with N_e values of 2.689 and 2.073, respectively. Shannon's information index (I) and polymorphic information content (PIC) were used to gauge genetic diversity within the markers. CMS 30 and CMS 47 exhibited high I values of 1.035 and 0.886, respectively, along with relatively elevated PIC values of 0.628 and 0.518, respectively, indicating their potential for genotype differentiation. This observation aligns with research in other citrus species, where SSR markers have demonstrated high utility in capturing genetic diversity (Barkley et al. 2006), (Jannati et al. 2009), (Biswas et al. 2011). Bhat and Jarret (1995) suggested that the number of polymorphisms might be more important than the number of primers for generating

a stable phenogram. They also indicated that the number of polymorphisms required to generate stable phenotypic analysis may vary with the plant material under investigation and the sequences amplified.

Nei's genetic distances were calculated using 11 polymorphic SSR locus, revealing varying genetic relationships among locations, with a minimum distance of 0.038 between Umsohme and Sokhwai and a maximum distance of 1.418 between Sokhwai and Umta. Cluster analysis based on Nei's genetic distances (unweighted pair group method with arithmetic mean [UPGMA]; NTSYS-pc) grouped the genotypes into two clusters (Fig. 4): Cluster I, consisting of genotypes from mid and high altitudes, and Cluster II, comprising those from lower altitudes, highlighting significant genetic divergence influenced by environmental conditions and altitude adaptation, similar to results in Assam lemon reported by Ahmed et al. (2023).

Analysis of molecular variance

The analysis of molecular variance (AMOVA) results indicated that a substantial portion (74%) of the total genetic diversity was attributed to differences among Khasi mandarin populations (Table 2), underscoring significant genetic differentiation based on location and altitude. However, 26% of

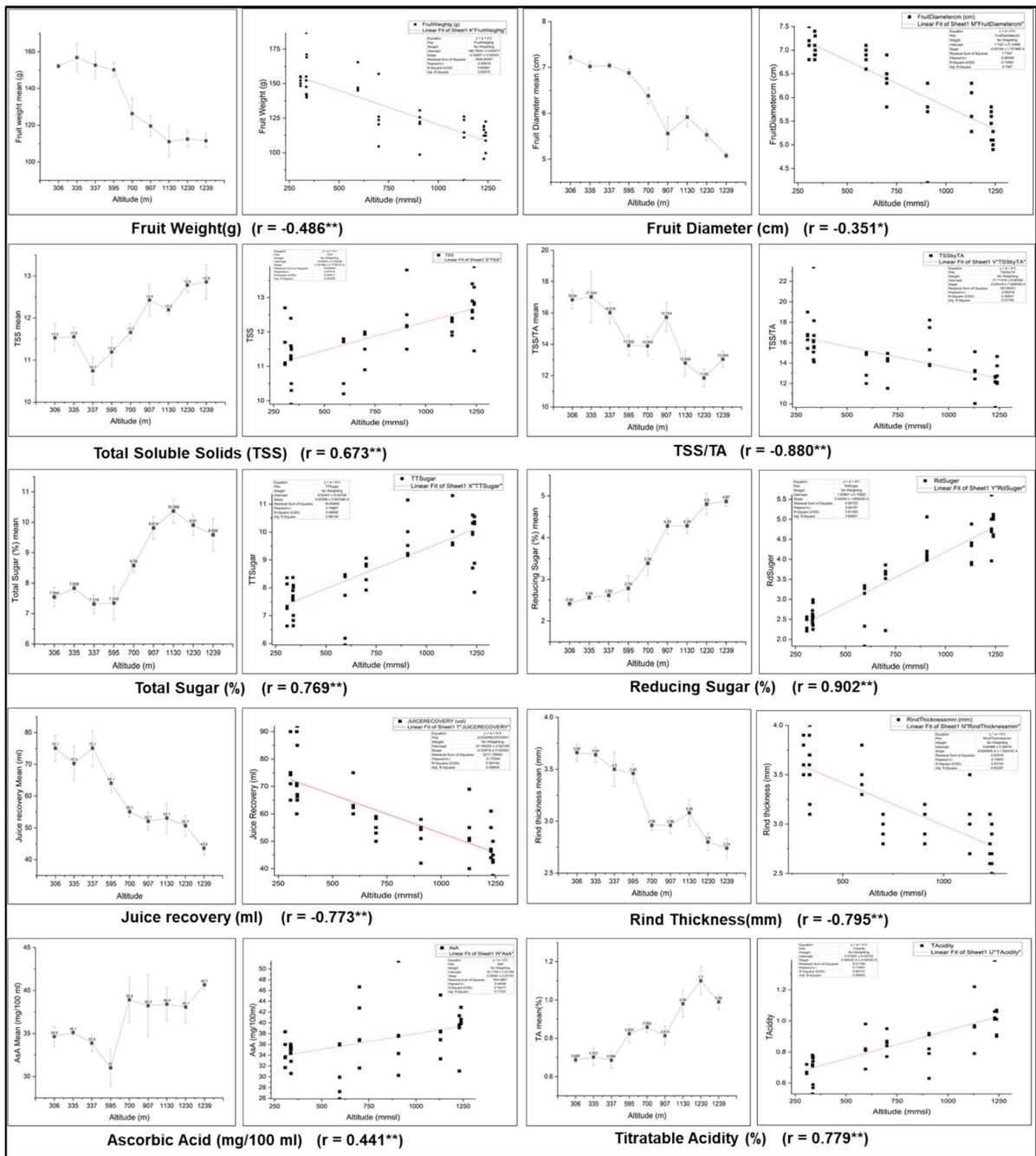


Fig. 3 Morphological and biochemical characteristics affected by altitude

the total genetic variability was found within each population, suggesting genetic diversity within the nine Khasi mandarin populations. These results were akin to those reported by Ahmed et al. (2023) for Assam lemon, where 84% of molecular variance

was observed among populations. In another study by Juibary et al. (2021) analysing sweet orange cultivars, 55% of the total genetic differences were attributed to differences among cultivars, while 45% occurred due to within-cultivar genetic variability.

Table 1 Characteristics of 11 simple sequence repeat (SSR) loci utilized for molecular diversity analysis across 45 Khasi mandarin genotypes

SI No	Marker	N	Major Allele frequency	Na*	Ne*	I*	PIC*
1	CMS 30	45	0.444	3	2.689	1.035	0.628
2	CMS 47	45	0.644	3	2.073	0.886	0.518
3	CT 19	45	0.511	2	1.999	0.693	0.500
4	CAT 01	45	0.533	2	1.991	0.691	0.498
5	CCSM 156	45	0.533	2	1.991	0.691	0.498
6	Ciclive 32,507	45	0.556	2	1.976	0.687	0.494
7	CT 21	45	0.600	2	1.923	0.673	0.480
8	Ciclive 32,846	45	0.733	2	1.642	0.580	0.391
9	CCSM 201	45	0.800	2	1.471	0.500	0.320
10	CCSM 40	45	0.800	2	1.471	0.500	0.320
11	CMS 4	45	0.933	2	1.142	0.245	0.124

N: number of samples or genotypes analyzed. Na: number of different alleles observed. Ne: effective number of alleles, which accounts for allele frequency and is a measure of genetic diversity. I: Shannon's information index, a measure of genetic diversity based on allele frequencies. PIC: polymorphic information content, a measure of the informativeness of a genetic marker, indicating how useful it is for distinguishing between genotypes

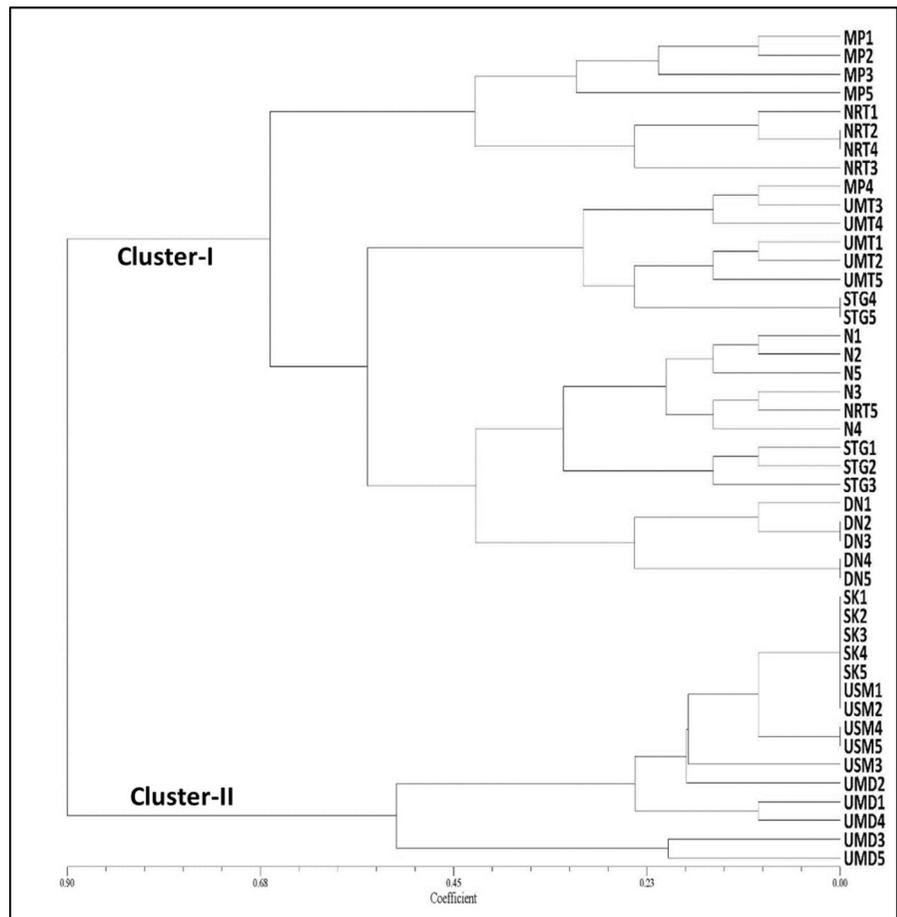
Fig. 4 Dendrogram based on Nei's genetic distance among 45 genotypes using 11 polymorphic simple sequence repeat (SSR) markers

Table 2 Summary of AMOVA

Source	df	SS	MS	Est. Var.	%
Among pops	8	65.778	8.222	1.538	74%
Within pops	36	19.2	0.533	0.533	26%
Total	44	84.978		2.071	100%

df: Degrees of freedom; SS: sum of squares; MS: mean square; Est. Var.: estimated variance; %: percentage of the total variance explained by each source

Correlation between geographical distance and genetic distance

Mantel z test revealed a statistically significant but weak positive correlation ($r=0.230$) between Nei's genetic distance and geographical distance (km) among Khasi mandarin populations at different locations. This suggests that geographic distance plays a role in genetic differentiation among Khasi mandarin genotypes, but it is not the sole or dominant factor. Other factors, such as environmental conditions, local adaptation, human cultivation practices, or historical seed exchange, might also contribute to genetic variation., which is consistent with the findings of Ahmed et al. (2023) for Assam lemon. Additionally, the influence of geographic and ecological variability on genetic diversity is well-documented in *Citrus reticulata* Blanco in Assam (Kashyap et al 2021).

Correlation between morphological and SSR marker-based genetic distance

The Mantel test indicated a statistically significant and moderately positive correlation ($r=0.506$) between genetic distance based on molecular data and genetic distance based on morphological data among the sampled populations. This suggests that genetic diversity within these Khasi Mandarin populations, as revealed by molecular markers, has a tangible impact on morphological diversity. These findings are consistent with those of Campos et al. (2005), who studied Mandarin (*Citrus reticulata* Blanco) and reported a weak positive correlation ($r=0.31$) between morphological and molecular data using the Mantel test.

In summary, molecular diversity analysis of Khasi mandarin genotypes using SSR markers revealed significant genetic differences influenced

by geographical factors and altitude. Moreover, the correlation between genetic and morphological distances reinforces the robustness of the findings and their potential relevance in citrus breeding and conservation efforts.

Conclusion

This comprehensive investigation into the genetic variability of elite Khasi mandarin plants across Meghalaya's altitudinal gradients revealed significant morphological and biochemical diversity, with altitude emerging as a crucial determinant of fruit quality attributes. Higher elevations demonstrated marked influences on key parameters, including enhanced TSS, titratable acidity, sugar profiles, and ascorbic acid content. Notably, an inverse relationship between altitude and fruit weight was observed, reflecting the complex interplay of environmental factors affecting fruit development. The study uncovered a negative correlation between TSS/TA ratio and altitude, indicating prolonged maturation periods at higher elevations—a finding with direct implications for harvest timing and post-harvest management. The observed positive correlation between ascorbic acid content and both TSS and total sugar levels illuminates the intricate relationship between sugar metabolism and AsA synthesis in these fruits. Molecular analysis utilizing SSR markers, particularly CMS 30 and CMS 47, revealed substantial genetic diversity among genotypes, with cluster analysis demonstrating altitude-based genetic grouping patterns. These comprehensive findings, integrating morphological, biochemical, and molecular analyses, provide valuable insights for cultivar selection, breeding programs, and conservation strategies for Khasi mandarin in the region, while offering practical applications for optimizing fruit production across various altitudinal zones.

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Author contributions AK, NTM, WT and MR conceptualized the study and designed the experiments. Funding acquisition was done by NTM. AK performed major experiments, analysed data and drafted the manuscript while SD also contributed in experimentation, edited the manuscript and improved the representation of the data and figures. All the authors have read and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

References

- AOAC (1995) Official Method of Analysis. Association of Official Analytical Chemists, Chapters 44, 45, Methods: 44.2.08, 45.1.14, p 16
- Ahmed R, Akhtar S, Das A, Begum K, Kashyap K, Banu S (2023) Delineating the degree of genetic divergence within Assam lemon (*Citrus limon* ‘Assam lemon’) accessions in Assam. Genetic Resources and Crop Evolution, India. <https://doi.org/10.1007/s10722-023-01606-8>
- Alhassan AF, Adjei PY, Muhammad D (2014) Effect of maturity stage and storage duration on physico-chemical properties of citrus (citrus sinensis var. Late Valencia). European Scientific J 10(36) ISSN: pp 1857–7881 (Print) e-ISSN 1857–7431
- Anand J, Rawat JS, Rawat V, Singh B, Khanduri VP, Riyal MK, Kumar P, Pinto MMSC, Kumar M (2022) Climatic and altitudinal variation in physicochemical properties of citrus sinensis in India. Land 11(11):2033. <https://doi.org/10.3390/land11112033>
- Barkley NA, Roose ML, Krueger RR, Federici CT (2006) Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theor Appl Genet 112(8):1519–1531
- Bhat KV, Jarret RL (1995) Random amplified polymorphic DNA and genetic diversity in Indian musa germplasm. Genet Resour Crop Eval 42:107–118
- Biswas MK, Chai L, Amar MH, Zhang X, Deng X (2011) Comparative analysis of genetic diversity in Citrus germplasm collection using AFLP, SSAP, SAMPL SSR Markers Sci Hortic 129(4):798–803. <https://doi.org/10.1016/j.scienta.2011.06.015>
- Campos TE, Espinosa GMA, Warburton ML, Varela SA, Monter VÁ (2005) Characterization of mandarin (*Citrus* spp.) using morphological and AFLP markers. Inter-ciencia 30:687–693
- Debbabi OS, Bouhlal R, Abdelaali N, Mnasri S, Mars M (2013) Pomological study of sweet orange (*Citrus sinensis* L. Osbeck) cultivars from tunisia. Int J Fruit Sci 13:274–284
- Deka S, Bhairavi KS, Singh S, Jose SK, Kakoti RK (2022) Diversity of spiders (Arachnida: Araneae) recorded in Khasi mandarin ecosystem of Northeastern India. Indian J Agric Res. <https://doi.org/10.18805/IJAR.A-5935>
- Deshmukh N, Patel R, Rymbai H (2016) Fruit maturity and associated changes in Khasi Mandarin (*Citrus reticulata*) at different altitudes in a humid tropical climate. Indian J Agric Sci 86:854–900
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15
- Hangsing H, Mathew B, Kalita DC (2016) Performance of Khasi mandarin in Garo Hills of Meghalaya. Int J Sci Environ Technol 5(5):3213–3223
- Hedge JE, Hofreiter BT (1962) In: Whistler RL, Be Miller JN (eds) Methods in Carbohydrate Chemistry, vol 17. Academic Press, New York, p. 420
- India production of MANDARIN (M. ORANG, KINNOW, ORANGE). (n.d.). Retrieved from https://agriexchange.apeda.gov.in/India%20Production/India_Productions.aspx?cat=fruitandhscode=1064
- IPGRI (1999) Descriptors for citrus. International Plant Genetic Resources Institute, Rome, Italy. ISBN 92-9043-425-2
- Jagota S, Dani HM (1982) A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Anal Biochem 127(1):178–182
- Jannati M, Fotouhi R, Abad AP, Salehi Z (2009) Genetic diversity analysis of Iranian citrus varieties using micro satellite (SSR) based markers. J Hortic for 1(7):120–125
- Juibary PL, Seyedmehdi FS, Sheidai M, Noormohammadi Z, Koohdar F (2021) Genetic structure analysis and genetic finger printing of sweet orange cultivars (*Citrus sinensis* (L.) Osbeck) by using SCoT molecular markers. Genetic Resour Crop Evolut 68(4):1645–1654
- Kashyap K, Bhagwat RM, Banu S (2021) Assessment of genetic variability amongst cultivated populations of Khasi mandarin (*Citrus reticulata* Blanco) detected by ISSR. Plant Genetic Resour: Charact Utilizat 19(2):122–132. <https://doi.org/10.1017/S1479262121000162>
- Kaur H, Sidhu GS, Sarao NK, Singh RP, Singh G (2022) Assessment of genetic diversity of mandarin cultivars grown in major citrus regions of world using morphological and microsatellite markers. Hortic Environ Biotechnol 63(3):425–437
- Khalid S, Malik AU, Saleem BA, Khan AS, Khalid MS, Amin M (2011) Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of ‘Kinnow’ mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora). Sci Hortic 135:137–144
- Li LJ, Tan WS, Li WJ, Zhu YB, Cheng YS, Ni H (2019) Citrus taste modification potentials by genetic engineering. Int J Mol Sci 20(24):6194

- Liu Y, Heying E, Tanumihardjo SA (2012) History, global distribution, and nutritional importance of citrus fruits. *Compr Rev Food Sci Food Saf* 11(6):530–545
- Mahato N, Sinha M, Sharma K, Koteswararao R, Cho MH (2019) Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. *Foods* 8:523
- Malik SK, Rohini MR, Kumar S, Choudhary R, Pal D, Chaudhury R (2012) Assessment of genetic diversity in sweet orange [*Citrus sinensis* (L.) Osbeck] cultivars of India using morphological and RAPD markers. *Agric Res* 1(4):317–324. <https://doi.org/10.1007/s40003-012-0045-3>
- Mapson LW (1970) The Biochemistry of Fruits and Their Products (vol 1). In: Hulme AC (ed) Academic Press, London, pp 369–384
- Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. *J Biol Chem* 153(2):375–380
- Pradhan A, Sharma L (2019) Biochemical profiling of peel and pomace of organically grown *Citrus reticulata* at different altitudinal gradient. *J Exp Biol Agric Sci* 7(5):442–451
- Ranganna S (1986) Handbook of Analysis and Quality Control for Fruit and Vegetable Products. Tata Mc Graw-Hill Publishing Company, New Delhi
- Rokaya PR (2017) Effect of altitude and various and postharvest factors on quality and shelf life of mandarin (*Citrus reticulata* Blanco). Agriculture and Forestry University, Rampur
- Rokaya PR, Baral DR, Gautam DM, Shrestha AK, Paudyal KP (2016) Effect of altitude and maturity stages on quality attributes of mandarin (*Citrus reticulata* Blanco). *Am J Plant Sci* 7(6):958–966
- Roussos PA, Gasparatos D, Kyriakou C, Tsihli K, Tsantili E, Haidouti C (2012) Growth, nutrient status, and biochemical changes of sour orange plants subjected to sodium chloride stress. *Commun Soil Sci Plant Anal* 44(1–4):805–816. <https://doi.org/10.1080/00103624.2013.749438>
- Shrestha N, Shrestha S, Bhattarai A (2016) Determination of ascorbic acid in different citrus fruits of Kathmandu Valley. *J Med Bio Sci Res* 2(1):9–14
- Shrestha RL, Dhakal DD, Gautam DM, Paudyal KP, Shrestha S (2012) Study of fruit diversity and selection of elite acid lime (*Citrus aurantiifolia* Swingle) genotypes in Nepal. *Am J Plant Sci* 3(8):1098–1104. <https://doi.org/10.4236/ajps.2012.38132>
- Singh AK, Meetei NT, Singh BK, Mandal N (2016a) Khasi mandarin: Its importance, problems and prospects of cultivation in North-eastern Himalayan region. *Int J Agric, Environ Biotechnol* 9(4):573
- Singh P, Singh A, Navraj KS, Gurupkar SS, Harinder SR (2016b) Genetic diversity and DNA fingerprinting of indigenous and exotic mandarin genotypes in India using SSR markers. *Aust J Crop Sci* 10(1):24–31
- Somogyi M (1952) Notes on sugar determination. *J Biol Chem* 195(1):19–23
- Susandarini R (2013) Assessment of taxonomic affinity of Indonesian Pummelo (*Citrus maxima* (Burm.) Merr.) based on morphological characters. *Am J Agric Biol Sci* 8(3):182–190
- Tarancón P, Tárrega A, González M, Besada C (2021) External quality of mandarins: influence of fruit appearance characteristics on consumer choice. *Foods* 10(9):2188
- Timilsina K, Tripathi KM (2019) Effect of altitude and fruit bearing position on physical quality attributes of mandarin (*Citrus reticulata* Blanco) in Kavre, Nepal. *Int J Hortic Agric Food Sci* 3(4):232–242. <https://doi.org/10.22161/ijhaf.3.4.12>

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