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Research Article

Genetic variability and association analyses of morphological and biochemical traits in *Tamarindus indica* **L. clones**

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Abstract

The study aimed to investigate the genetic variability and association of morphological and biochemical characters of 60 different Tamarind clones. The experiment was conducted in 10-year-old germplasm bank of Tamarind at ICFRE-IFGTB Field Research Station, Kurumbapatti, Salem, Tamil Nadu, India. Analysis of variance revealed significant variation among clones for the morphological and biochemical characters. High phenotypic and genotypic coefficients of variation were observed for the parameters like annual yield per tree, fruit weight, pulp weight, seed weight, shell weight, vein weight, number of seeds per fruit, fruit length, fruit thickness, fruit width and total sugar content. High broad-sense heritability and high genetic advance per cent mean were recorded for annual yield per tree, tree height, number of primary branches, fruit weight, pulp weight, seed weight, shell weight, vein weight, number of seeds per fruit, fruit length, fruit thickness, fruit width, ascorbic acid, total acidity, total sugar, reducing sugar, non-reducing sugar and protein. Phenotypic path analysis highlighted positive direct effect of fruit weight, pulp weight and seed weight on annual yield per tree. Mahalanobis D-square analysis clustered the 60 tamarind clones into ten groups with higher inter-cluster distances highlighting the substantial genetic diversity present among the genetic resources. This comprehensive assessment provides insights for the genetic improvement of tamarind, aiding in the selection of superior genotypes for breeding programs.

Keywords: Genetic variability, morphological trait, biochemical traits

INTRODUCTION

Tamarindus indica L., commonly known as Tamarind, belongs to the Leguminosae (Fabaceae) family . It is a monotypic genus with a chromosome number of 2n=24. It is a tropical fruit tree grown mainly for its fruits, which are consumed fresh or in processed forms (Purseglove, 1987). The fruit pulp is high in vitamins and minerals, making it ideal for commercial products such as soft drinks, jams, and confectioneries. Tamarind contains ascorbic acid (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 sugars (12.13 - 16.52%), total sugars (35 -50%), organic acids (8-18%), and tartaric acid (Ishola *et al*., 1990). It is cultivated in 54 countries, including 18 within its native range and 36 where it has become naturalized. In India the area under tamarind cultivation is estimated to be 44,056 hectares with fruit production of 1,62,148 metric tonnes and the average productivity of 32.21 Kg ha⁻¹, In Tamil Nadu, the area under tamarind during 2022-23 was14,669 hectares with and yield of 44,653 tonnes of fruits (Spices Board,

2024). Tamarind ranks as the sixth most valuable spice in terms of export revenue in India with an annual export of 25,870 tonnes of pulp in fresh, dry, and paste form and valued for Rs. 147.70 crores (APEDA, 2023). Despite being a multipurpose and largely cultivated species for livelihood improvement of rural population, very minimal effort made for genetic improvement of the species. It is due to the long gestation periods and time-consuming nature in tree improvement research. Indigenous farmers have traditionally selected planting materials from natural populations based on phenotypic traits. However, this phenotypic selection results in growing stocks that are essentially wild (El-Siddig *et al*., 2006). The variation in pod length and width shows genotypic similarities with other traits, the potential for improvement hinges on sampling the genetic variability within and between populations. Therefore, understanding the genetic variation, species structure, and genetic parameters of key traits is crucial for developing effective improvement and conservation strategies. The traditional crossing is not feasible due to the longer juvenile phase of the progeny and there is a need for more trait-specific research to enable provenance trials that can lead to selections combining desirable characteristics (Prasad *et al*., 1998). These selections should be developed into cultivars suited to various land-use systems such as agroforestry, orchards, plantations, and rehabilitating wastelands with inherent stress conditions (El-Siddig *et al*., 2006). The seedling progenies of tamarind exhibits wide variation in phenology, reproductive biology, bearing habit, fruit yield, fruit size, pulp color, acidity and sugar content. This extensive variation provides significant opportunity for genetic improvement of the species. Enhancing tamarind productivity and yield in orchards involves developing genotypes with desirable traits such as fast growth, good tree form, high yield, resistance or tolerance to major pests, diseases, and drought (Radhamani *et al*., 1993). Path analysis studies are vital for fruit crops such as tamarind, mango, and citrus, where improving both quantitative and qualitative traits is important. These studies provide insights into the interrelationships and contributions of independent characters to a dependent variable, helping tree breeders apply effective selection procedures in improvement programs. Understanding the nature and magnitude of interrelationships among yield and its contributing factors is essential for the simultaneous enhancement of traits and yield. In this context, the present investigation aims to assess genetic variability and association analysis based on yield and yield attributes of different Tamarind genotypes. This assessment will aid in conserving valuable germplasm and protecting it from genetic erosion.

Genetic variability and its components represent the genetic fractions of observed variability, providing measures of the transmissibility of variation and the response to selection. Understanding the inheritance patterns of various traits is crucial for determining the most suitable breeding procedures for any crop. A breeder's choice of material for improvement work depends on the extent of genetic variability present. Phenotypic expression often does not accurately reflect the genotype, as natural population variation is phenotypic variability resulting from genotypic value, environmental effects, and genotype-environment interactions. By combining path analysis with an assessment of genetic variability, this study aims to provide comprehensive insights to evaluate the genetic variability and association analysis of different morphological and biochemical traits of tamarind clones and to developed effective selection procedure and breeding strategies.

MATERIALS AND METHODS

The experiment was conducted on a clonal assemblage of tamarind at the Indian Council of Forestry Research and Education (ICFRE) - Institute of Forest Genetics and Tree Breeding (IFGTB) Field Research Station in Kurumbapatti, Salem, Tamil Nadu, India during 2019- 2020 and 2020-2021. The filed trial is located 11⁰45.140 North latitude and 78°09.417 East longitude, with an average elevation of 294 m above Mean Sea Level (MSL). The clonal assemblage was established with 60 sour, red, and sweet tamarind genetic resources collected from various regions of Tamil Nadu, Karnataka, and Andhra Pradesh during 2010. The tamarind clones were planted in Randomised Complete Block Design at a spacing of 5x5m with four replications, and three ramets per replication were maintained.

Biometric data of tamarind clones including tree height, girth at breast height, crown cover area, number of primary branches, number of secondary branches, and annual yield per tree (kg) were recorded. The ripened fruits were collected and observation recorded for fruit weight (g), pulp weight (g), seed weight (g), shell weight (g), vein weight (g), number of seeds per fruit, fruit length (cm), fruit thickness (cm), and fruit width (cm) in the laboratory. Various biochemical parameters were assessed to further enrich the analysis. Estimation of total acidity (%) was done by 1N alkaline (NaOH) modified AOAC (2010) procedure. Tartaric acid (%) were determined by the method described by Roopa and Kasiviswanatham (2013). Total soluble solids (Brix^o) of extracted juice was measured with an Erma Hand Refractometer (AOAC, 1990). Sugar contents, including reducing sugar, nonreducing sugar (%) and total sugar (%) were estimated using the Di-Nitro Salicylic Acid (DNSA) method (Miller, 1972). Protein (mg/g) content was estimated using the Lowry method (Lowry *et al*., 1951), and carbohydrates (%) were assessed by following AOAC (2010) procedures. Ascorbic acid (mg/g) content was determined using the Pearson (1976) method. The morphometric and biochemical data were subjected to pooled analysis of variance (ANOVA) and genetic parameters were estimated by using 'R' programme and D-Square and path analysis were calculated using Indostat package.

RESULTS AND DISCUSSION

Studies on genetic parameter: ANOVA was employed to assess the genetic variability among different clones of tamarind. The pooled analysis of variance indicated significant differences in the mean sum of squares of genotypes and genotype × environment interaction for all traits except tree height and number of primary branches (**Table 1**). The estimates of phenotypic coefficient variation (PCV) and genotypic coefficient variation (GCV), heritability and genetic advance per cent of mean is represented in **Table 2**.

High PCV and GCV was observed for annual yield per tree (36.18% and 35.74%), number of primary branches (21.69% and 21.60%), fruit weight (24.41% and 24.23%), pulp weight (24.95% and 24.09%), seed weight (25.86% and 24.43%), shell weight (24.90% and 23.50%), vein weight (27.78% and 24.80%), number of seeds per fruit (28.87% and 25.54%), fruit length (25.70% and 25.53%), ascorbic acid (24.18% and 24.01%), total acidity (31.21% and 29.54%), total sugar (42.05% and 34.22%), reducing sugar (44.27% and 35.43%), non-reducing sugar (41.19% and 29.91%) and protein (23.60% and 21.91%) indicating

the presence of high variation among the tamarind clones. Similarly, Singh and Nandini (2014) reported high PCV and GCV for fruit weight, pulp weight, shell weight, vein weight, seed weight and number of seed per fruit in tamarind. Moderate PCV and GCV was estimated for tree height (18.19% and 18.06%), fruit thickness (17.30% and 16.85%), fruit width (19.02% and 18.52%) and total soluble solid (17.17% and 11.17%). These findings confirms the reports of Algabal *et al*. (2012) for fruit thickness and fruit width. High and moderate PCV and GCV were observed for tartaric acid (30.33% and 19.70%), number of secondary branches (45.59% and 12.93%), and carbohydrate (28.65% and 18.90%), while, moderate and low PCV and GCV was observed for girth at breast height (19.92% and 6.00%), crown cover area (18.64% and 4.43%) indicating that variation existing in these traits is influenced not only by genotype but also by environmental factors. The PCV was higher than the GCV for all traits studied, indicating the influence of environmental factors, in addition to genetic makeup play a significant role in trait expression. Genetic variation refers to the inherent variability in genes that is unaffected by environmental conditions. High GCV values indicate

ns P > 0.05; * P <= 0.05; ** P <= 0.01

PCV-Phenotypic coefficient variation, GCV-Genotypic co-efficient variation, *h2 b-*Broad sense heritability, GA-Genetic advance, GAM-Genetic advance per cent of mean

that such traits can be enhanced through selection, while low GCV values suggest a significant influence of environmental factors on trait expression. These findings were consistent with Divakara *et al*. (2012), Singh and Nandini (2014), Singh and Singh (2021) and Raut *et al*. (2022) in tamarind.

In this study, high broad sense heritability coupled with high genetic advance was observed for annual yield per tree (97.59% and 72.73%), tree height (98.54% and 36.92%), number of primary branches (99.17% and 44.32%), fruit weight (98.58% and 49.56%), pulp weight (93.17% and 47.89%), seed weight (89.25% and 47.54%), shell weight (89.10% and 45.70%), vein weight (79.73% and 45.63%), number of seed per fruit (78.22% and 46.53%), fruit length (98.61% and 52.21%), fruit thickness (94.94% and 33.83%), fruit width (94.84% and 37.16%), ascorbic acid (98.64% and 49.13%), total acidity (89.56% and 57.58%), total sugar (66.20% and 57.35%), reducing sugar (64.03% and 58.40%) and protein (86.19% and 41.89%). These traits exhibit additive gene effects that play significant role in effective selection processes in breeding programme.

Bhogave *et al*. (2017) reported high heritability coupled with high genetic advance observed for pulp weight, fruit weight, seed weight, and total sugar. Similarly, Mamathashree *et al*. (2022) observed high heritability with high genetic advance in total soluble solids, reducing sugars, total sugars, tartaric acid, and total acidity in tamarind genotypes and revealed additive nature of these traits. Medium heritability coupled with medium genetic advance was observed in total soluble solids (42.35% and 14.98%). Medium heritability and high genetic advance per cent of mean was observed in non-reducing sugars (52.75% and 44.75%), tartaric acid (42.19% and 26.36%) and carbohydrate (43.51% and 25.68%). Low broad sense heritability coupled with low genetic advance percent of mean was observed in girth at breast height (6.30% and 3.11%), crown cover area (5.65% and 2.11%), number of secondary branches (8.04% and 7.55). High heritability with low genetic advance, or vice versa, indicates that the variability is due to non-additive gene interactions such as dominance or epistasis. The present findings aligned with the findings of Rajamanickam (2020) in tamarind; Sharma *et al*. (2011) in cashew nut; Rajan *et al*. (2009)

Table 3. Continued..

Residual = 0.27. Tree height (THT), Girth at breast height (GBH), Crown cover area (CC), No. of primary branches (NPB), No. of secondary branches (NSB), Fruit length (FL), Fruit thickness (FT), Fruit width (FWH), Fruit weight (FWT), Pulp weight (PWT), Seed weight (SDWT), Shell weight (SLWT), Vein weight (VWT), No. of seed/fruit (NOS), Total soluble solids (TSS), Tartaric Acid (TAC), Ascorbic acid (AA), Total acidity (TA), Reducing sugar (RS), Non-Reducing sugar (NRS), Total sugar (TS), Protein and Carbohydrate (CHO).

and Nayak *et al*. (2013) in mango; Rabha *et al*. (2013) in citrus; Jambhale *et al*. (2014) in papaya; Mohammed *et al*. (2014) and Rajamanickam and Rajmohan (2008) in banana and Bhat and Dhillon (2015) in pear.

Phenotypic path analysis: The phenotypic path analysis revealed significant direct effects of various traits on the annual yield per tree in positive and negative directions (**Table 3**). Fruit weight (0.24), pulp weight (0.40), number of secondary branches (0.02), ascorbic acid (0.11), total sugar (0.05), number of primary branches (0.03), fruit width (0.05), seed weight (0.10), vein weight (0.09), fruit length (0.08), fruit thickness (0.09) tree height (0.08) and girth at breast height (0.03) exhibited positive direct effect on annual yield per tree. In this context, direct selection of plants based on any of these independent traits can improve tamarind fruit yield per tree. Priyanka *et al*. (2021) and Pooja *et al*. (2018) revealed similar result that pulp weight, seed weight, vein weight, fruit weight had positive direct effect on annual yield per tree. Hence, direct selection based on any of these independent traits can lead to improvements in tamarind genotypes for annual yield per tree. Negative direct effects on annual yield per tree observed with shell weight (-0.12), carbohydrate (-0.09), reducing sugar (-0.08), non-reducing sugar (-0.01), protein (-0.05), number of seeds per fruit (-0.03), total soluble solids (-0.06), tartaric acid (-0.02), total acidity (-0.01), and crown cover area (-0.01). Therefore, these traits do not aid in selecting genotypes for yield improvement. Similar results were reported by Singh and Nandini (2014), Mayavel *et al*. (2018), Rajamanickam *et al*. (2020) and Pooja *et al*. (2022).

Mahalanobis D-square analysis of 60 tamarind clones: The 60 different tamarind clones were grouped into 10 clusters. Composition of different clusters along with number of accessions is presented in **Table 4**. Among the 10 clusters, the highest number of tamarind clones were observed in cluster I (13), followed by cluster III (9) and cluster VII (6). Inter and intra cluster genetic distance (D) values among ten clusters are presented in **Table 5**. Inter cluster distance values ranged from 1032.32 (Cluster I and Cluster VIII) to 11558.17 (Cluster IV and Cluster VII). The largest inter cluster distance value was observed between Cluster IV and Cluster VII (11558.17), followed Cluster II and VII (7165.63). Intra cluster values ranged from 233.02 (Cluster VI) to 1809.44 (Cluster X). Maximum intra cluster distances were recorded by Cluster X (1809.44), followed by cluster IX (1416.58) and Cluster VII (1089.85). The wide range between the highest and lowest inter-genotypic distances indicates substantial genetic diversity among the genotypes. Genotypes with greater cluster distances were more heterogeneous nature while smaller distances, indicate that the clusters were homogeneous themselves (Rajan *et al*., 2009).

Cluster mean values for the 24 characters are represented in **Table 6**. Cluster mean of annual yield per tree ranged from 54.23 kg (cluster VII) to 18.99 kg (cluster IV). For tree height cluster mean ranged from 7.21 m (cluster IX) to 3.96 m (cluster VI). Whereas, girth at breast height ranged from 46.23 cm (cluster VII) to 31.31 cm (cluster VI). Cluster mean of crown cover area ranged from 4.84 m (cluster VIII) to 4.12 m (cluster IV). The highest cluster mean of number of primary branches was recorded in cluster VII (5.17) while lowest was observed in cluster IV (3.20). Number of secondary branches varied from 22.48 (cluster VII) to 8.75 (cluster IV). Cluster VIII (29.20 g) and cluster IX (14.72 g) recorded highest and lowest cluster mean of fruit weight respectively. Pulp weight ranged from 14.30 g (cluster VII) to 7.45 g (cluster IV). Seed weight ranged from 6.13 g (cluster V) to 3.13 g (cluster IV). Maximum and minimum shell weight was observed in cluster VII (5.16 g) to cluster IV (2.77 g). Highest vein weight cluster mean observed in cluster VII (1.95 g) and lowest cluster mean of vein recorded in cluster IV

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Diagonal values represent the intra cluster distance

Annual yield per tree (AYT), Tree height (THT), GBH (Girth at breast height), Crown cover area (CC), No. of primary branches (NPB), No. of secondary branches (NSB), Fruit length (FL), Fruit thickness (FT), Fruit width (FWH), Fruit weight (FWT), Pulp weight (PWT), Seed weight (SDWT), Shell weight (SLWT), Vein weight (VWT), No. of seed/fruit (NOS), Total soluble solids (TSS), Tartaric Acid (TAC), Ascorbic acid (AA), Total acidity (TA), Reducing sugar (RS), Non-Reducing sugar (NRS), Total sugar (TS), Protein and Carbohydrate (CHO).

(1.00 g). Maximum number of seeds per fruit cluster mean was observed in cluster VII (10.96), while minimum number of seeds per fruit was observed in cluster IV (5.65). Cluster VII (14.78 cm) and cluster IV (6.06 cm) recorded maximum and minimum value of fruit length cluster mean respectively. Highest fruit thickness was observed in cluster VI (6.84 cm) to cluster VIII (4.17 cm). Cluster mean of fruit width varied from 1.98 cm (cluster VI) to 1.22 cm (cluster IV).

For total soluble solids, the maximum cluster mean was observed in cluster VI (15.09 Brix) and minimum was observed in cluster VII (9.71 Brix) Maximum and minimum cluster mean of tartaric acid were observed in cluster X (18.06 %) and cluster IX (8.12 %). Highest cluster mean of ascorbic acid was recorded in cluster X (6.66 mg/g) while the lowest was found in cluster IX (3.18 mg/g). For total acidity cluster mean ranged from 16.00 % (cluster VIII) to 6.90 % (cluster IV). Cluster mean of total sugar ranged from 49.65 % (cluster VI) to 20.83 % (cluster VII). Reducing sugar recorded maximum and minimum value in cluster IX (36.68 %) and cluster X (15.06 %). Cluster VI (13.02 %) and cluster X (6.00 %) recorded highest and lowest mean of non-reducing sugar. The highest and lowest cluster mean of protein was recorded in cluster VI (2.69 mg/g) and cluster IX (2.09 mg/g). Maximum and minimum value of cluster mean of carbohydrate was observed in cluster IV (2.69 %) and cluster VII (1.44 %). Significant differences in the cluster means for all characters among the clusters were observed. This clustering pattern can be used to select parent crosses and determine cross combinations that may generate the highest variability for various traits. Similar findings were reported by Singh and Nandini (2014) and Divakara *et al*. (2012) in Tamarind and Rajan *et al*. (2009) in mango.

The analysis of variance revealed significant variation across morphometric and biochemical traits in Tamarind clones. High phenotypic and genotypic variations were observed in the key traits like annual yield per tree, fruit weight, and total sugars and demonstrate exceptional potential for improvement through targeted selection. High heritability paired with genetic advance were presented in in traits such as annual yield, tree height, and fruit weight and suggests additive gene effects making them ideal for breeding. Path analysis highlighted the strong direct impact of fruit weight, pulp weight, and seed weight on annual yield. Mahalanobis D-square analysis grouped the 60 tamarind clones into 10 clusters, showcasing remarkable genetic diversity. These insights provide a robust foundation for breeding programs to significantly enhance tamarind productivity and quality.

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