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Research Article

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Posted Date: October 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11111185/v1>

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Additional Declarations: No competing interests reported.

Morphometric Diversity Assessment of Acid Lime (*Citrus aurantifolia* Swingle) Genotypes from Northeast India

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Abstract

Acid lime (*Citrus aurantifolia* Swingle) is a tropical citrus species valued for its fruit quality and adaptability. The Northeastern region of India has long been recognized as a secondary center of citrus diversity, harboring genetically rich and diverse acid lime genotypes. This investigation conducted from 2020-2023 at College of Horticulture and Forestry, CAU(I), Pasighat, Arunachal Pradesh, sought to quantify the morphological variation among 30 local acid lime genotypes collected across eight northeastern states of India. Morphological characterization followed the standardized descriptors of the International Plant Genetic Resources Institute for citrus, documenting tree architecture, leaf morphology, fruit attributes, and seed characteristics. Significant variability was recorded across all measured traits. Tree height ranged from 2.68 m to 6.56 m, stem girth from 31.00 cm to 64.55 cm, and canopy spread from 3.37 m to 7.05 m. Leaf lamina length varied between 40.00 mm and 71.22 mm, leaf area from 10.73 cm² to 36.44 cm², and fruit weight from 31.47 g to 91.60 g. Juice yield spanned 15.00 mL to 47.00 mL, while the number of fruits per plant ranged from 100 to 356. The average number of seeds per fruit ranged from 4.00 to 31.00. Genetic variability analysis revealed that the phenotypic coefficient of variation consistently exceeded the genotypic coefficient of variation for all traits. High broad-sense heritability (>90%) was observed for most quantitative traits, indicating strong genetic control. Correlation analysis identified significant positive associations among yield-contributing characters, while principal component analysis extracted five components jointly explaining 78.81% of total variation, with fruit weight, pulp weight, rind weight, and stem girth dominating PC1. Cluster analysis partitioned the 30 genotypes into two major clusters, with genotype CHF-AL-23 forming a uniquely distinct solitary group. The substantial morphometric diversity documented here underscores the considerable potential of Northeast Indian acid lime germplasm for breeding programs aimed at varietal improvement and conservation of genetic resources.

Keywords: Acid lime, *Citrus aurantifolia*, morphological characterization, genetic diversity, Northeast India, heritability, correlation, principal component analysis, cluster analysis

1. Introduction

Acid lime (*Citrus aurantifolia* Swingle) is one of the most commercially significant tropical citrus species, prized for its adaptability, juice quality, and rich vitamin C content (Lambani et al., 2018). The Northeastern region of India represents a well-documented secondary center of citrus diversity, harboring genetically rich and locally adapted acid lime genotypes that have evolved under diverse agro-climatic conditions (Govind & Yadav, 1999; Singh, 2004). The region's microclimatic heterogeneity, encompassing the buffer zones of the Nokrek Biosphere Reserve in the Garo Hills and the diverse hill ecosystems of Manipur, Mizoram, Nagaland, and Sikkim, has fostered considerable morphological and genetic variability among citrus species (Lanusenla et al., 2025; Singh, 2010; Uchoi et al., 2025).

Morphological characterization remains the foundation for evaluating genotypic variability and constitutes an indispensable initial step in crop improvement, germplasm conservation, and the documentation of genetic resources (Institute, 1999). Recent studies on acid lime germplasm from West Bengal, Nepal, Tamil Nadu, and Karnataka have consistently demonstrated the value of multivariate morphometric tools—variance components, correlation, PCA, and cluster analysis—for partitioning total variation into heritable and non-heritable fractions (Munankarmi et al., 2018; Nyamagoud et al., 2025; Rajamanickam et al., 2024; Sekhar & Kundu, 2021, 2025). Similar investigations on related citrus species, including Assam lemon and mandarins, reinforce the utility of IPGRI-based descriptors and multivariate statistics in deciphering genetic relationships (Akhtar et al., 2024; Singh et al., 2022).

The present study was therefore designed to quantify morphological variation among 30 regionally distinct acid lime genotypes drawn from eight northeastern states, estimate key genetic variability parameters for 21 quantitative traits, assess genotypic and phenotypic correlation structures, identify principal trait axes through PCA, and classify the germplasm into discrete morphometric clusters. Such integrated analyses furnish the empirical baseline required for identifying elite genotypes and formulating effective selection strategies in acid lime breeding (Rattanpal et al., 2022; Sekhar & Kundu, 2025).

2. Materials and Methods

2.1 Survey, Selection, and Identification of Superior Genotypes

An extensive survey was conducted across eight northeastern states of India: Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and Sikkim (Fig. 1). Well performing genotypes were identified and selected based on the IPGRI Citrus Descriptors, following the standardized methodology (Institute, 1999). A total of 30 genotypes (designated CHF-AL-01 through CHF-AL-30) were documented with precise GPS-coordinated collection sites (Table 1).

2.2 Collection of Fruits and Leaves for Morphological Assessment

Ten to fifteen fruits were harvested from each selected genotype. Concurrently, mature leaf samples were collected in labeled bags and transported to the laboratory for subsequent morphometric analyses, following standard IPGRI citrus documentation protocols (Institute, 1999).

Table 1. List of acid lime genotypes collected and their sources of collection

Genotypes code	Latitude (°N)	Longitude (°E)	Owner and Place of collection
CHF-AL-01	25.868293°	93.773871°	Neiphre, Diphupar, Nagaland
CHF-AL-02	25.54234°	91.34520°	Dohling, Nongspung, Meghalaya
CHF-AL-03	24.503246°	94.013201°	Sundari Devi, Kakching, Manipur
CHF-AL-04	24.81828°	93.981189°	Lawson-1, Kongpal, Manipur
CHF-AL-05	28.161762°	95.446917°	Riju Megu-1, Mebo, Arunachal Pradesh
CHF-AL-06	24.12554°	93.54097°	Unshani, Chakpikarong, Manipur
CHF-AL-07	25.7098°	94.090476°	Keze, Meriema, Nagaland
CHF-AL-08	23.495315°	91.347223°	Sandhya Debbarma, Chandigarh, Tripura
CHF-AL-09	24.727331°	93.954178°	Abdul Helim, Kyamgei Muslim, Manipur
CHF-AL-10	28.033505°	95.463454°	Toram Perme, Borguli, Arunachal Pradesh
CHF-AL-11	27.754069°	94.946708°	Bipul Pegu, Jonai, Assam
CHF-AL-12	24.817827°	93.981294°	Pankaj, Sawonbung, Manipur
CHF-AL-13	23.742310°	92.697415°	Tlana, Luangmual Venglai, Mizoram
CHF-AL-14	24.356043°	93.701123°	Kamboi, Churachandpur, Manipur
CHF-AL-15	25.871038°	93.779129°	Chothave, Dimapur, Nagaland
CHF-AL-16	28.164342°	95.46222°	Riju Megu-2, Mebo, Arunachal Pradesh
CHF-AL-17	24.818951°	93.981917°	Lawson-2, Imphal, Manipur
CHF-AL-18	24.340918°	93.706878°	Lalnunnem, Rengkai, CCpur, Manipur
CHF-AL-19	28.061467°	95.283346°	Getor Dai, Diking, Arunachal Pradesh
CHF-AL-20	25.470641°	92.367211°	Deikit, Shangpung, Meghalaya
CHF-AL-21	24.943904°	93.882704°	Shieddarth, Sekmai, Manipur
CHF-AL-22	27.248432°	88.59365°	Ambrush, Pakyong, Sikkim
CHF-AL-23	27.403309°	94.893752°	Ratneshwar, Dibrugarh, Assam
CHF-AL-24	27.46025°	95.018342°	Mohanbari, Assam
CHF-AL-25	28.061678°	95.282746°	Minu, Pasighat, Arunachal Pradesh
CHF-AL-26	27.239035°	88.315962°	Joel Lepcha, Mayong, Sikkim
CHF-AL-27	23.44127°	92.39469°	Jona, Aizawl, Mizoram
CHF-AL-28	28.349102°	95.023672°	Toto Tamut, Parong, Arunachal Pradesh
CHF-AL-29	28.061388°	95.282632°	Suman Jamoh, Balek, Arunachal Pradesh
CHF-AL-30	23.087246°	91.700507°	Uttam Tripura, Rupaichari, Tripura

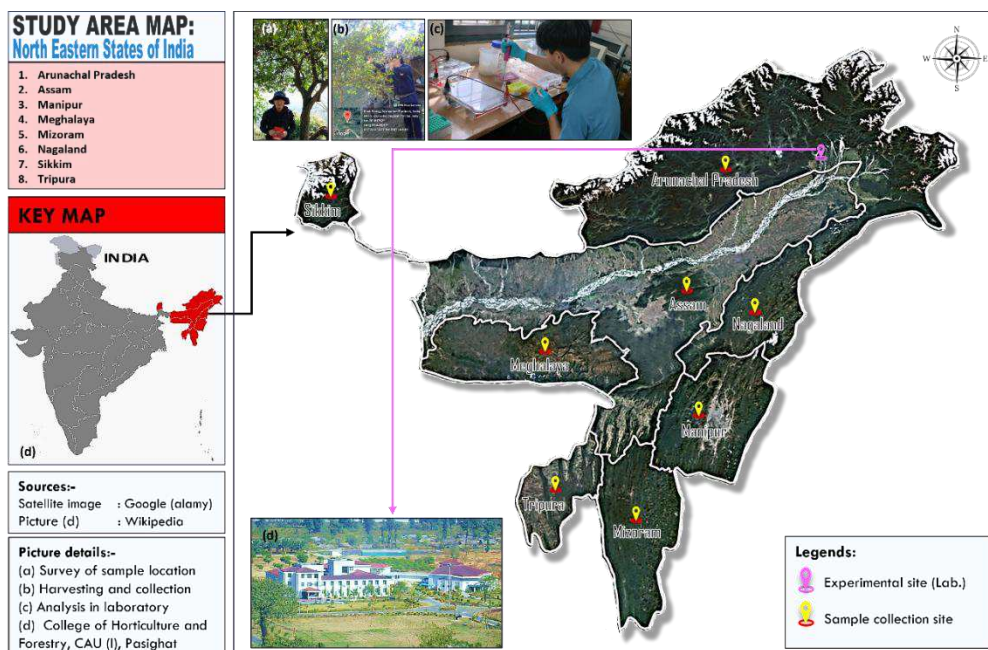


Figure 1: Geographical map representing the sources of genotypes collection

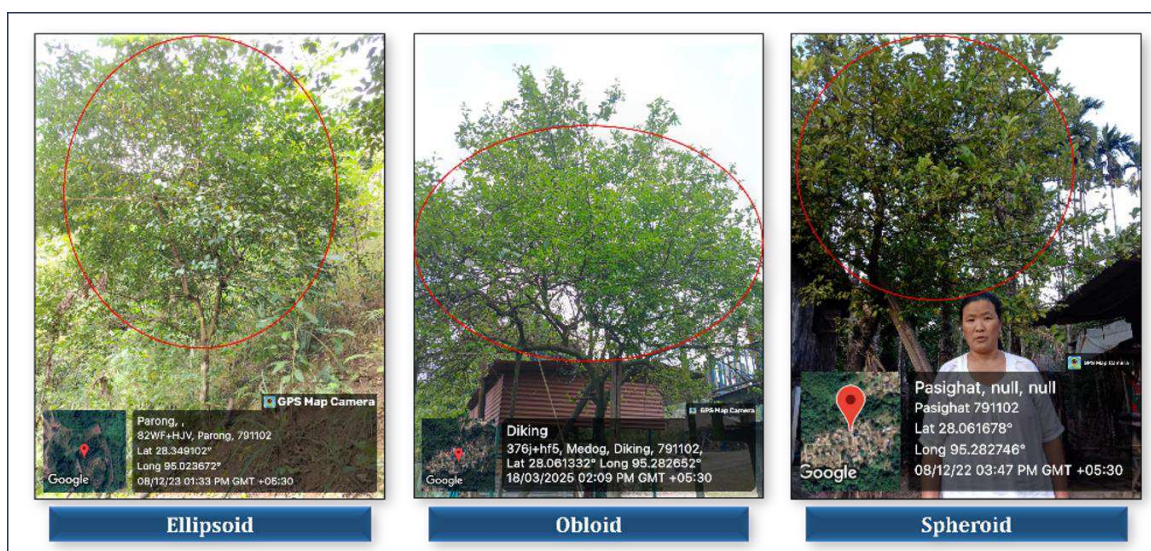


Plate 1: Different shapes of Acid lime trees

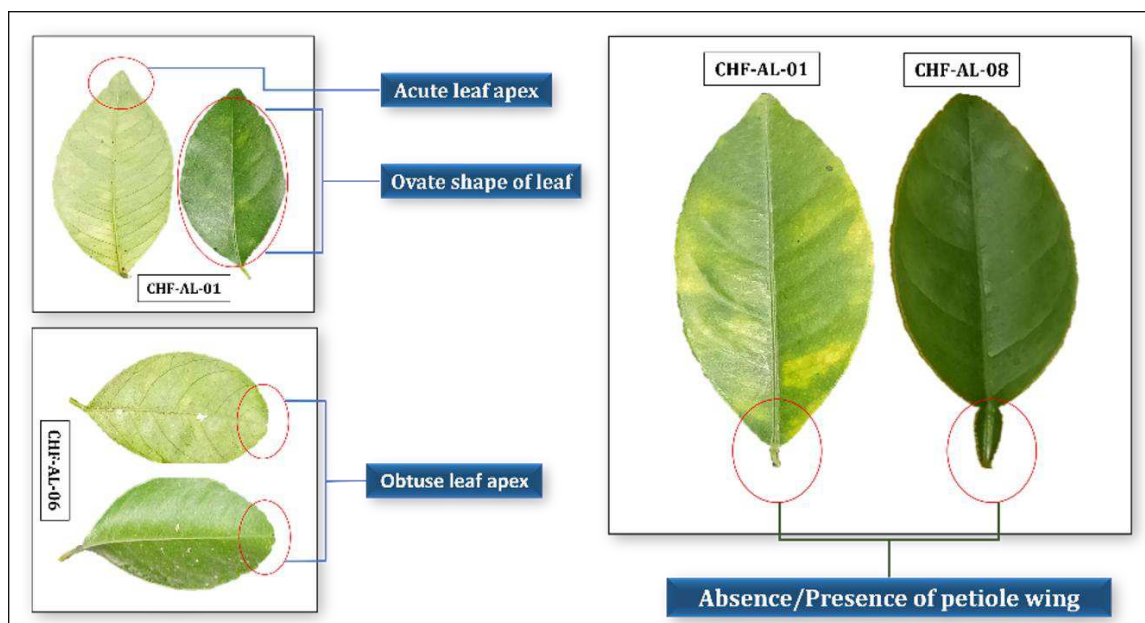


Plate 2: Leaf morphology of Acid lime

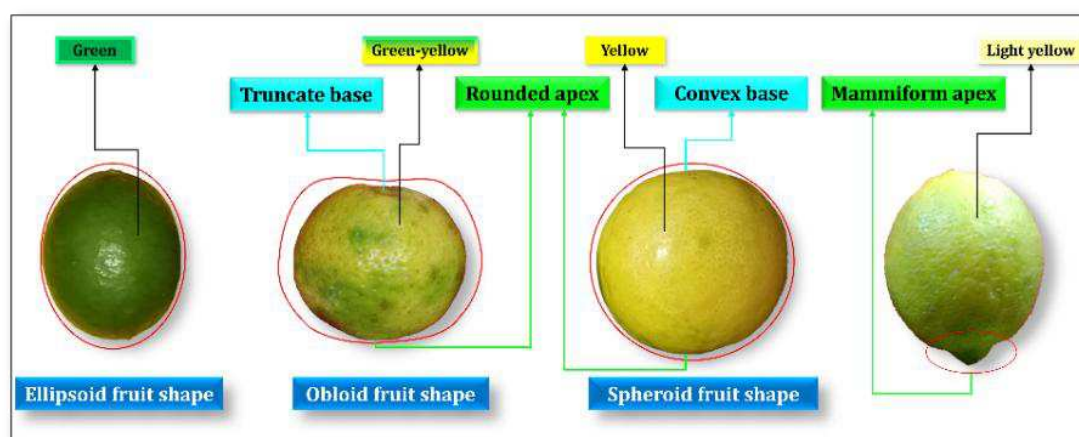


Plate 3: Fruit morphology of Acid lime



Plate 4: Seed morphology of Acid lime



Plate 5 : Variation in leaves of 30 acid lime genotypes

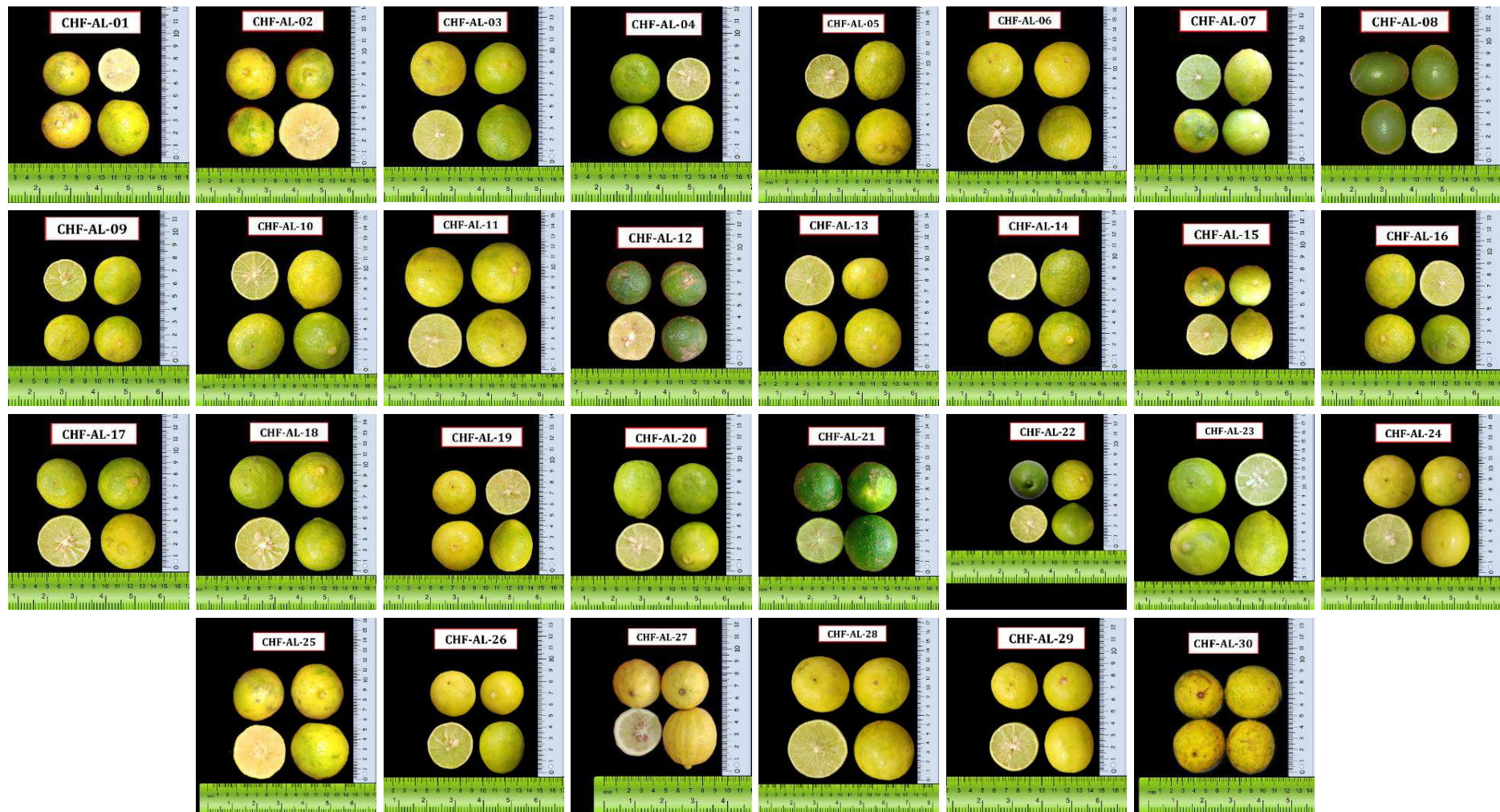


Plate 6: Variation in fruits of 30 acid lime genotypes (CHF-AL-1 to CHF-AL- 30)

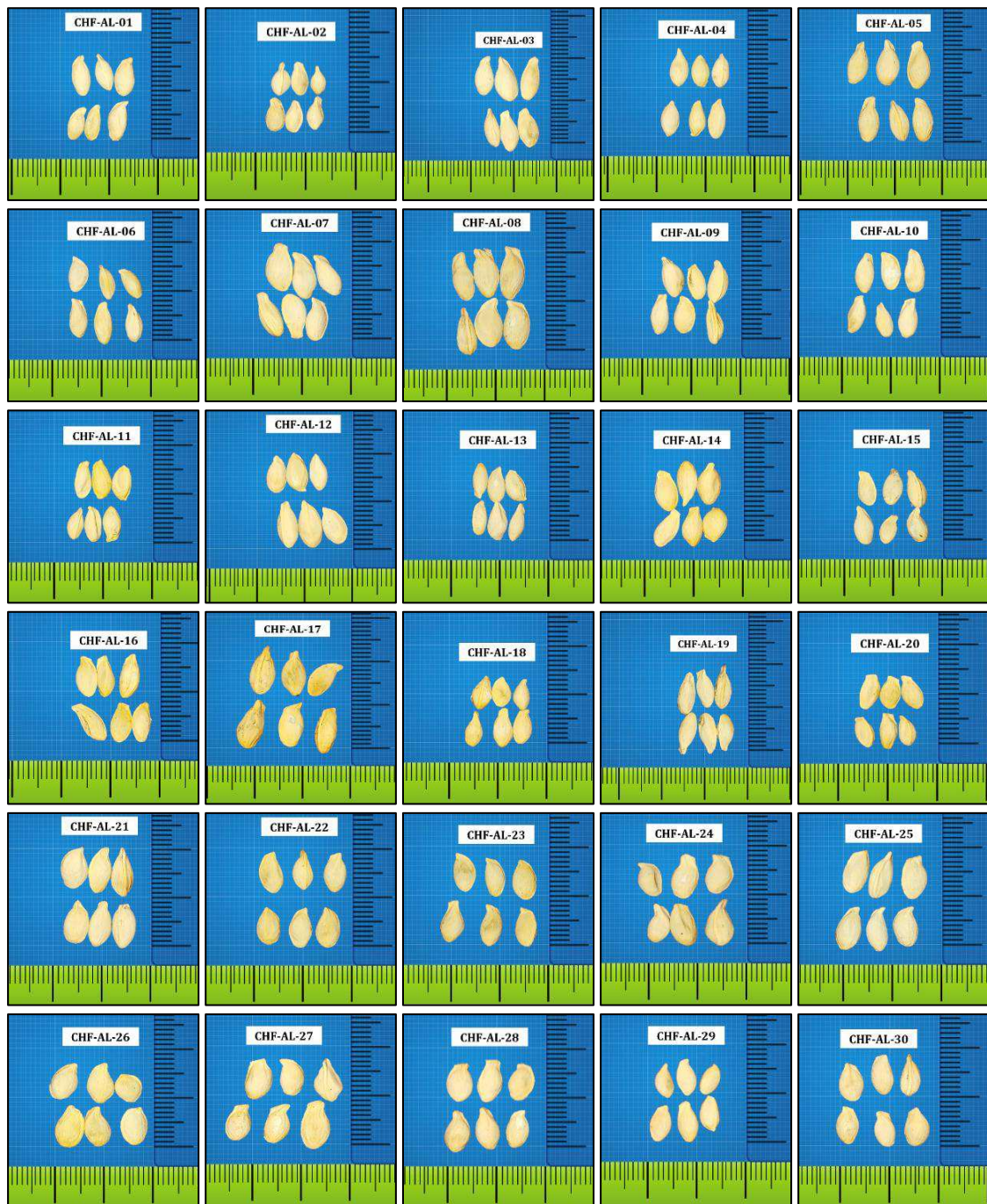


Plate 7: Variation in seeds of 30 acid lime genotypes

2.3 Morphological attributes

The IPGRI descriptors for *Citrus* spp. served as the operational reference framework for documenting tree characters (height, stem girth, canopy spread in N–S and E–W directions, tree shape, growth habit, branch density), leaf characters (lamina length, width, length:width ratio, lamina shape, apex shape, leaf area, petiole wing presence/absence), fruit characters (weight, length, diameter, number per plant, fruit shape, base shape, apex shape, stalk attachment, segment count, rind colour, rind thickness, pulp weight, rind weight, juice yield, specific gravity), and seed characters (average seed number per fruit, weight of ten seeds, seed length, seed width, seed shape, seed colour, cotyledon colour) (Institute, 1999). Specific gravity of fruit was computed as;

$$\text{Specific gravity} = \frac{\text{weight of fruit}}{\text{volume of the fruit}}$$

2.4 Statistical Analysis

Data were processed using Microsoft Excel and analyzed through OPSTAT, SPSS, XLSTAT, and Agri Analyze (Popat *et al.*, 2024) software packages. Components of genetic variability—including genotypic and phenotypic coefficients of variation, broad-sense heritability (H^2), and genetic advance as a percentage of mean—were computed for all quantitative traits. Pearson's genotypic and phenotypic correlation coefficients, principal component analysis, and hierarchical cluster analysis (using the average linkage method with between-group rescaled distances) were also computed to characterize diversity patterns among the 30 genotypes.

3. Results

3.1 Morphological Characterization of Acid Lime Genotypes

3.1.1 Tree Characters

Documentation of tree architectural traits in 2021 revealed substantial phenotypic variation, primarily attributed to differences in tree age and heterogeneous environmental conditions across collection sites (Govind & Yadav, 1999). Tree height ranged from 2.68 m to 6.56 m, with a mean of 4.81 m. Stem girth varied between 31.00 cm and 64.55 cm, with a population mean of 47.83 cm. Canopy spread (measured along north–south and east–west axes) ranged from 3.37 m to 7.05 m, averaging 5.06 m. Three distinct crown forms were identified: ellipsoid (15 genotypes: CHF-AL-01, -02, -03, -04, -05, -07, -08, -09, -10, -13, -18, -19, -20, -27, -30), spheroid (3 genotypes: CHF-AL-25, -28, -29), and obloid (12 genotypes). Regarding growth habit, 18 genotypes exhibited a spreading form, while 12 showed a drooping habit. Branching

density varied, with sparse branching observed in 6 genotypes, medium density in 14, and dense branching in 8 genotypes (Plate 1; Table 2).

3.1.2 Leaf Characters

Leaf lamina length ranged from 40.00 mm to 71.22 mm, with a mean of 48.34 mm, while lamina width varied between 30.00 mm and 65.14 mm, averaging 40.44 mm. The lamina length-to-width ratio ranged from 1.09 to 1.33, with a population mean of 1.20. Leaf area spanned 10.73 cm² to 36.44 cm², with a mean value of 15.70 cm². All 30 genotypes exhibited an ovate lamina shape. Regarding apex morphology, 27 genotypes displayed an acute apex, whereas three genotypes—CHF-AL-06, CHF-AL-13, and CHF-AL-15—presented an obtuse apex. Petiole wings were absent in all genotypes except CHF-AL-08, in which they were distinctly observed (Figure 4&5; Plate 2&5; Table 3).

Table 2. Variation in tree characteristics of 30 Acid Lime genotypes

Genotypes	Plant height (m)	Stem girth (cm)	Canopy spread N-S & E-W (m)	Tree Shape	Density of branches
CHF-AL-01	4.18	47.66	4.32	Ellipsoid	Sparse
CHF-AL-02	4.23	45.56	5.21	Ellipsoid	Medium
CHF-AL-03	4.76	53.10	5.79	Ellipsoid	Dense
CHF-AL-04	4.56	50.30	5.07	Ellipsoid	Sparse
CHF-AL-05	3.51	41.00	3.37	Ellipsoid	Sparse
CHF-AL-06	4.87	51.40	5.65	Obloid	Medium
CHF-AL-07	4.80	48.15	4.87	Ellipsoid	Medium
CHF-AL-08	4.45	43.11	4.27	Ellipsoid	Dense
CHF-AL-09	2.68	31.00	3.85	Ellipsoid	Dense
CHF-AL-10	5.40	41.36	5.91	Ellipsoid	Medium
CHF-AL-11	5.75	51.22	5.45	Obloid	Medium
CHF-AL-12	5.00	38.33	5.77	Obloid	Dense
CHF-AL-13	4.66	43.55	4.78	Ellipsoid	Sparse
CHF-AL-14	6.56	64.55	7.05	Obloid	Medium
CHF-AL-15	5.20	44.61	5.42	Obloid	Medium
CHF-AL-16	4.60	48.33	4.51	Obloid	Medium
CHF-AL-17	4.64	52.43	5.36	Obloid	Dense
CHF-AL-18	5.45	56.78	5.85	Ellipsoid	Dense
CHF-AL-19	3.40	41.33	3.85	Ellipsoid	Sparse
CHF-AL-20	4.44	39.43	4.11	Ellipsoid	Medium
CHF-AL-21	4.26	43.44	4.23	Obloid	Sparse
CHF-AL-22	4.12	38.66	4.25	Obloid	Dense
CHF-AL-23	5.19	61.54	6.65	Obloid	Medium
CHF-AL-24	5.38	60.33	5.56	Obloid	Medium
CHF-AL-25	5.80	53.12	5.18	Spheroid	Medium
CHF-AL-26	4.60	43.55	4.08	Obloid	Sparse
CHF-AL-27	5.23	44.16	4.29	Ellipsoid	Sparse
CHF-AL-28	5.78	57.25	5.83	Spheroid	Medium
CHF-AL-29	6.12	58.26	6.51	Spheroid	Dense
CHF-AL-30	4.67	41.44	4.67	Ellipsoid	Medium
Mean	4.81	47.83	5.06		

Table 3. Variation in Quantitative and Quantitative leaf characteristics of 30 acid lime genotypes

Genotypes	Leaf lamina length (mm)	Leaf lamina width (mm)	Leaf lamina length/leaf lamina width ratio	Leaf area (cm²)	Leaf lamina shape	Shape of Leaf apex	Presence or absence of petiole wing
CHF-AL-01	46.56	39.68	1.17	14.51	Ovate	Acute	Absent
CHF-AL-02	45.26	37.29	1.21	13.25	Ovate	Acute	Absent
CHF-AL-03	51.27	41.11	1.25	16.55	Ovate	Acute	Absent
CHF-AL-04	48.29	40.78	1.18	15.47	Ovate	Acute	Absent
CHF-AL-05	46.87	38.96	1.20	14.34	Ovate	Acute	Absent
CHF-AL-06	43.87	36.89	1.19	12.71	Ovate	Obtuse	Absent
CHF-AL-07	49.21	41.35	1.19	15.98	Ovate	Acute	Absent
CHF-AL-08	45.66	34.90	1.31	12.52	Ovate	Acute	Present
CHF-AL-09	45.88	38.98	1.18	14.05	Ovate	Acute	Absent
CHF-AL-10	49.66	38.78	1.28	15.12	Ovate	Acute	Absent
CHF-AL-11	51.23	42.98	1.19	17.29	Ovate	Acute	Absent
CHF-AL-12	49.29	41.87	1.18	16.21	Ovate	Acute	Absent
CHF-AL-13	40.00	34.14	1.17	10.73	Ovate	Obtuse	Absent
CHF-AL-14	71.22	65.14	1.09	36.44	Ovate	Acute	Absent
CHF-AL-15	41.39	37.30	1.11	12.12	Ovate	Obtuse	Absent
CHF-AL-16	45.28	37.39	1.21	13.30	Ovate	Acute	Absent
CHF-AL-17	47.77	40.29	1.19	15.12	Ovate	Acute	Absent
CHF-AL-18	50.12	38.77	1.29	15.26	Ovate	Acute	Absent
CHF-AL-19	44.88	37.98	1.18	13.39	Ovate	Acute	Absent
CHF-AL-20	41.89	35.88	1.17	11.80	Ovate	Acute	Absent
CHF-AL-21	43.88	36.18	1.21	12.47	Ovate	Acute	Absent
CHF-AL-22	41.89	34.00	1.23	11.19	Ovate	Acute	Absent
CHF-AL-23	68.13	60.16	1.13	32.19	Ovate	Acute	Absent
CHF-AL-24	62.19	46.79	1.33	22.85	Ovate	Acute	Absent
CHF-AL-25	45.89	38.99	1.18	14.05	Ovate	Acute	Absent
CHF-AL-26	43.79	36.98	1.18	12.72	Ovate	Acute	Absent
CHF-AL-27	48.34	38.30	1.26	14.54	Ovate	Acute	Absent
CHF-AL-28	50.48	43.89	1.15	17.40	Ovate	Acute	Absent
CHF-AL-29	47.29	40.40	1.17	15.01	Ovate	Acute	Absent
CHF-AL-30	42.78	36.99	1.16	12.43	Ovate	Acute	Absent
Mean	48.34	40.44	1.20	15.70	--	--	--
C.D.	1.91	1.71	0.05	0.58	--	--	--
±SE(m)	0.67	0.60	0.02	0.204	--	--	--
SE(d)	0.95	0.85	0.03	0.289	--	--	--
C.V.	2.41	2.58	2.61	2.255	--	--	--

Figure 3: Stem girth of 30 acid lime genotypes

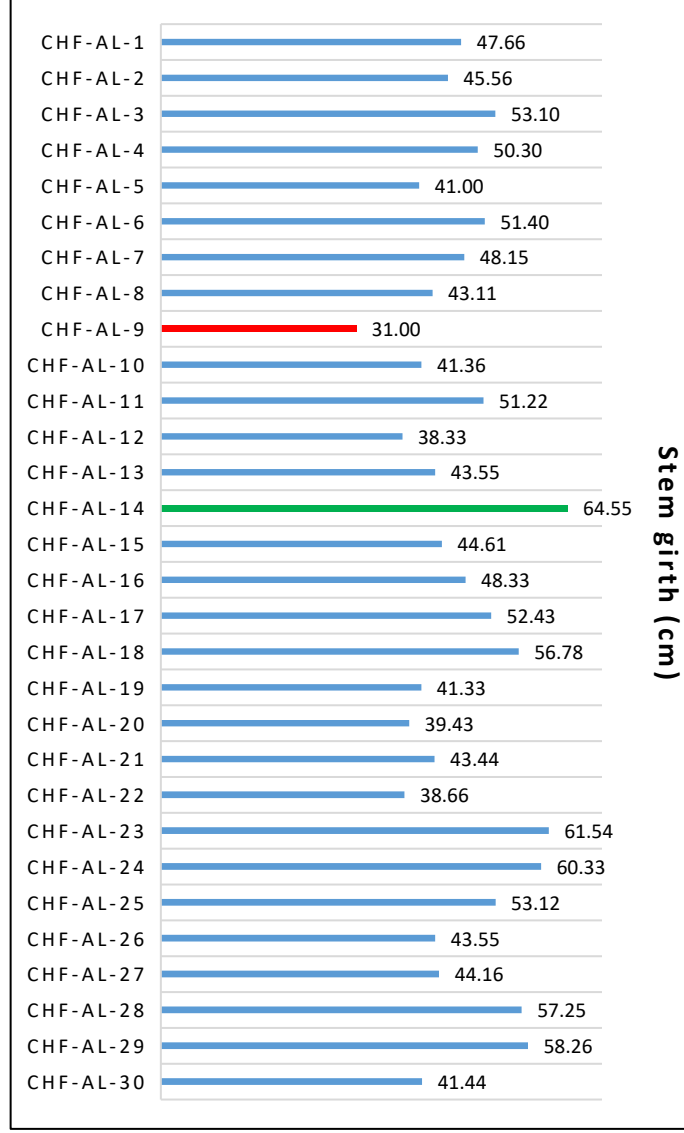


Figure 2: Plant height and canopy spread of 30 acid lime genotypes

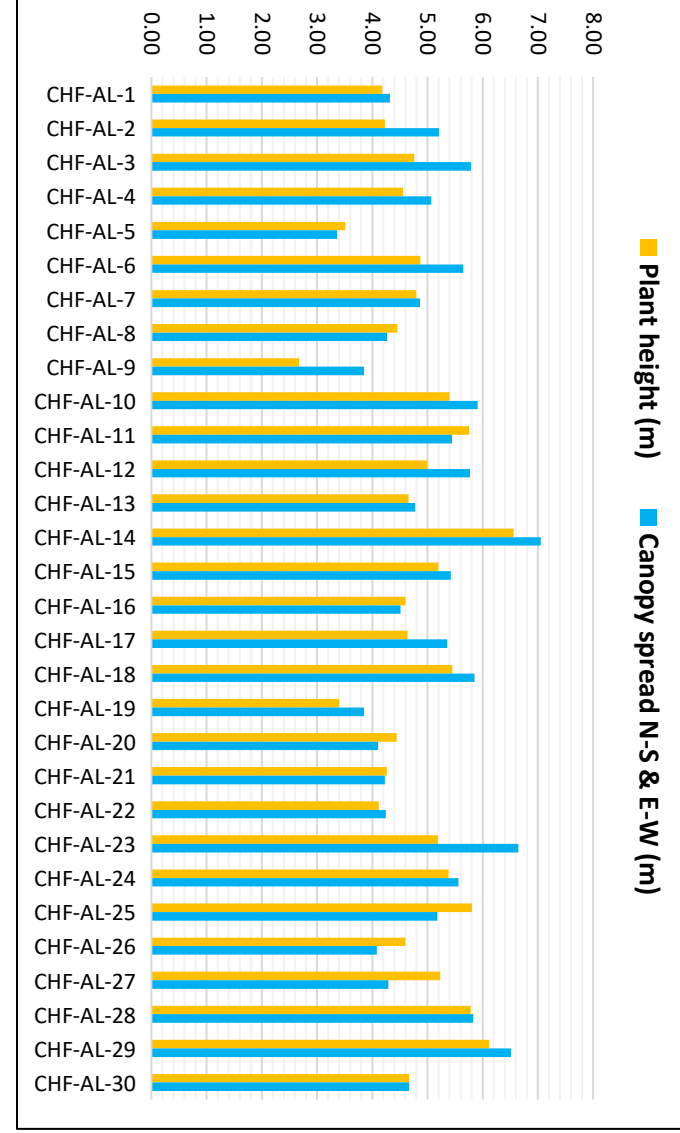


Figure 5: Leaf lamina length/width ratio and leaf area of 30 genotypes of acid lime

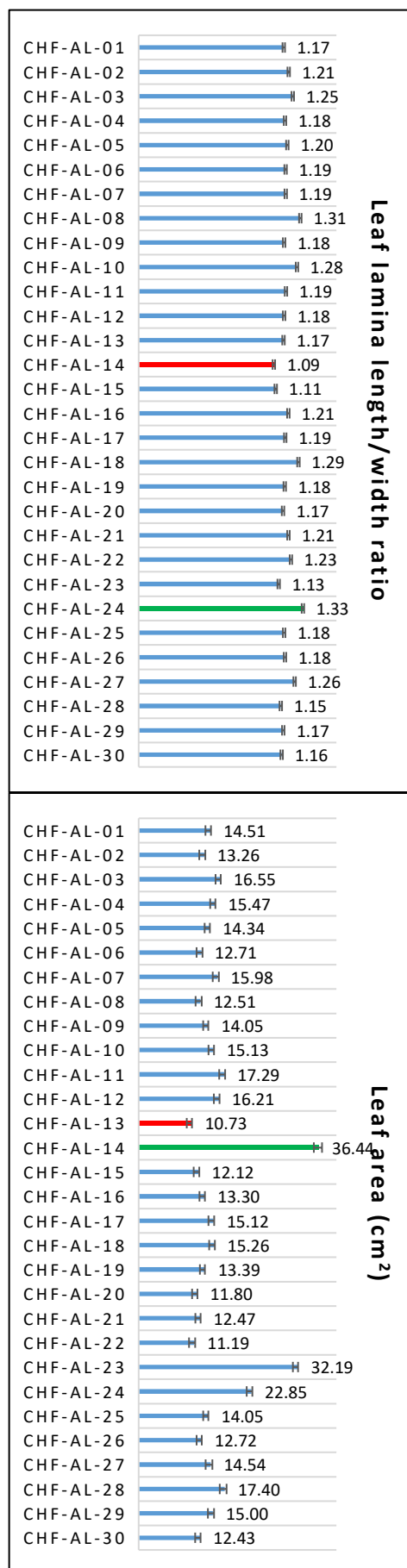
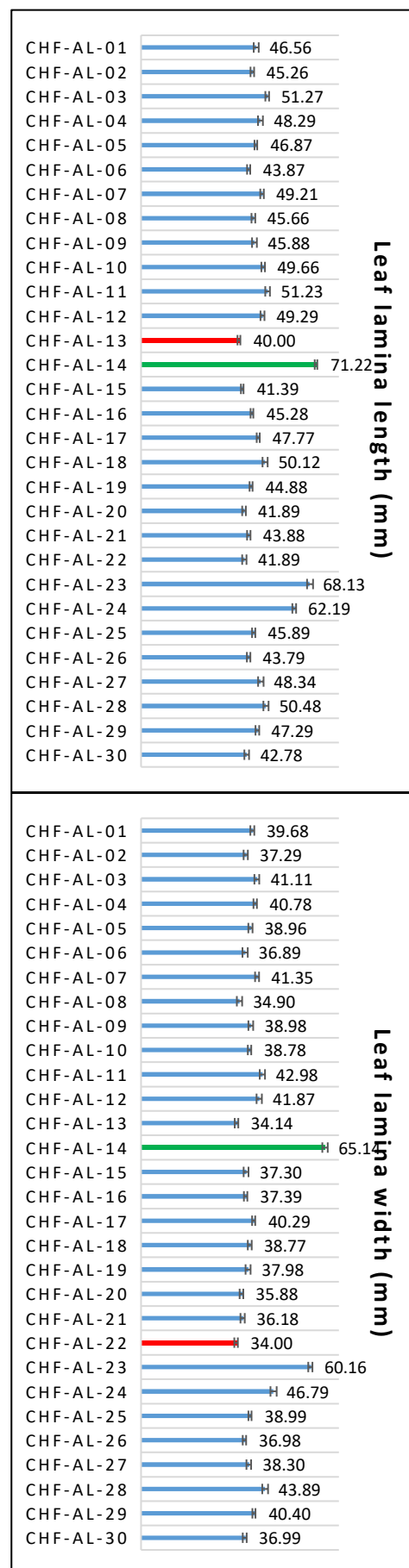


Figure 4: Leaf lamina length and leaf lamina width of 30 genotypes of acid lime



3.1.3 Fruit Characters

Substantial variability was observed across all fruit-related traits (Table 5). Twenty-six genotypes exhibited a spheroid fruit shape, while three (CHF-AL-01, -25, -30) were ellipsoid, and one displayed an obloid form (Plate 3&6; Table 5). Regarding fruit base morphology, eleven genotypes had a truncate base, whereas nineteen presented a convex base. Fruit apex shape was predominantly rounded (21 genotypes), with truncate (4 genotypes) and mammiform (5 genotypes) forms also recorded. Attachment strength varied, with five genotypes showing weak attachment, twenty-three exhibiting medium attachment, and two genotypes (CHF-AL-17 and CHF-AL-26) displaying strong attachment.

Four distinct epicarp colour variants were documented: yellow was most prevalent (17 genotypes), followed by green–yellow, light yellow, and green (1 genotype each) (Plate 3 & Table 5). Fruit weight ranged from 31.47 g to 91.60 g, with a mean of 55.51 g (Table 5 & Figure 6). Fruit length varied between 36.22 mm and 69.94 mm, averaging 48.57 mm, while fruit diameter ranged from 34.19 mm to 66.43 mm, with a population mean of 46.62 mm (Table 5 & Figure 6). Pulp weight spanned 26.87 g to 81.27 g (with CHF-AL-23 recording 82.70 g), averaging 49.38 g. Juice yield ranged from 15.00 mL to 47.00 mL, with a mean of 24.20 mL (Table 5 & Figure 7). The number of fruits per plant varied widely, from 100 to 356, averaging 168.97 (Table 5 & Figure 7). Rind thickness ranged from 1.02 mm to 3.20 mm (mean = 2.17 mm), while rind weight varied between 4.20 g and 10.23 g, with a mean of 6.47 g (Table 5 & Figure 8). The number of segments per fruit ranged from 7 to 11, with a mean of 9.33; the highest segment counts (11) were recorded in CHF-AL-01, -04, and -27 (Table 5 & Figure 9). Notably, the specific gravity of fruit remained remarkably stable, ranging narrowly from 1.03 to 1.09, with no significant differences detected among genotypes (Table 5 & Figure 9).

Table 5. Variation in quantitative and qualitative fruit characteristics of 30 acid lime genotypes

Genotypes	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Pulp weight (g)	Juice yield (mL)	No. of fruits per plant	Rind thickness (mm)	Rind weight (g)	No. of segments per fruit	Specific gravity of fruit	Fruit shape	Shape of fruit base	Shape of fruit apex	Fruit attachment to stalk	Fruit skin (epicarp) colour
CHF-AL-01	46.00	41.22	39.35	41.80	26.00	120.00	1.78	4.20	11.00	1.07	ellipsoid	truncate	rounded	weak	green
CHF-AL-02	49.17	44.00	44.31	42.51	25.00	145.00	2.10	6.66	9.00	1.05	obloid	truncate	rounded	medium	yellow
CHF-AL-03	56.67	44.16	44.79	50.27	22.00	150.00	1.88	6.40	10.00	1.04	spheroid	truncate	rounded	medium	yellow
CHF-AL-04	42.67	37.43	38.74	36.96	17.00	165.00	1.02	5.70	11.00	1.06	spheroid	convex	rounded	medium	green-yellow
CHF-AL-05	77.00	61.62	58.36	66.90	23.00	127.00	3.11	10.10	10.00	1.03	spheroid	convex	truncate	medium	yellow
CHF-AL-06	58.13	59.73	56.44	50.73	32.00	140.00	2.80	7.40	10.00	1.04	spheroid	truncate	rounded	medium	green-yellow
CHF-AL-07	37.83	40.83	36.53	32.94	20.00	155.00	1.88	4.89	9.00	1.07	spheroid	convex	mammiform	medium	yellow
CHF-AL-08	35.00	43.16	34.19	30.50	17.00	265.00	2.67	4.50	9.00	1.09	ellipsoid	convex	rounded	weak	light yellow
CHF-AL-09	34.05	36.22	39.15	29.65	20.00	120.00	1.54	4.40	10.00	1.08	spheroid	truncate	rounded	medium	yellow
CHF-AL-10	54.67	50.22	43.93	47.67	24.00	100.00	2.10	7.00	10.00	1.05	spheroid	convex	rounded	medium	green-yellow
CHF-AL-11	88.33	57.04	58.07	79.13	45.00	230.00	2.98	9.20	10.00	1.03	spheroid	convex	rounded	medium	yellow
CHF-AL-12	47.16	38.17	37.10	41.76	27.00	130.00	2.02	5.40	10.00	1.05	spheroid	truncate	rounded	medium	green-yellow
CHF-AL-13	48.00	49.43	52.90	51.70	27.00	144.00	1.89	4.30	8.00	1.05	spheroid	convex	truncate	medium	yellow
CHF-AL-14	66.67	52.34	48.72	58.57	25.00	265.00	2.76	8.10	10.00	1.04	spheroid	convex	rounded	medium	green-yellow
CHF-AL-15	40.67	40.93	38.53	35.57	18.00	137.00	2.04	5.10	8.00	1.07	ellipsoid	convex	mammiform	medium	green-yellow
CHF-AL-16	83.33	48.65	45.79	73.11	20.00	130.00	3.20	10.23	8.00	1.03	spheroid	convex	rounded	medium	green-yellow
CHF-AL-17	63.33	46.54	47.57	55.76	22.00	139.00	2.80	7.57	9.00	1.04	spheroid	convex	truncate	strong	light yellow
CHF-AL-18	63.33	49.49	51.46	55.33	20.00	270.00	2.27	8.00	8.00	1.04	spheroid	convex	rounded	medium	yellow
CHF-AL-19	52.00	47.92	44.08	46.80	15.00	115.00	1.27	5.20	8.00	1.05	spheroid	truncate	rounded	weak	yellow
CHF-AL-20	47.00	50.47	43.93	44.77	24.00	122.00	1.73	4.23	10.00	1.06	spheroid	convex	rounded	medium	green-yellow
CHF-AL-21	45.67	51.16	47.53	40.97	23.00	132.00	1.67	4.70	8.00	1.06	spheroid	convex	truncate	medium	yellow
CHF-AL-22	41.43	38.67	38.60	37.09	21.50	119.00	1.97	4.34	10.00	1.07	spheroid	convex	mammiform	medium	green
CHF-AL-23	91.60	69.94	66.43	82.70	47.00	356.00	2.90	9.40	10.00	1.03	spheroid	truncate	rounded	medium	green
CHF-AL-24	53.37	52.30	48.55	46.48	21.00	219.00	2.10	6.89	9.00	1.05	spheroid	truncate	rounded	medium	yellow
CHF-AL-25	90.67	56.16	58.21	81.27	42.00	148.00	2.91	8.90	10.00	1.03	ellipsoid	convex	rounded	medium	yellow
CHF-AL-26	55.00	44.89	43.02	47.65	23.00	146.00	2.23	7.35	7.00	1.05	spheroid	convex	mammiform	strong	light yellow
CHF-AL-27	57.17	46.93	48.73	49.67	18.00	154.00	2.17	7.50	11.00	1.04	spheroid	convex	mammiform	medium	yellow
CHF-AL-28	52.41	66.09	64.67	46.91	27.54	138.00	1.47	5.50	8.00	1.05	spheroid	truncate	rounded	weak	yellow
CHF-AL-29	55.67	46.92	44.66	49.47	18.00	258.00	1.87	6.20	10.00	1.05	spheroid	truncate	rounded	weak	yellow
CHF-AL-30	31.47	44.47	34.33	26.87	16.00	230.00	1.94	4.60	9.00	1.08	ellipsoid	convex	rounded	medium	green
Mean	55.51	48.57	46.62	49.38	24.20	168.97	2.17	6.47	9.33	1.05	--	--	--	--	--
C.D.	8.69	6.14	5.19	1.85	1.13	7.42	0.09	0.23	0.39	N/A	--	--	--	--	--
±SE(m)	3.07	2.16	1.83	0.65	0.40	2.62	0.03	0.08	0.14	0.02	--	--	--	--	--
SE(d)	4.34	3.06	2.59	0.92	0.56	3.70	0.04	0.12	0.20	0.02	--	--	--	--	--
C.V.	9.57	7.72	6.79	2.29	2.85	2.68	2.50	2.19	2.56	2.61	--	--	--	--	--

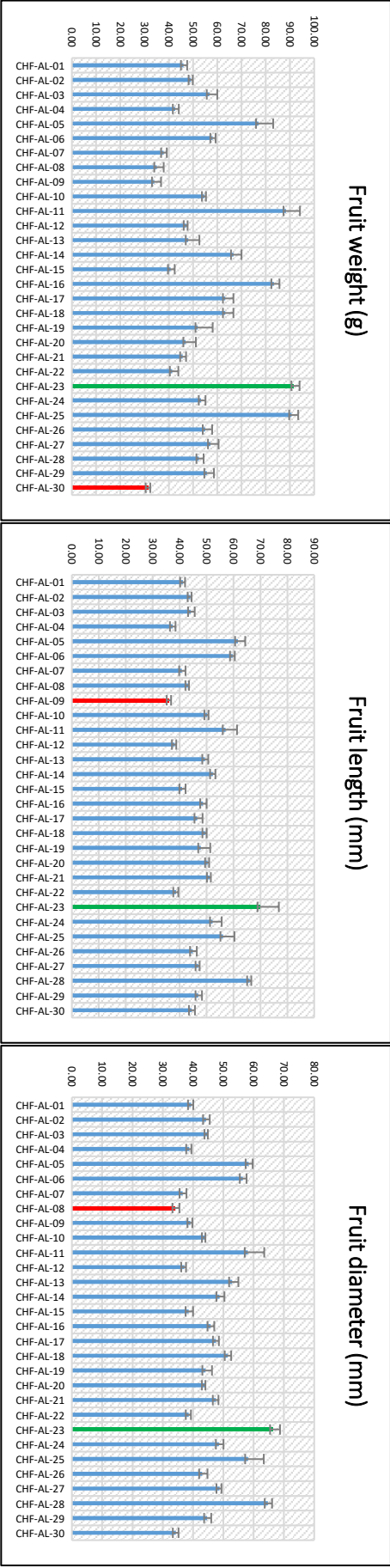


Figure 6: Fruit weight, fruit length and fruit diameter of 30 genotypes of acid lime

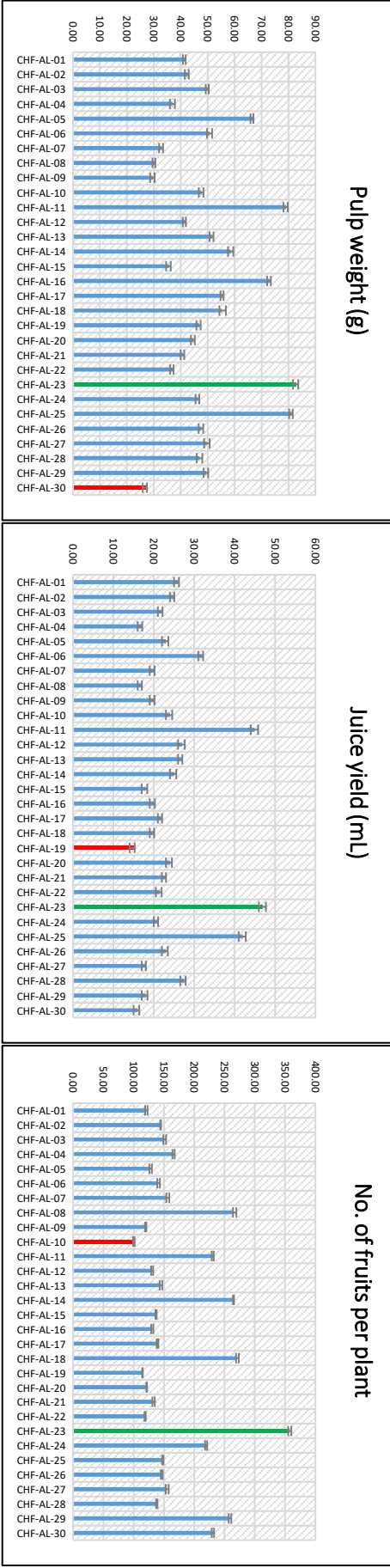


Figure 7: Pulp weight, juice yield and No. of fruits per plant of 30 genotypes of acid lime

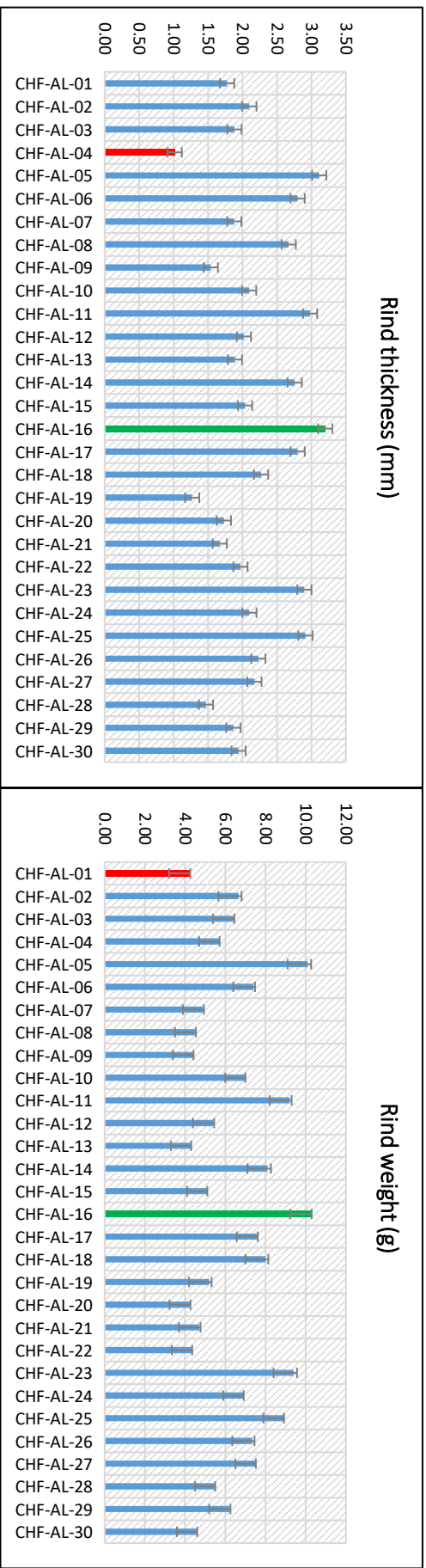


Figure 8: Fruit rind thickness and rind weight of 30 genotypes of acid lime

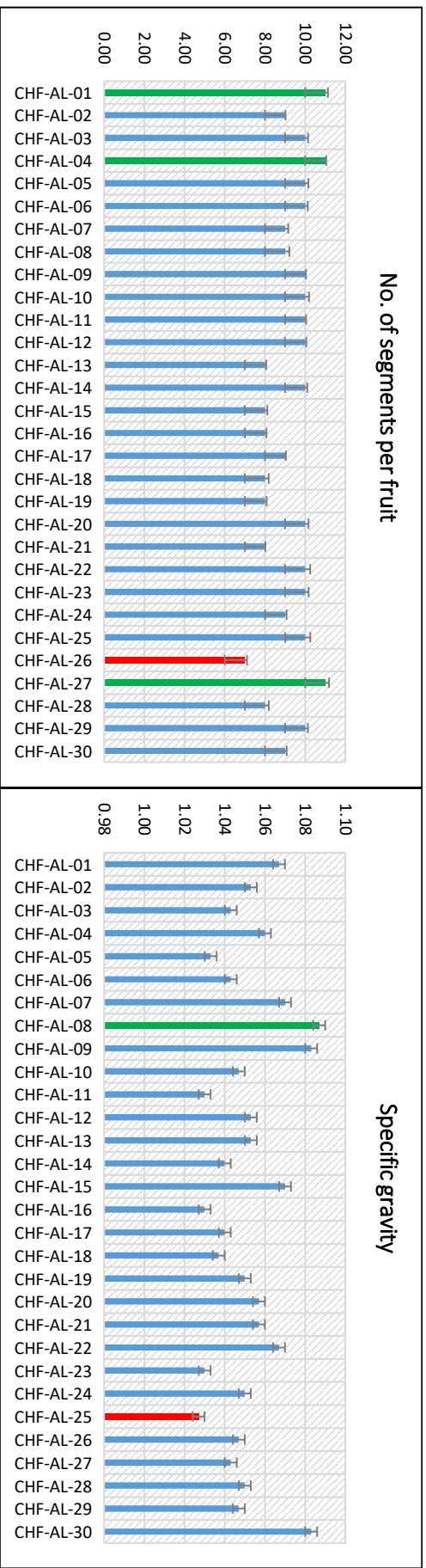


Figure 9: No. of segments per fruit and specific gravity of 30 genotypes of acid lime

3.1.4 Seed Characters

Seed traits also exhibited considerable variation. The average number of seeds per fruit ranged from 4.00 to 31.00, with a population mean of 18.20. The average weight of 10 seeds varied between 5.10 g and 8.70 g (with the highest values recorded in CHF-AL-08 and CHF-AL-25), averaging 6.68 g. Seed length ranged from 6.20 mm to 10.11 mm (mean = 7.82 mm), while seed width varied between 3.70 mm and 6.00 mm, with a mean of 4.71 mm. Three distinct seed shape categories were observed: clavate (15 genotypes), ovoid (10 genotypes), and semi-deltoid (5 genotypes). Seed colour was uniformly cream across all 30 genotypes, and cotyledon colour was consistently light green in all accessions (Table 6; Figure 10 & 11).

Table 6. Qualitative and quantitative characters of seed 30 acid lime genotypes

Genotypes	Average no. of seeds per fruit	Average weight of 10 seeds (g)	Seed length (mm)	Seed width (mm)	Seed shape	Seed colour	Cotyledon colour
CHF-AL-01	10.00	6.70	7.20	4.31	Clavate	Cream	Light green
CHF-AL-02	12.00	5.80	6.88	4.11	Clavate	Cream	Light green
CHF-AL-03	15.00	7.00	10.11	4.96	Clavate	Cream	Light green
CHF-AL-04	13.00	5.50	7.10	3.80	Ovoid	Cream	Light green
CHF-AL-05	12.00	8.00	9.12	5.00	Semi deltoid	Cream	Light green
CHF-AL-06	11.00	5.40	7.80	4.00	Clavate	Cream	Light green
CHF-AL-07	11.33	6.70	8.20	5.00	Semi deltoid	Cream	Light green
CHF-AL-08	10.33	8.70	10.00	5.60	Clavate	Cream	Light green
CHF-AL-09	30.67	5.80	6.99	4.00	Clavate	Cream	Light green
CHF-AL-10	20.67	6.70	7.20	3.70	Clavate	Cream	Light green
CHF-AL-11	27.00	6.60	7.32	4.00	Semi deltoid	Cream	Light green
CHF-AL-12	17.67	8.20	9.22	4.87	Ovoid	Cream	Light green
CHF-AL-13	17.33	6.00	7.02	3.89	Ovoid	Cream	Light green
CHF-AL-14	19.00	5.60	6.88	4.10	Ovoid	Cream	Light green
CHF-AL-15	20.00	5.10	6.20	4.00	Clavate	Cream	Light green
CHF-AL-16	23.00	7.00	7.10	4.60	Semi deltoid	Cream	Light green
CHF-AL-17	30.67	8.50	10.10	5.00	Semi deltoid	Cream	Light green
CHF-AL-18	21.33	5.90	6.80	4.00	Clavate	Cream	Light green
CHF-AL-19	11.00	5.30	7.10	4.70	Clavate	Cream	Light green
CHF-AL-20	15.33	5.40	6.80	4.30	Clavate	Cream	Light green
CHF-AL-21	11.67	7.30	8.90	5.13	Ovoid	Cream	Light green
CHF-AL-22	4.00	6.50	7.80	4.65	Ovoid	Cream	Light green
CHF-AL-23	31.00	6.60	7.66	4.70	Ovoid	Cream	Light green
CHF-AL-24	28.67	6.80	8.20	5.60	Clavate	Cream	Light green
CHF-AL-25	22.00	8.70	8.27	5.00	Ovoid	Cream	Light green
CHF-AL-26	19.00	6.00	7.88	6.00	Ovoid	Cream	Light green
CHF-AL-27	16.67	7.60	7.78	5.78	Clavate	Cream	Light green
CHF-AL-28	14.33	6.80	7.20	5.00	Clavate	Cream	Light green
CHF-AL-29	14.67	6.50	7.00	4.80	Ovoid	Cream	Light green
CHF-AL-30	21.33	6.90	7.12	5.60	Clavate	Cream	Light green
Mean	18.20	6.68	7.82	4.71	--	--	--
C.D.	3.67	0.27	0.32	0.17	--	--	--
±SE(m)	1.30	0.10	0.11	0.06	--	--	--
SE(d)	1.83	0.14	0.16	0.08	--	--	--
C.V.	12.66	2.50	2.53	2.20	--	--	--

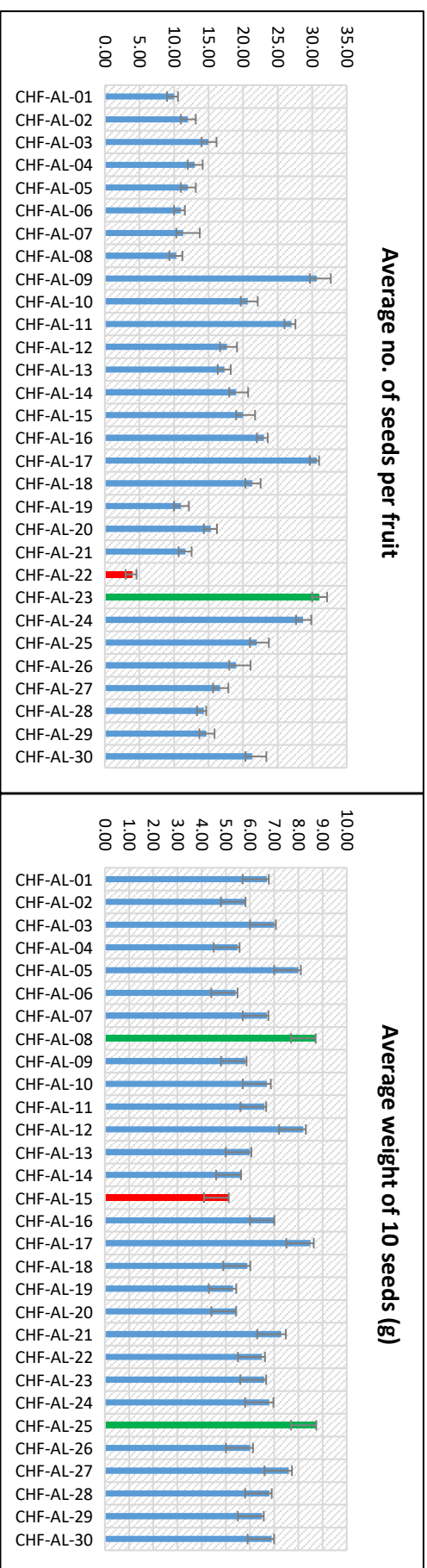


Figure 10: Average no. of seeds per fruit and weight of 10 seeds of 30 genotypes of acid lime

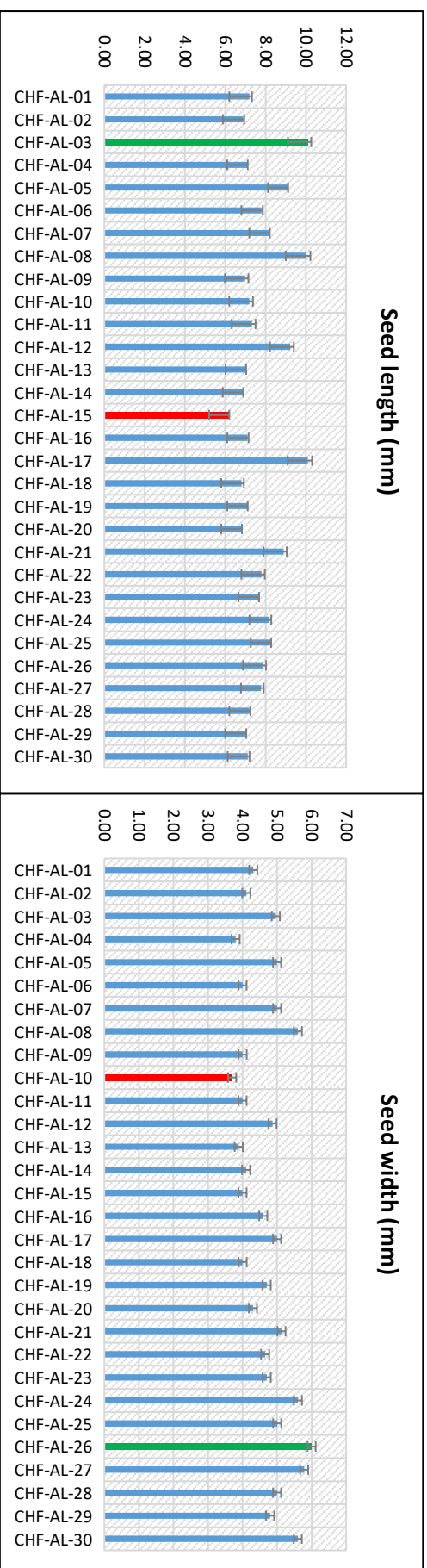


Figure 11: Seed length and seed width of 30 genotypes of acid lime

3.2 Components of Genetic Variability

Analysis of variance components furnishes the empirical basis for designing effective selection strategies (Rattanpal et al., 2022; Singh et al., 2022). PCV was consistently higher than GCV for every trait assessed, indicating that environmental factors contribute to—but do not dominate—phenotypic expression, confirming an underlying strong genetic control (Sekhar & Kundu, 2021, 2025).

The highest PCV and GCV were recorded for the average number of seeds per fruit (40.58% and 38.50%), followed by the number of fruits per plant (36.77% / 36.67%), leaf area, and juice yield (32.95% / 32.82%) (Table 7). The traits with the lowest coefficients of variation were specific gravity (2.60% / 0.43%) and the leaf lamina ratio, indicating strong genetic stability (Nyamagoud et al., 2025).

Broad-sense heritability ranged from 2.74% to 99.61%, with the highest values for leaf area (99.61%), followed by number of fruits per plant and rind weight (99.45%), pulp weight (99.41%), juice yield (99.24%), rind thickness (99.11%), canopy spread (98.88%), and tree height (97.92%). Specific gravity exhibited the lowest heritability (2.74%). Genetic advance was greatest for number of fruits per plant (127.28%), followed by fruit weight (31.80%) and pulp weight (30.37%), while specific gravity (0.00%) registered the lowest genetic advance (Rattanpal et al., 2022; Singh et al., 2022).

Table 7. Genetic variability components for 21 morphological traits of 30 acid lime genotypes

Sl. No.	Parameters	Variability		Heritability (%)	Genetic advance (%)
		GCV (%)	PCV (%)		
1.	Tree height (m)	16.97	17.15	97.92	1.66
2.	Stem girth (cm)	16.46	16.71	97.00	15.97
3.	Canopy spread (m)	17.92	18.02	98.88	1.86
4.	Leaf lamina length (mm)	14.73	14.93	97.34	14.47
5.	Leaf lamina width (mm)	16.49	16.69	97.61	13.57
6.	Leaf area (cm ²)	35.66	35.73	99.61	11.51
7.	Ratio of leaf lamina length:width	4.29	4.99	73.77	0.09
8.	No. of fruits per plant	36.67	36.77	99.45	127.28
9.	Fruit weight (g)	29.25	30.77	90.37	31.80
10.	Fruit length (mm)	16.47	18.24	81.49	14.87
11.	Fruit diameter (mm)	18.00	19.25	87.39	16.16
12.	Juice yield (ml)	32.82	32.95	99.24	16.30
13.	Av. no. of segment per fruit	11.27	11.56	95.02	2.11
14.	Pulp weight (g)	29.94	30.03	99.41	30.37
15.	Rind thickness (mm)	25.93	26.05	99.11	1.15
16.	Rind weight (g)	28.93	29.01	99.45	3.84
17.	Specific gravity of fruit	0.43	2.60	2.74	0.00
18.	Av. No. of seeds per fruit	38.50	40.58	90.00	13.33
19.	Av. Weight of 10 seeds	15.33	15.54	97.39	2.07
20.	Seed length (mm)	13.53	13.76	96.64	2.13
21.	Seed width (mm)	13.68	13.86	97.47	1.30

3.3 Correlation Analysis

Genotypic correlation coefficients were consistently higher in magnitude than their phenotypic counterparts across all trait pairs examined, suggesting that the observed associations are predominantly governed by genetic factors rather than environmental modulation (Table 8 & 9). The most pronounced positive and significant genotypic associations included: tree height with canopy spread ($r = 0.8087$) and stem girth ($r = 0.7509$); stem girth with canopy spread ($r = 0.7692$); leaf lamina length with leaf area ($r = 0.9815$) and leaf lamina width ($r = 0.9627$); leaf area with leaf lamina width ($r = 0.9977$); fruit weight with pulp weight ($r = 1.0094$), rind weight ($r = 0.9194$), and fruit diameter ($r = 0.7906$); fruit length with fruit diameter ($r = 0.9126$); pulp weight with fruit weight ($r = 1.0094$), fruit diameter ($r = 0.8020$), and juice yield ($r = 0.7294$); rind thickness with rind weight ($r = 0.7865$); average number of seeds per fruit with fruit weight ($r = 0.4307$) and pulp weight ($r = 0.4216$); and average weight of 10 seeds with seed length ($r = 0.7733$) and seed width ($r = 0.5749$).

Notable negative correlations were recorded between fruit weight and specific gravity ($r = -0.9103$) and between rind weight and specific gravity ($r = -0.8532$), indicating that fruits with denser internal composition tend to possess thinner, lighter rinds. Phenotypic correlations largely paralleled these patterns, though at slightly attenuated magnitudes, further confirming the predominantly genetic basis of these trait associations (Chuenwarin et al., 2024; Lamani & Murthy, 2022).

3.4 Cluster Analysis

Hierarchical cluster analysis based on 21 morphological traits partitioned the 30 acid lime genotypes into two major clusters, designated A (29 genotypes) and B (1 genotype) (Figure 12). Cluster A subdivided into two major subclusters: A1 (22 genotypes) and A2 (7 genotypes). Subcluster A1 further resolved into A1.1 (19 genotypes, the most morphologically cohesive group, with three sub-groups: A1.1.1, A1.1.2, A1.1.3) and A1.2 (3 genotypes). Subcluster A2 split into A2.1 (7 genotypes) and A2.2. Cluster B consisted solely of CHF-AL-23, separated at a substantially higher rescaled distance, indicating pronounced morphological divergence— allied to its superior fruit weight, juice yield, and fruit number. This unique grouping suggests CHF-AL-23 likely harbours distinct genetic attributes warranting priority attention in elite selection (Akhtar et al., 2024; Lambani et al., 2018; Sekhar & Kundu, 2025).

3.5 Principal Component Analysis

Principal component analysis (PCA) was performed on 21 morphological traits, and components with eigenvalues greater than 1.0 were retained following Kaiser's criterion. This yielded five principal components that collectively accounted for 78.81% of the total phenotypic variation (Table 10 & Figure 13) .

The first principal component (PC1) explained 40.50% of the total variance and was predominantly loaded by fruit weight (8.97%), pulp weight (8.76%), specific gravity (7.61%), rind weight (7.34%), stem girth (7.14%), fruit diameter (7.07%), leaf lamina length (6.44%), and leaf lamina width (6.41%). PC2, contributing 15.63% of the variation, was primarily driven by average weight of 10 seeds (12.06%), leaf lamina width (8.51%), canopy spread (8.45%), leaf area (7.98%), and rind thickness (6.47%). PC3 accounted for 11.22% of the variance and was associated with seed length (19.93%), seed width (19.46%), average weight of 10 seeds (15.96%), and leaf lamina length-to-width ratio (8.40%). PC4 explained 6.20% of the variation, with heavy loading on average number of segments per fruit (49.11%), while PC5, contributing 5.26%, was dominated by average number of seeds per fruit (35.96%).

The biplot of PC1 versus PC2, which together captured 56.13% of the total variation, revealed clear discrimination between genotypes possessing superior fruit quality attributes and those characterized predominantly by seed- and leaf-related traits. This separation underscores the agronomic relevance of the identified principal component structure and supports its utility in germplasm characterization (Nyamagoud et al., 2025; Sekhar & Kundu, 2025; Sonwane et al., 2020).

Table 8. Genotypic correlation coefficients for morphological traits of Acid lime genotypes

Character	TH (m)	SG (cm)	CS (m)	LLL (mm)	LLW (mm)	LA (cm ²)	RLLW	NFPP	FW(g)	FL (mm)	FD (mm)	JY (ml)	ANSmPF	PW (g)	RT (mm)	RW (g)	SGr	ANSPF	AW10S (g)	SL (mm)	SW (mm)
TH (m)	1	0.7509	0.8087	0.5005	0.4961	0.4934	-0.1027	0.4918	0.3977	0.4283	0.3657	0.3469	0.0606	0.384	0.2834	0.3547	-0.4417	0.216	0.0707	-0.1409	-0.0073
SG (cm)		1	0.7692	0.716	0.6895	0.6918	-0.0887	0.6313	0.5226	0.4761	0.5476	0.347	0.0255	0.4975	0.299	0.464	-0.5366	0.2626	-0.0156	-0.0278	0.0037
CS (m)			1	0.6581	0.6669	0.6571	-0.191	0.5472	0.3158	0.2254	0.3284	0.3642	0.132	0.3012	0.1795	0.2693	-0.3715	0.3257	-0.1039	-0.1145	-0.2727
LLL (mm)				1	0.9627	0.9815	-0.0804	0.6387	0.4341	0.2502	0.3922	0.3481	0.2469	0.396	0.2788	0.4079	-0.3758	0.4269	0.0153	0.0374	0.0133
LLW (mm)					1	0.9977	-0.3935	0.6039	0.4341	0.2542	0.3937	0.4	0.2342	0.4065	0.2588	0.3656	-0.3526	0.4021	-0.0817	-0.072	-0.0701
LA (cm ²)						1	-0.2787	0.6291	0.4239	0.2441	0.3852	0.3766	0.2289	0.3987	0.2783	0.3715	-0.3515	0.3974	-0.0652	-0.0433	-0.0468
RLLW							1	-0.008	-0.1205	-0.1086	-0.1562	-0.2918	-0.0128	-0.1494	0.0293	0.0372	0.0347	-0.0769	0.344	0.4046	0.2739
NFPP								1	0.3009	0.1494	0.253	0.2751	0.0513	0.2863	0.3256	0.2631	-0.1408	0.3524	0.0298	-0.0371	0.1196
FW(g)									1	0.7107	0.7906	0.7121	0.1015	0.9423	0.7397	0.9194	-0.9103	0.4307	0.2433	0.0588	-0.061
FL (mm)										1	0.9126	0.6307	-0.0714	0.7216	0.4925	0.6008	-0.6564	0.1652	0.2123	0.0285	0.0558
FD (mm)											1	0.7229	-0.0155	0.802	0.4455	0.636	-0.7552	0.299	0.0708	-0.0269	-0.0853
JY (ml)												1	0.2221	0.7294	0.4936	0.4632	-0.5463	0.3493	0.1437	0.0129	-0.1941
ANSmPF													1	0.0818	0.0335	0.0606	-0.0311	-0.0691	0.1519	0.0822	-0.2139
PW (g)														1	0.7039	0.8577	-0.9052	0.4216	0.2199	0.0347	-0.0918
RT (mm)															1	0.7865	-0.5554	0.3556	0.4227	0.2757	0.0985
RW (g)																1	-0.8532	0.4257	0.2472	0.0898	0.0245
SGr																	1	-0.2964	-0.4879	-0.1893	0.2653
ANSPF																		1	0.1017	-0.056	-0.0197
AW10S (g)																			1	0.7733	0.5749
SL (mm)																				1	0.5422
SW (mm)																					1

TH=Tree height, SG=Stem girth, CS=Canopy spread, LLL=Leaf lamina length, LLW=Leaf lamina width, LA=Leaf area, RLLW=Ratio of leaf lamina length:width, NFPP=No. of fruits/plant, FW=Fruit weight, FL=Fruit length, FD=Fruit diameter, JY=Juice yield, ANSmPF= Av. No. of segments per fruit, PW=Pulp weight, RT=Fruit rind thickness, RW=Rind weight, SGr=Specific gravity of fruit, ANSPF=Average number of seeds per fruit, AW10S=Average weight of 10 seeds, SL=Seed length, SW=Seed width.

Table 9. Phenotypic correlation coefficients for morphological traits of Acid lime genotypes

Charac ter	TH (m)	SG (cm)	CS (m)	LLL (mm)	LLW (mm)	LA (cm2)	RLLW	NFPP	FW(g)	FL (mm)	FD (mm)	JY (ml)	ANSmP F	PW (g)	RT (mm)	RW (g)	SGr	ANSPF	AW10S (g)	SL (mm)	SW (mm)
TH (m)	1	0.7348	0.7971	0.49	0.4879	0.4865	-0.0811	0.4854	0.3682	0.3851	0.3308	0.3408	0.0551	0.3793	0.2182	0.3491	-0.2658	0.1979	0.0692	-0.1373	-0.0075
SG (cm)		1	0.756	0.7002	0.6701	0.6792	-0.0732	0.6208	0.4839	0.4233	0.4954	0.3418	0.0296	0.4901	0.2899	0.4576	-0.3016	0.2482	-0.0185	-0.0197	-0.0006
CS (m)			1	0.6446	0.6579	0.6512	-0.1651	0.543	0.2967	0.1998	0.3073	0.36	0.1332	0.2993	0.1749	0.2668	-0.2005	0.3038	-0.1009	-0.115	-0.2745
LLL (mm)				1	0.9365	0.9655	-0.0556	0.6273	0.4023	0.211	0.3451	0.3448	0.2392	0.3931	0.2698	0.4028	-0.2328	0.4018	0.0083	0.0421	0.0096
LLW (mm)					1	0.9822	-0.3169	0.5963	0.3978	0.2182	0.3727	0.3938	0.2259	0.3988	0.2515	0.3591	-0.2056	0.3777	-0.0808	-0.0707	-0.0725
LA (cm2)						1	-0.2443	0.627	0.4011	0.2189	0.3605	0.373	0.219	0.3958	0.2771	0.3687	-0.2019	0.3762	-0.0642	-0.042	-0.0449
RLLW							1	0.0013	-0.0949	-0.066	-0.134	-0.241	-0.0155	-0.124	0.0296	0.0446	0	-0.0488	0.291	0.3285	0.2322
NFPP								1	0.2855	0.1323	0.2371	0.2734	0.0477	0.2845	0.3233	0.2615	-0.0882	0.3352	0.0276	-0.0365	0.1203
FW(g)									1	0.6434	0.7057	0.6735	0.0942	0.9577	0.7076	0.8774	-0.5154	0.4014	0.2322	0.0516	-0.048
FL (mm)										1	0.7912	0.5575	-0.0502	0.6494	0.4516	0.5503	-0.3711	0.1339	0.2116	0.0183	0.0673
FD (mm)											1	0.6693	-0.0271	0.747	0.4155	0.5886	-0.4192	0.2698	0.0754	-0.034	-0.0796
JY (ml)												1	0.217	0.7252	0.4882	0.4605	-0.3207	0.3272	0.1387	0.0161	-0.1913
ANSmP F													1	0.0831	0.0295	0.0618	-0.0339	-0.066	0.1408	0.0755	-0.206
PW (g)														1	0.6962	0.8542	-0.5421	0.3986	0.216	0.0327	-0.0913
RT (mm)															1	0.7787	-0.3234	0.3301	0.4162	0.2684	0.0984
RW (g)																1	-0.5259	0.4071	0.244	0.0898	0.023
SGr																	1	-0.1778	-0.0707	-0.0342	0.0564
ANSPF																		1	0.0912	-0.0466	-0.0232
AW10S (g)																			1	0.7507	0.5572
SL (mm)																				1	0.5274
SW (mm)																					1

TH=Tree height, SG=Stem girth, CS=Canopy spread, LLL=Leaf lamina length, LLW=Leaf lamina width, LA=Leaf area, RLLW=Ratio of leaf lamina length:width, NFPP=No. of fruits/plant, FW=Fruit weight, FL=Fruit length, FD=Fruit diameter, JY=Juice yield, ANSmPF= Av. No. of segments per fruit, PW=Pulp weight, RT=Fruit rind thickness, RW=Rind weight, SGr=Specific gravity of fruit, ANSPF=Average number of seeds per fruit, AW10S=Average weight of 10 seeds, SL=Seed length, SW=Seed width.

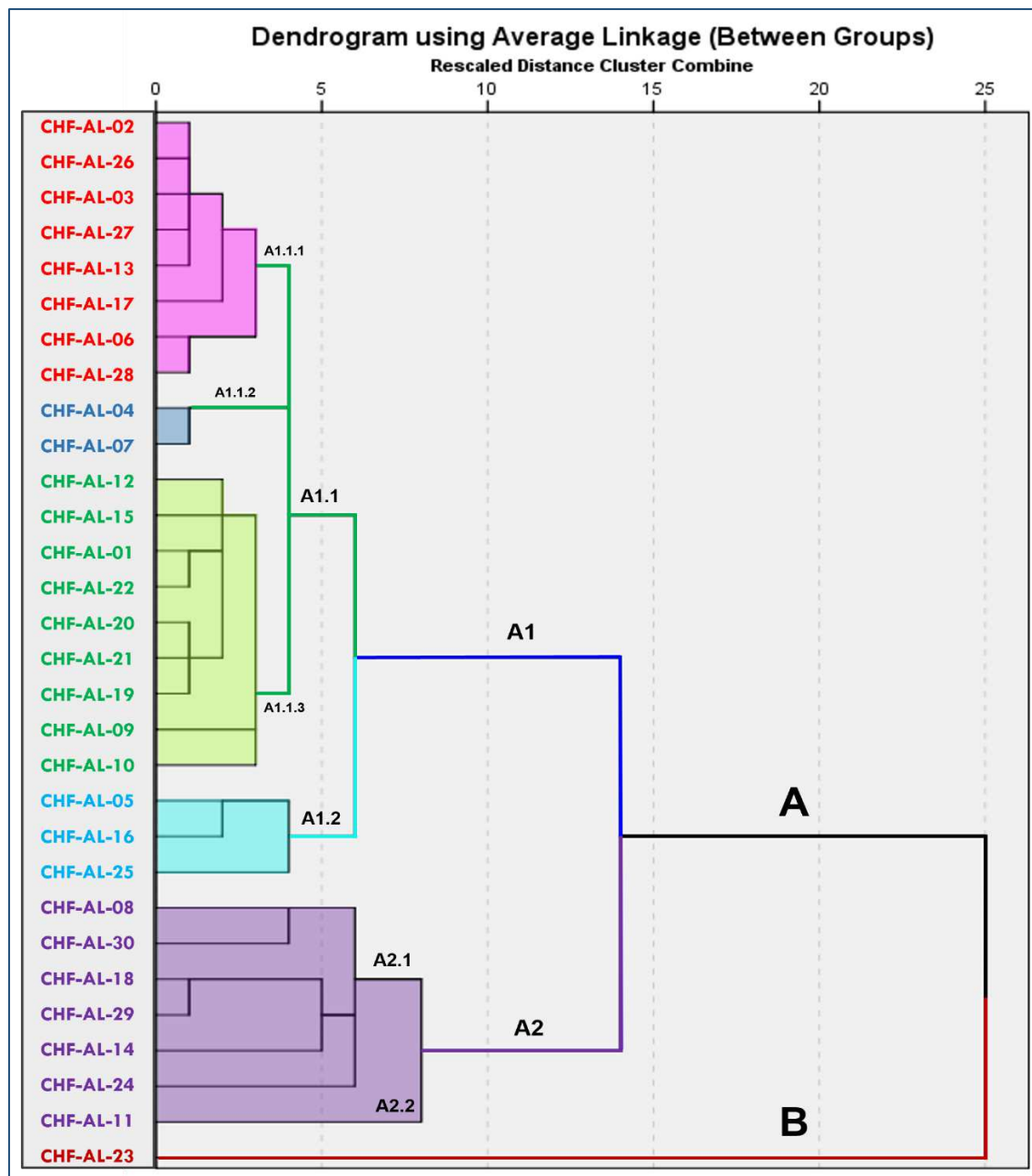


Figure 12: Dendrogram generated using Average Linkage (Between Groups) Rescaled Distance Cluster Combine

Table 10. Eigenvalues, variance contributions, and cumulative percentages of principal components

Components	PC1	PC2	PC3	PC4	PC5
Eigen values	8.51	3.28	2.36	1.30	1.11
Variability (%)	40.50	15.63	11.22	6.20	5.26
Cumulative %	40.50	56.13	67.35	73.55	78.81
Characters	Contribution of the variables (%)				
Tree height (m)	5.01	2.43	0.67	9.32	12.85
Stem girth (cm)	7.14	2.91	1.33	7.35	4.30
Canopy spread (m)	5.02	8.45	0.53	1.49	6.07
Leaf lamina length (mm)	6.44	6.11	5.55	1.42	0.79
Leaf lamina width (mm)	6.43	8.51	1.89	2.57	1.54
Leaf area (cm ²)	6.41	7.98	2.94	2.06	1.51
Ratio of leaf lamina length:width	0.37	3.89	8.40	2.45	2.47
No. of fruits per plant	3.75	4.16	6.27	1.79	1.90
Fruit weight (g)	8.97	4.32	1.64	0.46	0.46
Fruit length (mm)	5.39	4.36	2.43	3.87	4.73
Fruit diameter (mm)	7.07	1.89	3.97	1.06	1.10
Juice yield (ml)	5.89	0.77	2.79	5.27	0.60
Av. no. of segment per fruit	0.23	0.47	0.54	49.11	15.49
Pulp weight (g)	8.76	4.17	2.49	0.40	0.33
Rind thickness (mm)	4.94	6.47	0.36	0.66	3.38
Rind weight (g)	7.34	4.86	0.27	0.01	1.96
Specific gravity of fruit	7.61	3.52	2.43	0.40	0.78
Av. No. of seeds per fruit	2.91	0.05	0.15	0.11	35.96
Av. Weight of 10 seeds	0.27	12.06	15.96	1.74	1.16
Seed length (mm)	0.00	8.30	19.93	2.19	1.20
Seed width (mm)	0.04	4.32	19.46	6.28	1.43

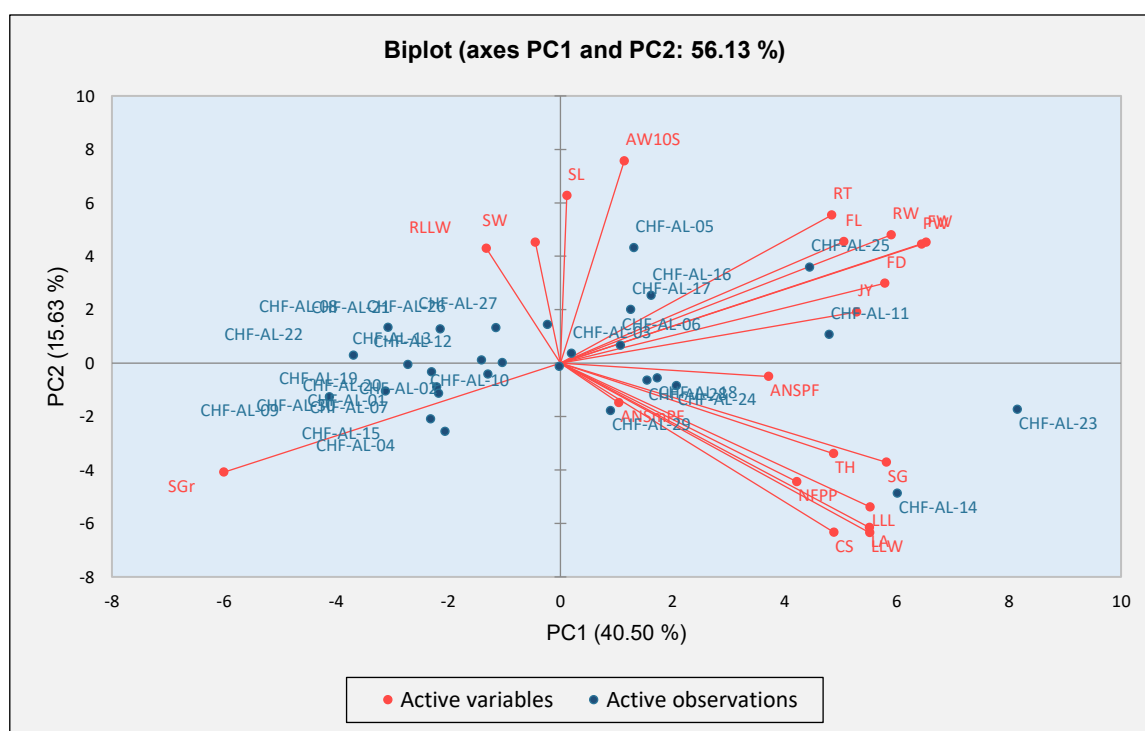


Figure 13: Biplot graphic with two principal components (PC1 and PC2: 56.13%) for 21 morphological characters of 30 acid lime genotypes

4. Discussion

4.1 Diversity in Tree Architecture

Tree architectural traits exhibited wide variability (height: 2.68–6.56 m; girth: 31.00–64.55 cm; canopy spread: 3.37–7.05 m), reflecting complex interactions between inherent genetic determinants and environmental modulation across collection sites. Tree height and girth variation is consistent with observations in Northeast Indian 'Kachai lemon' and other regional citrus landraces (Haokip et al., 2021; Munankarmi et al., 2018; Singh, 2004). Such variability offers valuable selection indices for breeding programs targeting orchard-design optimization and yield enhancement (Lambani et al., 2018; Sekhar & Kundu, 2025).

4.2 Diversity in Leaf Morphology

Leaf traits demonstrated striking variability, with leaf lamina length (40.00–71.22 mm), width (30.00–65.14 mm), and area (10.73–36.44 cm²) all recording substantial ranges. Notably, CHF-AL-14 achieved the largest leaf dimensions across all three metrics, suggesting its strong photosynthetic and vegetative vigor potential (Sekhar & Kundu, 2025). Genotype CHF-AL-23, similarly, exhibited large leaves (68.13 mm length). The leaf lamina length:width ratio ranged from 1.09 to 1.33, with all genotypes displaying an ovate lamina shape and predominantly acute apex morphology. Petiole wings were present only in CHF-AL-08, a rare trait that may serve as a diagnostic marker.

4.3 Diversity in Fruit Attributes

Fruit characters exhibited the most agronomically significant variation. CHF-AL-23 emerged as the premier genotype, recording the highest fruit weight (91.60 g), longest fruit (69.94 mm), largest diameter (66.43 mm), greatest pulp weight (82.70 g), and maximum juice yield (47.00 mL). CHF-AL-25 also produced heavy fruits (90.67 g) with high pulp weight (81.27 g) and juice (42.00 mL). The fruit count per plant ranged from 100 to an exceptional 356 in CHF-AL-23, indicating its strong reproductive efficiency and yield potential. These superior-performing genotypes merit priority status in commercial cultivar development (Haokip *et al.*, 2022; Nyamagoud et al., 2025; Rajamanickam et al., 2024; Sekhar & Kundu, 2021, 2025).

4.4 Diversity in Seed Attributes

Seed-related traits varied widely: seed count per fruit (4.00–31.00), weight of 10 seeds (5.10–8.70 g), seed length (6.20–10.11 mm), and seed width (3.70–6.00 mm). Genotype CHF-AL-23 produced the highest seed count (31.00), while CHF-AL-22 recorded the lowest (4.00), making

the latter a candidate of interest for breeding programmes targeting low-seeded or parthenocarpic cultivars (Munankarmi et al., 2018).

4.5 Genetic Variability and Heritability

The preponderance of high heritability (>90%) for most yield-contributory traits, especially leaf area, number of fruits per plant, rind weight, pulp weight, juice yield, rind thickness, canopy spread, and tree height, indicates these characters are under predominantly additive genetic control and are thus amenable to direct phenotypic selection. Concurrently, the high genetic advance for fruit number per plant (127.28%) and fruit weight (31.80%) confirms that selection pressure on these traits should yield rapid genetic gains. The low heritability and negligible genetic advance for specific gravity emphasize its conservation across genotypes and limited value as a selection index (Rattanpal et al., 2022; Sekhar & Kundu, 2025; Singh et al., 2022).

4.6 Multivariate Diversity Structure

Cluster analysis effectively partitioned the germplasm, isolating CHF-AL-23 into a unique solitary cluster—a strong indication of its genetic distinctiveness and adaptation to its Dibrugarh, Assam location (Akhtar et al., 2024; Sonwane et al., 2020). PCA identified fruit weight, pulp weight, rind weight, and stem girth as the principal discriminating variables, echoing similar trait axes identified in West Bengal and Nepalese acid lime germplasm (Munankarmi et al., 2018; Sekhar & Kundu, 2021, 2025). The contribution of seed-related traits to PC3 and PC4 highlighted the complementary nature of reproductive morphological markers in gauging overall diversity.

5. Conclusion

The present investigation establishes that Northeast India harbours substantial and exploitable morphometric diversity in acid lime germplasm across tree, leaf, fruit, and seed traits. Key quantitative characters—including tree height (2.68–6.56 m), fruit weight (31.47–91.60 g), juice yield (15.00–47.00 mL), and number of seeds per fruit (4.00–31.00)—displayed wide ranges of variation. Genetic analysis confirmed $PCV > GCV$ for all traits, with narrow $PCV - GCV$ gaps signifying tight genetic control. High broad-sense heritability (>90%) for leaf area, fruit number, pulp weight, juice yield, and rind weight, coupled with substantial genetic advance for number of fruits per plant (127.28%) and fruit weight (31.80%), points to additive gene action and the suitability of these traits for direct selection. Correlation analysis revealed strong positive genotypic linkages between fruit weight, pulp weight, rind weight, and juice

yield, providing robust indirect selection indices. PCA extracted five components jointly explaining 78.81% of total variation, with fruit weight and pulp weight emerging as principal discriminators. Cluster analysis partitioned the germplasm into two major clusters, with CHF-AL-23 forming a uniquely distinct solitary group distinguished by its highest fruit weight (91.60 g), juice yield (47.00 mL), and exceptionally high fruit number per plant. Together with CHF-AL-14, CHF-AL-25, and CHF-AL-11, this genotype represents an elite genetic resource of significant value for future breeding programs aimed at varietal development, commercial cultivation, and germplasm conservation. Future research employing molecular markers and biochemical profiling, complemented by multi-location field trials, will be essential for validating and sustainably deploying these genetic resources.

Declaration of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

Data Availability Statement: All data supporting the findings of this study are available within the paper and its Supplementary Information which can be obtained from corresponding author on request.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Clinical Trial Registration: This study is not a clinical trial and therefore does not require clinical trial registration.

Ethics, Consent to Participate, and Consent to Publish declarations: Plant material used in this study comprised 30 acid lime (*Citrus aurantifolia* Swingle) genotypes collected from cultivated plants/orchards across eight Northeastern states of India, namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim. The plant material was collected in accordance with applicable institutional, local and national guidelines and regulations governing the collection and use of plant genetic resources. No plant material was collected from protected or restricted wild habitats. The collected material was used solely for research and characterization purposes. No specific collection or access-and-benefit-sharing licence was applicable to the collection of these cultivated plant materials.

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