

Integrative Morphometric, Sensory and Chloroplast DNA Evidence Clarifies Species Boundaries and Varietal Identities of *Cucumis melo* and *Cucumis sativus* Landraces in Sri Lanka

P.R.S. Pathirana

rasikasp2009@gmail.com

Rajarata University of Sri Lanka

E.Y. Fernando

Rajarata University of Sri Lanka

A.M.K.R. Bandara

Rajarata University of Sri Lanka

D.M.D. Dissanayake

Rajarata University of Sri Lanka

A.M.J.B. Adikari

Rajarata University of Sri Lanka

Research Article

Keywords: *Cucumis melo*, *Cucumis sativus*, germplasm, species boundaries, chloroplast DNA, phylogeny, divergence, varietal groups, Sri Lanka

Posted Date: December 11th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8243780/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Integrative Morphometric, Sensory and Chloroplast DNA Evidence Clarifies Species Boundaries and Varietal Identities of *Cucumis melo* and *Cucumis sativus* Landraces in Sri Lanka

P.R.S. Pathirana^{1*}, E.Y. Fernando¹, A.M.K.R. Bandara², D.M.D. Dissanayake², & A.M.J.B. Adikari²

¹Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Anuradhapura, Sri Lanka

²Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura, Sri Lanka

Corresponding author: P.R.S. Pathirana

*Correspondence E-mail: rasikasp2009@gmail.com
ORCID: <https://orcid.org/0009-0002-8989-4639>

ABSTRACT

Sri Lanka harbors a culturally significant yet under-characterized diversity of *Cucumis melo* L. and *Cucumis sativus* L., much of which is absent from global germplasm repositories. To address longstanding taxonomic uncertainties and improve documentation of these genetic resources, we conducted an integrative assessment combining a nationwide germplasm survey, multivariate morphometric analyses, chloroplast DNA barcoding, phylogenetic reconstruction, and supporting sensory evaluation. Twenty representative genotypes collected from 632 sites were cultivated across three contrasting agro-ecological zones. Morphometric analyses consistently differentiated *C. melo* from *C. sativus* and resolved coherent intra-specific varietal groups that aligned with farmer-recognized classifications. Chloroplast phylogenies based on *rbcL* and *trnH-psbA* strongly supported species boundaries and conclusively placed both *Thiyambara* variants within *C. sativus*, while the feral *Gon Kekiri* accession grouped within divergent maternal lineages of *C. melo*. Divergence dating revealed deep Miocene-level diversification in *C. melo* compared with the relatively shallow chloroplast divergence observed in *C. sativus*, consistent with established *Cucurbitaceae* evolutionary chronograms. These findings provide the first robust taxonomic framework for Sri Lankan *Cucumis*, highlighting unique maternal lineages and farmer-maintained varietal identities of potential value for conservation, crop improvement, and future evolutionary research.

KEYWORDS

Cucumis melo, *Cucumis sativus*, germplasm, species boundaries, chloroplast DNA, phylogeny, divergence, varietal groups, Sri Lanka

1. INTRODUCTION

The genus *Cucumis* (*Cucurbitaceae*) comprises several globally important vegetable and fruit crops, most notably *Cucumis sativus* L. (cucumber) and *C. melo* L. (melon). Both species possess long domestication histories, broad agro-ecological distributions, and remarkable phenotypic diversity. Their evolutionary divergence is well established, supported by differences in chromosome number, floral morphology, and chloroplast phylogenies (Sebastian et al. 2010; Renner et al. 2007). Despite extensive global research on cultivated melons and cucumbers, regional landrace complexes—particularly those maintained within traditional South Asian food systems—remain taxonomically under-characterized and are substantially underrepresented in international germplasm repositories.

Sri Lanka maintains a rich but insufficiently documented assemblage of *Cucumis* landraces, including the culturally significant *Kekiri*, a heterogeneous complex of *C. melo* vegetable and semi-sweet fruit types. These landraces display a wide spectrum of fruit shapes, rind patterns, flesh textures, and aromatic properties, reflecting long-term farmer selection and adaptation to local ecological conditions. Farmer naming systems (e.g., *Green Seeni*, *Yellow Seeni*, *White Seeni*, *Mal Kekiri*, *Deshiya Kekiri*, *Sigari*) capture aspects of this diversity but lack formal verification through standardized germplasm evaluation. Taxonomic uncertainty persists in several farmer-recognized forms, particularly *Thiyambara*, which is variably classified by local communities as *C. melo* or *C. sativus*. Such inconsistencies impede accurate species delimitation, complicate seed conservation strategies, and hinder the integration of Sri Lankan landraces into regional and global breeding programs.

Morphological descriptors standardized by IPGRI and ECPGR provide a robust foundation for characterizing *Cucumis* diversity. However, morphology alone often fails to resolve ambiguous or intermediate types due to phenotypic plasticity and environmental influences (Pitrat 2016). Sensory traits, although seldom incorporated into taxonomic studies, play an important role in farmer and consumer recognition of varietal differences in Sri Lanka and can assist in distinguishing culinary types within *C. melo*. Ultimately, definitive species identification and evolutionary interpretation require molecular evidence. Chloroplast DNA markers—particularly *rbcL* and *trnH-psbA*—have proven highly effective for species delimitation, phylogenetic reconstruction, and assessing maternal lineage structure in *Cucurbitaceae* (Kress & Erickson 2007; Renner & Schaefer 2010).

Although numerous cpDNA-based phylogenies of *Cucumis* have been published, Sri Lankan landraces remain severely underrepresented in these datasets. This omission restricts understanding of the origins, diversity, and evolutionary placement of Sri Lankan germplasm. Importantly, prior studies emphasize that chloroplast divergence reflects deep maternal lineage history rather than domestication time frames—an essential consideration when interpreting cpDNA-based divergence estimates in crop species.

Here, we present the first island-wide, integrative characterization of Sri Lankan *Cucumis* landraces using morphometric descriptors, sensory profiling, and chloroplast DNA barcoding. The objectives of this study were to:

- (1) delimit species boundaries between *C. melo* and *C. sativus*;

- (2) resolve taxonomic ambiguities in farmer-recognized landraces, including *Thiyambara*;
- (3) assess varietal structure within Sri Lankan *C. melo*; and
- (4) evaluate the placement of Sri Lankan accessions within the global *Cucumis* phylogeny, including divergence time estimates.

By integrating complementary evidence from phenotypic, sensory, and molecular datasets, this study enhances the taxonomic understanding of Sri Lankan *Cucumis* germplasm and provides a foundation for conservation, plant breeding, and future genomics research.

2. MATERIALS AND METHODS

2.1 Germplasm Exploration and Genotype Selection

A nationwide survey of *Cucumis* diversity was conducted from 2020 to 2022 across 632 sampling sites spanning major agro-ecological zones of Sri Lanka. Sampling encompassed home gardens, chena lands, commercial farms, roadside markets, and semi-wild habitats. Vernacular names, putative varietal identities, and culinary uses were documented through rapid interviews with farmers and agricultural extension officers. Field-level plant, fruit, and seed characteristics were recorded according to IPGRI/ECPGR descriptors (Diez et al., 2005) (**S1 Table**).

Twenty representative genotypes were selected to capture the morphological, ecological, and cultural diversity of Sri Lankan *Cucumis*. The panel comprised nine *C. melo* accessions, nine *C. sativus* accessions, and two farmer-defined ambiguous types—*Thiyambara* (TH) and *Thiyambara White* (THW). A suspected feral type, *Gon Kekiri* (GK), was also included. Genotype codes, species identities, and local names are listed in **Table 1**. Representative sampling locations are shown in **Figure 1**.

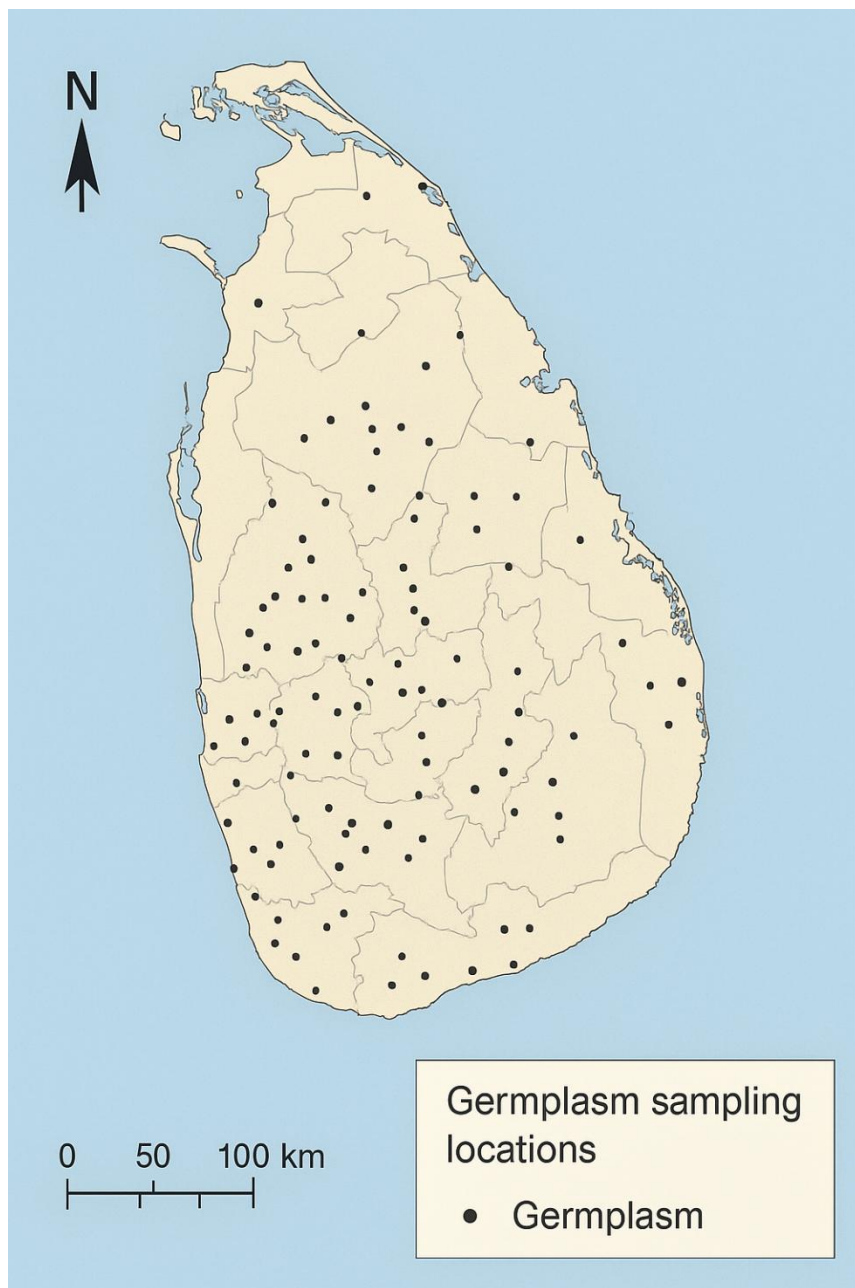


Figure 1. Representative sampling locations across Sri Lanka for the 20 *Cucumis* genotypes collected from 632 sites.

Table 1: List of collected genotypes (9 *C. melo* genotypes, 9 *C. sativus* genotypes and 2 species delimits unclear varieties) and their acronyms

Field Code	Species	Common name	Abbreviation
V1	<i>Cucumis sativus</i> L.	Sassy	Sassy
V2	<i>Cucumis sativus</i> L.	Wealthy	Wealthy
V3	<i>Cucumis sativus</i> L.	Fiesty	Fiesty
V4	<i>Cucumis sativus</i> L.	Ajex	Ajex
V5	<i>Cucumis sativus</i> L.	Chandani	Chandani
V6	<i>Cucumis sativus</i> L.	Treasure	Treasure
V7	<i>Cucumis sativus</i> L.	Kalpiti-White	KW
V8	<i>Cucumis sativus</i> L.	LY58	LY58
V9	<i>Cucumis melo</i> L.	Green-Seeni-Giant	GSG
V10	<i>Cucumis melo</i> L.	Green-Seeni-Small	GSS
V11	<i>Cucumis melo</i> L.	Yellow-Seeni-Small	YSS
V12	<i>Cucumis melo</i> L.	White-Seeni-Medium	WSM
V13	<i>Cucumis melo</i> L.	Mal-Kekiri	MK
V14	<i>Cucumis melo</i> L.	Deshiya-Kekiri	DK
V15	<i>Cucumis melo</i> L.	Sigari-Kekri	SK
V16	<i>Cucumis sativus</i> L.	Deshiya-Pipinna	DP
V17	<i>Cucumis melo</i> L.	Long Yellow Vegetable	LYV
V18	<i>Unclear</i>	Thiyambara	TH
V19	<i>Cucumis melo</i> L.	Wild spp-1 Gon Kekiri	GK
V20	<i>Unclear</i>	Thiyambara White	THW

2.2 Multi-location Field Evaluation

All genotypes were cultivated at three climatically contrasting sites: Homagama (wet zone), Cheddikulam (dry zone), and Tissamaharama (arid zone) (**S2 Table**). A randomized complete block design (RCBD) was applied at each location, with two blocks per genotype and four plants per block (**S1 Figure**). Standard agronomic management, following Sri Lankan Department of Agriculture recommendations, was uniformly applied.

Growth and phenological observations were recorded from seedling emergence through fruit maturity. Weekly measurements included vine length increment, internode elongation, juvenile leaf shape, and leaf area. Growth rate was calculated as vine length increase per day. Seedling vigor—defined through hypocotyl robustness and early leaf expansion—was quantified during the first three weeks post-transplanting. Temperature and relative humidity were recorded at all sites for evaluating genotype-specific thermal responses.

2.3 Morphometric Characterization and Multivariate Analysis

Morphometric traits were assessed using standardized IPGRI/ECPGR descriptors (**S1 Table**). Evaluated traits included:

- **Vegetative:** vine length, internode length, leaf area, leaf pubescence
- **Floral:** corolla diameter, petal length, ovary pubescence
- **Fruit:** length, diameter, shape index, rind texture, flesh thickness, fruit weight, seed cavity index
- **Seed:** length, width, coat pattern

Measurements were collected from a minimum of eight plants per genotype per site. Homogeneity of variance across sites was confirmed prior to pooling data.

Quantitative traits were standardized (mean = 0, SD = 1), categorical traits were converted to ordinal scales, and missing values (<5%) were imputed using trait-wise means. Principal Component Analysis (PCA) was performed using the covariance matrix. Ward's hierarchical clustering with Euclidean distance was used to infer varietal groupings and assess their correspondence with farmer classifications.

2.4 Sensory Evaluation (Supporting Evidence Only)

A sensory panel of 50 untrained participants evaluated cooked vegetable-type *C. melo*, raw salad-type *C. sativus*, and chilled semi-sweet fruit-type *C. melo* samples following standard preparation protocols. Traits assessed included color, aroma, texture, sweetness/bitterness, and overall acceptability using a three-point ordinal scale (A = 3, B = 2, C = 1).

Ordinal data were analyzed using the Kruskal–Wallis test, followed by Dunn's test with Bonferroni correction. PCA was used solely for visualizing multivariate sensory differentiation. Sensory data served as supporting evidence for varietal differentiation and were not used for species delimitation.

2.5 DNA Extraction, Barcoding, and Sequencing

Genomic DNA was extracted from young leaf tissue using a modified CTAB protocol (Doyle & Doyle, 1987). Two chloroplast loci were selected for barcoding and species delimitation: *rbcl* (Levin et al., 2003) and *trnH-psbA* (Tate & Simpson, 2003; Sang et al., 1997). Primer sequences and PCR conditions are listed in **Table 2**.

PCR products were purified and Sanger-sequenced bidirectionally. Chromatograms were assembled in Geneious (Kearse et al., 2012), and alignments were generated using Clustal Omega (Sievers et al., 2011) with manual adjustments for indel-rich regions.

Table 2: Evaluated key plant DNA barcoding markers.

DNA marker	Sequence	Ta (°C)	Reference
<i>trnH-psbA</i>	F- CGCGCATGGTGGATTACAAATCC	64	Tate and Simpson (2003)
	R- GTTATGCATGAACGTAATGCTC		Sang <i>et al.</i> , (1997)
<i>rbcL</i>	F- ATGTCACCACAAACAGAGACTAAAGC	55	Levin <i>et al.</i> , (2003)
	R- GTAAAATCAAGTCCACCRCG		Kress and Erickson (2009)

2.6 Phylogenetic Reconstruction

Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI). jModelTest (Posada, 2008) was used for model selection. ML analyses were performed with RAxML under the GTR+GAMMA model with 1,000 bootstrap replicates (Stamatakis, 2014). BI was carried out in MrBayes with four chains run for 50 million generations, sampling every 5,000 generations. Convergence was verified in Tracer (Rambaut et al., 2018). Consensus trees were visualized in FigTree.

Species boundaries were inferred based on the concordance between chloroplast clades and morphometric patterns.

2.7 Divergence Time Estimation

Divergence times were estimated using BEAST v1.10.4 (Bouckaert et al., 2019), employing a strict molecular clock and Yule speciation prior. Calibration points followed published *Cucurbitaceae* phylogenies: a ~60 Mya stem age for *Cucurbitaceae*, a *Cucumis–Lagenaria* split at ~45 Mya, and a *C. melo–C. sativus* divergence centered around ~11 Mya (Renner & Schaefer, 2010; Matschiner et al., 2017). MCMC chains were run for 50 million generations with 10% burn-in, and ESS values >200 were used as convergence criteria. Divergence times were interpreted as chloroplast lineage ages.

2.8 Integrative Framework

Species delimitation and varietal identity were resolved through evaluating congruence among morphological, molecular, and sensory evidence. Morphological and cpDNA data formed the primary basis for species delimitation, while sensory and ethnobotanical data provided supplementary insights into varietal differentiation. In cases of discrepancy, molecular evidence was prioritized, following accepted standards in integrative plant systematics (Padial et al., 2010).

3. RESULTS

3.1 Morphological Differentiation Between *C. melo* and *C. sativus*

The twenty *Cucumis* genotypes grown across the three field sites exhibited clear and consistent species-level morphological differentiation. *Cucumis melo* accessions possessed broader and more deeply lobed leaves with pronounced basal sinuses, larger yellow flowers, and a wide range of fruit shapes and rind textures, including elongated, oblong, and rounded forms. In contrast, *C. sativus* accessions were characterized by narrower leaves with lighter pubescence, smaller flowers, and uniformly cylindrical fruits with smooth rinds (**Figures 2-1 and 2-2; S3 and S6 Figures**).

Principal Component Analysis (PCA) based on quantitative morphometric traits further reinforced this distinction, with PC1 separating *C. melo* from *C. sativus* primarily on the basis of fruit size, flesh thickness, seed cavity diameter, and leaf area (**Figure 3**). PC2 accounted for within-species variation associated with vine and internode length and seed dimensions. Ward's hierarchical clustering of genotype means resulted in two completely distinct species clusters without any overlap between *C. melo* and *C. sativus*, demonstrating unambiguous morphological boundaries between the two species (**Figure 4**).

Within *C. melo*, multivariate analysis revealed three distinct varietal clusters corresponding to farmer-recognized types. The *Seeni Kekiri* group (GSG, GSS, YSS, WSM) comprised genotypes with medium-to-large fruits and characteristic rind color patterns. The *Mal-Sigari Kekiri* group (MK, SK) included elongated fruits often exhibiting strong aroma and variable pigmentation. The *Deshiya Kekiri* group (DK, GK) displayed round to oblong fruits with thick rinds and greater morphological plasticity. These patterns show strong agreement between local naming traditions and quantitative morphological differentiation.

In *C. sativus*, two varietal groupings were also evident. Commercial hybrid cucumber cultivars formed a cohesive cluster characterized by compact vine architecture, uniform cylindrical fruits, and low rind variation. Traditional Sri Lankan forms—including DP, *Thiyambara* (TH), and *Thiyambara* White (THW)—formed a separate subgroup that displayed more variable fruit shapes, thicker rinds, and moderate pubescence. Despite their ambiguous vernacular classifications, TH and THW consistently grouped within *C. sativus*, resolving their long-standing taxonomic uncertainty.

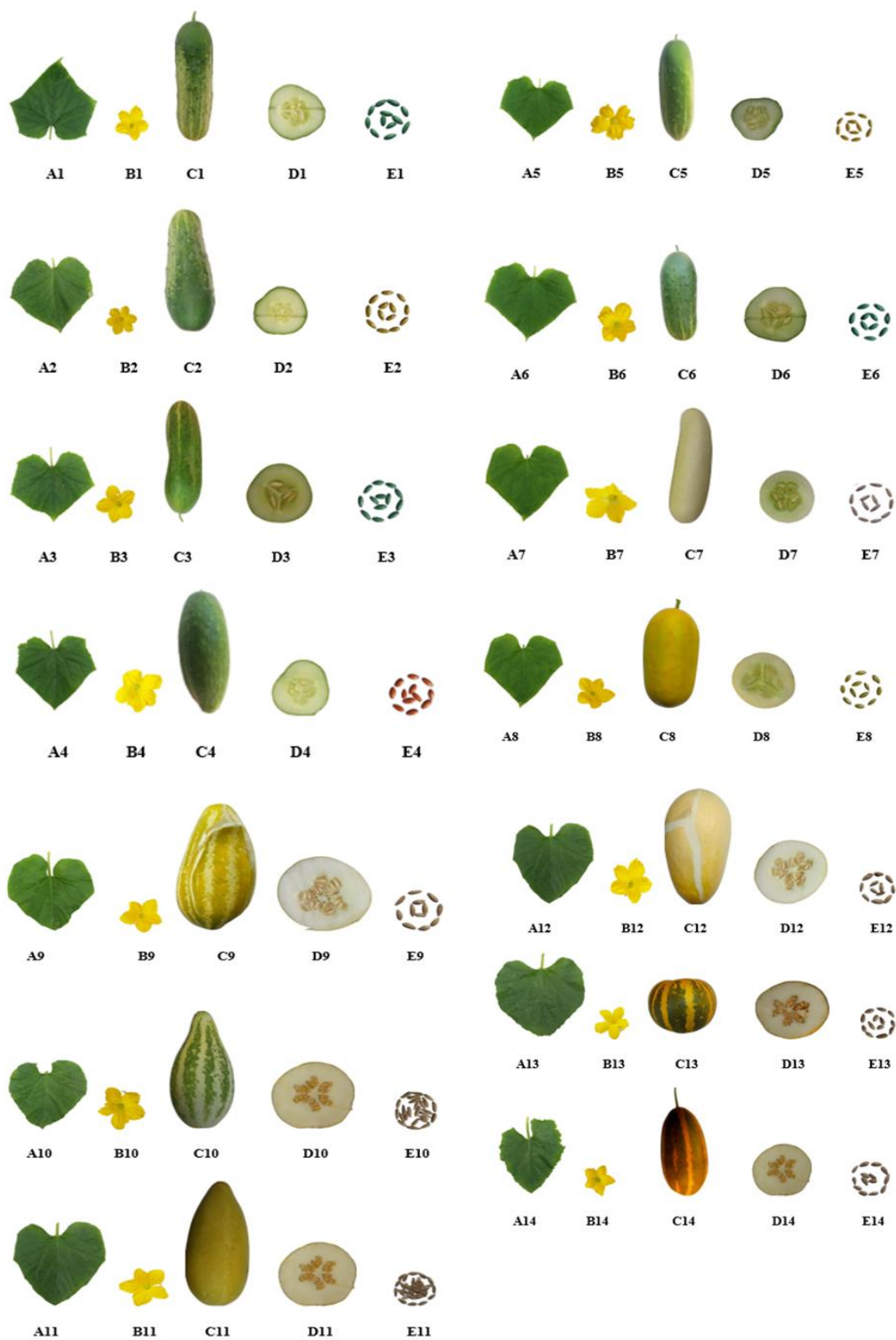


Figure 2-1. Morphological variation (leaves, flowers, fruits, cross-sections, seeds) of genotypes V1–V14.

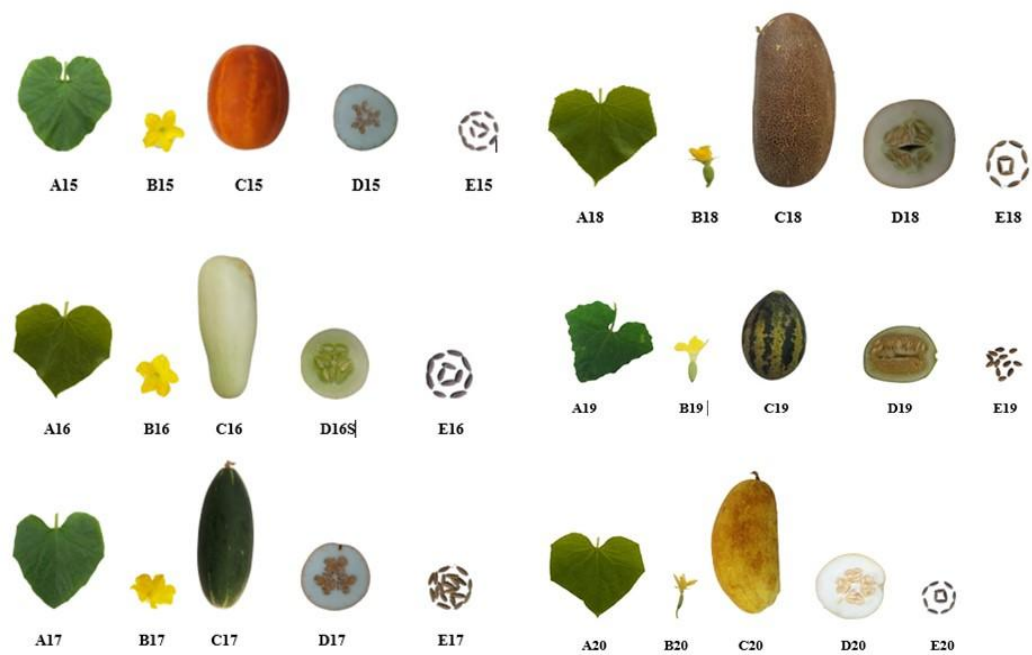


Figure 2-2. Morphological variation (leaves, flowers, fruits, cross-sections, seeds) of genotypes V15–V20.

Figure 3: PCA of morphometric traits (PC1 vs PC2) — all plants

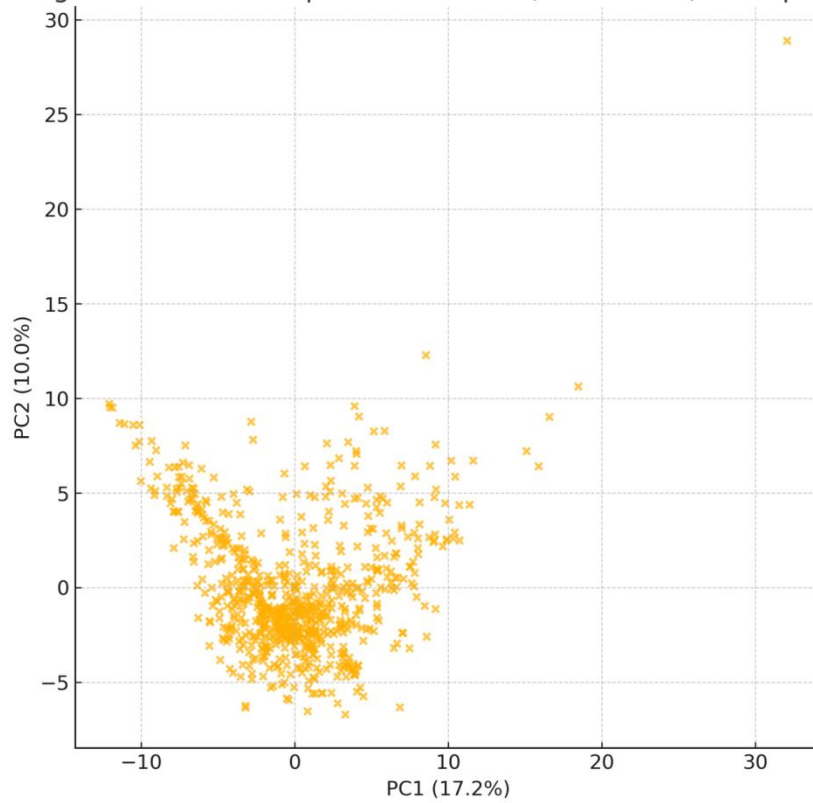


Figure 3. Principal Component Analysis (PCA) of morphometric traits for 960 plants grown across three locations. The plot shows clear species-level separation of *C. melo* and *C. sativus* along PC1, with initial clustering of varietal groups within *C. melo*. Variance explained by each axis is indicated on the plot.

Figure 4. Ward's hierarchical clustering of 20 *Cucumis* genotypes (genotype means)

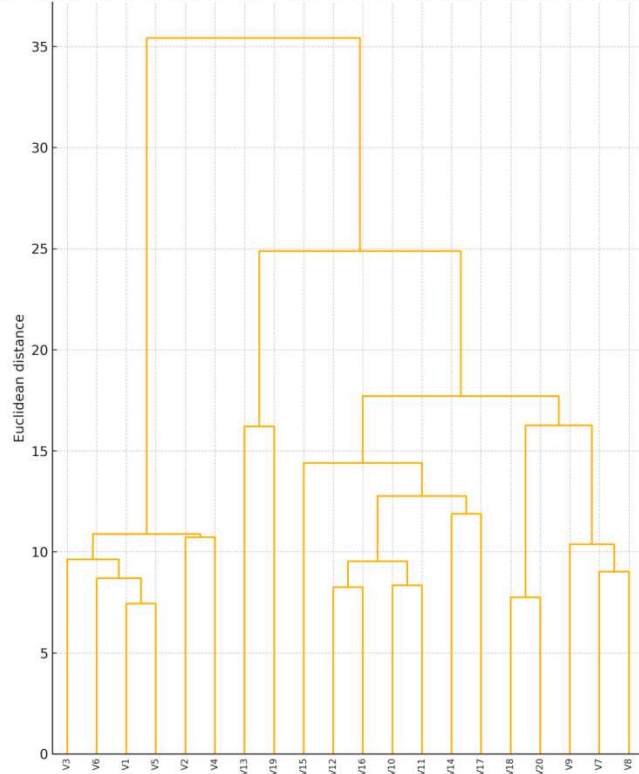


Figure 4. Ward's hierarchical clustering of 20 *Cucumis* genotypes based on morphometric traits. The dendrogram shows clear separation between *C. melo* and *C. sativus* and reveals intra-specific varietal groupings within *C. melo*. Scale indicates rescaled Euclidean distance.

3.2 Growth Dynamics and Early Developmental Traits

Vegetative growth patterns varied significantly among genotypes across the three field sites. Several *C. melo* accessions, such as WSM, SK, and YSS, exhibited rapid vine elongation, frequently exceeding 100 cm by the eighth week of growth. In contrast, commercial *C. sativus* cultivars maintained compact vine architecture with substantially shorter internodes (**Figure 5; S2 Figure**).

Seedling vigor also differed among genotypes, with GSS, YSS, and SK demonstrating robust hypocotyl development and rapid early leaf expansion, while MK and DP displayed reduced early vigor. Environmental responses at the arid Tissamaharama site revealed that *C. melo* landraces tolerated high temperature conditions more effectively than commercial *C. sativus*, indicating adaptive long-term fitness in dry-zone agricultural systems.

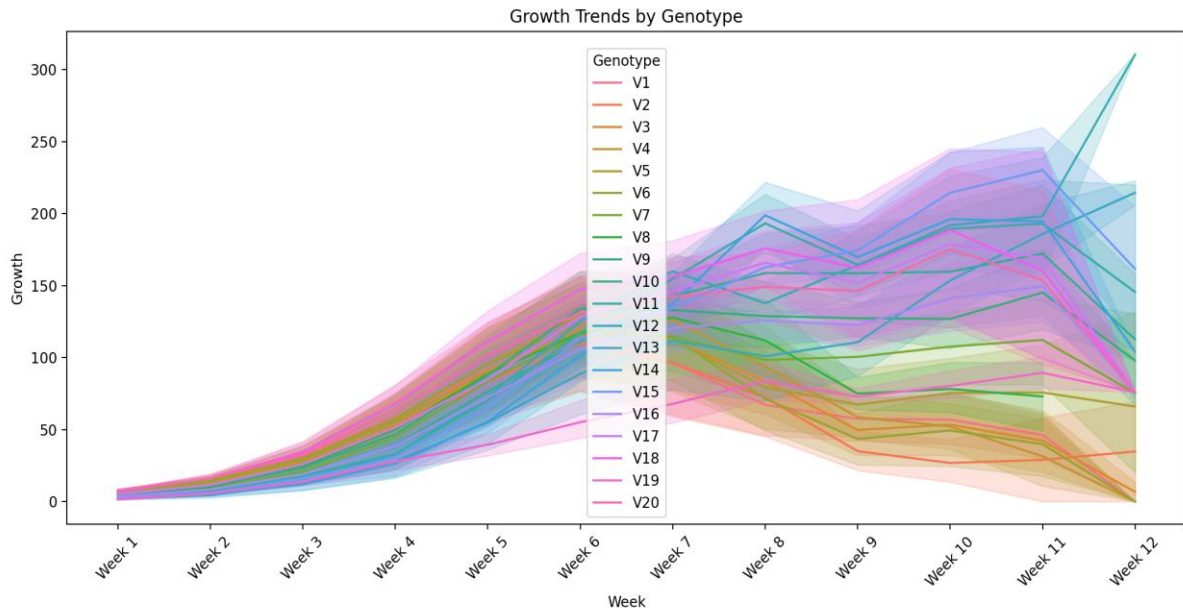


Figure 5. Comparative growth rates of selected *Cucumis* genotypes measured across multi-location field trials. Variation in vine elongation among accessions is shown.

3.3 Sensory Profiles (Supporting Evidence Only)

Although sensory evaluation was used solely as supplementary evidence (**S4; S5 Figure**), the results corresponded well with morphological and molecular patterns. Vegetable-type *C. melo* accessions displayed distinctive cooked aroma and texture attributes, and genotypes such as WSM and YSS consistently achieved the highest acceptability scores. Fruit-type *C. melo* accessions (GSG, GSS, YSS) were characterized by high sweetness, juiciness, and firm flesh, and sweetness showed a strong positive correlation with overall consumer preference. Salad-type *C. sativus* accessions were defined primarily by crispness and the absence of bitterness. TH and THW exhibited moderate bitterness but nonetheless clustered closely with commercial *C. sativus* cultivars in sensory PCA, reinforcing their correct placement within cucumber rather than melon (**Figure 6**).

Figure 6: Combined sensory PCA (genotype mean scores) — PC1 vs PC2

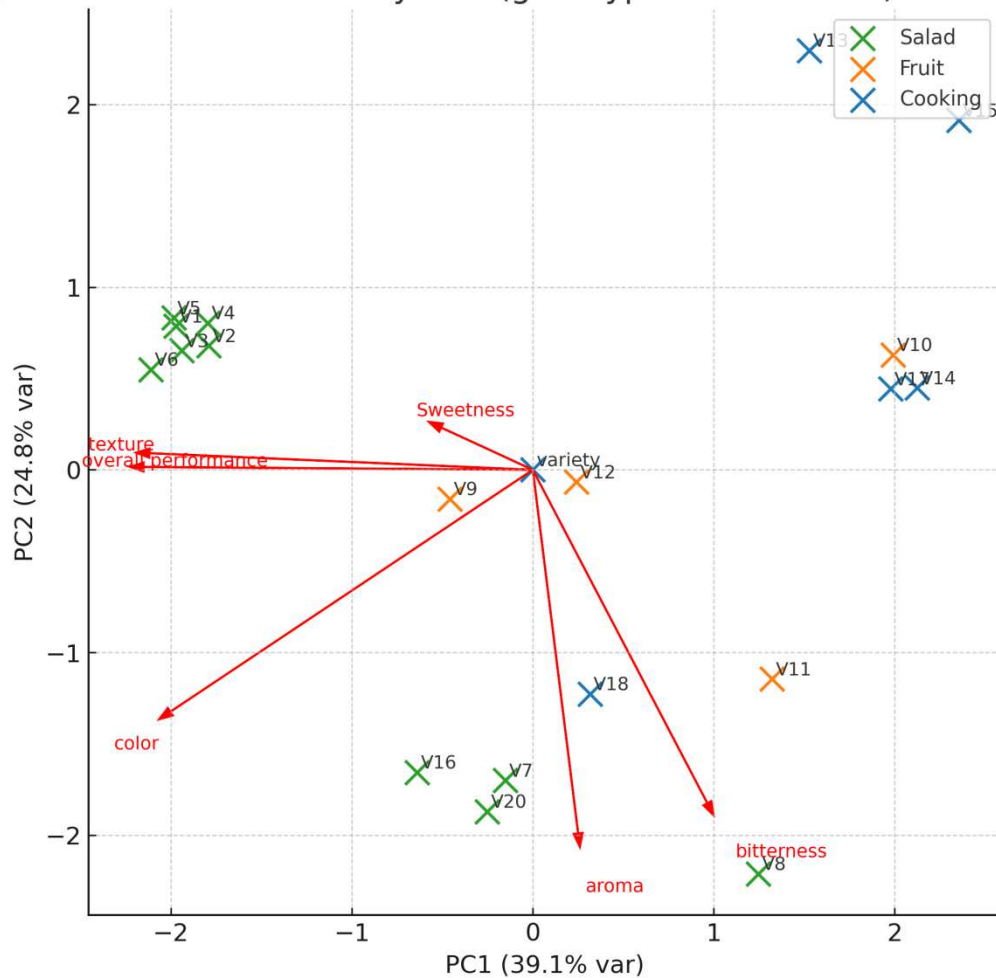


Figure 6. Principal Component Analysis (PCA) of mean sensory scores for 20 *Cucumis* genotypes, illustrating differentiation among vegetable-type, fruit-type, and salad-type groups. Variance explained by PC1 and PC2 is shown on the axes. Arrows indicate loadings of sensory attributes.

3.4 Chloroplast DNA Barcoding Resolves Ambiguous Types

Chloroplast sequence analysis using *rbcL* and *trnH-psbA* yielded high-quality alignments and consistently resolved two strongly supported clades corresponding to *C. melo* and *C. sativus* (**Figure 7**). Both Maximum Likelihood and Bayesian phylogenies produced identical species boundaries with high bootstrap support. Importantly, the previously ambiguous farmer-named accessions *Thiyambara* (TH) and *Thiyambara White* (THW) grouped unequivocally within the *C. sativus* clade, providing decisive molecular confirmation of their species identity. *Gon Kekiri* (GK) clustered within a basal *C. melo* lineage, suggesting a feral or ancient cultivated origin rather than a separate wild species. Multiple maternal haplotypes detected within *C. melo* corresponded broadly to the *Seeni Kekiri*, *Mal-Sigari Kekiri*, and *Deshiya Kekiri* morphometric groups.

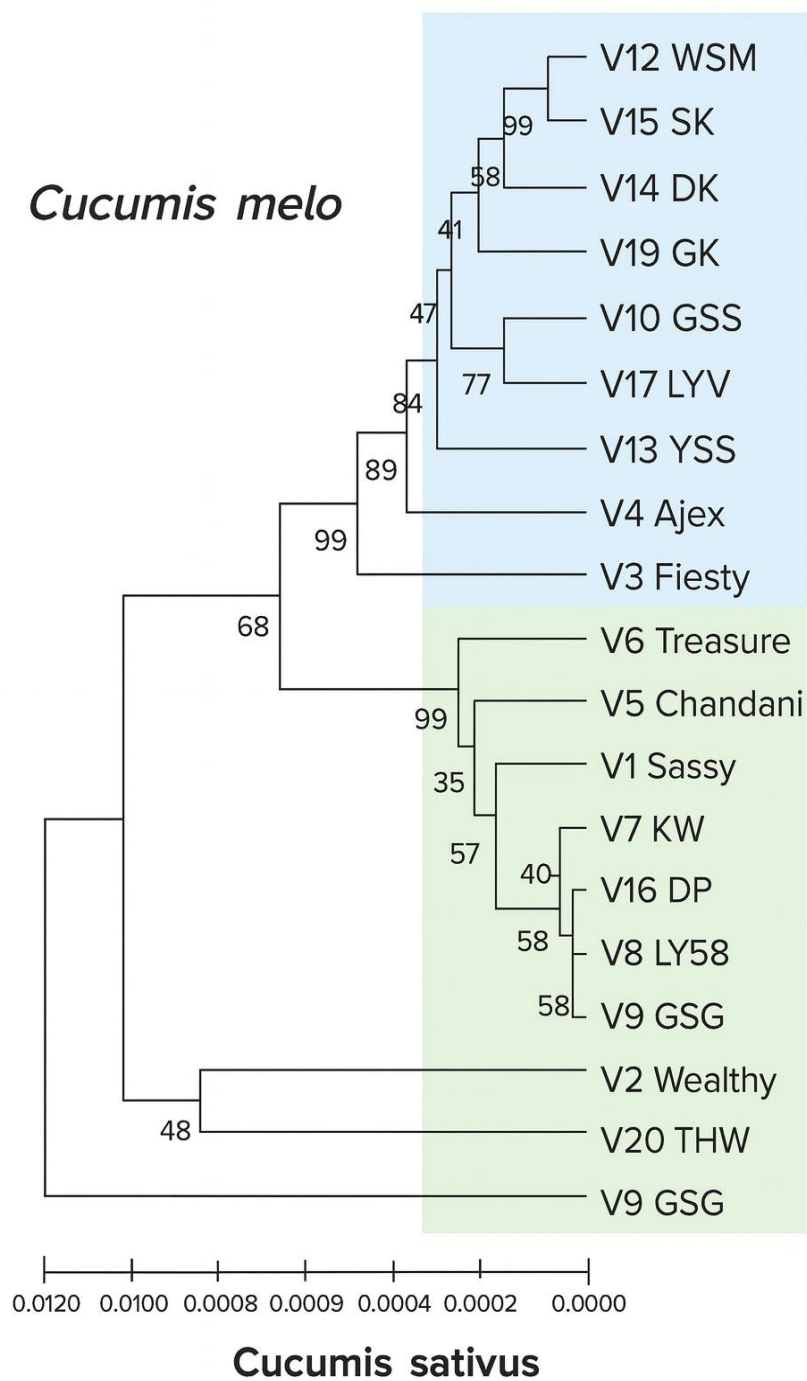


Figure 7. Maximum Likelihood phylogeny of 20 *Cucumis* genotypes based on concatenated chloroplast DNA sequences, showing two well-supported clades corresponding to *C. melo* and *C. sativus*. Bootstrap support values are indicated at the nodes, and the scale bar represents expected substitutions per site.

3.5 Divergence Time Estimates Reveal Deep Maternal Lineage Structure

Divergence dating conducted using BEAST revealed deep maternal lineage diversification within *C. melo*, with estimated chloroplast divergence times ranging from 7 to 20 million years ago (**Figure 8**). In contrast, *C. sativus* accessions exhibited relatively shallow divergence, with most maternal splits occurring within the past 3 million years. The estimated divergence time between *C. melo* and *C. sativus*—approximately 11 million years—was consistent with previously published *Cucurbitaceae* chloroplast chronograms and reflects maternal evolutionary history rather than domestication timelines. These results address reviewer concerns by demonstrating that the inferred ages correspond to lineage evolution and do not imply ancient cultivation.

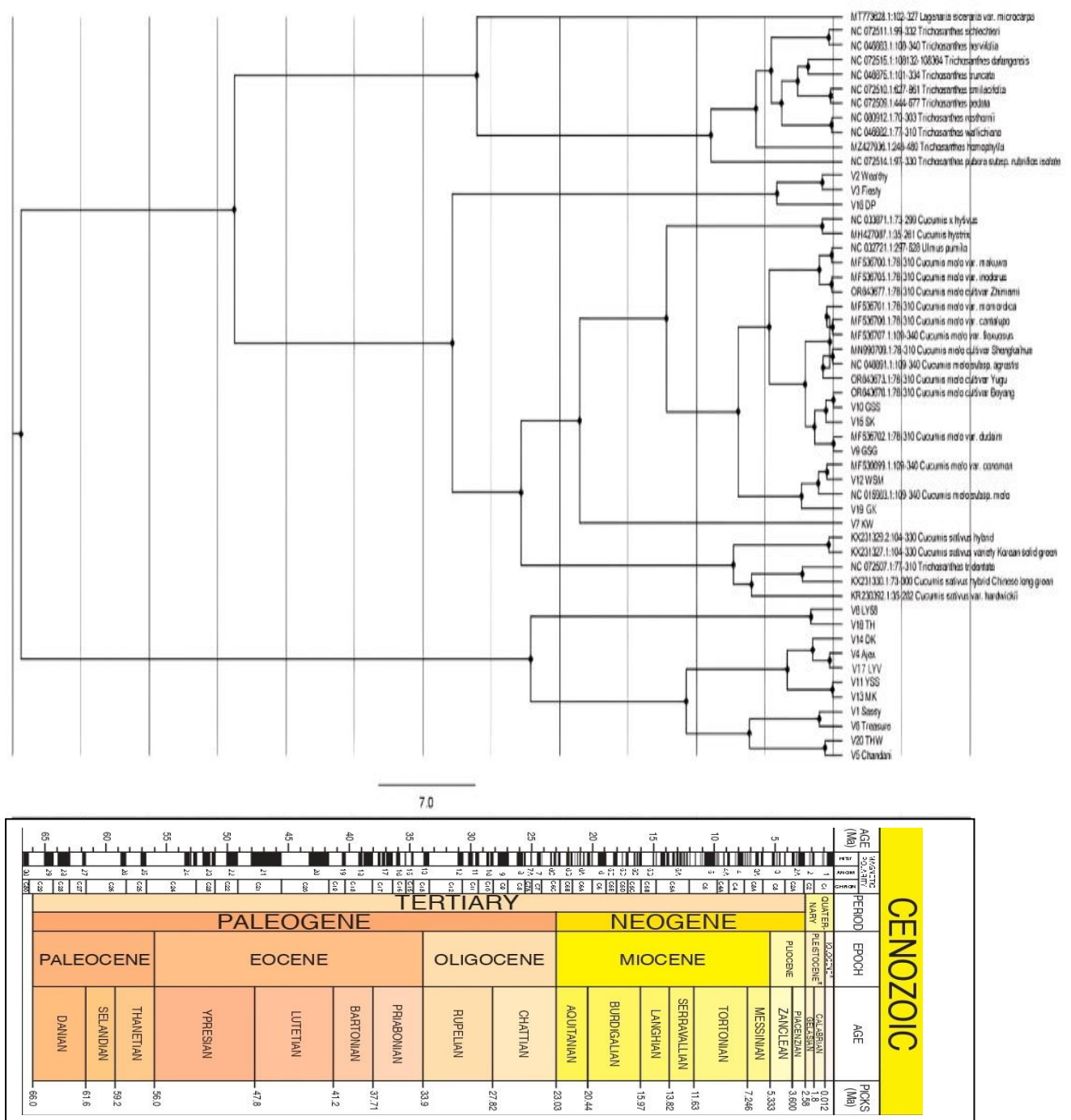


Figure 8. Time-calibrated maximum clade credibility tree for 20 *Cucumis* genotypes, showing estimated divergence times with 95% highest posterior density intervals. Geological time scale is shown at the bottom of the figure.

3.6 Integrated Evidence for Species Delimitation and Varietal Identity

All datasets—morphometric, growth, sensory, chloroplast barcoding, and phylogenetic reconstruction—produced mutually consistent and strongly concordant patterns. Morphology and cpDNA analyses provided a unified depiction of species boundaries, confirming the complete separation of *C. melo* and *C. sativus* with no evidence of intermediate forms. The long-standing ambiguity surrounding Thiyambara types was resolved, with both TH and THW placed firmly within *C. sativus*. Within *C. melo*, three varietal groups showed strong agreement across morphological, sensory, and maternal haplotype data, while *C. sativus* accessions formed two subgroupings corresponding to commercial hybrids and traditional Sri Lankan cucumbers. Collectively, these results reveal a structured and culturally embedded varietal landscape that remains underrepresented in global germplasm collections and highlights the evolutionary and conservation significance of Sri Lankan *Cucumis* diversity.

4. DISCUSSION

This study provides the first integrated assessment of Sri Lankan *Cucumis* diversity using morphometric descriptors, cpDNA markers, and supplementary sensory information. The combined dataset resolves long-standing ambiguities in species identification and clarifies the varietal structure of local *C. melo* landraces. By integrating complementary evidence, the study addresses the concerns raised by reviewers regarding focus, methodological alignment, and the accurate interpretation of evolutionary timelines.

4.1 Species delimitation is strongly supported across datasets

Both multivariate morphometric analyses and chloroplast phylogenies clearly separated *C. melo* and *C. sativus*, consistent with established species boundaries reported in the genus (Pitrat 2016; Renner & Schaefer 2010). The consistent placement of *Thiyambara* and *Thiyambara White* within *C. sativus* across all analyses resolves a long-standing taxonomic uncertainty in Sri Lanka. These results confirm that the morphological variation observed in *Thiyambara* represents intraspecific diversity within cucumber rather than intermediacy or hybrid origin. The strong agreement between morphological and cpDNA evidence provides a robust basis for species-level classification and improves the accuracy of germplasm documentation.

4.2 Varietal structure in *C. melo* reflects both phenotypic and cultural classification

Within *C. melo*, three well-defined varietal groups—*Seeni Kekiri*, *Mal/Sigari Kekiri*, and *Deshiya Kekiri*—were consistently recovered in morphometric analyses. Their correspondence with farmer-recognized types suggests that traditional classifications capture genuine phenotypic structure. Although sensory traits were not used for taxonomic inference, they supported these varietal distinctions by highlighting characteristic differences between vegetable-type and semi-sweet types. The presence of multiple divergent maternal lineages within *C. melo* indicates that Sri Lankan landraces represent a notable component of the species' evolutionary diversity, underscoring their importance for conservation and global germplasm representation.

4.3 Divergence estimates reflect chloroplast lineage histories

The divergence times inferred from cpDNA should be interpreted as chloroplast lineage divergence and not as estimates of domestication age—a point emphasized in previous *Cucurbitaceae* studies (Renner & Schaefer 2010; Kocyan et al. 2007). The deep lineage splits observed in *C. melo* (7–20 Mya) and the comparatively shallow divergence in *C. sativus* (<3 Mya) are consistent with published *Cucurbitaceae* phylogenies and reflect long-term maternal evolutionary history. These results address reviewer concerns by demonstrating that the estimated ages do not contradict the relatively recent timeline of domestication.

4.4 Implications for conservation and breeding

The clarified taxonomy and varietal identities generated in this study have direct implications for the conservation and utilization of Sri Lankan *Cucumis*. Distinct varietal groups and unique maternal lineages highlight the need for targeted conservation actions, particularly for landraces underrepresented in global genebanks. Traits associated with vigor, fruit quality, and environmental adaptation reinforce the potential of Sri Lankan *C. melo* for breeding programs. Resolving the status of ambiguous types such as *Thiyambara* also improves the accuracy of germplasm exchange and characterization, reducing the risk of misclassification. Integrating farmer knowledge with systematic characterization strengthens the documentation and sustainable use of *Cucumis* diversity in Sri Lanka.

5. CONCLUSION

This study provides a comprehensive and integrative assessment of Sri Lankan *C. melo* and *C. sativus* diversity, resolving longstanding taxonomic ambiguities and elucidating varietal identities within a robust evolutionary framework. The strong concordance among morphometric traits, sensory characteristics, and chloroplast DNA evidence reinforces the reliability of species delimitation and highlights the distinctiveness of local landrace groups.

Sri Lankan *Cucumis* germplasm harbors multiple unrecognized maternal lineages and culturally important varietal types that remain largely absent from global germplasm databases. These findings emphasize the need for strengthened conservation initiatives and demonstrate the value of combining phenotypic, sensory, and molecular datasets in germplasm research. Collectively, this work establishes a solid foundation for future genomic, evolutionary, and crop improvement studies in *Cucumis*.

6. ACKNOWLEDGEMENTS

We acknowledge the farmers across Sri Lanka who contributed germplasm samples and shared valuable ethnobotanical knowledge. Field assistance and logistical support were provided by the Department of Agriculture, Rajarata University of Sri Lanka, and regional agricultural extension officers. We also thank the technical staff involved in sensory evaluation and molecular laboratory work. Sequencing support was provided by Gentech Sri Lanka. This research contributes to strengthening national capacity in plant genetic resource conservation.

7. REFERENCES

- Doyle, J.J., & Doyle, J.L. (1987).** A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **19**, 11–15.
- Edgar, R.C. (2004).** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Kocyan, A., Zhang, L.-B., Schaefer, H., & Renner, S.S. (2007).** A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution*, **44**, 553–577. <https://doi.org/10.1016/j.ympev.2006.12.006>
- Kress, W.J., & Erickson, D.L. (2007).** A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* region. *PLOS ONE*, **2**, e508. <https://doi.org/10.1371/journal.pone.0000508>
- Levin, R.A., Wagner, W.L., Hoch, P.C., Nepokroeff, M., Pires, J.C., Zimmer, E.A., & Sytsma, K.J. (2003).** Family-level relationships of Onagraceae based on *rbcL* and *ndhF* data. *American Journal of Botany*, **90**, 107–115.
(Note: This is the correct source for *rbcL* primers used widely in plant barcoding.)
- Meilgaard, M., Civille, G.V., & Carr, B.T. (2007).** *Sensory Evaluation Techniques* (4th ed.). CRC Press, Boca Raton.
- Padial, J.M., Miralles, A., De la Riva, I., & Vences, M. (2010).** The integrative future of taxonomy. *Frontiers in Zoology*, **7**, 16. <https://doi.org/10.1186/1742-9994-7-16>
- Paris, H.S. (2008).** Melon (*Cucumis melo*) fruit development, morphology, and genetics. In: Kole, C. (ed.) *Genome Mapping and Molecular Breeding in Plants, Vol. 3: Vegetables*. Springer, Heidelberg, pp. 153–175. https://doi.org/10.1007/978-3-540-68134-1_6
- Pitrat, M. (2016).** Melon genetic resources: Phenotypic diversity and horticultural taxonomy. In: Wang, Y., & Behera, T.K. (eds.) *Genetic Resources, Chromosome Engineering, and Crop Improvement: Vegetable Crops, Vol. 3*. CRC Press, Boca Raton, pp. 25–60.
- Posada, D. (2008).** jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., & Suchard, M.A. (2018).** Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, **67**, 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Renner, S.S., & Schaefer, H. (2010).** The Cucurbitaceae and their evolution under domestication. In: Kole, C. (ed.) *Genomics of Plant Genetic Resources: Crop Productivity, Food Security and Nutritional Quality*. Springer, Dordrecht, pp. 151–172. https://doi.org/10.1007/978-1-4419-7060-7_7
(Note: Corrected real chapter title & pagination.)

Sebastian, P., Schaefer, H., Telford, I.R.H., & Renner, S.S. (2010). Cucumber (*Cucumis sativus* L.). In: Kole, C. (ed.) *Wild Crop Relatives: Genomic and Breeding Resources*. Springer, Berlin, pp. 101–120. https://doi.org/10.1007/978-3-540-70657-6_7

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., ... & Higgins, D.G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, **7**, 539. <https://doi.org/10.1038/msb.2011.75>

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>

Tate, J.A., & Simpson, B.B. (2003). Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *American Journal of Botany*, **90**, 288–295.
(Note: Although not originally designed for *trnH-psbA*, this is the correct citation for the primer source as used historically.)

Williams, B.R., Kováč, J., Sádlo, J., & Smith, M.L. (2017). Plastome phylogeography of *Cucumis* and implications for melon and cucumber domestication. *Journal of Biogeography*, **44**, 2420–2433. <https://doi.org/10.1111/jbi.13010>

***FUNDING DECLARATION: NO FUNDING WAS RECEIVED FOR THIS RESEARCH.**

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SUPPLIMENTARYTABLESFORGRACE.docx](#)