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Principal Component based Dissection of Growth and Leaf Trait Variation in River Red Gum (*Eucalyptus camaldulensis*) Accessions

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Abstract: This study investigated the morphological and growth variation among 21 accessions of *Eucalyptus camaldulensis* (River Red Gum) using multivariate statistical techniques under tropical conditions in South Gujarat, India. Eleven morphometric descriptors were assessed following the DUS (Distinctness, Uniformity and Stability) testing guidelines. Principal Component Analysis (PCA) revealed that the first three principal components accounted for 84.57% of the total variation. PC1, explaining 49.64% of the variance, was primarily influenced by leaf elongation traits such as leaf length, blade ratio, roundness and petiole length along with tree volume. PC2 (18.50%) captured variation related to clear bole height and broader leaf size parameters, while PC3 contributed 16.42%. The analysis highlights significant phenotypic diversity among the accessions, with implications for biomass selection, breeding programs, and the refinement of descriptors for varietal registration. These findings demonstrate the utility of PCA in trait prioritization and support the strategic selection of superior genotypes for tree improvement initiatives.

Keywords: Principal component analysis, Morphological variation, Growth traits, Leaf traits, River Red Gum, *Eucalyptus camaldulensis*, Tree improvement, DUS testing

Eucalyptus camaldulensis Dehnh., commonly known as River Red Gum, is one of the most extensively planted *Eucalyptus* species due to its fast growth, adaptability and multiple utility as fuelwood, timber, pole, pulp and plywood raw material. *E. camaldulensis* is native to Australia and has been widely introduced across diverse ecological regions of India, particularly in semi-arid and tropical zones, where its robust nature supports afforestation, agroforestry and industrial plantation programs. Apart from other species like Poplar, *Melia dubia*, *Leucaena* spp. *etc*., (Luna et al., 2011, Thakur et al., 2023), *Eucalyptus* is in growing demand as a raw material for pulp and particle board industries, further emphasizing its importance as an industrial species in many Indian states (Luna et al., 2009) including Gujarat (Huse et al., 2024). Since it is highly used for pole production by the construction industries, especially in South Gujarat, there is a great demand for good genotypes that provide high strength as poles (Huse et al., 2024).

Despite its ecological and economic significance, limited attention has been given to the characterization of intra-specific morphological variation among accessions maintained for breeding and clonal deployment. Understanding morphological and growth variability among genotypes is essential for effective selection and improvement programs. In long-lived tree species the evaluation of both qualitative and quantitative traits, particularly those related to leaf structure and growth form, branching pattern and bark features, provides a foundation for identifying superior and distinct genotypes. Moreover, under the framework of Distinctness, Uniformity and Stability (DUS) testing, prescribed by the Protection of Plant Varieties and Farmers' Rights Authority (PPVFRA), New Delhi, morphological descriptors form the basis for varietal registration, intellectual property protection and germplasm conservation. Recent studies have demonstrated the utility of multivariate statistical techniques such as Principal Component Analysis (PCA) in identifying trait combinations that account for the majority of variability among genotypes. Sharma et al. (2018), Kumar et al. (2020) and Lakshmi et al. (2020) highlighted the PCA as a multivariate analysis tool for characterization of crops. In this context, the present investigation was undertaken during 2024–2025 at Navsari Agricultural University (NAU), Navsari, Gujarat, India to evaluate the phenotypic variability among 21, five-year-old *Eucalyptus camaldulensis* accessions using multivariate analysis – PCA to identify the most influential traits contributing to variability and to support DUS characterization and thereby identifying the superior genotypes for tree improvement and varietal registration of the University. The present study aims to assess the morphological variation among selected superior individuals of *Eucalyptus camaldulensis*, selected from a broader base population, with the long-term goal of supporting breeding and genetic improvement programs.

MATERIAL AND METHODS

Study area and environmental conditions: The present investigation was conducted during 2024-2025 at the

Forestry Farm, Navsari Agricultural University (NAU), Navsari, Gujarat, India. The study site is geographically located at 20°55′19″ N latitude and 72°53′11″ E longitude, with an elevation of 11.98 meters above mean sea level. The region falls under a tropical climate zone characterized by hot summers, humid monsoons and mild winters. The site receives an average annual rainfall of 1500–1600 mm, primarily during the southwest monsoon (June to September).

Plant material, experimental design and selection strategy: The study was conducted on a population of 128 individual trees, representing 36 families of *Eucalyptus camaldulensis* Dehnh, established at the Department of Forest Biology and Tree Improvement, NAU, Navsari during 2020. These families were raised from the germplasm collected from ICFRE – Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore and the trees were spaced at 2 x 2 m and maintained under identical silvicultural practices, thereby minimizing environmental variability and allowing phenotypic differences to reflect underlying genetic variation. Initial assessments were conducted on all 128 individuals for growth and morphological traits. Based on family level performance, 21 families showing superior growth and vigour were shortlisted. From each selected family, one phenotypically superior tree was chosen, resulting in a total of 21 accessions for detailed morphological characterization. This corresponds to a selection intensity of approximately 16%. The selective sampling was designed to identify superior genotypes for tree improvement, it is acknowledged that it does not represent the full genetic variability within the entire population. This has been taken into consideration when interpreting the results.

Morphological and growth trait assessment: Comprehensive set of 11 morphological descriptors (identified as in the DUS test guidelines for *E. camaldulensis* and *E. tereticornis*, prescribed by the PPVFRA (2013) including growth and leaf traits were used to evaluate the different accessions. The growth traits include tree height (m), clear bole height (CBH, m), diameter at breast height (DBH, cm at 1.37 m) and stem volume (m³), whereas the leaf morphometric traits *viz*., leaf length (LL, cm), leaf breadth (LB, cm), leaf area (LA, cm²), leaf perimeter (LP, cm), leaf blade ratio (LBR), leaf roundness (LR) and petiole length (LPL, cm).

Height and mid diameter measurements were performed using dendrometers, digital callipers were used to estimate the DBH. The data were recorded following the standard procedures. For leaf morphometric parameters, 25 fully grown leaves were collected from the middle of the canopy and were scanned along with a reference scale using common scanner. The images were then analysed using the *Image J* software (Schneider et al 2012).

Statistical and multivariate analysis: All quantitative traits were subjected to descriptive statistical analysis, including calculation of means, standard deviations and coefficients of variation to assess the extent of phenotypic diversity among accessions. To investigate the underlying structure of trait variation, Principal Component Analysis (PCA) was performed using the correlation matrix, thus standardizing the influence of measurement units across traits (Jackson 2003). PCA was conducted using KAU Grape software (Gopinath et al., 2020). Only components with eigenvalues greater than 1.0 were retained in accordance with Kaiser's criterion (Kaiser 1960). Trait loadings with absolute values ≥ 0.30 were considered significant contributors to the respective components. The percentage of variance explained by each principal component was used to identify the dominant axes of phenotypic variation. Visualization tools such as scree plots, loading plots and biplots were generated to interpret the clustering of accessions and relative importance of traits across components. The PCA results were then integrated with phenotypic profiles to identify key traits relevant to DUS characterization and potential selection criteria for tree improvement programs.

RESULTS AND DISCUSSION

The first three principal components (PCs) had eigenvalues greater than 1.0 and collectively accounted for 84.57% of the total variance, indicating a high level of data reduction while retaining essential variability. Specifically, PC1 exhibited the highest eigenvalue (5.46), contributing 49.64% of the total variability. PC2 accounted for 18.50% of the variance with an eigenvalue of 2.03, whereas PC3 contributed 16.42% with an eigenvalue of 1.81, together representing a robust cumulative variance suitable for multivariate trait interpretation (Table 1, 2 and Fig. 1). The coefficients of the first two principal components (PC1 and PC2), which together explained 68.14% of total variability (Table 3 , Fig. 2). PC1 emerged as the most influential component, with high positive loadings observed for leaf-related traits such as leaf length (LL = 0.41), leaf perimeter (LP = 0.41), leaf blade ratio (LBR = 0.40) and leaf roundness (LR = 0.40). Other notable contributors included leaf petiole length (LPL = 0.32) and tree volume (Vol = 0.25). The high positive loadings of these traits indicate that PC1 primarily captures variation related to leaf elongation and overall tree biomass production. Accessions with higher PC1 scores are likely to exhibit longer, more elongated leaves and greater volume accumulation - features that assist in genotypic differentiation and DUS characterization. PC2 showed strong positive loadings for clear bole height (CBH = 0.57), leaf area (LA = 0.51) and leaf breadth (LB = 0.40). The high loading of CBH suggests that this component reflects variation in tree architecture, particularly stem form and canopy lift, while the leaf traits point to differences in leaf size rather than shape. Leaf breadth (LB) displayed a negative loading on PC1 (-0.27) but a positive loading on PC2 (0.40), indicating its inverse relationship with leaf elongation traits yet positive association with general leaf size. Similarly, diameter at breast height (DBH) and volume contributed moderately to PC1 but less to PC2, suggesting that these growth traits are more closely aligned with leaf elongation and biomass rather than tree architecture. Overall, PC1 was primarily influenced by leaf elongation and tree volume traits, whereas PC2 represented variation in stem height and broader leaf characteristics. This differentiation implies that both morphological and growth parameters contribute significantly to the observed diversity among the studied accessions and can be effectively utilized in selection and breeding programs.

The PCA results demonstrate substantial morphological diversity among the 21 accessions of *Eucalyptus camaldulensis*, particularly in leaf morphology and architectural traits. The high cumulative variance (84.57%) aligns closely with findings from previous studies on *Eucalyptus* and other forest tree species. Lakshmi et al. (2020), working on *Eucalyptus* clones, identified three principal components (eigenvalues >1.0) that together explained 85.62% of total variation, with leaf length, blade ratio, petiole length and leaf area being the most discriminating traits. Vishnu et al. (2022) also observed that leaf morphometric descriptors significantly contributed to accession differentiation in teak, reinforcing the effectiveness of morphological traits in DUS characterization and multivariate diversity analysis. Their study confirmed that mature leaf descriptors strongly contribute to genotypic distinctness and serve as reliable parameters for DUS-based varietal characterization.

In a comparable study, Vinothkumar et al. (2021) on *Tectona grandis* reported three PCs with eigenvalues above 1.0, accounting for 92.8% of total variation among 122 clones, where quantitative traits such as leaf area, perimeter and stem form were dominant contributors. Sahoo and

Table 1. Summary of variation in growth and leaf morphological traits among 21 *Eucalyptus camaldulensis* accessions

Trait	Mean ± SD (m)
Tree height	15.80 ± 1.35
Clear bole height	8.24 ± 1.92
Diameter at breast height (DBH)	11.49 ± 2.02
Tree volume	0.11 ± 0.05
Leaf blade area	41.46 ± 8.80
Leaf perimeter	42.64 ± 9.76
Leaf length	18.55 ± 4.49
Leaf breadth	3.55 ± 0.80
Leaf blade ratio*	5.68 ± 2.13
Leaf roundness**	3.46 ± 1.22
Leaf petiole length	2.01 ± 0.55

* Leaf blade ratio = Leaf length / Leaf breadth, ** Leaf roundness = Leaf perimeter^2 / (4π × Leaf area)

Table 2. Principal component analysis-Eigen value and percentage of variance studied on quantitative traits of 21 accessions of *Eucalyptus camaldulensis*

PC No.	Eigen value	Percentage of variance	Cumulative percentage
PC 1	5.46	49.64	49.64
PC 2	2.03	18.50	68.14
PC 3	1.81	16.42	84.57
PC 4	0.82	7.47	92.04
PC 5	0.42	3.78	95.82
PC 6	0.35	3.21	99.03
PC 7	0.06	0.57	99.60
PC 8	0.03	0.27	99.87
PC 9	0.01	0.10	99.97
PC 10	0.0029	0.03	100.00
PC 11	0.0001	0.00	100.00

Table 3. Principal component analysis- Coefficients of PC1 and PC2

Variable	Coefficients of PC1	Coefficients of PC2
Height	0.18	0.11
CBH	0.08	0.57
DBH	0.18	-0.33
Vol	0.25	-0.22
LL	0.41	0.10
LA	0.17	0.51
LB	-0.27	0.40
LPL	0.32	0.18
LBR	0.40	-0.11
LP	0.41	0.13
LR	0.40	-0.09

Height: Tree height (m); CBH: Clear bole height (m); DBH: Diameter at breast height (cm at 1.37 m); Vol: Volume (m^3); LL: Leaf length (cm) LA: Leaf area (m^2); LB: Leaf breadth (cm); LPL: Leaf petiole length (cm); LBR: Leaf blade ratio; LP: Leaf perimeter (cm); LR: leaf roundness

Kumar (2022) and Sahoo et al. (2023) in *Eucalyptus tereticornis* demonstrated that three major components (eigenvalues >1.0) captured 86.2% and 89.5% of total variance respectively, with tree height, biomass, leaf area and leaf length-to-width ratio being the most influential traits. The close agreement between these studies and the present findings highlights the robustness of PCA as a multivariate tool in capturing complex morphological variation and

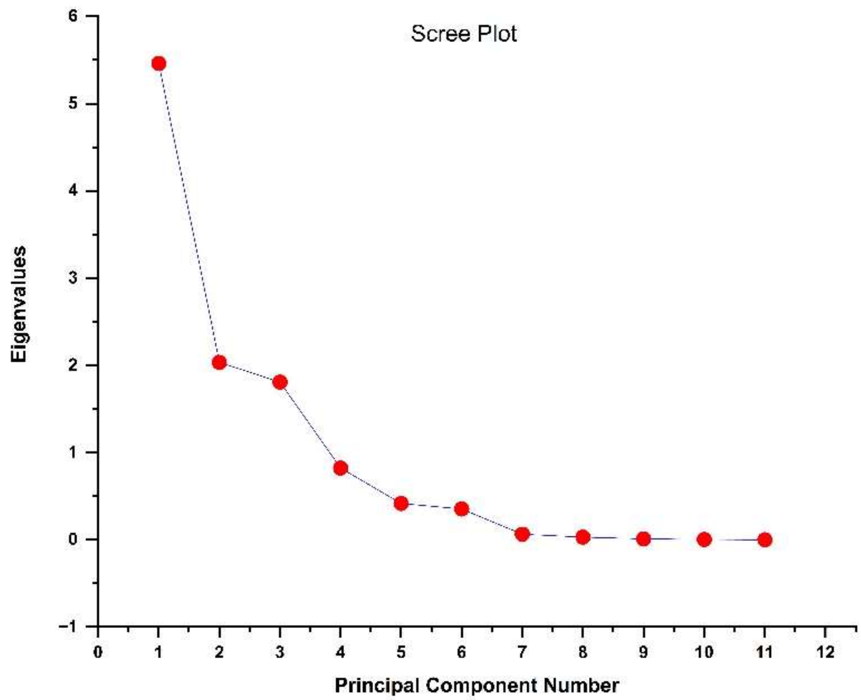

Fig. 1. Principal component analysis-scree plot showing Eigen values

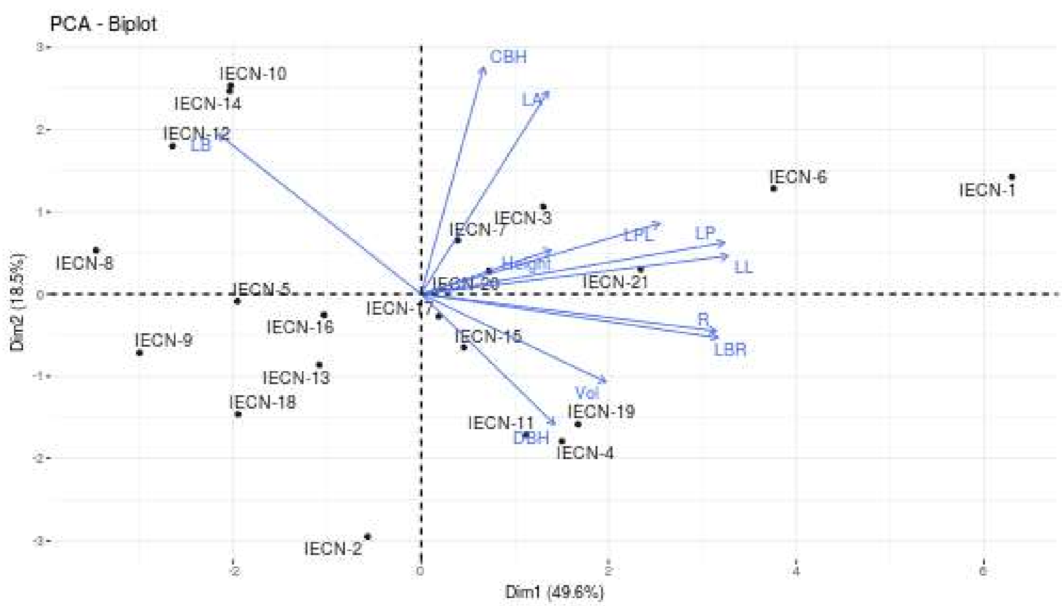

Fig. 2. Principal component analysis- Coefficients of PC1 and PC2

identifying the most informative traits for varietal differentiation and improvement.

The prominence of leaf morphological traits particularly leaf length, perimeter, blade ratio and roundness in PC1 of the present study further validates the role of foliar descriptors in expressing genetic and phenotypic divergence. Lakshmi et al. (2020) and Sahoo et al. (2023), also emphasized that mature leaf traits are heritable, easily observable and stable across developmental stages, making them suitable for varietal registration and breeding programs. The integration of leaf morphology with growth parameters such as tree height and volume enhances discriminative efficiency under multivariate frameworks, as mirrored in the present findings, where both sets of traits exhibited strong joint loadings under PC1-suggesting their combined utility in DUS testing and tree improvement strategies. The strong loadings of clear bole height and leaf breadth in PC2 indicate their effectiveness in distinguishing accessions based on tree form and canopy structure-traits that are directly associated with timber quality and stand management in plantation forestry. The moderate loadings of volume and DBH within PC1 further suggest that accessions with higher PC1 scores are likely to exhibit superior biomass accumulation and faster early growth. These findings validate the usefulness of PCA not only for quantifying phenotypic diversity but also as an indirect selection tool for breeding and clonal improvement programs. Similar interpretations was reported in *Tectona grandis* (Prasetyawati and A'ida 2019) and *Eucalyptus* (Couto et al., 2013), where multivariate analysis effectively distinguished genotypes based on foliar and architectural traits.

The observed variation among the 21 superior *Eucalyptus camaldulensis* accessions reveals a broad spectrum of growth and leaf morphological traits even among phenotypically selected individuals. Traits such as tree height, diameter, leaf area and blade ratio exhibited considerable variability, providing valuable insights for identifying elite genotypes suitable for further breeding. This diversity underscores the potential within selected material and the importance of robust characterization in advancing genetic improvement programs. The approach adopted in this study - combining field-based phenotypic selection of superior accessions with multivariate statistical analysis through PCA- provided a two-tiered strategy for refining genotype selection. Initial family level screening identified superior individuals under uniform field conditions, while PCA enabled the evaluation of trait combinations contributing most significantly to observed variation. This dual approach validated phenotypic observations and objectively identified the most influential traits contributing to accession differentiation, thus enhancing selection precision. Such integration of classical and modern methodologies improves the efficiency of genotype identification for both tree improvement and varietal registration. Similar strategies have been successfully implemented in *Populus* (Monclus et al., 2005), *Acacia nilotica* (Ndoye-Ndir et al., 2008), *Dalbergia sissoo* (Kumar et al., 2012) and *Eucalyptus tereticornis* (Couto et al., 2013; Sahoo and Kumar 2022). Collectively, these studies affirm that PCA-based morphometric evaluation is a powerful tool for identifying distinct and superior genotypes.

CONCLUSION

The present investigation revealed substantial morphological diversity among the phenotypically superior *Eucalyptus camaldulensis* accessions, with significant variation observed in both growth and leaf morphometric traits. The combined use of phenotypic selection and principal component analysis proved effective in identifying key traits particularly leaf elongation parameters and tree volume that contributed most to the observed variability. This dual approach not only enhanced the precision of genotype differentiation but also provided a robust basis for characterizing accessions under the DUS testing framework. Integrating field-based assessment with multivariate analysis, the study offers a practical model for refining genotype selection in tree improvement programs. This approach supports targeted germplasm evaluation and selection, which can further facilitate varietal registration under DUS guidelines and ultimately guide clonal deployment of superior *E. camaldulensis* material with enhanced productivity and adaptability for commercial forestry and agroforestry systems.

AUTHOR'S CONTRIBUTIONS

Conceptualization: SAH, Investigation: NM, SAH. Data collection: NM, Data analysis: SAH, NV, AYG. Writing: SAH, NM, RPG. Review and editing: SAH, RPG.

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