



Exploring Morpho-biochemical Diversity in Tamarind (*Tamarindus indica* L.) for Advanced Breeding Approaches

A. Mayavel¹, M. Amaravel¹, C. Bagathsingh¹, G. Radha Krishnan¹, B. Nagarajan¹

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ABSTRACT

Background: *Tamarindus indica* L., commonly known as Tamarind, is a unique species in the Leguminosae (Fabaceae) family. It is known for its economic and nutritional importance. The genetic diversity of Tamarind is crucial for breeding and conservation purposes. Despite its significance, there is limited research on the phenotypic variations among Tamarind clones, particularly concerning their morphological and biochemical traits. Understanding these variations is essential for improving Tamarind cultivars in terms of yield and quality. This study aims to fill this gap by assessing the phenotypic diversity in Tamarind clones using a range of morphological and biochemical parameters.

Methods: The study was conducted on a 10-year-old clonal assemblage of Tamarind. A total of 60 Tamarind clones were evaluated over two consecutive years (2019-2020 and 2020-2021). The experimental design was a randomized complete block design. The correlation, multiple linear regression, principal component analysis and cluster analysis were analysed in the morphological and biochemical traits to understand the contribution variation and relationship among these clones.

Result: The results indicated significant diversity among the 60 Tamarind clones. High coefficient of variation was observed in annual yield per tree. The PCA revealed that the first five principal components accounted for 81.77% of the total variation, with the first two components explaining 65.89% of the variance. A strong positive correlation and relationship were found between fruit weight and annual yield per tree. The hierarchical cluster analysis resulted in the categorization of the genotypes into three distinct clusters, each characterized by unique morphological and physiochemical traits. These findings are crucial for breeders focusing on enhancing Tamarind cultivars for better yield and quality.

Key words: Cluster analysis, Correlation, Multiple linear regression, Principle component analysis, Tamarind.

INTRODUCTION

Tamarindus indica L., commonly known as Tamarind, belongs to the Leguminosae (Fabaceae) family and it is a monotypic tree with a chromosome number of $2n=24$. Tropical fruit tree has diverse applications, primarily for its fruits, which are consumed either fresh or processed. It is widely cultivated in tropical and subtropical regions of world and in India it is largely grown in Karnataka, Madhya Pradesh, Bihar, Chhattisgarh, Andhra Pradesh and Tamil Nadu.

Almost every part of the tree has utility, with the fruit pulp being high in vitamins and minerals, making it suitable for commercial products like soft drinks, jams and confectioneries. Tamarind contain ascorbic acid content (2 to 20 mg/100 g), moisture (20.15 to 24.50%), total soluble solids (18 to 48°Brix), reducing sugars (25-45%), non-reducing sugar (12.13-16.52%), total sugar (35-50%), organic acids (8-18%) and tartaric acid (Ishola *et al.*, 1990).

Morphological descriptors are fundamental for plant identification, breeding and commercialization, involving cluster analysis to comprehend genetic similarities and dissimilarities (Santos *et al.*, 2012; Cervantes and Diego, 2010). Morphology characters such as fruit size, shape, color and overall appearance play a crucial role in plant description (Nasution and Chinawat, 2017). However, morphological descriptors have limitations in distinguishing between subfamilies and tribes due to the

¹Division of Genetics and Tree Improvement, ICFRE-Institute of Forest Genetics and Tree Breeding, Coimbatore-641 002, Tamil Nadu, India.

Corresponding Author: A. Mayavel, Division of Genetics and Tree Improvement, Indian Council of Forestry Research and Education-Institute of Forest Genetics and Tree Breeding, Coimbatore-641 002, Tamil Nadu, India.
Email: mayavelscientist@gmail.com

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similarity of traits (Swenson and Anderberg, 2005 and Kidaha *et al.*, 2019). Tamarind trees exhibit morphological variations in fruit, crown, foliage, trunk, seed and flower characteristics (Algabal *et al.*, 2012). Nagarajan *et al.* (1998) observed variations in reproductive and fruit traits among different Tamarind populations, influenced by environmental factors or genetic makeup. Pod yield, a complex economic trait, results from various factors related to plant genetics, linkage and environmental conditions. Understanding the associations among yield and quality parameters is crucial for effective selection in tree

improvement programs. Evaluation and characterization of yield and quality parameters play a pivotal role in determining the marketability and acceptability of fruits, making such studies valuable for breeders and nutritionists. With this background, the current study aimed to explore the prevalent variations and associations among the pulp biochemical traits in the selected Tamarind clones.

MATERIALS AND METHODS

The research experiment was carried out in clonal assemblage of *Tamarindus indica* at ICFRE-IFGTB Field Research Station, Kurumbapatti, Salem, Tamil Nadu, India during 2019-2020 and 2020-2021. Clonal assemblage was established with 60 sour, red and sweet Tamarind genetic resources collected from different parts of Tamil Nadu, Karnataka and Andhra Pradesh. The clonal assemblage established (2010) in randomised complete block design at a spacing of 5 × 5 m with four replication and three ramets /replication were maintained.

The data tree biometric data viz., tree height, girth at breast height, crown cover area, number of primary branches, number of secondary branches and annual yield/tree (kg) were recorded in the clonal assemblage.

Fully matured ripened fruits were harvested with identity and data on fruit morphology viz., fruit weight, pulp weight, seed weight, shell weight, vein weight, number of seeds per fruit, fruit length, fruit thickness and fruit width measured the laboratory.

To further enrich the analysis, various biochemical parameters were assessed. The pH value was obtained with a pH meter (Model 20 pH Conductivity Meter, Denver Instrument, United National Inventory Database), as described by AOAC (2010). Total acidity was estimated by 1N alkaline (NaOH) according to the modified procedure of AOAC (2010). TSS (Total Soluble Solids) of extracted juice was determined with an Erma Hand Refractometer (AOAC, 1990). The sugar contents (Reducing sugar, Non-Reducing sugar, and Total sugar) were estimated through the Di-Nitro Salicylic Acid (DNSA) method (Miller, 1972). The Lowry method was used to estimate protein (Lowry *et al.*, 1951). Carbohydrates, lipids, and tannin were extracted following AOAC (2010) procedures. Flavonoids were extracted according to Tan *et al.* (2014). The ascorbic acid content was determined following the Pearson (1976) method.

Table 1: Quantitative characteristics of Tamarind fruits summarized through descriptive statistics.

Characters	Minimum	Maximum	Mean	SD	CV
Annual yield/tree (AYT) (kg)	16.47	57.82	32.55	11.39	34.98
Tree height (THT) (m)	3.76	9.24	6.01	1.37	22.72
GBH (Girth at breast height) (cm)	18.02	63.93	38.32	9.05	23.63
Crown cover area (m) (CC)	2.96	5.83	4.43	0.81	18.28
No. of primary branches (NPB)	2.00	6.00	4.10	0.83	20.28
No. of secondary branches (NSB)	5.70	38.28	14.57	6.33	43.46
Fruit length (FL) (cm)	5.42	16.51	10.44	2.41	23.10
Fruit thickness (FT) (cm)	2.90	7.50	5.17	0.97	18.73
Fruit width (FWH) (cm)	1.02	2.36	1.59	0.30	18.76
Fruit weight (FWT) (g)	13.59	31.19	20.18	4.77	23.61
Pulp weight (PWT) (g)	6.86	15.85	10.12	2.45	24.18
Seed weight (SDWT) (g)	2.97	7.03	4.44	1.11	24.93
Shell weight (SLWT) (g)	2.72	6.30	3.93	0.93	23.70
Vein weight (VWT) (g)	0.97	2.08	1.36	0.32	23.88
No. of seed/fruit (NOS)	4.92	14.17	8.99	2.01	22.32
Total soluble solids (TSS)(Brix°)	8.30	16.04	11.89	1.98	16.64
Tartaric acid (TAC)(%)	4.68	19.69	13.29	4.07	30.61
pH	2.70	3.51	3.06	0.28	9.19
Ascorbic acid (AA)(%)	3.13	7.10	4.94	1.18	23.83
Total acidity (TA)(%)	5.74	15.82	9.62	2.77	28.79
Total sugar (TS)(%)	16.12	54.93	32.02	13.21	41.24
Reducing sugar (RS)(%)	11.02	41.06	22.75	9.90	43.50
Non reducing sugar (NRS)(%)	4.01	17.52	9.11	3.50	38.45
Protein (PR)(%)	1.32	4.48	2.52	0.55	21.65
Carbohydrate (CHO) (mg/g)	1.03	2.83	2.00	0.48	23.78
Lipids (LP) (mg/g)	1.22	2.85	1.97	0.39	19.82
Flavonoid (FLV) (mg/g)	3.18	6.87	5.41	0.95	17.58
Tannins (TAN) (mg/g)	0.20	0.91	0.53	0.18	33.37

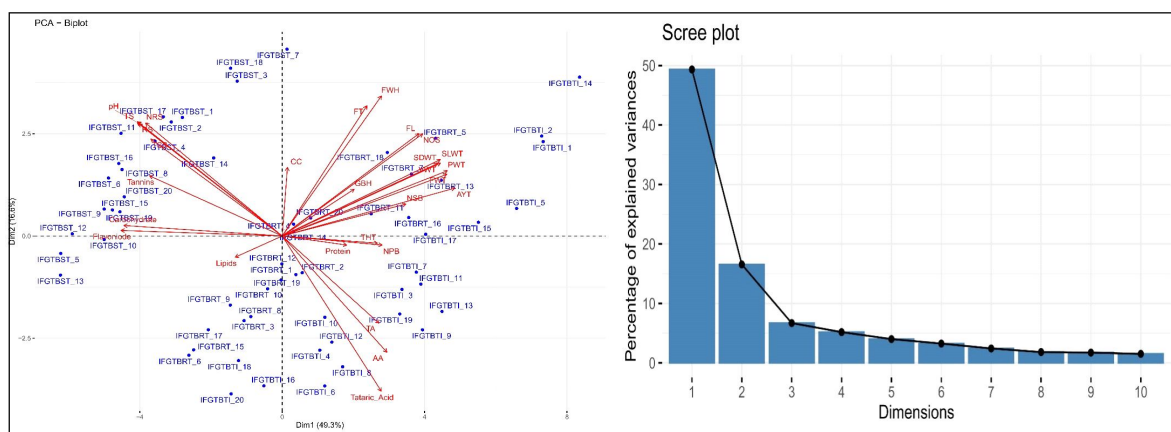


Fig 1: Bi-plot in principal component analysis for morphology and biochemical traits of 60 Tamarind clones.

Statistical analysis

In statistical analysis, calculation of mean, standard deviation and coefficient of variation were done in R programme. Multivariate analysis such as PCA, HCA, correlation analysis and multiple linear regression analyses were carried out using the package “Factoextra” and “FactoMiner” packages in R Statistical Software.

RESULTS AND DISCUSSION

Assessment of phenotypic variations

Analyzing phenotypic diversity highlighted substantial variation in different morphology and biochemical traits of *T. indica* (Table 1 and Fig 1). The coefficient of variation (CV %) was used as a measure to indicate the degree of dispersion of different traits around the mean. Consequently, a higher CV suggests a greater variability among the traits. The majority of the analysed traits displayed relatively higher CV values. The highest coefficient variation was observed in reducing sugar (43.50%) followed by no. of secondary branches (43.46%), total sugar (41.24%), non-reducing sugar (38.45%), annual yield/tree (34.98%) and tannin (33.37%), whereas the pH value (9.19%) of the Tamarind fruit pulp exhibited the lowest variation. Genetic diversity tends to be higher in widely distributed species compared to endemic woody species, known for their lower genetic variability (Luan *et al.*, 2006). Similar variations in Tamarind fruit traits have been reported by Prasad *et al.* (2009) and Sharma *et al.* (2015).

In the present investigation, the wide range of variation was recorded in total soluble solids ranged (8.30 to 16.04°), tartaric acid (4.68 to 19.69%), pH (2.70 to 3.51), ascorbic acid (3.13 to 7.10%), total acidity (5.74 to 15.82%), total sugars (16.12 to 54.93%), reducing sugars (11.02 to 41.06%), non-reducing sugar (4.01 to 17.52%), protein (1.32 to 4.48%), carbohydrate (1.03 to 2.83 mg/g), lipids (1.22 to 2.85 mg/g), flavonoid (3.18 to 6.87 mg/g) and the tannin (0.20 to 0.91 mg/g), with a mean of 0.53 mg/g) were higher compared to various reports (Praveena kumar *et al.*, 2020,

Table 2: Principal component analysis of quantitative traits of Tamarind in pooled over environment.

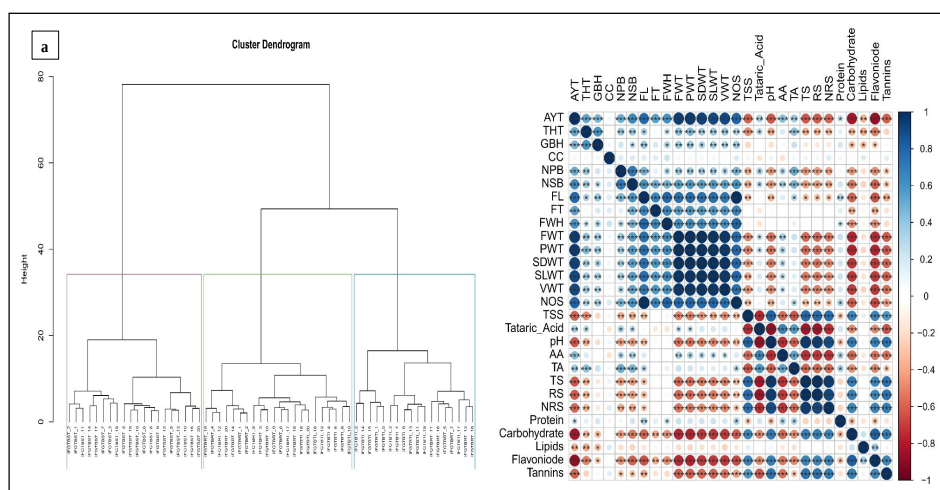
Principle component	Eigen value	Proportion of variation (%)	Total variation explained across axis
PC1	13.81	49.33	49.33
PC2	4.64	16.55	65.89
PC3	1.88	6.70	72.59
PC4	1.45	5.18	77.76
PC5	1.12	4.01	81.77
PC6	0.91	3.25	85.02
PC7	0.68	2.43	87.45
PC8	0.51	1.82	89.27
PC9	0.48	1.73	91.00
PC10	0.43	1.52	92.52
PC11	0.36	1.27	93.79
PC12	0.32	1.16	94.95
PC13	0.27	0.98	95.93
PC14	0.22	0.78	96.71
PC15	0.20	0.73	97.44
PC16	0.18	0.65	98.09
PC17	0.16	0.57	98.66
PC18	0.11	0.41	99.07
PC19	0.10	0.36	99.43
PC20	0.06	0.22	99.65
PC21	0.03	0.12	99.77
PC22	0.03	0.10	99.87
PC23	0.01	0.05	99.92
PC24	0.01	0.03	99.95
PC25	0.01	0.03	99.98
PC26	0.00	0.01	99.99
PC27	0.00	0.01	100.00
PC28	0.00	0.00	100.00

Hazarika and Lalrinpui, 2020 and Mamathashree *et al.*, 2022).

Principle component analysis (PCA)

Table 3: Principal component value for 28 characters in Tamarind pooled over environment (Factor loadings).

Variables	PC1	PC2	PC3	PC4	PC5	Total load
Annual yield/tree (AYT) (kg)	0.95	0.22	0.03	-0.11	-0.09	1.00
Tree height (THT) (m)	0.52	-0.03	0.54	-0.3	-0.29	0.44
GBH (Girth at breast height) (cm)	0.4	0.22	0.52	-0.28	-0.16	0.70
Crown cover area (CC) (m)	0.03	0.33	0.1	0.65	-0.28	0.83
No. of primary branches (NPB)	0.55	-0.04	0.41	0.43	-0.19	1.16
No. of secondary branches (NSB)	0.68	0.15	0.3	0.34	-0.02	1.45
Fruit length (FL) (cm)	0.75	0.49	0.07	0.17	0.18	1.66
Fruit thickness (FT) (cm)	0.46	0.62	-0.14	0.11	0.2	1.25
Fruit width (FWH) (cm)	0.54	0.67	0.04	0.15	0.18	1.58
Fruit weight (FWT) (g)	0.9	0.3	-0.19	-0.11	-0.1	0.80
Pulp weight (PWT) (g)	0.91	0.31	-0.17	-0.14	-0.11	0.80
Seed weight (SDWT) (g)	0.87	0.35	-0.24	-0.11	-0.09	0.78
Shell weight (SLWT) (g)	0.87	0.37	-0.22	-0.11	-0.09	0.82
Vein weight (VWT) (g)	0.85	0.35	-0.21	-0.17	-0.12	0.70
No. of seed/fruit (NOS)	0.77	0.49	0.04	0.16	0.17	1.63
Total soluble solids (TSS)	-0.72	0.46	-0.1	0.01	0.02	-0.33
Tartaric Acid (TAC)	0.55	-0.74	-0.04	0.08	-0.01	-0.16
pH	-0.8	0.54	0.06	-0.03	-0.01	-0.24
Ascorbic acid (AA)	0.58	-0.56	-0.02	0.01	0.25	0.26
Titrate acidity (TA)	0.53	-0.42	0.48	0.3	0.12	1.01
Total sugar (TS)	-0.8	0.55	0.12	-0.04	0.07	-0.10
Reducing sugar (RS)	-0.79	0.54	0.13	-0.05	0.09	-0.08
Non reducing sugar (NRS)	-0.75	0.54	0.1	0	0.03	-0.08
Protein (PR)	0.35	-0.04	0.2	-0.06	0.78	1.23
Carbohydrate (CHO)	-0.87	0.05	0.05	0	-0.07	-0.84
Lipids (LP)	-0.26	-0.1	-0.56	0.51	-0.04	-0.45
Flavonoid (FLV)	-0.89	0.02	-0.08	0.08	-0.04	-0.91
Tannins (TAN)	-0.73	0.29	0.4	0.02	0.01	-0.01

**Fig 2:** (a) Dendrogram analysis of 60 Tamarind clones based on morphological and biochemical traits. (b) Correlation between morphological biochemical characters of Tamarind clones

Principal component analysis (PCA) of morphological and biochemical traits in Tamarind clones (Tables 2 and 3) revealed significant phenotypic variation. The first five principal components (PCs), each with an Eigenvalue over

1.0, accounted for 81.77% of total variation. These PCs are crucial in differentiating Tamarind clones. The PCA bi-plot (Fig 2) shows the first two PCs explaining 65.89% of the variance. Positive loadings on PC1 included traits like

Table 4: Cluster mean for morphology and physiochemical characters among Tamarind genotypes.

Cluster	AYT	THT	GBH	CC	NPB	NSB	FL	FT	FWH	FWT	PWT	SDWT	SLWT	VWT
Cluster 1	47.91	7.03	43.93	4.44	4.59	20.49	12.59	5.78	1.79	25.89	13.17	5.76	5.01	1.76
Cluster 2	28.16	5.72	35.08	4.30	4.05	12.69	9.49	4.74	1.43	18.91	9.32	4.10	3.65	1.24
Cluster 3	23.57	5.42	36.85	4.58	3.70	11.30	9.54	5.10	1.59	16.45	8.27	3.63	3.26	1.14
Cluster	NOS	TSS	TAC	pH	AA	TA	TS	RS	NRS	PR	CHO	LP	FLV	TN
Cluster 1	10.85	10.15	15.02	2.83	5.67	11.12	21.43	14.88	6.46	2.68	1.44	1.66	4.24	0.40
Cluster 2	8.21	11.41	16.43	2.90	5.40	10.11	24.59	17.08	7.36	2.58	2.06	2.24	5.65	0.46
Cluster 3	8.18	13.97	8.29	3.44	3.77	7.74	49.73	36.07	13.42	2.31	2.44	1.95	6.20	0.73

Table 5: Multiple linear regression model for Tamarind annual yield per plant.

Variables	(Intercept)	THT	GBH	CC	NPB	NSB	FL	FT	FWH	FWT	PWT	SDWT	SLWT	VWT	NOS
Estimate	12.85	0.16	0.11*	0.51	0.64	0.06	-0.62	0.21	-0.71	1.32*	0.40	2.07	-5.77*	5.09	1.51
Std. Error	16.55	0.32	0.04	0.37	0.53	0.08	1.18	0.42	1.81	0.60	1.32	2.02	1.98	4.22	1.52
t value	0.78	0.51	2.82	1.40	1.20	0.85	-0.53	0.49	-0.39	2.19	0.30	1.03	-2.92	1.21	1.00
Pr(> t)	0.44	0.61	0.01	0.17	0.24	0.40	0.60	0.63	0.70	0.04	0.76	0.31	0.01	0.24	0.33
Multiple R-squared = 0.98															
Adjusted R-squared = 0.97															

ns: P > 0.05; * P <= 0.05; ** P <= 0.01. (THT): Tree height, (GBH): Girth at breast height, (CC): Crown cover area, (NPB): Number of primary branches, (NSB): Number of secondary branches and (AYT): Annual yield/tree (kg), (FWT): Fruit weight, (PWT): Pulp weight, (SDWT): Seed weight, (SLWT): Shell weight, (VWT): Vein weight, (NOS): number of seeds per fruit, (FL): Fruit length, (FT): Fruit thickness and (FWH): Fruit width, (TAC): Tartaric acid, (AA): Ascorbic acid, (TA): Total acidity, (TS): Total sugar, (RS): reducing sugar, non-reducing sugar, (PR): Protein, (CHO): Carbohydrate, (LP): Lipids, (FLV): Flavonoid and (TAN): Tannin.

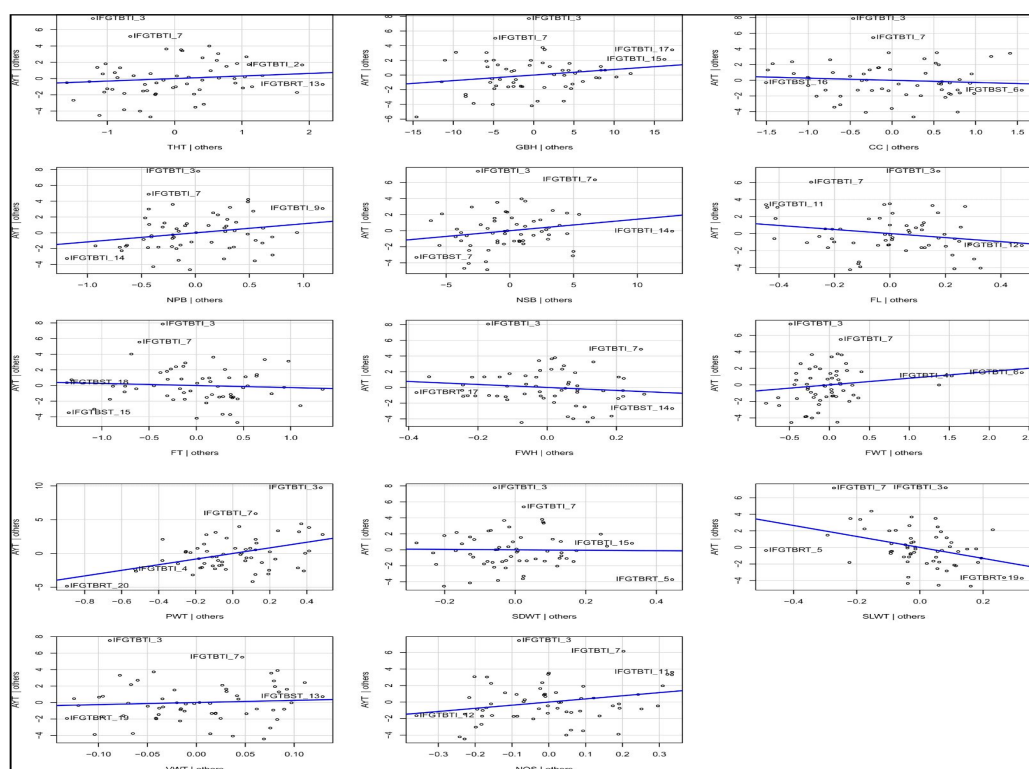


Fig 3: Multiple linear regression for yield and yield attributing traits of Tamarind clones.

annual yield per tree, tree height, crown cover, fruit dimensions, pulp and seed weights and certain acids and protein, indicating their strong influence. Conversely, traits like total soluble solids, pH, sugars, carbohydrates, lipids, flavonoids and tannins showed negative loadings on PC1. PC2 was influenced by fruit dimensions and sugars; tree height and girth affected PC3; crown cover and branch numbers influenced PC4 and protein, ascorbic acid and total acidity impacted PC5.

These findings aligned with Ayala-Silva *et al.* (2016) and Kanupriya *et al.* (2023), who found similar significant contributions of PCs in Tamarind variability. Mishra *et al.* (2022) reported comparable findings in guava, with PCs representing nutritional/bioactive compounds and fruit yield attributes. Key traits like annual yield/tree, branch numbers, fruit dimensions, tartaric acid and protein were most influential in phenotypic variation among *T. indica* clones.

Hierarchical cluster analysis

The exploration of similarities and distinctions among the accessions utilized Ward's dendrogram, validating the clusters (Fig 2a), further branching into numerous sub-clusters based on their similarities. Cluster I, Cluster II and Cluster III contained 22, 18 and 20 genotypes, respectively. Cluster I predominantly included sour Tamarind genotypes with high mean values for annual yield per tree, tree height, girth at breast height, fruit weight, pulp weight, seed weight, vein weight, number of seeds and high levels of total acidity and ascorbic acid (Table 4). In

Cluster II, with both sour and red Tamarind genotypes, there were notable mean values for tartaric acid and pH. Cluster III consisted of sweet Tamarind genotypes with lower mean values for all morphological traits but higher mean values for all physiochemical traits, excluding tartaric acid, ascorbic acid, total acidity and pH. Ayala-Silva *et al.* (2016) analysed the pomological diversity of 13 Tamarind genotypes in Miami, Florida, considering both qualitative and quantitative traits. Through cluster analysis, the Tamarind genotypes were classified into three major clusters. Cluster 'A' grouped semi sour genotypes, cluster 'C' included sour genotypes and cluster 'B' consisted of genotypes primarily characterized by their sweet taste, dark pulp, and smaller fruit size.

Correlation studies

In a study on Tamarind trees, significant correlations were found between various traits and their impact on yield and fruit quality. Annual yield per tree positively correlates with traits like tree height, girth at breast height and all fruit morphometric traits, as highlighted in studies by Pooja *et al.* (2018), Mayavel *et al.* (2018) and Raut *et al.* (2022). Tartaric acid, ascorbic acid and total acidity also show positive correlations with yield and fruit weight, aligning with findings by Challapilli *et al.* (1995).

Pulp weight is identified as a key economic trait, strongly associated with fruit size and seed count. The study also notes a positive correlation between total soluble solids and sugars, proteins and other nutrients.

However, tartaric acid negatively correlates with these components, as observed by Divakara (2009), indicating a trade-off in Tamarind's biochemical makeup. Overall, the study underscores the complex relationships between various traits in Tamarind, offering insights for breeding and cultivation strategies.

Multiple linear regression

The fruit weight exhibited a strong and positive correlation with the annual yield per plant, emphasizing the role of fruit weight in increasing the annual yield per tree (Table 5) (Fig 3). Fruit morphology characteristics, including fruit thickness, pulp weight, seed weight, vein weight and the number of seeds per fruit, also showed a positive relationship with the annual yield per tree. In this context, these traits constitute fruit morphology traits that contribute to the enhancement of the annual yield per tree. Similarly Fandohan *et al.* (2011) reported strong positive relationship between fruit weight and pulp mass.

CONCLUSION

The coefficient of variation (CV %) highlighted high variability in reducing sugar, number of secondary branches, total sugar, non-reducing sugar, annual yield per tree, tannin, and tartaric acid. Principal component analysis (PCA) identified five components contributed for 81.77% of total variation. Correlation studies revealed strong relationships among most traits, revealing insights for genetic enhancement strategies. Additionally, multiple linear regression analysis underscored a significant positive relationship between fruit weight and annual yield per tree. Hierarchical cluster analysis effectively grouped the accessions into three distinct clusters, reflecting similarities in morphological and physiochemical traits. These findings enhance our understanding of *Tamarindus indica* trait interplay and lay a base for targeted breeding and selection programs to improve desirable characteristics in this vital fruit crop.

Conflict of interest

On behalf of all manuscript authors, I am providing the conflict statement that there is no conflict of interest.

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